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Autocrine Interactions of Keratinocyte Growth Factor, Hepatocyte Growth Factor, and Kit-Ligand in the Regulation of Normal Ovarian Surface Epithelial Cells*

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ABSTRACT

Ovarian tumors are primarily derived from the layer of epithelium surrounding the ovary termed the ovarian surface epithelium (OSE). Although extensive research has focused on established ovarian tumors, relatively little is known about the normal biology of the OSE that gives rise to ovarian cancer. The local expression and actions of growth factors are likely involved in both normal and tumorigenic OSE biology. The current study investigates the expression and action of keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and kit-ligand (KL) in normal ovarian surface epithelium (OSE). The actions of various growth factors on KGF, HGF, and KL expression are examined. Observations indicate that freshly isolated normal OSE express the genes for KGF, HGF, and KL and expression is maintained *in vitro*. KGF messenger RNA expression in OSE was found to be stimulated by KGF and HGF, but not KL. HGF expression in OSE was found to be stimulated by KGF, HGF, and KL. KL expression in OSE was also found to be stimulated by KGF, HGF, and KL. Therefore, the various growth factors can regulate the mRNA expression of each other in OSE. Effects of growth factors on OSE growth were examined. KGF, HGF, and KL stimulated OSE growth to similar levels as the positive control epidermal growth factor. Observations suggest that KGF, HGF, and KL interact to promote OSE growth and growth factor expression. The ability of these growth factors to interact in a positive autocrine feedback loop is postulated to be important for normal OSE biology. Paracrine interactions with the adjacent stromal cells will also be a factor in OSE biology. Abnormal interactions of these growth factors may be involved in the onset and progression of ovarian cancer. (*Endocrinology* **141:** 2532–2539, 2000)

ALTHOUGH a small number of ovarian cancers originate from cells associated with the ovarian follicle, greater that 95% of ovarian cancers originate in the epithelial cells on the surface of the ovary (1). The epithelial cells that cover the surface of the ovary are most commonly referred to as the ovarian surface epithelium (OSE). The OSE is a modified mesothelium covering the surface of the ovary. It is a simple epithelium separated from underlying ovarian stromal tissue by a basal lamina. The OSE appears to contribute to the formation of various extracellular matrix components (2). During normal ovarian function, the OSE undergoes cyclic changes. It can release enzymes that contribute to the breakdown of the underlying stroma that are adjacent to the preovulatory follicle (3, 4). After ovulation, the OSE proliferates and covers the area affected by follicular rupture (5). The hypothesis has developed that repetitious ovulation contributes to the etiology of ovarian cancer through the altered cellular activity of the OSE at ovulation (6). Although extensive research has focused on established ovarian tumors, relatively little is known about the normal biology of the OSE that gives rise to ovarian cancer. The local expression and actions of growth factors are likely involved in normal OSE biology and may be involved in ovarian cancer. Three growth factors have recently been shown to be expressed by normal OSE and ovarian cancer cells: keratinocyte growth factor (KGF) (7), hepatocyte growth factor (HGF) (8), and kit-ligand (KL) (9). The receptors for these specific factors are also all expressed by the OSE (7–9). An understanding of the factors that regulate expression of KGF, HGF, and KL in OSE cells may help explain some of the factors involved in the normal OSE biology and development of ovarian cancer.

KGF is a 28-kDa protein that is a member of the fibroblast growth factor family (FGF-7) (10). KGF is primarily produced by stromal- or mesenchymal-derived cells in many tissues and acts as an epithelial cell-specific mitogen (11). KGF has not previously been shown to be expressed by epithelial cells. The receptor to KGF (KGFR) is a splice variant of the FGF receptor family (FGFR-2) that is primarily localized on epithelial cells (12, 13). In the ovary, KGF mediates cell-cell interactions between theca cells and granulosa cells (14). Theca cell KGF expression in the follicle can be regulated by estradiol and gonadotropins (14).

HGF is an 87-kDa protein composed of a 69-kDa α-subunit and a 34-kDa β-subunit that is important for the organogenesis and morphogenesis of various tissues and organs (15, 16). HGF is primarily produced by mesenchymal-derived cells in many tissues and acts as an epithelial-cell-specific mitogen. The receptor to HGF (HGFR) is the product of the c-met proto-oncogene (p190MET) that is primarily localized to epithelial cells (17, 18) but can also be expressed by macrophages, neurons, endothelial cells, muscle cells, and cytotrophoblasts (19, 20). In the ovary, HGF mediates cell-cell

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interactions between theca cells and granulosa cells and expression can be regulated by estradiol and the LH-like factor human CG (hCG) (21). Overexpression of HGF or its receptor, c-met, has been observed in tumors from a variety of organs (22–24). A subset of ovarian cancers express high levels of the HGF receptor c-met (17, 25). HGF can stimulate motility, chemotaxis, and mitogenesis in ovarian carcinoma cells that over-express c-met (26) and may provide a selective growth advantage to these cells. Expression of c-met has been studied in ovarian cancer, but expression of HGF in ovarian tumors has been limited. In addition, little information is available on the expression and action of HGF in normal OSE biology (8).

The KL (also named stem cell factor, mast cell factor, or steel factor) and its tyrosine kinase receptor c-kit are encoded at the steel (SI) and white spotting (W) loci of the mouse, respectively (27, 28). In adult ovaries, KL is expressed in granulosa cells and has been shown to be important for oocytes (29, 30) and theca cells (31) during follicular development. Because OSE cells and granulosa cells are thought to arise from a common cellular lineage, KL may also be important for OSE cells. KL can have a wide range of activities on germ cells, melanocytes, mast cells, and primitive hematopoietic cells of the myeloid, erythroid, and lymphoid cell linages (32). Many of these cell types initiate their developmental program and differentiate in response to KL. It also appears that KL can cause many of these cell types to proliferate. KL and c-kit also play a role in several human cancers such as lung (33), thyroid (34), breast (35), and testicular malignancies (36). Coexpression of KL and c-kit has been reported in gynecological tumors including serous adenocarcinomas and germ cell tumors of the ovary (37). The potential role of KL and c-kit in normal OSE biology and in the onset of ovarian cancer remains to be elucidated.

Studies involving OSE have been limited by the lack of an efficient experimental model. Normal OSE of the rat, rabbit, and human have been isolated and cultured (38–40), but the size and availability of tissue often limit the use of these models. Bovine ovaries present a useful model for normal OSE biology. The bovine ovary has a similar physiology and size as the human ovary. The cow is a mono-ovulator that ovulates regularly and has an ovarian cycle similar to that of the human. Bovine ovarian cancer has been reported (41) suggesting that bovine OSE has similar tumorigenic potential as human OSE. Bovine ovarian tumors also appear of epithelial origin. Therefore, the bovine ovary provides a useful model for examining the factors that regulate normal OSE (42). Once information is established in the bovine model, these studies can be compared with human OSE and ovarian tumor cells.

The current study was designed to examine the factors that regulate expression and interactions of KGF, HGF, and KL in bovine ovarian surface epithelium. The hypothesis is tested that locally produced growth factors interact in a positive feedback autocrine manner to regulate normal OSE. Information regarding the regulation of KGF, HGF, and KL expression may provide insight into the factors that regulate normal OSE biology and ovarian tumor progression.

Materials and Methods

Ovarian surface epithelium (OSE) cell isolation and cell culture

Bovine ovaries were obtained from young nonpregnant cycling heifers less that 10 min after slaughter. Ovaries were shipped on ice. OSE cells were isolated fresh (for QRT-PCR studies) or cultured(for QRT-PCR and growth studies). OSE cells were scraped from the surface of the ovary with a rubber policeman as previously described (42, 43). Sheets of epithelial cells were suspended in HBSS and then pelleted and washed before suspension for plating. Cells were plated with an initial density of approximately 10^6 cells/2 cm^2, and were maintained at 37 C in 5% CO2 atmosphere in Ham's F-12 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% calf serum. Medium was changed every 48–72 h. Once the cells had grown to confluence, the cells were trypsinized and split into appropriate plates. For isolation of RNA from cultured cells, OSE cells were plated in 100 mm large culture plates (Nunc, Noskilde, Denmark) and maintained in Ham's F-12 supplemented with 10% calf serum. The purity of OSE isolated by this procedure is greater than 98% by keratin staining with no detectable stromal contamination (42).

For analysis of growth factor treatment of KGF, HGF, and KL expression, OSE cells were plated in six-well plates Ham's F-12 supplemented with 10% calf serum. When cells achieved confluence, cells were washed in Ham's F-12 and maintained in Ham's F-12 in the absence of calf serum. OSE cells were treated with no growth factor (control, 50 ng/ml KGF, 50 ng/ml HGF, 50 ng/ml KL, or 50 ng/ml basic FGF (bFGF) (R&D Systems Inc.). Treated cells were cultured for 48 h and harvested for total RNA.

RNA preparation

Total RNA was prepared from freshly isolated or cultured cells using Trizol reagent (Life Technologies, Inc.). Trizol was added directly to freshly isolated cells or 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.* IB 15) 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 (14, 21, 31). 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 GGGGTA 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′-GGACCA 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; 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. These primers were found not to generate nonspecific PCR products under the conditions used. These primers were designed to span an intron to eliminate the possibility of nonspecific priming to residual genomic DNA. Before RT tubes containing total RNA and specific 3′-primers were heated to 65 C for 10 min to facilitate denaturing and cooled to room temperature to facilitate annealing. Total RNA (1 μg) was reverse transcribed for 1 h at 37 C using to following conditions: 1 μg total RNA, 1 μM specific 3′-primers of interest (up to 4 different primers including 1B15), 0.1mMd NTP, 10 mM DDT, 40 U RNase inhibitor (Promega Corp., Madison, WI), and 200 Units M-MLV reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD) in 40 μl RT buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 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 DNA's (*i.e.* Bluescript) containing bovine KGF, HGF, KL, or 1B15 subclones were used to generate standard curves from 1 attogram/μl (10^{-15}) to 10 pg/μl (10×10^{-9}) each containing 10 ng/μl Bluescript carrier DNA. Identical 10 μl aliquots of each sample and standard were pipetted in duplicate in a 96 well reaction plate (Marsh Biomedical Products, Rochester, NY) and sealed with adhesive film (Marsh Biomedical Products) for PCR amplification. But this design it was possible to simultaneously assay 5 known standard concentration and 40 unknown samples for each gene. Amplification was preformed in a Perkin-Elmer Corp. 9600 equipped with a heated lid using the following conditions: 0.4 μM each primer, 16 μM dNTPs, and 1.25 U AmpliTaq polymerase in 50 μl GeneAmp PCR Buffer (containing 1.5 mM Mg Cl_2, Perkin-Elmer Corp.). 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 of ^{32}P-labeled dCTP (Redivue, Amersham Pharmacia Biotech, 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, Inc., 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.

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).

Growth assays

Cell growth was analyzed by quantifying (^{3}H) thymidine incorporation into newly synthesized DNA. OSE cells were plated at approximately 50% confluence in 0.5 ml DMEM containing 0.1% calf serum. After 48 h, cells were treated with no growth factor (control), 50 ng/ml KGF, 50 ng/ml HGF, 50 ng/ml KL, 40 ng/ml epidermal growth factor (EGF), or 10% calf serum (10% CS). Cells were plated for 48 h and then treated for 20 h. After treatment, 0.5 ml DMEM containing 2.5 μCi (^{3}H) thymidine was added to each well, and the cells were incubated for 4 h at 37 C and the sonicated. The quantity of (^{3}H) thymidine incorporated into DNA was determined, as previously described (42). Data were normalized to total DNA per well using an ethidium bromide procedure previously described.

Statistical analysis

All data were analyzed by a JMP 3.1 statistical analysis program (SAS Institute, Inc., Cary, NC). Effects of growth factors on (^{3}H) thymidine incorporation into DNA and growth factor expression as well as differences between fresh *vs.* cultured OSE growth factor expression were analyzed by a one-way ANOVA. Observed significance probabilities of 0.05 (Prob > 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 the Dunnett's test which guards against the high α-size (Type I) error rate across the hypothesis. Significant differences among fresh *vs.* cultured OSE growth factor expression were determined using the Tukey-Kramer HSD (honestly significant difference) test, which protects the significance tests of all combinations of pairs (44). Data were obtained from a minimum of three different experiments performed in replicate with three different cell preparations.

Results

Gene expression of KGF, HGF, and KL in normal OSE cells isolated from bovine ovaries was examined using sensitive quantitative RT-PCR assays (14, 21, 31). Total RNA was isolated from 8 to 12 different preparations of normal OSE that were freshly isolated or cultured. Previously, the cultured OSE have been shown to be viable with no change in cell number under the culture conditions used (42). Samples were reverse transcribed using the specific 3′ primers of the KGF, HGF, KL, and cyclophilin (*i.e.* 1B15) genes. Samples were then simultaneously amplified by PCR along with known KGF, HGF, KL, or 1B15 standard plasmids to quantitate gene expression. Steady-state levels of mRNA expression were determined and normalized for potential the constitutively expressed gene cyclophilin. Normalization for 1B15 expression corrected for potential changes in cell number, for the amount and the integrity of initial mRNA and for small difference in the efficiency of RT between samples. Gene expression for KGF, HGF, and KL was observed in freshly isolated and cultured OSE cells (Fig. 1, A–C). The level of KGF expression was similar between fresh OSE cells and cultured OSE cells, but steady-state KGF expression was slightly elevated in cultured cells (Fig. 1A). No difference in HGF and KL expression was observed between freshly isolated and cultured OSE cells (Fig. 1, B–C). A comparison of the levels of expression in relation to 1B15 expression demonstrated KGF mRNA levels were approximately 100-fold higher than HGF and 10-fold higher than KL. These results demonstrate that OSE cells express appreciable levels of the KGF, HGF, and KL genes that are maintained in cell culture. This extends previous observations of the expression of KGF, HGF, and KL by OSE using Northern blots and immunocytochemistry (7–9). Therefore, cultured bovine OSE cells provide a useful model for studying the expression and potential action of KGF, HGF, and KL in normal OSE.

The ability of KGF, HGF, and KL to influence the growth of bovine OSE cells was investigated. Subconfluent OSE cells were treated with KGF, HGF, and KL for 20 h followed by a 4 h incubation with (^{3}H) thymidine. Cells were treated with 40 ng/ml EGF or 10% bovine calf serum as positive controls. KGF, HGF, and KL were all found to stimulate DNA synthesis in bovine OSE (Fig. 2) to similar levels as EGF. The combined treatment of KGF, HGF, and/or KL was found to be additive up to the level of stimulation observed with 10% calf serum, but not surpass the 10% calf serum stimulation (data not shown). The stimulation of OSE growth was confirmed by an increase in OSE cell number after a 72-h treatment with the growth factors (data not shown) (42). Therefore KGF, HGF, and KL can act as growth factors for normal OSE. The ability of these growth factors to promote the growth of OSE suggests that KGF, HGF, and KL may be involved in the normal growth of OSE.

The ability of OSE cells to express and respond to KGF, HGF, and KL suggests that these factors may provide autocrine stimulation of OSE cells. Effects of KGF, HGF, and KL on OSE gene expression were investigated. OSE cells were grown to confluence in six-well plates and maintained in Ham's F-12 in the absence of calf serum. OSE cells were treated with no growth factor (control), 50 ng/ml KGF, 50

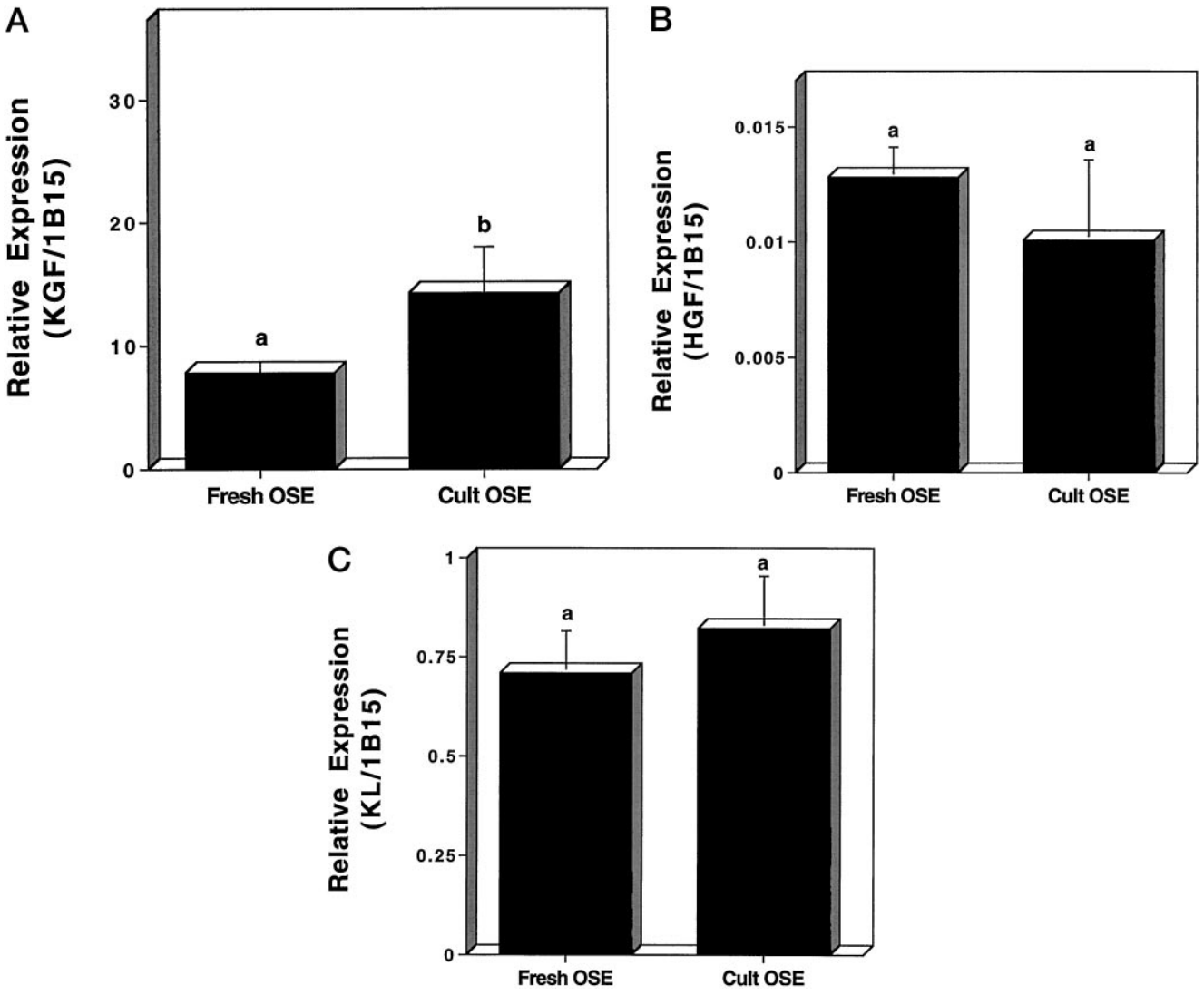

FIG. 1. Quantitation of steady-state KGF mRNA expression in OSE cells. Bovine OSE cells were isolated fresh or grown to confluence as described in *Materials and Methods*. Total RNA was extracted from freshly isolated cells or cultured cells. Steady-state levels of KGF (A), HGF (B), and KL (C) mRNA were determined in replicate using quantitative RT-PCR as described in *Materials and Methods*. Levels of KGF, HGF, and KL mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (*e.g.* fg KGF mRNA/fg 1B15 mRNA). Data are presented as the mean ± SEM of duplicate determinations from 8–12 different sets of RNA from each cell type. An ANOVA was performed and significant differences among cell types were determined using the Tukey-Kramer HSD test. *Bars with different superscript letters* differ from each other ($P < 0.05$).

ng/ml HGF, 50 ng/ml KL, or 50 ng/ml bFGF. Treated OSE cells were cultured for 48 h and harvested for total RNA. The OSE cell number does not change under these conditions due to the serum-free culture conditions. Gene expression for KGF, HGF, and KL were determined using quantitative RT-PCR as described in *Materials and Methods*. Both KGF and HGF stimulated KGF gene expression (Fig. 3). In contrast, KL and bFGF had no effect on KGF gene expression in OSE. HGF gene expression was stimulated by KGF, HGF, and KL (Fig. 4). No effect was observed on HGF gene expression by bFGF. All treatments stimulated KL gene expression including KGF, HGF, KL, and bFGF (Fig. 5). These results demonstrate that locally produced growth factors can stimulate expression of other growth factors. The interactions between these growth factors appear to act at least in part as a positive feedback loop (Fig. 6). Interactions *in vivo* will also likely involve paracrine interactions between OSE and adjacent stromal cells.

Discussion

The autocrine actions of growth factors to stimulate OSE proliferation have been proposed to be involved in the onset and progression of ovarian cancer (45). The local production of these growth factors may also be important for the normal biology of the OSE. The current study demonstrates normal OSE cells express the genes KGF, HGF, and KL that may be important for normal OSE growth. Other growth factors are also produced by OSE cells including bFGF and transforming growth factors. The results in the current study suggest that

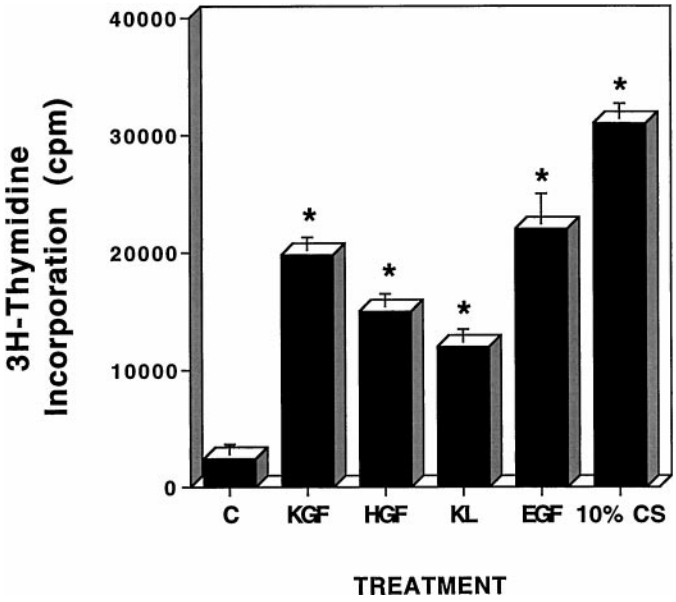

FIG. 2. Regulation of DNA synthesis in bovine OSE cells. Bovine OSE cells were isolated as described in *Materials and Methods*. Cells were deprived of serum when approximately 70% confluent ^{3}H-thymidine incorporation into DNA was determined after 20 h of treatment with 50 ng/ml KGF, 50 ng/ml HGF, 50 ng/m; KL, 40 ng/ml EGF or 10% bovine calf serum (CS). Cells were incubated with ^{3}H-thymidine for 4 h. Data are representative of four different experiments done in replicate. An ANOVA was performed and significant differences from control were determined using the Dunnett's test. *Bars with asterisks* differ from control ($P < 0.05$).

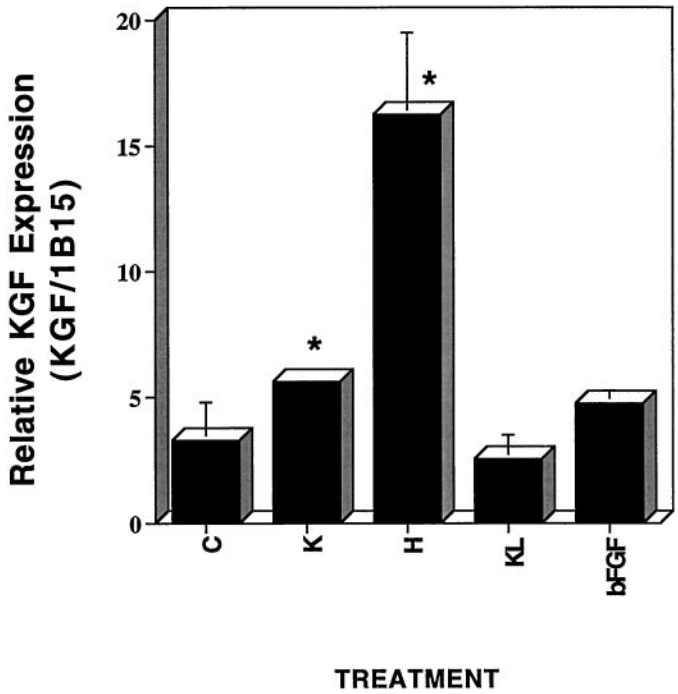

FIG. 3. Regulation of KGF mRNA expression in OSE cells. Bovine OSE cells were grown to confluence as described in *Materials and Methods*. Cells were deprived of serum and treated with 50 ng/ml KGF, 50 ng/ml HGF, 50 ng/ml KL, or 50 ng/ml bFGF for 48 h. Steady-state levels of KGF mRNA were determined in replicate using quantitative RT-PCR as described in *Materials and Methods*. Levels of KGF mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (fg KGF mRNA/fg 1B15 mRNA). Data are presented as the mean ± SEM of duplicate determinations from duplicate wells. Data are representative of three different experiments. An ANOVA was performed and significant difference from control were determined using the Dunnett's test. *Bars with asterisks* differ from control ($P < 0.05$).

all of these growth factors can interact in a feedback loop that may be important for normal OSE functions (Fig. 6), as well as ovarian cancer.

Growth control of both normal and tumorigenic OSE is a critical cellular parameter to consider in understanding ovarian cancer. The majority of information available on ovarian growth factors relates to the developing ovarian follicle (46). Several growth factors, however, have been shown to influence OSE. Normal OSE cells express the EGF receptor, and a large number of tumorigenic OSE cells also express the EGF receptor (47 48). EGF can stimulate the proliferation of normal human OSE (48) and bovine OSE (42) cells. Transforming growth factor-α (TGFα) has been associated with ovarian cancer and may act as an autocrine growth factor to induce cell proliferation in both normal and tumorigenic OSE (49). bFGF and its receptor are expressed by human ovarian epithelial neoplasms (50) suggesting that bFGF may also regulate ovarian cancer proliferation through an autocrine mechanism. Several ovarian cancer cell lines proliferate in response to bFGF (51). TGFβ is a multifunctional protein that has a major role in inhibiting the actions of growth stimulators such as EGF/TGFα, bFGF, and KGF. TGFβ has been shown to be produced by OSE (52), and TGFβ can inhibit the growth of normal OSE cells and some tumorigenic OSE cells (51, 52). In the current study, OSE cells were stimulated to grow by KGF, HGF, and KL treatments. These results suggest that these growth factors may be important for the normal growth cycle of OSE cells. Local expression of KGF, HGF, and KL by OSE cells (7–9) provides an autocrine stimulation of OSE growth that may be involved in the development and progression of ovarian cancer. This autocrine feedback loop suggested that these and other growth factors may interact to promote growth factor expression and regulate OSE proliferation. *In vivo* the paracrine interactions between OSE and the adjacent stromal cells will also likely be involved in the regulation of OSE proliferation (Fig. 6).

Analysis of KGF, HGF, and KL expression provided several significant observations. KGF gene expression was stimulated by KGF and HGF, but not by KL or bFGF. HGF gene expression was stimulated by all growth factors except bFGF. KL gene expression was stimulated by all the growth factors examined. Interestingly, KGF, HGF, and KL were able to stimulate expression of themselves. All of these growth factors have the ability to stimulate OSE growth. The inability of KL to influence KGF mRNA levels, while KGF and HGF did stimulate expression suggests the signal transduction of the specific receptors are distinct and regulate OSE differently. Similar observations can be made with the ability of bFGF to stimulate KL, but not KGF or HGF expression. The distinct growth factors and their cellular signaling creates a complex network of factors and signaling in the regulation of OSE growth. Elucidation of the details of how this network of factors control OSE cells will provide insight into normal OSE biology and develop a better understanding of how

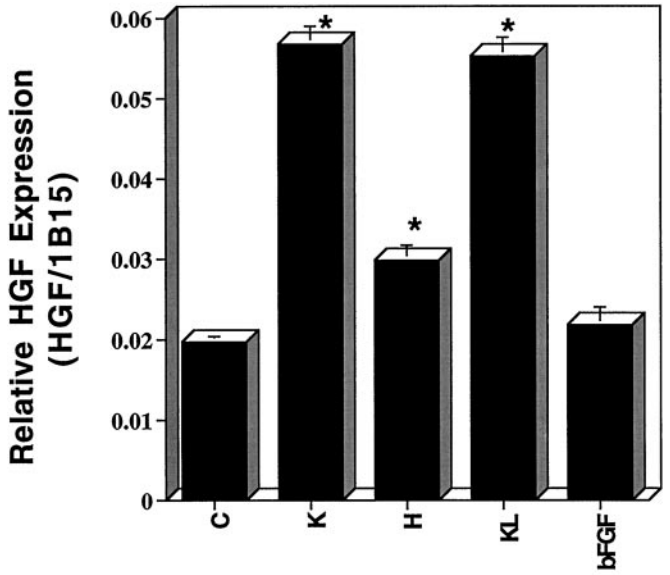

FIG. 4. Regulation of HGF mRNA expression in OSE cells. Bovine OSE cells were grown to confluence as described in *Materials and Methods*. Cells were deprived of serum and treated with 50 ng/ml KGF, 50 ng/ml HGF, 50 ng/ml KL, or 50 ng/ml bFGF for 48 h. Steady-state levels of HGF mRNA were determined in replicate using quantitative RT-PCR as described in *Materials and Methods*. Levels of HGF mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (fg HGF mRNA/fg 1B15 mRNA). Data are presented as the mean ± SEM of duplicate determinations from duplicate wells. Data are representative of three different experiments. An ANOVA was performed and significant differences from control were determined using the Dunnett's test. *Bars with asterisks* differ from control ($P < 0.05$).

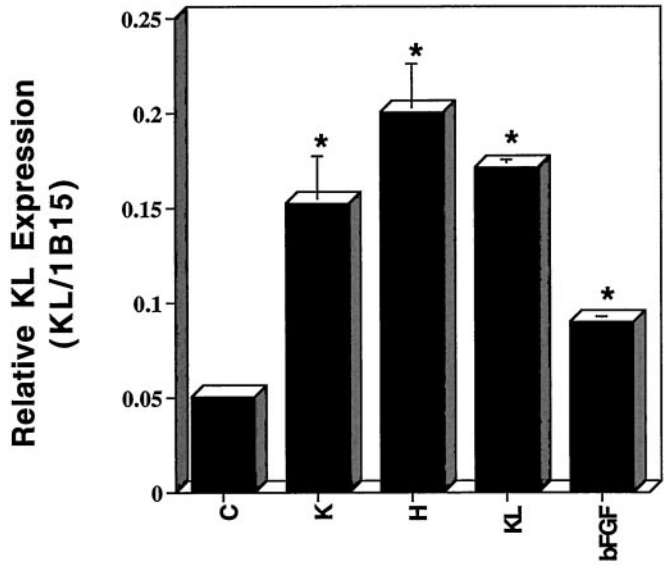

FIG. 5. Regulation of KL mRNA expression in OSE cells. Bovine OSE cells were grown to confluence as described in *Materials and Methods*. Cells were deprived of serum and treated with 50 ng/ml KGF, 50 ng/ml HGF, 50 ng/ml KL, or 50 ng/ml bFGF for 48 h. Steady state levels of KL mRNA were determined in replicate using quantitative RT-PCR as described in *Materials and Methods*. Levels of KL mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (fg KL mRNA/fg 1B15 mRNA). Data are presented as the mean ± SEM of duplicate determinations from duplicate wells. Data are representative of three different experiments. An ANOVA was performed and significant differences from control were determined using the Dunnett's test. *Bars with asterisks* differ from control ($P < 0.05$).

alterations in this regulatory pathway may be involved in the onset and progression of ovarian cancer. It is important to clarify that the analysis of mRNA levels for the growth factors does not directly correlate to protein production. Therefore, the elucidation of OSE biology requires a wider variety of growth factors to be examined at both the gene expression (*i.e.* mRNA levels) and protein levels. The current study suggests that a network of local autocrine and paracrine interactions is likely involved in the control of OSE biology.

The expression of both KGF and HGF by this "epithelial" cell may be a significant marker of this unusual phenotype. The biology of KGF and HGF has demonstrated that KGF and HGF expression are limited primarily to cells of mesenchymal or stromal origin. OSE cells are modified peritoneal mesothelial cells that are derived from the coelomic epithelium which overlies the gonadal ridge in the embryo (53). Therefore, the OSE is of mesodermal origin and developmentally is closely related to the underlying stromal fibroblasts. The OSE has characteristics of a classic epithelial cell such as keratin, mucin, desmosomes, apical microvilli, and basal lamina. However, OSE cells also coexpress vimentin, a mesenchymal intermediate filament protein. During postovulatory repair, OSE cells reversibly modulate to a more fibroblast-like form. In culture, these cells produce epithelial (*e.g.* laminin and collagen type IV) and mesenchymal (*e.g.* collagen types I and III) components of extracellular matrix (54). A variety of environmental cues cause OSE cells to change from an epithelial to mesenchymal morphology

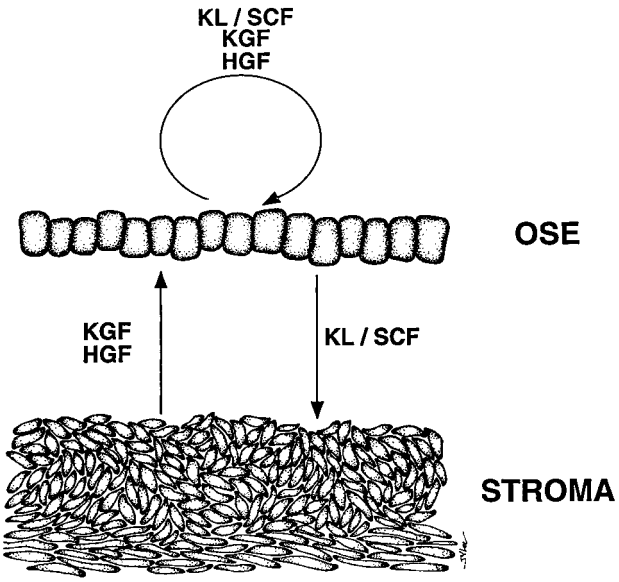

FIG. 6. Schematic of growth factor interactions. The proposed autocrine and paracrine interactions between OSE and ovarian stromal cells involving KGF, HGF, and KL/stem cell factor.

(40, 55). Thus, these cells may be relatively immature, uncommitted cells which express a dual, epithelio-mesenchymal phenotype (55). The uncommitted differentiated state of this cell may be a factor in its susceptibility to become transformed and develop tumors.

A significant observation in the current study is the ability of OSE cells to not only express KGF, HGF, and KL, but to respond to these growth factors in a autocrine manner (Fig. 6). Another significant observation is the ability of several growth factors to regulate expression of KGF, HGF, and KL in OSE cells. This observation is proposed to represent a general model of growth factor expression and action on OSE cells. Autocrine stimulation of growth, as well as growth factor expression, may be important for normal OSE biology and suggests this cell may be somewhat self sufficient in its growth regulation. Although paracrine interactions with adjacent stromal cells will likely be critical, OSE have the capacity to become independent of stromal control. The growth factor receptors also may be a factor in the regulation of OSE and will need to be considered in future analysis. Abnormal regulation of growth and growth factor expression is postulated to be involved in the development and progression of ovarian cancer. The current study establishes the concept that locally produced growth factors may interact in a complex feedback loop that affects both OSE growth and differentiated functions (*i.e.* growth factor expression).

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