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Characterization of Bovine Ovarian Surface Epithelium and Stromal Cells: Identification of Secreted Proteins[1]

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ABSTRACT

The majority of ovarian cancers are derived from a single layer of epithelial cells that covers the surface of the ovary termed the ovarian surface epithelium (OSE). Ovarian surface stromal cells underlie the OSE and have a morphology distinct from that of the interstitial stromal cells between follicles. Because of the similarities between bovine and human ovarian physiology, and to allow an adequate supply of tissue and cells, bovine OSE and stromal cell cultures were established to investigate the cell biology of these cell types. Morphological analysis of bovine ovarian sections revealed that several layers of ovarian surface stromal cells can be identified and are structurally distinct from interstitial stromal cells. Both OSE and stromal cells can be isolated and cultured for weeks in the absence or presence of serum. The cell populations were found, through use of a keratin immunocytochemical stain for OSE, to be highly purified. To investigate the functional properties of the two cell types, radiolabeled secreted proteins were collected and electrophoretically analyzed. The radiolabeled secreted protein profiles of OSE and stromal cells were found to be distinct with a discrete number of secreted proteins. Major OSE secretory products were obtained from serum-free concentrated conditioned medium, electrophoretically separated, blotted, and sequenced. Two OSE secretory products of 28 kDa and 40 kDa were sequenced and found to match a sequence in the computerized database. The 28-kDa OSE protein was identified as a tissue inhibitor of metalloproteinase, TIMP. The 40-kDa OSE protein was identified as the insulin-like growth factor (IGF) binding protein-2 (IGFBP2). The TIMP may play a role in regulating the local proteolytic activity of the OSE, and IGFBP2 in regulating IGF-I actions on OSE. These proteins provide biochemical markers for OSE that can be used to further investigate the regulation of OSE function. The growth properties of cultured bovine OSE were also investigated. OSE proliferation was stimulated by epidermal growth factor (EGF) and was not influenced by keratinocyte or hepatocyte growth factors. Transforming growth factor-β was found to inhibit the growth stimulatory actions of EGF on OSE. Concentrated serum-free stromal cell-conditioned medium was also found to influence OSE growth. Therefore, the ovarian surface stromal cells appear to produce factors that can regulate the growth of the OSE. Combined results indicate that a culture system has been established to investigate the biology of OSE and ovarian surface stromal cells. Two OSE secretory products were identified as TIMP and IGFBP2. Preliminary data are presented that the ovarian surface stromal cells can regulate OSE growth. This stromal-epithelial cell interaction may have an important role in the normal cell biology of the OSE and be involved in the transformation of this cell to form an ovarian cancer.

INTRODUCTION

Ovarian cancer accounts for approximately 6% of cancer deaths among women in the United States. This year more than 20 000 cases of ovarian cancer will be diagnosed, and greater than 13 000 women will die of this disease. The incidence of ovarian cancer has increased continuously, and it is now the most common cause of death from gynecologic cancer (52%) [1]. Although a small number of ovarian cancers originate from cells associated with the ovarian follicle, greater than 90% of ovarian cancers originate in the epithelial cells on the surface of the ovary [1, 2].

The epithelial cells that cover the surface of the ovary have been most commonly referred to as the ovarian surface epithelium but have also been termed the ovarian germinal epithelium and ovarian epithelial cells. Throughout this report, these cells will be referred to as the ovarian surface epithelium (OSE). The OSE is a modified mesothelium that overlies the ovary. It is a simple epithelium separated from underlying ovarian stromal tissue by a basal lamina of dense collagenous connective tissue [3]. The OSE appears to contribute to the formation of various extracellular matrix components [4]. The OSE undergoes cyclic changes that are important in normal ovarian function. It can release enzymes that contribute to the breakdown of the underlying stroma that overlies the preovulatory follicle [5, 6]. After ovulation, the OSE proliferates and covers the area affected by follicular rupture [7]. Recently, the hypothesis has been proposed that repetitious ovulation contributes to the etiology of ovarian cancer through the altered cellular activity of the OSE at ovulation [8].

The alteration in OSE function and growth at ovulation implies that cellular association with the underlying stroma influences the OSE. Both OSE and stroma contribute to the extracellular matrix that separates the two cell types [4]. The cellular associations between OSE and stroma also have been shown to influence the intermediate filaments in the OSE that may be compared with the early stages of neoplastic progression [9]. Tumorigenic tissue derived from the OSE also has close associations with stromal tissue. Tumor invasion often requires an association with host stromal tissue, and most ovarian tumors have a stromal-like component [10, 11]. Therefore, stromal-epithelial interactions may have a critical role in the function and growth of normal

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and tumorigenic OSE. This stromal-epithelial interaction remains to be defined.

In spite of its clinical importance, studies of cellular interactions involving OSE and their role in ovarian carcinogenesis have been limited by the lack of an experimental system. OSE of the rat, rabbit and human has been isolated and cultured [12–15]. The size and amount of tissue are factors limiting the use of these models. Therefore, in the current study an alternative bovine animal model was developed. The bovine ovary has essentially the same physiology and size as the human ovary. The cow is a monoovulator that ovulates regularly and has an ovarian cycle similar to that of the human. Bovine ovarian endocrinology is also similar to that of the human. Importantly, bovine ovarian cancer has been documented [16]. A large amount of fresh tissue can be obtained routinely from local abattoirs. The bovine ovarian cell types can easily be isolated and cultured to maintain their hormone responsiveness [17–19]. Bovine ovarian cells also can be isolated to examine the gene expression of growth factors [20, 21]. These bovine cells proliferate in culture in response to appropriate growth factors [20–22]. Therefore, the bovine ovary provides a useful animal model for investigating and establishing specific cell-cell interactions involving normal OSE. Once these cellular interactions have been established, it will be essential to compare the observations with those of human OSE and ovarian tumor cells. However, the large amount of bovine tissue available for cellular and molecular studies will be useful to efficiently develop an understanding of the normal OSE.

The current study was designed to establish cultures of bovine ovarian surface epithelial cells and stromal cells. A functional analysis of the cells was investigated through the identification of secreted proteins. The growth properties of OSE were also investigated. An in vitro system has been established that will facilitate the investigations of stromal-epithelial interactions that regulate OSE.

MATERIALS AND METHODS

Tissue Isolation and 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, Inc. (Fresno, CA). OSE cells were scraped from the surface of the ovary with a rubber policeman as previously described [15]. Sheets of epithelial cells were suspended in Hanks' buffered salt solution and then pelleted and washed prior to suspension for plating. After the removal of OSE cells, the ovarian surface stromal cells were microdissected from areas of the ovary devoid of follicles. A section of surface stromal cells 1–2-mm wide by 5–8-mm long and 1-mm deep was collected. Only the stromal cells on the surface of the ovary were collected. The tissue piece was minced and digested with 1 mg/ml collagenase and 1 mg/ml hyaluronidase for 2 h at 37°C or 18 h at 4°C. Cells were plated with an initial density of approximately 10^6 cells/2 cm^2, and were maintained at 37°C in a 5% CO_2 atmosphere in Ham's F-12 (Gibco Labs., Grand Island, NY) supplemented with 10% calf serum. Medium was changed every 48–72 h. Once the cells had grown to confluence, the serum was removed and the cells were washed repeatedly with serum-free medium. Cells subsequently were maintained under serum-free conditions for 2–3 wk with 48–72 h medium collections. If required, serum-free conditioned medium was concentrated 100-fold by ultrafiltration with a 3-kDa exclusion limit membrane.

Gel Electrophoresis

Radiolabeled conditioned medium from cell cultures was collected after 1–3 days of serum-free culture. When required, the medium was concentrated by ultrafiltration using a 3-kDa exclusion limit membrane. Electrophoresis was performed on 7.5–15% gradient polyacrylamide gels under reducing conditions with use of the Laemmli sodium dodecylsulfate buffer system [23]. For radiolabeling, cells were maintained in cystine- and methionine-free medium containing 5 μCi/ml [^{35}S]methionine and 5 μCi/ml [^{35}S]cystine. Gels were fluorographed with diphenyloxazole in acetic acid, as previously described [24].

Peptide Microsequencing

Concentrated serum-free OSE-conditioned medium (100×) was mixed with radiolabeled OSE-conditioned medium, electrophoresed, and transferred to polyvinyldene fluoride membrane (PVDF; BioRad, Richmond, CA) in 10 mM CAPS (3-[cyclohexylamino-propane-sulfonic acid]) buffer, pH 11.0) and 10% methanol. Transferred proteins were stained in 0.1% Coomassie Blue R-250 dye in 50% methanol and destained in 40% methanol and 10% acetic acid. Bands of interest were cut out and microsequenced on a Beckman-Porton PI 2090 peptide sequencer using the Edman degradation procedure [25]. Sequences were searched for homology in PIR/Swiss-Prot protein data banks. Coomassie staining was correlated with the presence of radioactivity by determining the presence of radioactivity in adjacent sections in a scintillation counter.

Growth Assays

Cell growth was analyzed by quantifying [^{3}H]thymidine incorporation into newly synthesized DNA. OSE cells were plated (approximately 1 million cells/cm^2 providing 50% confluence) in 0.5 ml DMEM medium containing 0.1% calf serum. After 48 h, cells were treated with no growth factor (control), 10–100 ng/ml keratinocyte growth factor (KGF), 5–50 ng/ml hepatocyte growth factor (HGF), 100 ng/ml basic fibroblast growth factor (bFGF), or 10–40 ng/ml epidermal growth factor (EGF). Cells were plated for 48 h and then treated for 24 h. After treatment, 0.5 ml DMEM con-

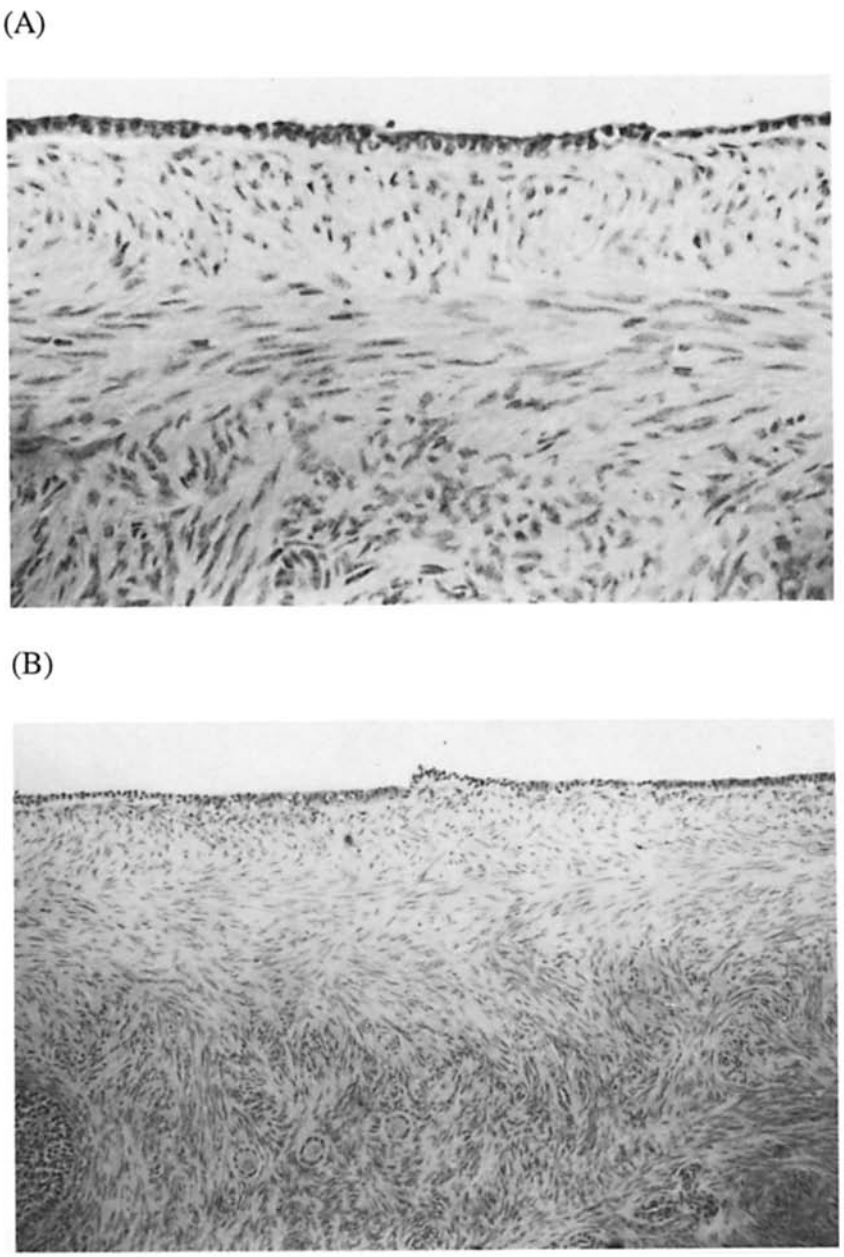

FIG. 1. Morphology of surface of bovine ovary. Paraffin sections of bovine ovary were stained with hematoxylin and eosin and photographed at ×25 (**A**) and ×16 (**B**) magnification. A single layer of OSE on surface of ovary is adjacent to layers of stromal cells. Data are representative of five different experiments.

taining 2.5 μCi [^{3}H]thymidine was added to each well, and the cells were incubated for 4 h at 37°C and then sonicated. The quantity of [^{3}H]thymidine incorporated into DNA was determined, as previously described [26]. Data were normalized to total DNA per well using an ethidium bromide procedure previously described [18].

Histology and Histochemistry

Cells were plated in 1-ml multi-well dishes over plastic coverslips (Thermanox, 15 mm round; Lux, Vangard, Neptune, NY). Optimal cell counts were obtained when cells were plated at approximately 1×10^6 cells/well. For hematoxylin and eosin staining, cells were fixed with 4% buffered formalin. The rinsed cells were then stained with Gill hematoxylin (Fisher, Atlanta, GA), rinsed with tap water, and counterstained with acidic eosin. The stained cells were dehydrated through graded ethanol washes, cleared with xylene, and mounted onto glass slides with Permount (Fisher).

Keratin immunocytochemistry was performed according to an immunoperoxidase technique. Briefly, cells were fixed with cold methanol and then incubated for 30 min in 0.3% H_2O_2 in methanol, for 30 min in PBS with 1% horse serum

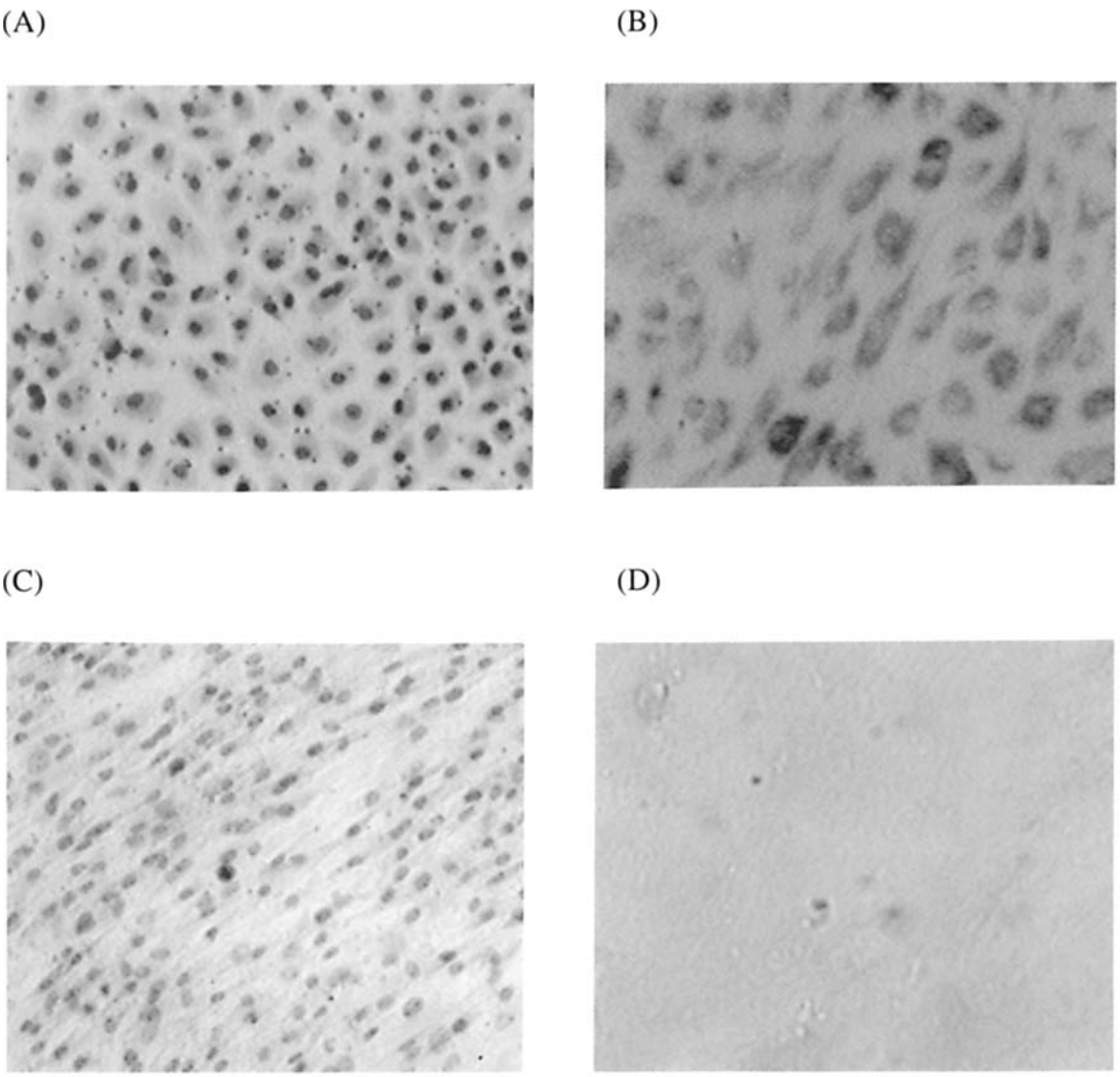

FIG. 2. Morphology and keratin immunocytochemistry of bovine OSE (**A** and **B**) and stromal cells (**C** and **D**) in culture. Hematoxylin and eosin stain of cultured OSE (**A**) and stromal cells (**C**); keratin immunocytochemistry (higher magnification) of OSE (**B**) and stromal cells (**D**). Data are representative of five different experiments. Magnification = 10×.

(heat-inactivated) and for 1 h with rabbit polyclonal keratin antibody (DAKO, Capintena, CA). Incubations (30–60 min) with biotinylated goat anti-rabbit IgG (1:250) in PBS and Vectastain ABC Reagent (Vector, Burlingame, CA) were followed by an incubation with the peroxidase substrate (0.5 mg/ml diaminobendzydine tetrahydrochloride prepared in 50 mM Tris buffer, pH 7.6, with 0.03% H_2O_2) until reaction product was visualized. The rinsed cells were then counterstained with Gill hematoxylin and rinsed with tap water. The cells were rinsed extensively with PBS between incubations. The cells were dehydrated, cleared, and mounted as outlined above. Keratin-positive cells are stained brown.

DNA and Protein Assays

DNA was measured fluorometrically with ethidium bromide as previously described [22]. An aliquot of the sonicated cell suspension was added to an equal volume of ethidium bromide solution (0.25 mM ethidium bromide, 100 U/ml heparin in ethidium bromide buffer [EBB:20 mM sodium chloride, 5 mM ethylene diamine tetracetate, 10 mM Tris, pH 7.8]; Sigma), was diluted 1:2 with EBB, and was allowed to incubate at room temperature for 30 min. Fluorescent emission at 585 nm with 350 nm excitation was then monitored. A standard curve with calf thymus DNA was used to quantify DNA levels in the culture wells. This assay has a sensitivity of approximately 0.1 μg DNA. Total protein concentration was measured according to the method of Bradford [27].

RESULTS

The morphology of the bovine ovary is very similar to that of the human ovary. The surface morphology of the

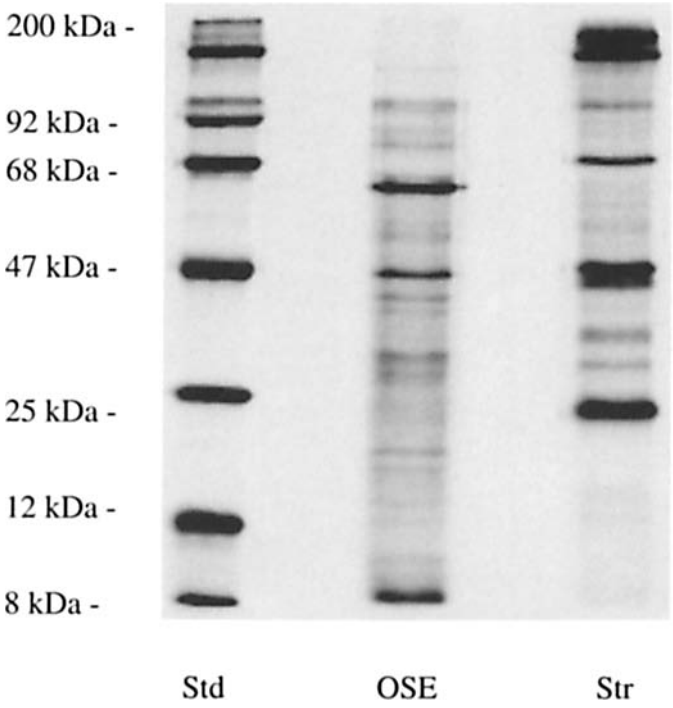

FIG. 3. Radiolabeled secreted protein profiles of bovine OSE and surface stromal cells (Str). Cultured cells were incubated in presence of ^{35}S-met and ^{35}S-cys for 24 h; then secreted proteins were collected, electrophoretically separated by SDS PAGE, and fluorographed. Radiolabeled molecular size standards (Std) were also electrophoretically separated. Data are representative of three different experiments.

bovine ovary is shown in Figure 1. A single layer of OSE are present on the outer surface of the ovary. Adjacent to the OSE are multiple layers of ovarian surface stromal cells. Immediately adjacent to the OSE is a layer of stromal cells somewhat perpendicular to the surface approximately 10 cells thick. A second layer of stromal cells organized parallel to the surface is approximately 8–10 cells thick. Below these perpendicular and parallel layers of cells are stromal cells with random organization that are similar to the interstitial stromal cell population throughout the ovary between follicles. Both the perpendicular and parallel layers of stromal cells generally surround the entire ovary unless disrupted by an ovulatory follicle or corpus luteum. These two stromal layers differ in nuclear morphology, with the perpendicular layer having a more rounded nuclear structure and the parallel layer an elongated structure (Fig. 1). These perpendicular and parallel layers of stromal cells are more organized than the interstitial stromal cells and were the primary stromal cells isolated for subsequent culture.

Bovine OSE and stromal cells were isolated and cultured as described in the *Materials and Methods* section. Cell yields from 10–15 ovaries vary between 10^7 and 10^8 cells. The morphologies of the cultured OSE and ovarian surface stromal cells are shown in Figure 2. As previously described for other species, the bovine OSE have a cuboidal epithelial-type morphology. The stromal cells have a more

TABLE 1. Amino acid sequence of bovine OSE secreted proteins and exact matches with proteins in the computerized data base.

Protein (size kDa)	N-Terminal sequence	Database match
OSE-P28	GTLVPPHPQFAFXNSDDVIR	TIMP
OSE-P40	VLFRXPPXYKGSLAAXKPPPG	IGFBP-2

fibroblastic elongated morphology in culture. The OSE were found to stain positive with a rabbit polyclonal wide spectrum anti-keratin antibody (Dako), whereas the stromal cells had no detectable staining (Fig. 2). After the cells were counted, the purity of the cell population was estimated to be greater than 98%. Both cell populations grow well in 10% calf serum and can be maintained for 4–6 wk in cell culture. Cells were not subcultured for the current study. After the cells have been grown to confluence, the serum can be removed and the cells maintained under serum-free conditions for several weeks.

Investigation of secreted proteins was used as a general functional analysis of the two different cell types. Radiolabeled secreted proteins were obtained from serum-free cultures of both OSE and stromal cells. An electrophoretic separation and fluorographic profiles of the radiolabeled secreted proteins are shown in Figure 3. The profiles of the OSE- and stromal cell-secreted proteins are distinct and provide additional support for the purity of the cell populations. Stromal cells were found to produce more proteins of high molecular mass and OSE more of lower molecular mass. Both cell types produced a discrete number of proteins. The profiles were not found to vary when taken at different culture durations, and the profiles were similar between different preparations of cells.

The functional identification of specific secreted proteins provides biochemical markers for a cell and insight into the physiological significance of a cell. The large number of bovine OSE cells and the large amount of serum-free conditioned medium available provided the material to examine the identity of the major OSE-secreted proteins shown in Figure 3. Concentrated serum-free OSE-conditioned medium was electrophoretically separated and blotted to PVDF membrane. Radiolabeled OSE-secreted proteins were processed in a similar manner. The membrane was stained with Coomassie Blue dye, and the location of radioactive bands was determined by counting of excised bands. A correlation was made to prominent Coomassie-stained proteins with the presence of radioactive proteins. A number of these major OSE secretory products were then cut from the blot and sequenced. Several secreted proteins did not provide a consensus sequence. Other secreted proteins did provide a consensus sequence, but did not match any known sequence in the database. Two proteins of 40 kDa and 28 kDa did provide a consensus sequence from three different OSE preparations that matched proteins in the computerized protein database, Table 1. The 28-kDa protein was found to provide an exact match with the tissue metalloproteinase

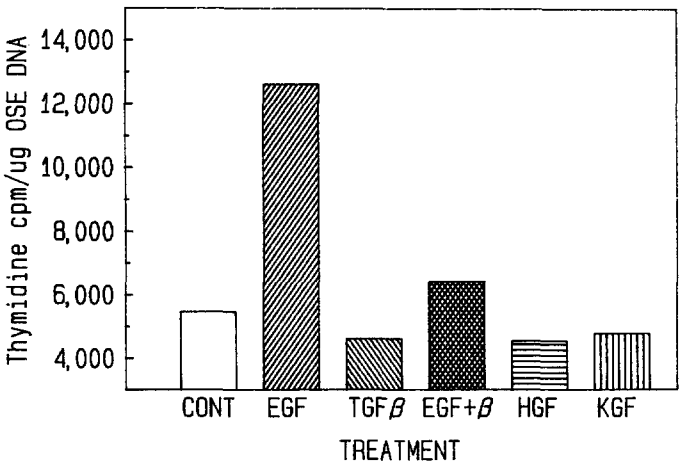

FIG. 4. Actions of growth factors on OSE proliferation. Cultured cells were not treated (CONT) or were treated with EGF, 10 ng/ml; TGFβ, 2 ng/ml; EGF plus TGFβ; HGF, 10 ng/ml; or KGF, 10 ng/ml for 18 h. Cells were then incubated with [^{3}H]thymidine for 4 h, and [^{3}H]thymidine incorporation/μg DNA was determined. Data are representative of three different experiments done in replicate.

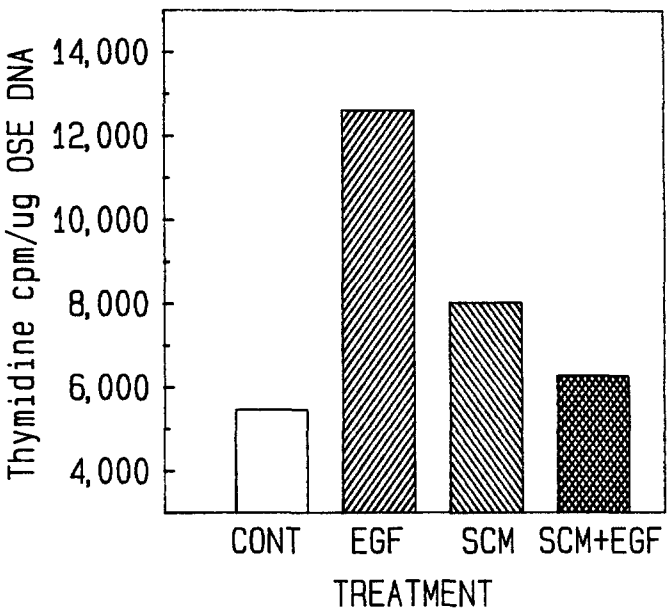

FIG. 5. Effect of SCM on OSE growth. OSE was cultured in absence or presence of EGF and SCM (50 μg/ml) for 18 h followed by a 4-h incubation with thymidine to assess [^{3}H]thymidine/μg DNA. Data are representative of three different experiments done in replicate.

inhibitor, TIMP-1 [28]. The 40-kDa protein was found to provide an exact match with the insulin-like growth factor binding protein-2 IGFBP2 [29]. Therefore, OSE appear to secrete TIMP-1 and IGFBP-2 (Table 1), and these correlate to the 28-kDa and 40-kDa bands shown in Figure 3. The potential physiological significance of the secretion of these proteins by OSE is discussed.

The growth properties of the bovine OSE were investigated by examining the effects of various growth factors on the cells in culture. OSE were plated at 50% confluence and maintained in 0.1% calf serum for 48 h. Cells were then treated for 48 h with various growth factors followed by a 4-h incubation with [^{3}H]thymidine. The standard error within a given experiment and treatment group was less than 10%. EGF was found to stimulate OSE growth (Fig. 4). Transforming growth factor β (TGFβ) had no effect alone but did inhibit the actions of EGF. HGF and KGF had no effect on OSE growth under the conditions utilized. KGF did influence the morphology of the cells in culture, promoting a more elongated and flattened structure (data not shown). Therefore, bovine OSE are stimulated to proliferate in culture by EGF and inhibited by TGFβ. The effects of ovarian surface stromal cell-conditioned medium on OSE growth were investigated to extend the above growth observations and address the possibility that stromal cells may produce factors that influence OSE growth. Concentrated serum-free stromal conditioned medium (SCM) was used to treat OSE cells. SCM alone caused a small stimulation in OSE growth (Fig. 5). SCM was also found to inhibit the stimulatory actions of EGF. Therefore, SCM did have the ability to influence the growth of OSE and probably contains a mixture of positive and negative growth regulators.

DISCUSSION

Cell-cell interactions have a critical role in the regulation of cell growth and differentiation for all tissues. Growth factors mediate a large number of both autocrine and paracrine interactions between cells. One of the most common cell-cell interactions present in essentially every organ is between mesenchymal/stromal cells and epithelial cells. Almost every functioning epithelial cell is associated with an adjacent mesenchymal/stromal cell. During embryogenesis, the mesenchymal cells of most organs appear to direct the differentiation of their adjacent epithelium [30, 31]. Mesenchymal/stromal induction of epithelial morphogenesis also appears to be present in the adult to maintain epithelial cell growth and differentiation. Interactions between abnormal mesenchymal/stromal cells and epithelial cells have been associated with a number of cancers [32]. OSE also is associated with a mesenchymal/stromal cell type. Whether the ovarian stromal cells underlying the OSE are different functionally from the stromal-interstitial cells between follicles remains to be elucidated. It is likely that these two ovarian stromal populations are distinct since the adjacent epithelium may also modulate mesenchymal/stromal cell function. Morphologically, the surface ovarian stromal cells underlying the OSE in vivo are different from the stromal-interstitial cells between follicles. The dramatic structural difference between the perpendicular and parallel layers of ovarian surface stromal cells implies a potential functional difference between the cells that remains to be elucidated. Because of the importance of mesenchymal/stromal cells in promoting and maintaining optimal

epithelial cell differentiation and the observation that apparent abnormal mesenchymal-epithelial cell interactions are present in some tumors, investigation of interactions between the ovarian surface stromal cells and OSE is anticipated to provide insight into the regulation of normal and tumorigenic OSE.

An ovarian mesenchymal-epithelial cell interaction previously investigated is between theca cells and granulosa cells of the developing follicle. The stroma-derived theca cells, but not the epithelial-derived granulosa, were found to express TGFα [20]. This locally produced TGFα acts as an autocrine factor for theca cells and a paracrine factor for granulosa cells to stimulate cell proliferation and expansion of the follicle [20]. Theca cells also are the predominant site of TGFß expression [21]. TGFß inhibits the growth promoting activity of TGFα on both theca and granulosa cells [21, 22]. Growth inhibition is postulated to have a role in arresting cell growth in atretic follicles and preventing cell growth of primordial follicles. Therefore, the local production and inverse actions of TGFα and TGFß provide an efficient mechanism for controlling follicle growth. Recently, two additional mesenchymal growth factors, KGF and HGF, have also been shown to be expressed by theca cells; and both KGF and HGF can act on granulosa cells to promote cell growth [33]. KGF is in the FGF superfamily, termed FGF-7, is specifically expressed by mesenchymal cells, and acts specifically on epithelial cells [34–36]. HGF is a unique growth factor, is primarily expressed by mesenchymal cells, and acts specifically on epithelial cells [37–40]. The receptor for KGF is an FGF receptor splice variant of FGFR2 that specifically binds KGF and is primarily localized on epithelial cells [41–43]. The receptor for HGF has been identified as the c-met proto-oncogene, which is a tyrosine kinase receptor primarily localized on epithelial cells [44–46]. Both KGF and HGF appear to mediate a mesenchymal-epithelial cell interaction in the ovarian follicle [33]. Therefore, theca cells express TGFα, TGFβ, KGF, and HGF, which can all regulate granulosa cell growth during ovarian follicle development. Interestingly, this appears to be a mesenchymally controlled growth process mediated by the theca cells [47]. The possibility that a similar interaction exists between OSE and the underlying stromal/mesenchymal cells remains to be investigated. Although ovarian follicle cells could potentially interact with OSE in a paracrine manner, it is likely that the underlying ovarian stromal cells will have the critical regulatory role due to proximity and mesenchymal-epithelial association. The ovarian surface stromal cells and theca cells are derived from the same ovarian interstitial stem cell population. Therefore, some similarities may exist between theca-granulosa cell interactions and ovarian surface stromal-OSE interactions.

The current study has established cultures of bovine OSE and stromal cells that can be used to further investigate the cell biology of these cell types and potential interactions between the cells. Viable cultures were obtained that can be maintained under serum-supplemented or serum-free conditions. The purity of the cell populations using keratin as a marker of OSE implied that the cell populations were highly purified with minimal cell contamination. Further analysis of cell purity with a positive marker for stromal cells, such as isoactin, will help confirm this observation and is being investigated. The distinct radiolabeled secreted protein profiles provide additional support of the purity of the cell populations and the lack of cross-contamination. As previously discussed, the bovine ovarian physiology is similar to the human in size, its mono-ovulatory nature, and endocrinology. Because of the availability of large quantities of normal bovine ovaries, the bovine OSE and stromal cell cultures will allow studies not previously possible due to the lack of sufficient normal tissue. Therefore, cellular parameters such as normal cellular functions and growth properties of OSE can be investigated.

As an initial investigation of cellular function, the radiolabeled secreted protein profiles of both OSE and stromal cells were examined. A discrete and reproducible number of secretory products were identified. Although the blotting and sequencing approach is not likely to identify regulatory factors secreted by the cell that are not abundant, major secretory products can be potentially identified. Currently, very little is known regarding the specific cellular functions or secretory products of OSE. In the current study, two major secretory products were identified. A 28-kDa metalloproteinase inhibitor, TIMP, was identified. TIMP comprises a family of inhibitors of metalloproteinases expressed by a wide variety of tissues [48]. TIMPs play a critical role in tissue remodeling and the control of extracellular matrix degradation. Abnormal TIMP expression has been associated with tumor invasion and metastasis [49]. The production of TIMP by OSE may have a role in the tissue remodeling required during ovulation when the OSE could affect the underlying layer of cells. The control of proteolytic activity may also be critical to maintain appropriate OSE-stromal contact. Abnormal TIMP expression could promote abnormal morphology and release of the cells into the peritoneum. Another secretory protein of 40 kDa was identified as IGFBP2. IGFBP2 is a commonly expressed protein that binds and transports IGF-I [50]. IGFBP2 is the most common tissue-derived form of IGFBP. The production of IGFBP2 by OSE may assure localization of IGF-1 to the OSE environment. IGF-I is essential for all cells to grow and undergo DNA synthesis. IGFBP-2 production also can sequester IGF-I and control the local actions of IGF-I. The production of TIMP and IGFBP-2 by OSE needs to be confirmed by determining the presence of mRNA in the cells. These secretory proteins, however, may provide useful biochemical markers of the OSE. Currently, little is known regarding the regulation of OSE differentiation and function. Cellular functions such as TIMP and IGFBP2 may be used to investigate the regulation of OSE. Further examination of these and other OSE secretory products will provide insight into

the physiological significance of this cell. This information can eventually be used to investigate the changes that occur during transformation of OSE.

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 primarily relates to the developing ovarian follicle [51, 52]. 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 [53, 54]. EGF receptor expression is not related to the histologic stage of ovarian tumors but, as with other cancers, EGF receptor expression is correlated with a poor prognosis [55, 56]. EGF can stimulate the proliferation of OSE [53] and influence the action of regulatory agents on the cells [57]. TGFα is a member of the EGF family and acts at the EGF receptor. TGFα has been associated with ovarian cancer [58, 59] and may act as a marker in effusions from cancer patients [60, 61]. TGFα may act as an autocrine growth factor to induce cell proliferation in both normal and tumorigenic OSE [62, 63]. Cellular associations with OSE involving both normal and abnormal cell-cell interactions may also involve EGF/TGFα [64]. Another member of the EGF family, amphiregulin, also has been shown to be expressed by normal and tumorigenic OSE and can modulate OSE growth through actions on the EGF receptor [65]. The ability of OSE to express growth stimulators such as EGF/TGFα suggests that a growth inhibitor may be required to control the actions of the growth stimulator. TGFβ is a multifunctional protein that has a major role in inhibiting the actions of growth stimulators such as EGF/TGFα. TGFβ has been shown to be produced by OSE [66]. Both the TGFβ-1 and TGFβ-2 isoforms appear to be expressed. TGFβ can inhibit the growth of normal OSE cells and some tumorigenic OSE cells [66–68]. The concept that the local production and inverse actions of TGFα and TGFβ may have a role in OSE growth and ovarian cancer has previously been speculated on [62].

In the current study, the roles that EGF/TGFα and TGFβ may have in mediating cell-cell interactions involving bovine OSE were investigated. Bovine OSE cells were stimulated to grow with EGF, and TGFβ inhibited the actions of EGF. These results confirm previous observations and imply that this mechanism of growth regulation through EGF/TGFα and TGFβ is present in the normal OSE. To extend this growth analysis, the reactions to two mesenchymal specific growth factors, KGF and HGF, were investigated. Neither KGF nor HGF influenced the growth of OSE under the conditions utilized. KGF did influence OSE morphology. Further analysis of the actions of KGF and HGF are needed, in particular the combined effects with other growth factors. The possibility that the ovarian surface stromal cells may produce factors that influence OSE growth was examined here. Concentrated serum-free SCM did influence OSE growth. SCM is likely to contain multiple regulatory agents that stimulate and/or inhibit OSE growth. The current study indicates that stromal cells can secrete factors that influence OSE growth. The identification of the factors involved and their role in normal and tumorigenic OSE growth and differentiation remains to be elucidated. The bovine culture system established will be useful in the future to investigate ovarian surface stromal-OSE interactions.

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