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Transforming Growth Factor Production and Action in the Ovarian Follicle: Theca Cell–Granulosa Cell Interactions

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Introduction

Cell-cell interactions have an important role in the physiology of an individual cell and a whole organ. Interactions between cells result in the maintenance or alteration of several parameters, including growth, function and differentiation. It is unlikely that any individual interaction could maintain and regulate all cellular processes. Therefore, a number of different types of cell-cell interactions are required. The types of specific cellular interactions possible have previously been categorized into environmental, nutritional and regulatory cell-cell interactions (1). The current manuscript will review the interactions between different cell types in the ovarian follicle which regulate cell growth.

Cell proliferation within the ovarian follicle is required for both the maintenance of ovarian function and the endocrine status of the female. The progression of a primordial follicle to become an ovulatory follicle is a process that involves a rapid rate of somatic cell proliferation. The two primary somatic cell types in the ovarian follicle are granulosa cells and theca cells. Granulosa cells help form the follicle and provide the cytoarchitectural support for the developing oocyte. Theca cells surround the follicle and provide structural support. A combination of granulosa cell growth, theca cell growth and antrum formation result in the expansion of the ovarian follicle. Although stimulation of cell growth is required for the ovulatory follicle to develop, the vast majority of follicles undergo atresia in which cell growth is arrested at various stages of follicle development. Therefore, in addition to the need for a growth stimulator, a growth inhibitor may also be required. The regulation of ovarian cell proliferation is an important and complex process that will require an array of externally and locally derived regulatory agents.

Investigations into the regulation of ovarian cell growth have primarily focused on the factors which regulate granulosa cell proliferation (2). Several growth factors have been shown to stimulate granulosa cell proliferation including fibroblast growth factor (FGF)

(3,4), insulin-like growth factor (IGF) (5), and epidermal growth factor (EGF) (3,4). Granulosa cells contain high affinity EGF receptors (6) and respond in vitro to EGF through an increase in cell proliferation (3,4). In addition, EGF has been shown to alter the hormonal regulation of granulosa cell function (2) by having an inhibitory effect on the ability of FSH to stimulate estrogen biosynthesis (6). These observations have led to the proposal that EGF may have an important role in regulating ovarian cell growth and differentiation (2). Because circulating levels of EGF are negligible (7), the local production of an EGF-like substance in the ovarian follicle would appear to be needed. Studies to determine the potential local production of an EGF-like substance in the ovary will be reviewed.

Transforming growth factor-alpha (TGF-alpha) is a protein that has structural homology with EGF (8), binds to the EGF receptor (9), and has similar biological activities as EGF (10). TGF-alpha is a unique gene product that is produced as a precursor integral membrane protein which is processed into a soluble extracellular protein (11). TGF-alpha was initially isolated from the conditioned medium of virally transformed fibroblasts (12) and has subsequently been shown to be produced by a large number of neoplastic cells (13). Cells of embryonic origin have also been shown to produce TGF-alpha (14). These observations have led investigators to propose that TGF-alpha may function during transformation as an autocrine growth factor (10). Reports have recently demonstrated that TGF-alpha is produced by normal adult cell types, including bovine pituitary cells (15) and human keratinocytes (16). These observations imply that TGF-alpha may also be a growth regulator in normal adult tissue. Therefore, TGF-alpha is a candidate for an EGF-like growth regulator in the ovarian follicle.

Since the majority of developing follicles undergo atresia and growth arrest, the potential presence of a growth inhibitor needs to be considered. Transforming growth factor-beta (TGF-beta) is a protein that has both stimulatory and inhibitory effects on cell proliferation (17). TGF-beta generally inhibits the growth of epithelial cell types, particularly if they are responsive to EGF (18). Although TGF-beta was initially isolated from the conditioned medium from virally transformed fibroblasts (19), it has subsequently been shown to be produced by a large number of neoplastic and normal cell types (20). This highly conserved protein is produced by a number of different species and acts via unique cell surface receptors (21). TGF-beta has also been shown to influence the differentiation and functions of a number of cell types (20). Granulosa cells are an epithelial cell type that respond to EGF; therefore, it was postulated that TGF-beta may influence granulosa cell growth. Recent observations that TGF-beta can regulate granulosa cell steroidogenesis (22,23) support this hypothesis.

Evidence for the local production of transforming growth factors in the ovary and data regarding the regulation of ovarian cell growth will be reviewed. Discussion of the proposed physiological significance of transforming growth factors in the ovary and their relationship to other growth regulators will also be presented.

Methodology

The experimental design used to determine the local production of growth factors in the ovary utilized the isolation and culture of individual cell types. The use of a purified cell population provides a direct means to determine both the sites of synthesis and action of locally produced growth factors. Results obtained with impure cell populations must be

qualified because of the potential presence of cell-cell interactions between the different cell types. Although purified populations of granulosa cells can be isolated from rat ovaries (24), homogeneous populations of theca cells are not easily obtained. Because of this fact and the observation that rat granulosa cells do not readily proliferate in vitro, an alternate animal model was utilized. The bovine is a mono-ovulator and the size of the ovary allows for the isolation of homogeneous populations of both granulosa cells and theca cells. These bovine cell types proliferate in vitro and can be cultured under serum-free conditions (3,4,25). Serum-free conditioned medium can be obtained from these cell cultures and used as a potential source for locally produced growth factors. Alternatively, these cell cultures can also be used to determine potential sites of action of specific growth regulators.

Quantitation of the presence of an EGF-like substance can be accomplished with an EGF radioreceptor assay and a bioassay that relies on the growth of an EGF-dependent cell line (7). TGF-alpha can also be analyzed with these same EGF assays. TGF-beta is analyzed with radioreceptor and radioimmunoassays, as well as a bioassay that relies on colony formation on soft agar (25). Confirmation of the presence of a growth factor with a bioassay demonstrates the biological activity of the components detected.

Transforming Growth Factors

Initial observations utilized a rat theca/interstitial cell culture which was a mixed population of cells depleted of granulosa cells. Serum-free conditioned medium from this culture system contained growth-promoting activity for a number of cell types, including bovine granulosa cells (26). The growth-promoting activity from this rat theca/interstitial cell conditioned medium was found to be a heat-stable protein of approximately 20 kDa by size exclusion chromatography (26). These observations demonstrated the local production of a growth factor in the ovary by a theca/interstitial cell population in culture. This growth-promoting activity was subsequently identified as an EGF-like substance with an EGF radioreceptor assay (27). It was found that rat granulosa cell-secreted proteins, obtained from serum-free conditioned medium, did not contain detectable EGF activity with an EGF radioreceptor assay. Secreted proteins from theca/interstitial cell cultures, however, did contain a component that specifically bound to the EGF receptor (27). Theca/interstitial cell-secreted proteins also stimulated the growth of an EGF-dependent cell line (27). The EGF-like substance was isolated by reverse phase hydrophobic chromatography and found to be a single molecular species by both the EGF radioreceptor assay and EGF growth assay (27). These observations indicated that a population of rat theca/interstitial cells, but not rat granulosa cells, produce an EGF-like substance that can promote the growth of granulosa cells and other EGF responsive cells (26,27).

To determine more precisely the site of synthesis of the EGF-like substance, bovine theca cells and granulosa cells were isolated and cultured (25). Theca cell and granulosa cell-secreted proteins were prepared from serum-free conditioned medium that was concentrated by ultrafiltration. Theca cell-secreted proteins were found to contain a component that specifically bound to the EGF receptor as determined by an EGF radioreceptor assay (28). As was found with rat granulosa cell-secreted proteins, bovine granulosa cell-secreted protein preparations were not found to contain an EGF-like substance (28). To confirm these observations, a bioassay for EGF was utilized that is dependent on the growth of an

EGF-sensitive cell type (7). Theca cell-, but not granulosa cell-secreted protein preparations were found to contain EGF-like bioactivity with this EGF growth assay. These combined observations demonstrate that theca cells are the site of synthesis for an EGF-like substance produced locally in the ovarian follicle.

Several biochemical characteristics of the EGF-like substance produced by theca cells were found to be different from those of authentic EGF. The molecular weight of the EGF-like substance was between 20-30 kDa under both denaturing and physiological conditions (27). The molecular weight of authentic EGF is approximately 6,000 (29). The hydrophobicity of the EGF-like substance was also found to be greater than that of authentic murine EGF (27). Therefore, either a large molecular weight form of EGF was produced, as has previously been identified in several physiological fluids (29), or a different EGF-like protein is present. To determine whether a different EGF-like protein is produced, a TGF-alpha molecular probe was obtained. This probe was a complimentary RNA (cRNA) previously described (16). Polyadenylated RNA was prepared from bovine theca cells and granulosa cells and analyzed by Northern analysis. Theca cells, but not granulosa cells, were found to express the TGF-alpha gene with a 4.5 kb RNA species being detected (28). This observation demonstrated that theca cells can produce TGF-alpha and imply that the EGF-like substance detected in theca cell-secreted protein preparations is TGF-alpha. Similar analysis with a human EGF cDNA probe indicated the absence of EGF gene expression in theca cells or granulosa cells (28). Since granulosa cells do not appear to produce TGF-alpha, EGF or any detectable EGF-like substance, the TGF-alpha produced by theca cells may have a paracrine role in regulating granulosa cell growth (28).

During the analysis of the presence of the EGF-like substance in theca cell-secreted protein preparations, it was found that a component was also present which could inhibit the ability of EGF to promote cell growth. The presence of an apparent growth inhibitor indicates that the growth assay may not provide an accurate estimate of the amount of EGF-like material present (27). The growth inhibitory substance was found to be separated from the EGF-like substance by reverse phase chromatography (27). These observations indicated that an EGF growth inhibitory substance was present in theca cell-secreted protein preparations that was distinct from the EGF-like substance or TGF-alpha. A protein that has previously been shown to inhibit the ability of EGF to promote cell growth is TGF-beta (17,20). Therefore, the possible presence of TGF-beta production by theca cells was examined. TGF-beta was detected in theca cell-secreted protein preparations using both radioimmunoassays and radioreceptor assays. TGF-beta biological activity was also detected with an assay based on colony formation on soft agar (25). Granulosa cell-secreted protein preparations did not contain any detectable TGF-beta (25). To demonstrate active synthesis and secretion of TGF-beta by theca cells, a TGF-beta antisera was used to immunoprecipitate radiolabeled theca cell-secreted proteins. A 25 kDa radiolabeled protein that co-migrated with authentic TGF-beta was specifically immunoprecipitated with the antisera (25). These observations demonstrated that theca cells, but not apparently granulosa cells, produce TGF-beta as a potential growth inhibitor for granulosa cell growth.

Growth Regulation

To examine the physiological significance of TGF-alpha and TGF-beta production by theca cells, the effects of transforming growth factors on bovine follicle cell growth were examined. TGF-alpha was found to stimulate granulosa cell growth (28) which confirms previous observations made on the effects of EGF on granulosa cell growth (2-4). Since the circulating levels of EGF are negligible (7), the local production of TGF-alpha by theca cells provides a source of an EGF-like substance in the ovarian follicle. TGF-alpha may have an important role in promoting and maintaining follicle cell growth during the growth phase of the ovary when a primordial follicle develops into an ovulatory follicle. Although TGF-beta alone had no effect on granulosa cell growth, TGF-beta does inhibit the ability of TGF-alpha and EGF to promote bovine granulosa cell growth (25). Demonstration of TGF-beta production by theca cells provides a source for a locally produced growth inhibitor (25). TGF-beta may be required to inhibit cell growth in the atretic follicle and also to prevent premature cell growth in the primordial follicle. Therefore, both TGF-alpha and TGF-beta can influence granulosa cell growth which provides a physiological function for the transforming growth factors produced by theca cells. Previous observations have also indicated that EGF can influence theca/interstitial cell steroidogenesis (30); therefore, the transforming growth factors may also influence theca cells. Theca cells were found to contain high-affinity EGF receptors using a Scatchard analysis (28). This data correlates with the need during follicle development for both granulosa cell and theca cell growth. TGF-alpha and TGF-beta production by theca cells may, therefore, have both an autocrine role in influencing theca cell growth and a paracrine role in regulating granulosa cell growth. The inverse actions of TGF-alpha and TGF-beta provide an efficient mechanism to control the rapid growth stimulation and inhibition required in the ovarian follicle.

In addition to the cell proliferation-related effects, growth factors have also been shown to influence the differentiation of granulosa cells. EGF has previously been shown to have inhibitory effects on the ability of FSH to promote estrogen biosynthesis (2,6). Subsequently these observations have been confirmed with TGF-alpha (31). Therefore, EGF-like substances that promote granulosa cell growth have inhibitory effects on the ability of agents to stimulate a functional parameter such as estrogen biosynthesis. It is not known whether the EGF-like substances directly inhibit granulosa cell steroidogenesis or indirectly inhibit steroidogenesis by altering the rate of granulosa cell proliferation. It is speculated that the reduced ability of regulatory agents to stimulate a differentiated function such as steroidogenesis in the presence of a growth factor may be due to the ability of the growth factor to promote cell proliferation. Promoting granulosa cell proliferation puts the cell into the growth phase of the cell cycle in which differentiated functions such as steroidogenesis may not be readily stimulated. This is supported by the actions of TGF-beta on the ability of FSH to stimulate granulosa cell steroidogenesis. TGF-beta augments the actions of FSH to stimulate granulosa cell estrogen production (22). TGF-beta inhibits cell growth and puts the cell into a nonproliferative differentiated state. Due to the cell being in this stage of the cell cycle, regulatory agents have an enhanced ability to stimulate granulosa cell-differentiated functions such as steroidogenesis. Therefore, it is speculated that the inhibitory and stimulatory effects of TGF-alpha and TGF-beta, respectively, on granulosa cell steroidogenesis are an indirect effect of the growth-promoting ability of these growth factors.

Any agent which could inhibit growth and thus promote a more differentiated state of the cells will likely have a stimulatory effect on granulosa cell functions. Alternatively, any agent which promotes the growth of granulosa cells will likely reduce the differentiated state of the cell and have inhibitory effects. This type of separation of differentiated functions and the growth of granulosa cells has previously been postulated (32). The physiological significance of the effects of transforming growth factors on granulosa cell hormonal regulation are currently unknown.

A number of different growth factors have been shown to influence granulosa cell growth (2-4) which may cooperate in the control of follicle cell expansion (Table 1). Insulin like-growth factor (IGF) has previously been shown to be produced by granulosa cells (33) and influence the growth (34) and functions of granulosa cells (5). Due to the local production of IGF, it has been proposed that IGF may have an important role in regulating granulosa cell growth and differentiation (5). IGF has been shown to be produced by the majority of cell types examined and is involved in the regulation and maintenance of cellular differentiation for many cell types. Therefore, it is likely that IGF will also play an integral role in the control of ovarian cell function, differentiation and hormonal regulation. Examination of cell growth has shown that IGF is a progression factor that regulates the DNA synthesis phase of cell proliferation (35). It is the combined actions of a growth initiator such as EGF and a progression factor such as IGF that maintain optimal cell growth within a tissue (35). From these observations it is speculated that the synergistic actions of TGF-alpha and IGF will be required to maintain optimal follicle cell proliferation. Fibroblast growth factor (FGF) has also been shown to promote granulosa cell growth (3,4). FGF has a number of functions which include being an angiogenic factor (36). Whether FGF will be a physiologically important growth factor for follicle cells such as granulosa cells or theca cells remains to be elucidated. However, the high degree of vascularization of the ovarian follicle, particularly in the corpus luteum, suggests that FGF may have an important role as an angiogenic factor for the follicle (36). Although it is possible that different growth factors have similar functions in the ovary, it is proposed that the primary roles for the different growth factors will be distinct. Therefore, TGF-alpha may function as a growth initiator and through the synergistic actions of IGF maintain optimal follicle cell growth. FGF may primarily function as an angiogenic factor to promote vascularization of the follicle and TGF-beta may function to inhibit cell growth when growth arrest is required. Although a number of growth factors have been identified to be involved in ovarian cell growth, investigations are now required to determine the physiological functions of these different growth factors.

Theca Cell-Granulosa Cell Interactions

The types of cellular interactions possible between theca cells and granulosa cells are numerous. These interactions will be divided into three general categories of environmental, nutritional and regulatory cell-cell interactions (1). The first category deals with the extracellular environment of the cell. Interactions mediated by an extracellular matrix and specific proteins such as cell adhesion molecules make up the environmental category. This type of interaction has an important role in providing the proper structural support and surroundings for the cell to maintain a normal morphology and differentiated state. This interaction is also very important during development and morphogenesis. The second category, called nutri-

Table 1. Ovarian growth regulators

Growth Factor	Site of Synthesis	Site of Action	Response/Function
TGFα	Theca	Theca/granulosa	Growth stimulator
EGF	—	Theca/granulosa	Growth stimulator
TGFβ	Theca	Theca/granulosa	Growth inhibitor
IGF	Granulosa/ theca?	Granulosa/theca?	Cell differentiation and growth stimulator
FGF	—	Granulosa/ vasculature	Angiogenic factor and growth stimulator

tional, deals with a cell obtaining essential components needed for survival. The maintenance of normal cellular functions requires many externally derived components such as energy metabolites. When an essential component such as a sugar metabolite, vitamin, or metal is derived from one cell type and delivered to a different cell type, a nutritional type of cell-cell interaction has occurred. The third category of cell-cell interaction deals with a component that is produced by one cell type that acts on a second cell type to cause a signal transduction event. This action induces a cellular response on the molecular level associated with the differentiation, function, or growth of the cell. This type of interaction is called regulatory. Interactions of this type are important in the control and maintenance of many cellular parameters. Regulatory agents involved in this type of interaction are paracrine factors. This interaction generally requires a receptor-mediated event to induce a second messenger that alters cellular parameters on the molecular level.

Examples of several theca cell-granulosa cell interactions are shown in Table 2. Environmental interactions are mediated by an extracellular matrix between the outer layer of mural granulosa cells and inner layer of theca interna. This extracellular matrix is speculated to be produced cooperatively by both cell types as has been shown in other tissues like the testis (1). One of the primary functions of this extracellular matrix is to provide structural support to the follicle. Additional effects of environmental cellular interactions on cell morphology and function have not been thoroughly investigated (38). A nutritional interaction between theca cells and granulosa cells that has been established is mediated through the production of androgens by theca cells. Testosterone produced by theca cells can be utilized by granulosa cells for the biosynthesis of estrogen (24). This interaction is a classic example of a nutritional interaction where a substrate, testosterone, is provided to an enzyme, aromatase. This interaction is critical for the maintenance of ovarian function and the endocrine status of the female. Regulatory interactions require a receptor-mediated signal transduction event on the molecular level. A recently identified regulatory interaction between theca cells and granulosa cells involves progestin production by granulosa cells. Progestins produced by granulosa cells can act on theca cells to influence cellular differentiation and function (39). Evidence has also been provided that estrogens may also influence theca cell functions (39). These steroid-mediated interactions may have an important role in mediating cellular differentiation in the follicle.

Table 2. Theca cell-granulosa cell interactions

Cell-Cell Interaction Category	Mediator	Ovarian Site of Synthesis	Response/Function
Environmental	Extracellular matrix	Granulosa/theca	Structural support for follicle
Nutritional	Androgen	Theca	Estrogen biosynthesis by granulosa
Regulatory	Progestin	Granulosa/theca	Theca cell differentiation
	TGFα	Theca	Growth stimulator for granulosa/theca
	TGFβ	Theca	Growth inhibitor for granulosa/ theca

The production of transforming growth factors by theca cells also provide mediators of cell-cell interactions in the follicle. TGF-alpha and TGF-beta produced by theca cells have a paracrine role in the regulation of granulosa cell growth and a possible autocrine role in the regulation of theca cell growth (Fig. 1). Growth factor-mediated paracrine interactions within a tissue provide an efficient mechanism for different cell types to form a functional unit to control tissue growth. The evolution of this process indicates the importance of cell-cell interactions between different cell types within a tissue and implies that few cell types will be autonomous in the regulation of growth and differentiation. Theca-granulosa cell interactions provide an example of mesenchymal-epithelial cell interactions. The importance of mesenchymal-epithelial cell interactions have been demonstrated during development (37), but remain to be elucidated in adult tissue. TGF-alpha and TGF-beta production by theca cells and subsequent actions on granulosa cells are examples of growth factor-mediated mesenchymal-epithelial cell interactions. The synthesis of TGF-alpha by theca cells also provides an example of TGF-alpha production by a normal adult mesenchymal cell type. Previous studies have implicated TGF-alpha as an important growth regulator during transformation (10,13) and embryonic development (14). Recently the demonstration of TGF-alpha by normal epithelial cell types has led to the proposal that TGF-alpha may also have an important role as a growth regulator in normal adult tissue (15,16). The observation that TGF-alpha is produced by theca cells supports this proposal and indicates that TGF-alpha may be an important growth regulator in EGF responsive tissues requiring rapid cell proliferation (28).

The observations reviewed indicate that theca cells produce both TGF-alpha and TGF-beta which may function as a growth stimulator and growth inhibitor, respectively, in the ovarian follicle (Fig. 1). The speculation is made that TGF-alpha production by theca cells would be predominate during the growth phase of the follicles and TGF-beta production would be predominate in the atretic and primordial follicle when growth is inhibited. These inverse actions of TGF-alpha and TGF-beta provide an efficient mechanism to control the

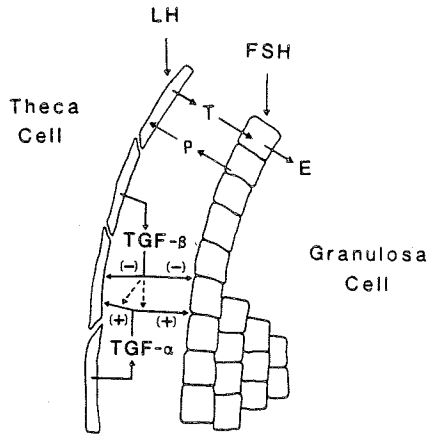

Fig. 1. Schematic of theca cell-granulosa cell interactions mediated by TGF-alpha, TGF-beta, testosterone (T), progestin (P) and estrogen (E).

rapid stimulation and inhibition of cell growth required in the ovarian follicle. Therefore, the local production of transforming growth factors will have an important role in the regulation of ovarian cell growth.

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