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Growth Factor-Mediated Cell–Cell Interactions in the Ovary

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I. Introduction

The local production and integrated actions of various growth factors are required for the growth and development of all tissues. Growth factors mediate critical cell–cell interactions that control cell proliferation and organ development and the number of specific growth factors identified has increased dramatically. Growth factors often exist in families composed of unique gene products that have similarities in structure and function, but often differ in the way gene expression is controlled. The existence of multiple members in a growth factor family allows for unique sites of expression and more precise developmental and hormonal regulation of growth factor production. Table 1 contains a partial list of several of the major types of growth factors, including nomenclature and major functions attributed to them.

Ovarian development also requires growth factor-mediated cell–cell interactions as a general mechanism for controlling cellular proliferation. Most of the information available on growth factors and the ovary deals with follicular development and adult ovarian function. These are the primary stages of development discussed here. However, it is likely that many of the same cell–cell interactions and growth factors are also important for other stages of ovarian development (e.g., embryonic or prepubertal stages). Since research has been focused primarily on identifying the sites of production and actions of specific growth factors, this chapter emphasizes the growth factors potentially involved in ovarian cell–cell interac-

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TABLE 1 Properties and Nomenclature of Several Common Growth Factors

Growth Factor		Approx. size (kDA)	Examples of physiological action
Insulin-like growth factor-I	IGF-I	7.5	Skeletal growth
Insulin-like growth factor-II	IGF-II	7.5	Fetal development
Epidermal growth factor	EGF	6	Tissue growth
Transforming growth factor α	TGFα	5	Tissue growth
Transforming growth factor β	TGFβ	25/dimer	Growth inhibition/tissue repair
Fibroblast growth factor	FGF	17	Angiogenesis/tissue growth
Vascular endothelial growth factor	VEGF	25–50/dimer	Angiogenesis/tissue growth
Nerve growth factor	NGF	13	Neuronal development
Interleukin-1	IL-1	17	Immune response/ inflammation
Platelet-derived growth factor	PDGF	30/dimer	Tissue growth
Stem cell factor (c-kit ligand)	SCF	30	Tissue growth/fetal development

tions. The specific functions of these growth factors *in vivo* in large part remain to be elucidated.

II. Growth Factors in the Ovary

Ovarian physiology requires rapid and continuous regulation of the growth associated with folliculogenesis. Growth factor-mediated interactions among theca cells, granulosa cells, and the oocyte are needed to maintain ovarian function and oogenesis. Granulosa cells provide the cytoarchitectural support for the developing oocyte and also help to form the follicle and antrum. Theca cells surround and form the exterior wall of the follicle. The interactions between theca cells and granulosa cells provide an example of a mesenchymal (stromal)–epithelial cell interaction. The effects of this cellular interaction on oocyte development and the influence of the endocrine system on this cellular interaction are essential to female reproductive biology. The theca cells and granulosa cells of the preantral and small antral follicles must undergo extensive proliferation and functional differentiation prior to ovulation (Hsueh *et al.*, 1984). In most large animals, follicle size increases from millimeters to centimeters. The primary expansion in cell number takes place in both the granulosa and theca cell populations. In addition to the cell proliferation required during follicle development, follicles at various stages of development become atretic and cell growth is arrested. Therefore, the regulation of cell proliferation in the follicle requires both stimulatory and inhibitory growth factors.

TABLE 2 Growth Factors in the Ovary

Growth factor	Proposed site of synthesis	Proposed site of action	Proposed function[a]
IGF-1	Granulosa	Granulosa	+Growth/+differentiation
		Theca	+Growth/+differentiation
FGF	Granulosa	Granulosa	+Growth
		Endothelium	Angiogenesis
TGFβ	Theca	Granulosa	−Growth/+differentiation
	Granulosa	Theca	−Growth/+differentiation
TGFα	Theca	Granulosa	+Growth/−differentiation
		Theca	+growth
VEGF	Granulosa	Endothelium	Angiogenesis/+growth
NGF	Ovary	Neurons	Innervation
SCF	Granulosa	Oocyte	Oocyte maturation

[a]A plus sign denotes an increase and a minus sign indicates a decrease.

Follicle-stimulating hormone (FSH) and estrogen have been shown to stimulate proliferation of follicle cells *in vivo* (Goldenberg *et al.*, 1972; Louvet and Vaitukaitis, 1976; Richards, 1979). These hormones, however, have negligible effects on cell growth *in vitro*. This implies that *in vivo* hormone actions are most likely indirectly mediated by the local production of growth factors. The mechanisms and specific growth factors involved in the control of ovarian cell proliferation remain to be fully elucidated (Carson *et al.*, 1989). However, several major growth factors have been identified as being produced and/or acting in the ovary. These are summarized in Table 2.

A. Insulin-like Growth Factor

The insulin-like growth factors (IGF) derive their name from their structural similarity to insulin (Froesch *et al.*, 1985). IGF-I (previously termed somatomedin C) is considered essential for cellular replication and is a progression factor for cell growth and DNA synthesis. Production and secretion of IGF-I by the liver accounts for the high levels of IGF-I in serum and interstitial fluid (Daughaday and Rotwein, 1989). IGF-II is another member of this family and may act as a growth factor during fetal development.

IGF-I is produced by granulosa cells under the control of growth hormone (Davoren and Hsueh 1986), FSH, and estradiol (Hammond et al., 1985; Hsu and Hammond, 1987). The gene expression of IGF-1 has also been localized in granulosa cells (Hernandez *et al.*, 1989; Oliver *et al.*, 1989) but not theca cells. The receptors for IGF-1 have been localized to granulosa cells (Baranao and Hammond, 1984; Davoren *et al.*, 1986; Adashi *et al.*,

1988b) and are affected by the actions of FSH (Adashi *et al.*, 1986, 1988c,d). The regulation of IGF receptor gene expression has also been examined (Hernandez *et al.*, 1991, 1992). IGF-1 stimulates granulosa cell oxytocin production (Schams *et al.*, 1988), the P450 side chain cleavage enzyme (Veldhuis *et al.*, 1986), the aromatase gene expression (Steinkampf *et al.*, 1988), lipoprotein metabolism (Veldhuis *et al.*, 1987), adenylate cyclase activity (Adashi *et al.*, 1986), plasminogen activator production (Tilly and Johnson, 1990), and LH receptor induction (Adashi *et al.*, 1985b).

In addition to affecting cellular function, IGF stimulates the proliferation of bovine (Savion *et al.*, 1981) and porcine (Baranao and Hammond, 1984) granulosa cells, but not rat granulosa cells *in vitro* (Adashi *et al.*, 1984). Although theca cells do not appear to produce IGF-1 (Oliver *et al.*, 1989), IGF-II gene expression has been localized to theca cells and not granulosa cells (Hernandez *et al.*, 1990a). Theca cells also contain IGF receptors and respond to IGF through an alteration in steroidogenesis (Hernandez *et al.*, 1988) and LH receptor binding (Cara *et al.*, 1990). Potential interactions between granulosa cells and theca cells through the local production and action of IGF have been suggested (Adashi *et al.*, 1985a; Geisthovel *et al.*, 1990). The localization of IGF-1 expression to granulosa and not theca cells implies a potential IGF-1-mediated paracrine interaction between granulosa and theca cells. IGF-1 can also play a role as an autocrine factor for granulosa cells. A physiological parameter to consider, however, is the high circulatory levels of liver-derived IGF-1 (>100 ng/ml) available to both cell types. This is an additional source of IGF-1 that needs to be considered in understanding the importance of IGF-mediated cell–cell interactions.

IGF binding proteins (IGFBP) are produced by ovarian cell types and are present in the follicle. These binding proteins can reduce the effective concentration and modulate the actions of IGF. Several forms of IGFBP have been identified and are present in the ovary. Both granulosa cells and theca cells produce IGFBP(2) (Samares *et al.*, 1992; Ricciarelli *et al.*, 1991; Nakatani *et al.*, 1991), IGFBP(3) (Ricciarelli *et al.*, 1992; Samares *et al.*, 1992; Mondschein *et al.*, 1990), and IGFBP(4), which appears to be species-specific for cellular localization. IGFBP(4) and IGFBP(5) appear to be expressed primarily by granulosa cells (Erickson *et al.*, 1992a,b; Nakatani *et al.*, 1991). All the forms are present in follicular fluid at various stages of development. Although the specific function(s) of these IGFBPs remains to be elucidated, it has been postulated that they may inhibit or control the actions of IGF (Ui *et al.*, 1989).

B. Transforming Growth Factor-α/Epidermal Growth Factor

Transforming growth factor-α (TGFα) is one of the structurally related peptides belonging to the epidermal growth factor (EGF) family (Derynck, 1988; Carpenter and Cohen, 1990). Because they have a similar protein

structure, these factors act at the same receptor to stimulate cell growth (Carpenter, 1987). TGFα is synthesized as a transmembrane precursor, which may activate EGF receptors on neighboring cells or be proteolytically cleaved to release mature peptide. TGFα appears to be produced by non-transformed cells, and may play an important role as a growth regulator in normal tissues.

Although EGF was not found to be produced in the ovary, an EGF-like substance was found in theca cells (Skinner *et al.*, 1987b) and was identified as TGFα (Skinner and Coffey, 1988; Kudlow *et al.*, 1987). Granulosa cells do not express TGFα (Skinner and Coffey, 1988; Lobb *et al.*, 1989) but have been shown to contain the EGF receptor (Mondschein and Schomberg, 1981; Chabot *et al.*, 1986; Feng *et al.*, 1986). EGF generally is inhibitory for adenylate cyclase (Dodson and Schomberg, 1987), LH receptor activity (Mondschein and Schomberg, 1981; Knecht and Catt, 1983a; May *et al.*, 1987), and FSH-induced aromatase activity (Hsueh *et al.*, 1981; May *et al.*, 1982). TGFα has inhibitory effects on granulosa cells (Adashi *et al.*, 1987) that are similar to those of EGF. The effects of TGFα, however, can vary among species (Gangrade *et al.*, 1991). Theca cells also contain the EGF receptor (Skinner and Coffey, 1988), and EGF/TGF-α influences theca cell steroidogenesis (Erickson and Case, 1983). One of the initial observations on growth regulation in the ovary was the ability of EGF to stimulate granulosa cell proliferation (Gospodarowicz *et al.*, 1977). The growth of theca cells can also be stimulated by TGFα/EGF (Skinner and Coffey, 1988). The actions of TGFα/EGF and the potential presence of EGF in the ovaries of various species can vary and remain to be fully elucidated.

Circulatory levels of EGF/TGFα are negligible, therefore, the factors must be produced locally within a specific organ. The ability of the theca cell to produce TGFα that can stimulate the growth of both granulosa and theca cells implies that TGFα may have an important role in promoting cell proliferation during follicle development. TGFα has been localized in developing ovarian follicles (Lobb *et al.*, 1989; Chegini and Williams, 1992). An interesting observation is that this appears to be a mesenchymal/stromal-controlled growth process (Skinner, 1990). Therefore, TGFα is postulated to mediate a paracrine interaction between theca and granulosa cells and an autocrine interaction between theca cells. The ability of hormones to influence TGFα production remains to be elucidated and may provide a mechanism through which hormones can regulate ovarian follicle cell growth.

C. Transforming Growth Factor-β

Transforming growth factor-β (TGFβ) is a multifunctional regulatory molecule that can stimulate or inhibit aspects of cellular growth and differentiation (Roberts and Sporn, 1988). TGFβ acts as a growth inhibitor by

inhibiting the actions of growth factors such as EGF/TGFα. TGFβ can also promote cellular differentiation, extracellular matrix production, and chemotaxis. Different subtypes of TGFβ are produced as latent secreted precursors. Most cell types contain receptors for this ubiquitous factor.

Skinner et al. (1987a) and Gangrade and May (1990) demonstrated that ovarian theca cells express and produce TGFβ *in vivo*, immunocytochemical localization of TGFβ is primarily confined to the theca cell layer (Thompson et al., 1989). Several recent studies have confirmed the immunocytochemical localization of TGFβ isoforms in follicle cells (Chegini and Williams, 1992; Chegini and Flanders, 1992); this may vary with follicle development and hormone treatment (Roy *et al.*, 1992). Although freshly isolated bovine granulosa cells do not appear to express TGFβ (Skinner *et al.*, 1987a), cultured rat granulosa cells produce TGFβ which can be suppressed by FSH (Kim and Schomberg, 1989; Mulheron and Schomberg, 1990). Therefore, theca cells appear to be a predominant source of ovarian TGFβ, but granulosa cells also have the capacity to express TGFβ. The specific types of TGFβ expressed and their hormonal and developmental regulation remain to be fully elucidated (Mulheron *et al.*, 1991, 1992).

Local production of TGFβ allows it to act on various ovarian cell types. TGFβ stimulates a number of granulosa cell functions, including FSH-induced LH receptors (Knecht *et al.*, 1986; Dodson and Schomberg, 1987), EGF actions (Feng *et al.*, 1986), FSH-induced aromatase activity (Ying *et al.*, 1986; Hutchinson *et al.*, 1987), IGF-1 production (Mondschein *et al.*, 1988), and inhibin production (Zhiwen *et al.*, 1988). TGFβ can also influence theca cell function and steroidogenesis (Magoffin *et al.*, 1989; Caubo *et al.*, 1989; Hernandez *et al.*, 1990b), and oocyte maturation (Feng *et al.*, 1988; Tsafriri *et al.*, 1989). However, it is not known if TGFβ acts directly or indirectly on oocytes. In addition to effects on cellular differentiation, TGFβ can also influence ovarian cell growth. TGFβ has been shown to inhibit TGFα/EGF-induced bovine and porcine granulosa cell growth (Skinner *et al.*, 1987a; Mondschein *et al.*, 1988). Conflicting data were found with rat granulosa cells (Dorrington *et al.*, 1988). TGFβ can also inhibit TGFα/EGF-induced theca cell growth (Roberts and Skinner, 1991). Observations suggest that TGFβ may have an important role as a growth inhibitor in the ovary. The ability of TGFβ to inhibit cell growth allows for a more differentiated state of the cell that is reflected in the generally stimulatory effects of TGFβ on cell function. Therefore, the influence of TGFβ on cell function may be indirectly mediated through the inhibition of cellular proliferation. Growth inhibition may be important in preventing premature cell growth of the preantral follicle, arresting cell growth during atresia, and controlling cell growth during follicle cell expansion.

The local production and action of TGFβ within the developing ovarian follicle implies that TGFβ is an important paracrine and autocrine factor for ovarian cell–cell interactions. The hormonal regulation of TGFβ production

(Bendell and Dorrington, 1991) may also have a role in the endocrine regulation of ovary growth. The physiological significance of TGFβ in the ovarian follicle remains to be elucidated.

D. Fibroblast Growth Factor

Fibroblast growth factor (FGF) can influence aspects of both cellular growth and differentiation (Gospodarowicz *et al.*, 1987). Aside from growth stimulation, recent studies indicate that FGF may play a critical role in angiogenesis and tissue repair. FGF has many cellular targets and is important in many organ systems, including the ovary (Gospodarowicz and Ferrara, 1989).

Basic FGF is produced by granulosa cells in the developing embryonic gonad (Gonzalez *et al.*, 1990) and in the adult ovary (Neufeld *et al.*, 1987; Koos and Olson, 1989; Guthridge *et al.*, 1992). The angiogenic factor in the ovary and corpus luteum has been identified as FGF (Gospodarowicz *et al.*, 1985). FGF can act on granulosa cells to alter the steroidogenic capacity of the cell (Baird and Hsueh, 1986; Adashi *et al.*, 1988a), gonadotropin receptors (Mondschein and Schomberg, 1981), and plasminogen activator expression (LaPolt *et al.*, 1990; Tilly and Johnson, 1990). In addition to these effects on cell function, FGF can stimulate granulosa cell proliferation (Gospodarowicz *et al.*, 1977; Gospodarowicz and Bialecki, 1979), and may indirectly cause many of the effects observed on cell function. An additional role for FGF production in the ovary is to act as an angiogenic factor and promote vascularization of the developing follicle and corpus luteum.

E. Platelet-Derived Growth Factor and Vascular Endothelial Growth Factor

Platelet-derived growth factor (PDGF) is a common growth factor that allows cells to become competent to enter the growth cycle. PDGF acts on granulosa cells to enhance FSH-induced progesterone secretion, adenylate cyclase activity (Knecht and Catt, 1983b), plasminogen activator production (Tilly and Johnson, 1990), and LH receptor induction (Knecht and Catt, 1983b; Mondschein and Schomberg, 1984). The effects of PDGF on ovarian cell growth remain to be elucidated, but action as a potential competence factor for cell proliferation is a plausible activity. The local production of PDGF in the ovary remains to be examined. A factor that is structurally related to PDGF has been identified as vascular endothelial growth factor (VEGF) (Leung *et al.*, 1989; Conn *et al.*, 1990). This growth factor is expressed in the ovary, particularly in luteal tissue, and is postulated to have a role in angiogenesis of the follicle (Phillips *et al.*, 1990; Ravindranath *et al.*, 1992).

F. Nerve Growth Factor

Nerve growth factor (NGF) is another mitogen that may mediate intercellular interactions involving growth (Yanker and Shooter, 1982). NGF is important for the development and maintenance of sympathetic neurons in the peripheral nervous system and cholinergic neurons in the central nervous system. Its expression typically correlates with the amount of sympathetic innervation.

NGF is also expressed in the ovary (Lara *et al.*, 1990a) and is affected by ovarian innervation (Lara *et al.*, 1990a,b). NGF antibodies inhibit ovarian sympathetic innervation (Lara *et al.*, 1990c). The low-affinity NGF receptor is expressed in the ovary and is regulated upon ovulation (Dissen et al., 1991). NGF production in the ovary is therefore likely to have actions on ovarian function through sympathetic innervation.

G. Additional Growth Factors

Several additional types of growth factors act and/or are produced by ovarian cells. One such factor is stem cell factor (SCF)/c-kit ligand. Zsebo *et al.* (1990a) characterized stem cell factor and found that it influences stem cell growth and development. SCF acts at the c-kit tyrosine kinase receptor (Zsebo *et al.*, 1990b) and therefore is also referred to as the c-kit ligand. The c-kit tyrosine kinase receptor is expressed in the ovary by oocytes at various stages of development (Manova *et al.*, 1990; Horie *et al.*, 1991). The c-kit receptor expression appears to decline with the onset of meiotic maturation, suggesting a role for SCF in meiotic arrest (Horie *et al.*, 1991). Expression of SCF by follicular cells suggests a role for SCF to mediate cell–cell interaction with the oocyte. It is anticipated that many additional growth factors will be identified upon further investigation. For example, keratinocyte growth factor and hepatocyte growth factor are present in the ovary and appear to mediate theca-granulosa all interactions (unpublished observation).

III. Summary

It is apparent that a large number of growth factors are produced and act in the ovary. Most of the research to date has focused on how specific growth factors affect differentiated functions of gonadal cell types. Factors that promote cell growth generally have suppressive effects on differentiation and attenuate hormone responsiveness. Factors that inhibit growth generally enhance differentiation and increase hormone responsiveness. When considering the function and physiology of locally produced growth factors, a distinction needs to be made between growth and differentiation. A factor that promotes cell proliferation and the cell cycle will indirectly

reduce the differentiated state of the cell, while the inverse is true of a factor that arrests cell proliferation and inhibits the cell cycle. Although specific growth factors may have a role in regulating differentiated functions, the possibility exists that many of the actions observed may be the indirect result of effects on cell growth. Therefore, the physiological importance of growth factor regulation of differentiated function remains to be elucidated. The control of cell growth, however, is a major function of specific growth factors. The integrated actions of various factors such as TGFα and TGFβ could provide an efficient mechanism for regulating the cell proliferation required in gonadal development. Further investigation of the developmental regulation of the expression, production, and action of individual growth factors will provide insight into the potential physiological roles for the various growth factors. Evidence obtained to date implies that growth factors will be critical regulatory agents involved in ovarian cell–cell communication.

The endocrine regulation of ovarian cell growth has been well documented *in vivo*. The actions of gonadotropins and reproductive steroids, however, are distinct from the pharmacology of most growth factors. In addition, these hormones often have negligible effects on cell proliferation *in vitro*. The possibility that the actions of reproductive hormones on gonadal cell growth are indirectly mediated through alterations in the expression of locally produced growth factors needs to be seriously considered. Current work suggests that hormones may regulate growth factor production. Further investigation of the hormonal regulation of the production and actions of growth factors will help elucidate the mechanisms involved in the endocrine regulation of gonadal development.

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