Cellular Interactions That Control Primordial Follicle Development and Folliculogenesis

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Specific factors that mediate local cell–cell interactions in the ovary related to the initiation and progression of follicle development will be discussed. Recently, several factors produced locally by the primordial follicle have been shown to induce primordial follicle development from a quiescent state to promote follicle development. Kit ligand/stem cell factor (KL/SCF) produced by the immature granulosa cells appears to promote theca cell organization. Basic fibroblast growth factor produced predominately by the oocyte, but by all cells at reduced levels, also was found to induce primordial follicle development similar to KL. It is likely that numerous locally produced factors will mediate cellular interactions and interact between each other to control the induction of primordial follicle development and influence processes such as the onset of puberty and menopause. After follicle development has been induced, theca cells and granulosa cells interact through classical mesenchymal–epithelial type interactions to influence the progression of follicle development. Mesenchymally derived theca cells have been shown to produce transforming growth factor alpha (TGF-α), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and transforming growth factor beta to regulate granulosa cell growth and function. The epithelial granulosa cells have been show to produce KL/SCF that can feed back on the theca cells to regulate theca cell growth and stimulate the production of the theca cell factors (TGF-α, KGF, and HGF). Therefore, a positive feedback loop between the theca cells and granulosa cells appears to exist to promote the dramatic cell growth required during folliculogenesis. Interestingly, hormones such as estrogen and gonadotropins stimulate the expression of these paracrine growth factors. Therefore, the actions of hormones to stimulate follicle development and growth are mediated in part through altering these local cell–cell interactions. In summary, the locally produced paracrine factors that mediate cell–cell interactions involved in primordial follicle development and the progression of follicle development during folliculogenesis are starting to be elucidated. (J Soc Gynecol Investig 2001;8:S17–S20)

KEY WORDS: Basic fibroblast growth factor, kit ligand, primordial, follicle, ovary, development, mesenchymal-epithelial, cell–cell interactions.

Some crucial aspects of ovarian biology, which are just beginning to be understood, are the initiation of primordial follicle development and the control of subsequent folliculogenesis. Females are born with a pool of oocytes organized into primordial follicles. This pool of primordial follicles represents the complete supply of oocytes that may potentially ovulate. During each menstrual cycle waves of primordial follicles initiate follicular development. Most of these developing follicles degenerate through the specific process of atresia involving apoptosis. In a mono-ovulator one follicle is selected as the dominant follicle and eventually ovulates. When the supply of oocytes (ie, primordial follicles) is diminished menstrual cyclicity ends and humans enter menopause. Therefore, the factors that control initiation of primordial follicle development and the subsequent progression of follicle development ultimately determine reproductive fitness and the age of the menopausal transition in humans.

The role of the locally acting growth factors kit ligand/stem cell factor (KL), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), and hepatocyte growth factor (HGF) is reviewed. These are factors known for their role in mesenchymal–epithelial cell interactions. It has long been recognized that growth and differentiation of epithelial cells are directed by adjacent mesenchymal cells during embryonic development and are optimally maintained by adjacent stroma in adult tissues. The ability of epithelial cells to feed back and communicate with mesenchymal cells is equally important. Both mesenchymal cells and adjacent epithelial cells produce factors that act in a paracrine manner to regulate cellular functions.

Kit ligand and its receptor c-kit are essential for oocyte migration during embryonic development[1–3] and follicular development in the adult ovary.[4–6] KL and c-kit are the products of the Steel and White Spotting loci in mice, respectively.[7–13] A number of mutations at Sl or W have been described that cause sterility owing to defects in oocyte migration or follicular development. Ovaries in mice carrying steel panda (Sl^{Pan}), steel t (Sl^{t}), and steel contrast (Sl^{con}) mutations contain follicles that arrest at early stages of follicular development.[14–16] The pool of primordial follicles is estab-

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Published by Elsevier Science Inc.

1071-5576/01/$20.00
PII S1071-5576(00)00099-X

lished in these mutant mice, but initiation and progression of primordial follicle development are inhibited. Granulosa cells in developing follicles produce KL[4,17] which can act on theca cells, stromal cells, and oocytes. Differentiated theca cells, undifferentiated stromal cells, and developing oocytes express the receptor c-kit.[4,17–19] KL has a variety of effects on isolated oocytes including the promotion of growth and maintenance of meiotic arrest.[5,20–23] These observations suggest that KL may be essential for initiation and/or progression of primordial follicle development in the ovary.

Experiments examined the effect of KL on initiation of primordial follicle development.[24] Ovaries from day 4 postnatal rats were cultured for 5 or 14 days using a floating filter culture system. These cultured ovaries were treated with KL (100 ng/mL) or with the c-kit receptor blocking antibody ACK-2 (1:100 dilution).[25–27] Primordial follicle development was characterized in these ovaries after culture and in freshly collected 4-day-old rat ovaries. Follicles were counted and classified as undeveloped primordial follicles (stage 0), early primary follicles (stage 1), primary follicles (stage 2), transitional follicles (stage 3), and preantral follicles (stage 4) using a previously described procedure.[28] Sections of fresh 4-day-old rat ovaries contained 68% primordial follicles (stage 0), 12% early primary follicles (stage 1), 11% primary follicles (stages 2), and 9% transitional and preantral follicles (stages 3 to 4). Under control conditions, untreated ovaries cultured for 14 days showed a significant reduction in the percentage of primordial follicles (stage 0) per section to 50% which was coupled to an increase to 50% in the percentage of developing follicles (stages 1 to 4). Over 83% of the follicles per section initiated development after 14 days of KL treatment. The ability of KL to promote development of follicles suggests that KL is sufficient to initiate primordial follicle development in the ovary.[24] ACK-2 antibody treatment completely blocked the spontaneous follicle development that occurred in control cultures. The percentage of primordial (stage 0) and early primary follicles (stage 1) were identical in freshly collected ovaries and ovaries cultured in the presence of ACK-2. This inhibition of follicle development by ACK-2 suggests that endogenous KL is necessary for the spontaneous development of primordial follicles in these cultures.

Basic fibroblast growth factor has been localized to the oocytes of primordial and primary follicles of many species[29] but not to putative granulosa cells of human primordial follicles.[30] The bFGF is also localized to granulosa cells of preantral follicles. Theca cells of developing follicles also stain positive for bFGF,[29,30] although Wordinger et al[31] observed no theca staining in antral mouse follicles. Receptors for bFGF have been reported in rat[32] and bovine[33] granulosa cells. bFGF is important in regulating a wide range of ovarian functions including granulosa cell mitosis,[34–37] steroidogenesis,[38] differentiation,[39] and apoptosis.[40] In addition, bovine granulosa cells have been shown to produce bFGF[41] in the preantral and antral follicle stages.

In organ culture experiments in this laboratory, the ability of bFGF to promote development of primordial follicles was investigated.[42] Ovaries cultured with bFGF showed a significant decrease in the number of primordial follicles and a corresponding increase in developing stage 1 to 4 follicles compared with controls. This demonstrates that bFGF, like KL, induces primordial follicle development to initiate folliculogenesis. How KL and bFGF may interact in this process of primordial follicle development remains to be determined. Because bFGF is expressed in the oocyte whereas KL is expressed in granulosa cells, the potential oocyte–granulosa cell interactions involving these factors may be important in the process (Figure 1). The potential ability of bFGF to directly regulate theca cell or ovarian stromal cell function was investigated.[42] bFGF was found to stimulate growth of bovine theca and stroma cells in culture as measured by [^{3}H]thymidine incorporation assay. Previous research has demonstrated that bFGF also stimulates granulosa cell growth.[34–37] Therefore, all the somatic cell types surrounding an oocyte respond to bFGF. Localization of bFGF by immunocytochemistry demonstrated high levels of bFGF in the oocytes of early stage (ie, primordial) follicles. These results suggest that oocyte-derived bFGF may promote ovarian granulosa, stromal, and theca cell growth during primordial and early follicular development. bFGF appears to induce primordial follicle development in part, by directly acting on adjacent ovarian cells to promote somatic cell growth.

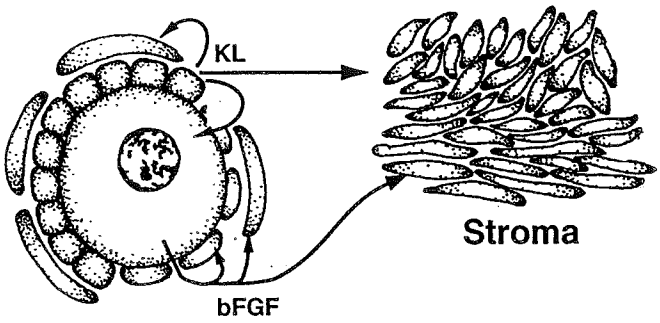

Figure 1. Summary of primordial follicle induction.

Other growth factors are also involved in the progression of follicular development once initiation of primordial follicle development has occurred. Cell–cell interactions between thecal and granulosa cells are essential for follicular development in the ovary. These mesenchymal–epithelial interactions are in part mediated by KGF, HGF, and KL. KGF and HGF are mesenchymal-derived growth factors that act on adjacent epithelial cells in a number of tissues.[43–47] KGF is a member of the fibroblast growth factor family (FGF7) and HGF is the ligand for the c-met receptor. Mesenchymal-derived thecal cells express the KGF and HGF genes and secrete the proteins.[44,49,50] KGF and HGF can regulate granulosa cell function and growth.[44,49,50] Epithelial-derived granulosa cells express the KL gene and secrete the protein.[48] KL can regulate thecal cell function and growth.[48] The expression of all of these factors increases to the highest levels in large antral follicles.[48–50] Therefore, the actions of KGF, HGF, and KL are postulated to be involved in folliculogenesis, particularly during later stages of follicular development.

A positive feedback loop has been identified between thecal cells and granulosa cells that is mediated by KGF, HGF, and

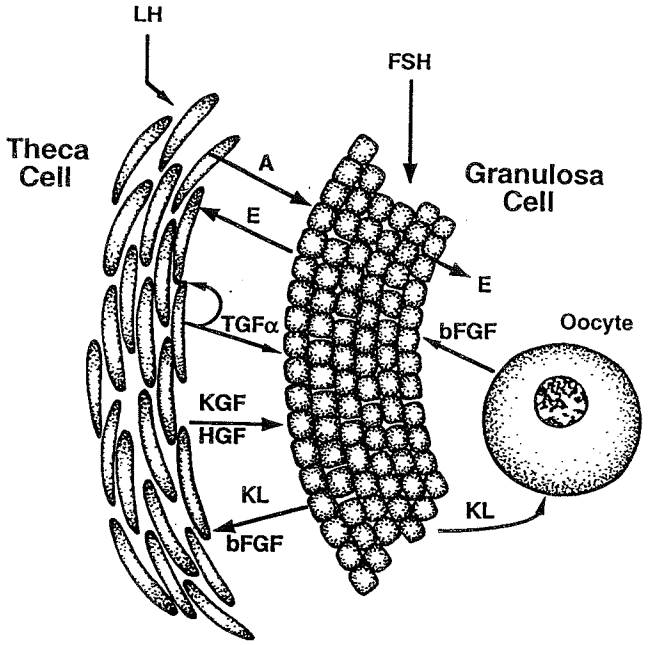

Figure 2. Summary of antral follicle control.

KL.[51] Treatment of thecal cells with granulosa cell–derived KL stimulated both KGF and HGF gene expression. Thecal cell-derived KGF and HGF also stimulate KL expression in granulosa cells. As the expression of KGF, HGF, and KL is greatest in large antral follicles,[48–50] the positive feedback among these factors may be particularly important during later stages of follicular development.[51]

Hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone stimulate follicular growth in vivo.[52–56] The actions of such hormones in the ovary are necessary for follicular development and reproductive viability. Although these hormones increase follicular growth in vivo, no proliferative effect is apparent on purified cells in vitro. As a result, the hypothesis has developed that gonadotropins and steroids indirectly stimulate follicular growth by influencing local mesenchymal–epithelial cell interactions in the ovary. Our previous observations suggest that hormones can directly regulate KGF and HGF expression in thecal cells.[48–51] It is also demonstrated that FSH and human chorionic gonadotropin directly stimulate KL expression in granulosa cells. The ability of gonadotropins to influence KGF, HGF, and KL gene expression provides an indirect mechanism for gonadotropins to regulate folliculogenesis.

The current hypothesis involving mesenchymal–epithelial cell interactions and these growth factors during folliculogenesis is summarized in Figure 2. This schematic of the cells in a follicle depicts both the endocrine and paracrine regulation of thecal, granulosa, and oocyte cell functions. It is well established that gonadotropins regulate thecal and granulosa cell–differentiated functions. Steroid-mediated thecal cell–granulosa cell interactions through androgen and estrogen are critical and are influenced by gonadotropins. Locally produced growth factors such as KGF, HGF, bFGF, and KL expression in the ovary are important in the local mediation of follicular development.

In summary, the cell–cell interactions involved in both the induction of primordial follicles to initiate folliculogenesis (Figure 1) and subsequent control of folliculogenesis (Figure 2) are starting to be elucidated. Clearly, local cellular interactions mediated by specific growth factors will be essential for both processes. Hormone control of ovarian function appears to promote a cascade of cell–cell interactions to mediate a physiologic response. Therefore, further investigation of the local cellular interactions will be essential to understand ovarian physiology and the abnormalities associated with disease states such as premature ovarian failure and polycystic ovarian disease.

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