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Kit ligand and basic fibroblast growth factor interactions in the induction of ovarian primordial to primary follicle transition

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Abstract

Ovulated eggs during a female's reproductive life are derived from a pool of primordial follicles arrested in prophase of the first meiotic division. When follicles leave the resting pool they undergo a primordial to primary follicle transition and will grow and develop until either ovulation occurs or follicles undergo atresia. Several growth factors have been implicated as acting locally within the ovary to regulate the primordial to primary follicle transition. How these growth factors may interact and cooperate to perform this vital function remains to be elucidated. The objective of the current study is to investigate interactions between kit ligand (KL) (i.e. stem cell factor) and basic fibroblast growth factor (bFGF) that promote the primordial to primary follicle transition in rat ovaries. Ovaries were removed from 4-day-old rat pups and cultured for 2 weeks with KL alone or with KL and a neutralizing antibody against bFGF. The ability of KL treatment to increase primordial follicle transition was blocked with a bFGF neutralizing antibody. In addition, ovary cultures were treated with bFGF alone or with bFGF and an anti-c-kit receptor antibody which blocks KL signaling. The ability of bFGF treatment to increase primordial follicle transition was blocked with an anti-c-kit receptor antibody. Observations indicate that both KL and bFGF must be active in order to optimally promote the changes that occur in oocytes, granulosa cells, and stromal/interstitial cells when primordial follicles initiate development. Cultured ovaries were treated with either KL or bFGF for 3 days and then bFGF and KL mRNA expression levels in the whole ovary were measured. KL was not found to regulate bFGF expression. In contrast, bFGF treatment was found to increase KL mRNA expression in cultured ovaries. These observations suggest that one function of the oocyte-derived bFGF is to increase the granulosa derived KL expression and that both KL and bFGF are required to optimally promote primordial to primary follicle transition. Elucidating the cell–cell interactions that mediate this network of specific locally derived growth factors is critical to understanding the physiology of the primordial to primary follicle transition. © 2003 Elsevier Ireland Ltd. All rights reserved.

Keywords: Ovary; Primordial follicle; Growth factors; Cell–cell interactions; KL; bFGF; Oocyte; Granulosa cell

1. Introduction

Female reproduction depends upon the growth and development of ovarian follicles leading to the ovulation of viable oocytes. The source for ovulated eggs during a female's reproductive life is the pool of primordial follicles arrested in prophase of the first meiotic division. A primordial follicle is composed of an oocyte arrested in the diplotene stage of prophase one of meiosis and surrounded by squamous pre-granulosa cells. When follicles leave the resting pool they undergo a primordial to primary follicle transition and the surrounding squamous pre-granulosa cells become cuboidal granulosa and begin to proliferate (Peters

et al., 1975; Cran and Moor, 1980; Hirshfield, 1991; Rajah et al., 1992). As follicles continue to develop through the primary, secondary and pre-antral stages they gain successive layers of granulosa cells, the oocyte increases in size and theca cells surround the follicle. When the available pool of primordial follicles is depleted female reproductive function ceases and humans enter menopause (Gosden et al., 1983; Richardson et al., 1987; Faddy et al., 1992; Faddy, 2000). An understanding of the role of growth factors in controlling this orderly follicular progression will lead to improved therapies for infertility and control of the menopausal transition.

Several growth factors have been identified as acting locally within the ovary to regulate the primordial to primary follicle transition. Bone morphogenetic protein-7 (BMP-7) has been shown to promote the primordial to primary follicle transition and to increase granulosa cell proliferation (Lee

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et al., 2001). Leukemia inhibitory factor (LIF) has also been shown to promote the primordial to primary follicle transition and to up-regulate granulosa cell expression of kit ligand (KL) (Nilsson et al., 2002). Bone morphogenetic protein-15 (BMP-15) is a growth factor expressed in the oocytes of developing follicles that plays a role in early follicle progression (Dube et al., 1998; Laitinen et al., 1998). BMP-15 stimulates proliferation of pre-antral granulosa cells and inhibits FSH-stimulated progesterone production in later stage granulosa (Otsuka et al., 2000). Müllerian inhibitory substance (MIS or AMH) is a growth factor produced by the granulosa cells of developing pre-antral and small antral follicles which inhibits the primordial to primary follicle transition (Baarends et al., 1995; Durlinger et al., 1999, 2002).

Both kit ligand and basic fibroblast growth factor (bFGF) have also been shown to be important in promoting the primordial to primary follicle transition in the rat (Parrott and Skinner, 1999; Nilsson et al., 2001). KL is produced by the granulosa cells of developing ovaries (Manova et al., 1993; Motro and Bernstein, 1993) and KL receptors (c-kit) are present on oocytes and theca cells (Manova et al., 1990, 1993; Horie et al., 1991; Motro and Bernstein, 1993). Theca cells from antral follicles will proliferate in culture in response to KL treatment (Parrott and Skinner, 1997) and KL acts to recruit theca cells from surrounding ovarian stroma during folliculogenesis (Parrott and Skinner, 2000). Therefore, KL is thought to act as a signal from the granulosa cells around primordial follicles to the oocyte and surrounding stroma to promote the events of the primordial to primary follicle transition (Parrott and Skinner, 1999, 2000). Basic FGF is expressed by the oocytes of primordial follicles and the granulosa cells of developing follicles (van Wezel et al., 1995; Yamamoto et al., 1997; Nilsson et al., 2001). Receptors for bFGF are found on granulosa cells (Shikone et al., 1992; Wandji et al., 1992). Granulosa and theca from antral follicles, as well as ovarian stromal cells, will proliferate in response to bFGF (Lavranos et al., 1994; Rodgers et al., 1996; Roberts and Ellis, 1999; Nilsson et al., 2001). Therefore, oocyte-derived bFGF is thought to signal to surrounding granulosa and stromal cells to promote the primordial to primary follicle transition (Nilsson et al., 2001).

Each of the above mentioned growth factors has been shown separately to regulate the primordial follicle transition. The current study investigates interactions between KL and bFGF that promote the primordial to primary follicle transition in rat ovaries.

2. Materials and methods

2.1. Organ cultures

Postnatal 4-day rat ovaries were dissected from freshly euthanized rat pups. All animal procedures were reviewed

and approved by the Washington State University Institutional Animal Care and Use Committee. Whole ovaries were cultured as previously described (Nilsson et al., 2001) on floating filters (0.4 μ m Millicell-CM, Millipore, Bedford, MD) in 0.5 ml Dulbecco's Modified Eagle's Medium (DMEM)-Ham's F-12 medium (1:1, v/v) containing 0.1% bovine serum albumin (BSA, Sigma, St. Louis, MO), 0.1% Albumax (Gibco-BRL, Gaithersburg, MD), 27.5 μ g/ml transferrin, 1 μ g/ml insulin (human recombinant, Sigma), and 0.05 mg/ml L-ascorbic acid (Sigma) in four well culture plate (Nunc plate, Applied Scientific, South San Francisco, CA). Ovaries were randomly assigned to treatment groups with 2–3 ovaries per floating filter in each well. Treatments during organ culture included recombinant human basic fibroblast growth factor (R&D Systems, Inc., Minneapolis, MN) at 40 ng/ml, or rat kit ligand (KL or SCF) (Amgen, Thousand Oaks, CA) at 50 ng/ml, or anti-bFGF neutralizing antibody at 20 μ g/ml, or c-kit receptor blocking antibody (ACK-2, Gibco-BRL, Carlsbad, CA) at 15 μ g/ml. These concentrations of reagents were determined to be optimal in previous experiments demonstrating higher concentrations did not enhance activities found. Anti-bFGF neutralizing antibody (R&D systems, cat AB-233-NA) was found by the manufacturer not to cross-react with human or mouse KL, or to the human c-kit receptor. ACK-2 (Gibco-BRL, cat 13314-018) was found to recognize the extracellular domain of the c-kit receptor (Horie et al., 1991; Nishikawa et al., 1991; Ogawa et al., 1991; Rolink et al., 1991; Yoshinaga et al., 1991). Medium was supplemented with penicillin and streptomycin to prevent bacterial contamination. After culture, ovaries were fixed, sectioned and stained with hematoxylin/eosin for use in morphological analysis. Alternatively, if mRNA levels were to be measured from cultured ovaries, then after culture two ovaries were pooled and homogenized in 500 μ l Trizol (Gibco-BRL, Rockville, MD) and stored at -20°C until RNA isolation.

2.2. Histology

The number of follicles at each developmental stage were counted in two serial sections and averaged from the largest cross-sections through the center of the ovary. The oocyte nucleus had to be visible in a follicle in order to be counted. Normally, 150–200 follicles were present in a cross-section. It has previously been demonstrated that total follicle number per section does not change after 2 weeks of culture compared to freshly isolated 4-day-old ovaries (Parrott and Skinner, 1999). Follicles were classified as either primordial (stage 0), or as one of the developing pre-antral stages (stages 1–4) as described previously (Parrott and Skinner, 1999). Briefly, primordial follicles consist of an oocyte partially or completely encapsulated by flattened squamous pre-granulosa cells. Developing (stages 1–4) follicles contain successively more cuboidal granulosa cells in layers around the oocyte (Parrott and Skinner, 1999; Nilsson et al., 2001).

2.3. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cultured ovaries homogenized in Trizol reagent as per the protocol provided (Gibco-BRL, Rockville, MD). RNA was stored at -70°C until use. As described previously (Parrott et al., 2000) the RNA samples were reverse transcribed to cDNA by adding 1 μg RNA to 1 μl each of 1 μM 3' kit ligand, basic fibroblast growth factor and cyclophilin (1B15) primers. Primer sequences are detailed below. Before reverse transcription tubes containing total RNA and 3'-primers were heated to 65°C for 10 min and cooled to room temperature. Total RNA was reverse transcribed for 1 h at 42°C using the following conditions: 1 μg total RNA, 1 μM specific 3'-primers, 0.1 mM dNTPs, 10 mM DTT, 40 U RNase inhibitor (Promega, Madison, WI), and 200 U M-MLV reverse transcriptase in 40 μl RT buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl_2). After 1 h samples were heated to 95°C for 5 min to inactivate the reverse transcriptase enzyme. Steady state levels of KL, bFGF, and cyclophilin (i.e. 1B15) mRNAs were analyzed using a specific quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay for each gene (Parrott et al., 2000). These quantitative RT-PCR assays have previously been described in detail (Parrott and Skinner, 1997). The primers used were: KL, 5'-GGA CAA GTT TTC GAA TAT TTC TGA AGG CTT GAG TAA TTA TTG-3' (5'-primer; 42-mer) and 5'-AGG CCC CAA AAG CAA ACC CGA TCA CAA GAG-3' (3'-primer; 30-mer), which generated a 452-bp KL PCR product for the soluble KL-1 isoform; bFGF, 5'-GTT GGT ATG TGG CAC TGA AAC-3' (5'-primer, 21-mer) and 5'-TGG GTC ACA ACC AAG CTA TA-3' (3'-primer, 20-mer), which generated a specific 271-bp bFGF PCR product; and 1B15, 5'-ACA CGC CAT AAT GGC ACT GGT GGC AAG TCC ATC-3' (5' primer, 33-mer) and 5'-ATT TGC CAT GGA CAA GAT GCC AGG ACC TGT ATG-3' (3'-primer, 33-mer) which generated a specific 1B15 105-bp product. Amplification was performed using the following conditions: 0.4 μM each primer, 25 μM dNTPs, and 0.5 U AmpliTaq polymerase in 25 μl GeneAmp PCR buffer (containing 1.5 mM MgCl_2 , Perkin-Elmer). Each PCR amplification consisted of an initial denaturing reaction (5 min, 95°C); 26–30 cycles of denaturing (1 min, 95°C), annealing 2 min, 55°C (KL) or 2 min, 54°C (bFGF) or 1 min, 60°C (1B15), and elongation 3 min (KL) or 2 min (bFGF, 1B15), 72°C reactions; and a final elongation reaction (10 min, 72°C). 1.0 μCi of ^{32}P -labeled dCTP (Redivue, Amersham Life Sciences, Arlington Heights, IL) was included in each sample during amplification for detection purposes. Specific PCR products were quantified by electrophoreses of all samples on 4–5% polyacrylamide gels, exposing the gels to a phosphor screen for 8–24 h, followed by quantifying the specific bands on a phosphor imager (Molecular Dynamics, Sunnyvale, CA). Each gene was assayed in separate PCR reactions from the same RT-PCR of unknown samples was

performed in parallel with known amounts of each gene inserted into a plasmid to use as a standard curve. Absolute quantities of each cDNA were read from the standard curves and then normalized per well for 1B15 in order to adjust for different numbers of cells per sample. The optimal number of cycles for amplification was determined for each assay in order to achieve maximum sensitivity while maintaining linearity (i.e. logarithmic phase of PCR reactions). KL and bFGF PCR products were amplified for 30 cycles and 1B15 PCR products were amplified for 26 cycles. The sensitivity of each quantitative PCR assay was below 1 fg, which corresponds to less than 125 fg target mRNA/ μg total RNA.

2.4. Statistics

Pairs comparisons were performed using Student's *t*-test. Multiple comparison tests were performed using Dunnett's analysis after a significant difference had been found with ANOVA. Groups were considered significantly different if $P \leq 0.05$. The *n*-values for each treatment group were the total number of samples pooled from three separate experiments. Each separate whole ovary assayed independently was defined as the experimental unit. All statistics were calculated with the help of GraphPad Prism, version 3.0a for Macintosh, GraphPad Software (San Diego CA USA).

3. Results

Experiments were performed to determine if the growth factors KL and bFGF interact to regulate the primordial to primary follicle transition and early follicle development. Ovaries from 2- to 4-day-old rats contain mostly primordial follicles. In order to determine if bFGF plays a role in the process by which KL promotes the primordial to primary follicle transition, the ovaries were removed from 4-day-old rat pups and cultured for 2 weeks with 40 ng/ml KL alone or with KL and a neutralizing antibody against bFGF. As expected (Parrott and Skinner, 1997), KL treatment caused an increase ($P < 0.001$) in the proportion of developing follicles (i. e. those having undergone the primordial to primary follicle transition) compared to untreated controls. When ovaries were treated with both KL and anti-bFGF antibody the proportion of developing follicles was not different than that of controls (Fig. 1). This indicates that anti-bFGF neutralizing antibody interferes with the ability of KL to promote follicle transition and progression. Anti-bFGF antibody alone had no effect on primordial follicle transition.

In order to determine if KL plays a role in the process by which bFGF promotes the primordial to primary follicle transition, the converse experiment to that described above was performed. Four-day-old rat ovaries were cultured for 2 weeks with bFGF alone or with bFGF and a blocking antibody against the c-kit receptor that binds KL. As expected (Nilsson et al., 2001), bFGF treatment caused an increase ($P < 0.001$) in the proportion of developing follicles. When

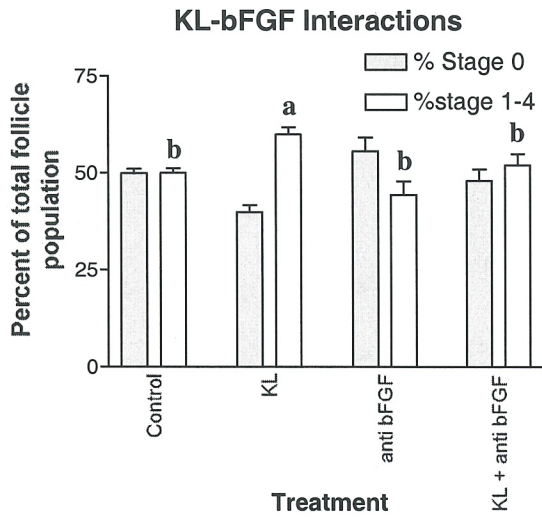


Fig. 1. Effect of anti-bFGF neutralizing antibody on the ability of KL treatment to promote the primordial to primary follicle transition in cultured ovaries. Ovaries from 4-day-old rats were placed into culture for 14 days. Cultured ovaries were incubated with 40 ng/ml KL alone or with KL and 20 μ g/ml anti-bFGF antibody. After culture all ovaries were fixed, stained, and subjected to morphological analysis. The follicles per ovary cross-section were categorized as being either primordial or developing (which includes all follicles having undergone the primordial to primary transition). Data is presented as the mean \pm S.E.M. from three separate experiments with $n = 4-10$ per treatment group. The proportion of developing follicles was significantly higher with KL treatment compared to any other treatment ($P \leq 0.05$ by Dunnett's test with KL treated set as control). Different letter superscripts indicate a statistical difference.

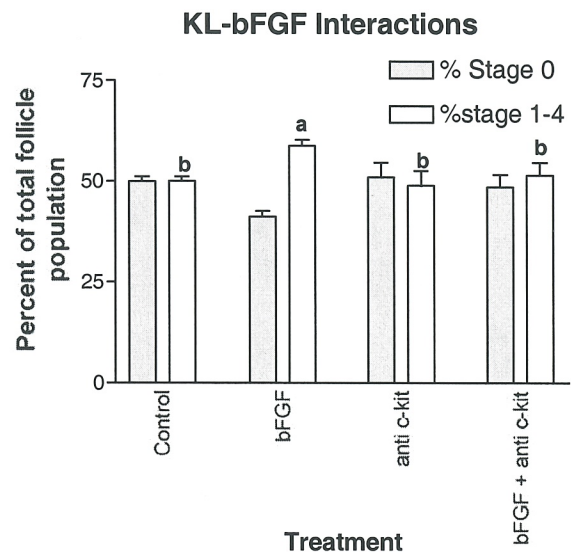


Fig. 2. Effect of anti-c-kit blocking antibody on the ability of bFGF treatment to promote the primordial to primary follicle transition in cultured ovaries. Ovaries from 4-day-old rats were placed into culture for 14 days. Cultured ovaries were incubated with 50 ng/ml bFGF alone or with bFGF and 15 μ g/ml anti-c-kit antibody. After culture all ovaries were fixed, stained, and subjected to morphological analysis. The follicles per ovary cross section were categorized as being either primordial or developing (which includes all follicles having undergone the primordial to primary transition). Data is presented as the mean \pm S.E.M. from three separate experiments with $n = 4-10$ per treatment group. The proportion of developing follicles was significantly higher with bFGF treatment compared to any other treatment ($P \leq 0.05$ by Dunnett's test with bFGF treated set as control). Different letter superscripts indicate a statistical difference.

ovaries were treated with both bFGF and anti-c-kit receptor antibody the proportion of developing follicles was not different than that of controls (Fig. 2). This indicates that anti-c-kit receptor blocking antibody interferes with the ability of bFGF to promote the primordial to primary follicle transition. Treatment with anti-c-kit antibody alone had no effect on primordial follicle transition.

The effects of KL, bFGF, and the neutralizing antibodies on total follicle number was investigated to confirm that no change in total follicle number occurs which might effect data interpretation. None of the growth factor or antibody treatments affected the total number of follicles per ovarian section (Fig. 3). Therefore, the effects observed on specific populations are related to a developmental transition from a primordial follicle to a primary follicle stage and not a reflection of changes in total follicle numbers.

Possible mechanisms by which the growth factors KL and bFGF might interact would be if KL induced bFGF expression in ovarian cells, or conversely if bFGF induced KL expression. In order to test if KL regulates the expression of bFGF, 4-day-old rat ovaries were cultured for 3 days in the presence or absence of 50 ng/ml KL. At the end of culture the amount of bFGF mRNA was measured using quantitative RT-PCR. KL treatment was not found to cause a change in bFGF mRNA expression in whole neonatal rat ovaries (Fig. 4). KL treatment was also not found to result in a

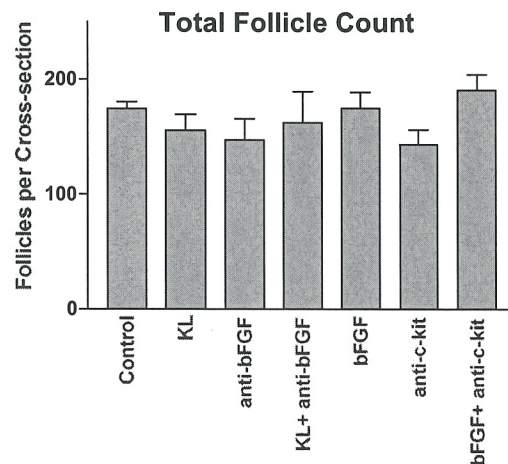


Fig. 3. Effect of growth factor and neutralizing antibody treatment on total follicle number in cultured ovaries. Ovaries from 4-day-old rats were placed into culture for 14 days. Cultured ovaries were incubated with 50 ng/ml bFGF, 15 μ g/ml anti-c-kit Ab, both bFGF and anti-c-kit Ab, 40 ng/ml KL, 20 μ g/ml anti-bFGF Ab or both KL and anti-bFGF Ab. After culture all ovaries were fixed, stained, and subjected to morphological analysis. The total number of follicles per ovary cross section were counted. Data is presented as the mean \pm S.E.M. ANOVA showed no significant difference in total follicle number between treatment groups.

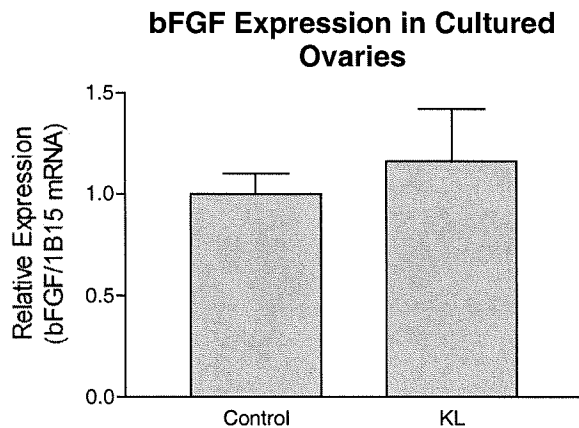


Fig. 4. Basic fibroblast growth factor mRNA expression in cultured ovaries in response to KL treatment. Ovaries from 4-day-old rats were placed into culture and treated with 50 ng/ml KL or were not treated for 3 days. RT-QPCR was performed on RNA from whole ovaries to determine levels of bFGF mRNA expression. Data are presented as relative expression as bFGF mRNA/1B15 mRNA (pg/well) normalized to untreated control values. Data are from three separate experiments ($n = 8-9$) with the mean \pm S.E.M. presented.

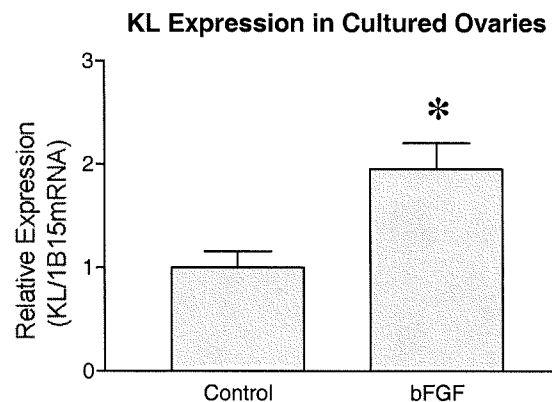


Fig. 5. KL mRNA expression in cultured ovaries in response to bFGF treatment. Ovaries from 4-day-old rats were placed into culture and treated with 40 ng/ml bFGF or were not treated for 3 days. RT-QPCR was performed on RNA from whole ovaries to determine levels of KL mRNA expression. Data are presented as relative expression as KL mRNA/1B15 mRNA (pg/well) normalized to untreated control values. Data are from three separate experiments ($n = 5-6$) with the mean \pm S.E.M. presented. Asterisk indicates a significant difference ($P < 0.05$) between treatments by Student's *t*-test.

change in KL mRNA expression (data not shown). Therefore, the interaction that occurs between KL and bFGF in the control of primordial to primary follicle transition is not the result of KL regulating bFGF expression.

In order to determine if bFGF regulates KL expression, 4-day-old rat ovaries were cultured for 3 days in the presence or absence of 40 ng/ml bFGF. At the end of culture the amount of KL mRNA in the cultured ovaries was measured using quantitative RT-PCR. It was found that bFGF treatment results in a significant ($P < 0.01$) increase in ovarian KL expression (Fig. 5). This indicates that bFGF positively regulates KL mRNA expression in whole ovaries. Basic FGF treatment was not found to result in a change in bFGF mRNA expression (data not shown).

4. Discussion

Several growth factors have been found that can regulate the primordial to primary follicle transition. The objective of the current study was to characterize how two of these factors, KL and bFGF, might interact to regulate this vital reproductive process. Experiments were performed to determine if the simultaneous presence of both KL and bFGF were required in order for primordial follicle transition to occur optimally. KL alone modestly increased the amount of primordial to primary follicle transition and the presence of anti-bFGF antibody abolished the ability of KL to promote the primordial follicle transition. This indicates that bFGF needs to be present in order for KL to maximally promote primordial follicle transition. From this experiment alone it is not known whether both KL and bFGF must be present simultaneously, or whether KL induces bFGF expression and

it is the action of bFGF which is responsible for primordial follicle transition. Basic FGF is not absolutely required for primordial transition to occur, as bFGF knock-out mice are fertile (Ortega et al., 1998). KL and other growth factors may compensate for the lack of bFGF in these mice.

Basic FGF alone also increased the amount of primordial to primary follicle transition, while the presence of c-kit blocking antibody abolished the ability of bFGF to promote primordial follicle transition. This indicates that KL must also be present in order for bFGF to maximally exert its actions. Taken together these experiments demonstrate that both KL and bFGF need to be present in order for the primordial to primary follicle transition to occur optimally. This argues against the idea that signaling from one of these growth factors simply induces expression of the other growth factor and that it is the second downstream factor that signals the primordial follicle to initiate follicle transition. Rather both KL and bFGF must act in parallel to optimally promote the primordial to primary follicle transition. However, this result does not eliminate the possibility that signaling from one of these growth factors might induce expression of the other.

Experiments were performed to determine whether KL treatment regulated bFGF expression. Ovaries were cultured with or without KL treatment and then bFGF mRNA expression was measured. KL was not found to regulate bFGF expression. However, in the converse experiment bFGF treatment was found to significantly increase KL expression. Therefore, bFGF induces an increase in KL expression, but both bFGF and KL must be present in the follicle to maximally promote the primordial to primary follicle transition. These findings are illustrated and summarized in Fig. 6.

KL is produced by pre-granulosa cells of primordial follicles and has been shown to act on the oocyte and on

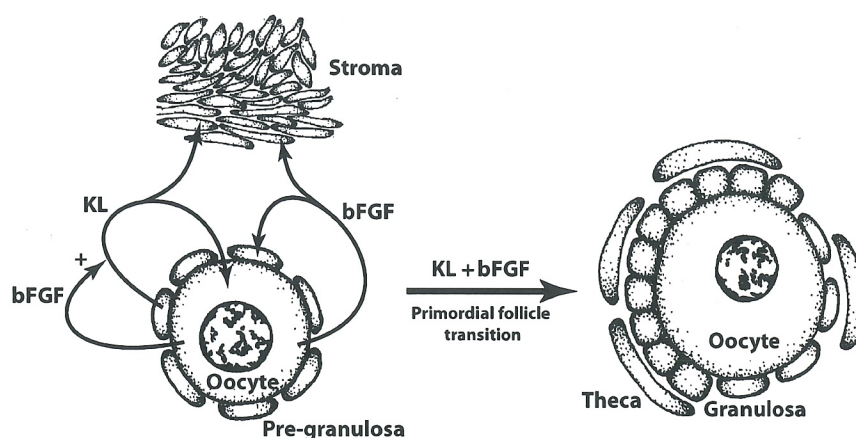


Fig. 6. Model of KL and bFGF actions and interactions as they relate to the primordial to primary follicle transition and early follicle development. Left image represents the primordial follicle. Right image represents a primary stage follicle. Arrows indicate the origins and targets of growth factor and hormonal actions. Arrows with a '+' at their head denote that they promote the activity to which they point.

surrounding stromal/interstitial cells (Manova et al., 1990, 1993; Horie et al., 1991; Motro and Bernstein, 1993; Parrott and Skinner, 2000). In contrast, bFGF is expressed by the oocytes of primordial follicles and acts on granulosa cells as well as stromal/interstitial cells (van Wezel et al., 1995; Nilsson et al., 2001). Since these two growth factors act on different cell types of the primordial follicle, and since oocytes and granulosa cells and surrounding stromal/interstitial cells must all undergo change during the primordial to primary follicle transition, it is not surprising that both factors must be active to optimally promote primordial to primary follicle transition.

The network of growth factors that interact to regulate primordial follicle transition remains to be elucidated. LIF has been shown to increase KL mRNA expression in granulosa cells from antral follicles (Nilsson et al., 2002), but this has not been confirmed in early stage follicles. Similarly, BMP-15 has been shown to regulate KL expression in antral-stage granulosa cells (Otsuka and Shimasaki, 2002), but again this has not been confirmed using early stage follicles. Both c-kit receptors and FGF receptors have been demonstrated on thecal/interstitial cells. Both these receptors are tyrosine kinase type receptors (Manova et al., 1990; Levy et al., 1992; Merino and Jaffe, 1993; Ito et al., 2001; Nilsson et al., 2001). Therefore, it is possible that in these cell types the stimulation of one receptor type could activate intracellular signaling molecules such as PI3 Kinase or ERK that are common to both receptors. These intracellular signaling pathways cross-talk such that KL and bFGF might interact on specific cells such as thecal/interstitial cells to synergize with each other. Further studies are needed to characterize the interactions that occur between all these growth factors to regulate the primordial to primary follicle transition.

In summary, experiments were performed using cultured 4-day-old rat ovaries to determine how the growth factors KL and bFGF interact to promote the primordial to primary follicle transition. It was found that neutralizing the activ-

ity of endogenous bFGF interfered with the ability of KL to promote primordial follicle transition. Conversely, it was also found that blocking the activity of endogenous KL interfered with bFGF's ability to promote primordial follicle transition. This indicates that both KL and bFGF must be active in order to maximally promote the changes that occur in oocytes, granulosa cells and stromal/interstitial cells when primordial follicles undergo transition to primary follicles. In addition, it was found that bFGF treatment induced an increase in KL mRNA expression. Characterizing the interactions that regulate the primordial to primary follicle transition is essential in order to understand and potentially manipulate this vital step in female reproductive physiology.

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