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Interaction and signal transduction between oocyte and samatic cells in the ovary

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1. ABSTRACT

Follicular development and differentiation are sequential events which are tightly regulated by endocrine hormones, intraovarian regulators and cell-cell interactions. Balanced cell proliferation and apoptosis play an important role in the selection of dominant follicle. The formation of primordial follicle is initiated after a migratory primordial germ cell establishes a transitory close contact with the somatic cells in the genital ridge. Primordial germ cell migration and homing within the gonadal ridge requires integrated signals involving contact of primordial germ cells with extra-cellular matrix proteins and cellular substrates and attraction by the developing gonads. The oocyte-secreted polypeptide growth factors, such as the growth and differentiation factor 9, bone morphogenetic proteins and the gap junction participate in cell-cell communication direct growth and differentiation of granulosa cells and cross-talk between oocyte and somatic cell in the ovary. Maturation of cumulus-oocyte complexes which is controlled by lutenizing hormone requires activation of mitogen-activated protein kinase in granulosa cells. In this review the insights gained on cell-cell interaction and signal transduction during follicular development and differentiation is summarized, mainly focusing on signaling factors produced by oocyte and somatic cells which regulate primordial follicular growth initiation and development.

2. INTRODUCTION

During embryogenesis, primordial germ cells migrating from the yolk sac through the dorsal mesentery of the hindgut to the genital ridge, and the somatic cells deriving from the mesenchyme of the genital ridge, both of the germ cells and the somatic cells proliferate at the genital ridge rapidly (1). Then each germ cell is enclosed by one layer of somatic cells to form the primordial follicle. After mitosis occurred in the somatic cells, the germ cells undergo the first meiotic division, called primary oocytes. The primary oocytes become arrested in diplotene stage of meiosis, until the primordial follicles start to grow and finally reach the ovulatory stage (2,3).

During onset of primordial follicle growth, flattened pre-granulosa cells become cuboidal and begin to proliferate, the enclosed oocyte begins to grow at the same time (4,5). It is interesting to know why and how some primordial follicles are capable of starting to grow, while their neighbor sisters remain quiescent. The signal(s) for selection of primordial follicle growth is not clearly known. At the earliest onset of primordial follicle growth, receptors for the gonadotropin (FSH) is not present in the follicular cells. Therefore, the initiation of primordial follicle growth is independent of FSH regulation. Crosstalk and interaction between follicular cells and oocyte may be important for onset of the primordial follicle growth.

During both mitosis and meiosis, large numbers of germ cells are culled from the ovary for as yet unknown reasons, resulting in less than one-third of the total number of potential germ cells being endowed in the ovary within primordial follicles shortly after birth (2,3). At birth, the number of germ cells in the primordial follicles has decreased markedly due to germ cell apoptosis occurring before formation of ovarian follicles (6).

Folliculogenesis is a complex process involving dramatic morphological and functional changes in granulosa and theca cells. This process is sequential and dictated by specifically regulated response to endocrine hormones and intraovarian regulators. Follicular selection dominantly depends on cell-cell interaction and apoptosis (4-6). A balance of cell proliferation and apoptosis plays an important role in the follicular dominate selection.

This review briefly summarized the latest data in the literature, including the data achieved in our laboratory, mainly focusing on signaling factors produced by oocyte and somatic cells to regulate folliculogenesis, the related molecular mechanisms and signal pathways were also discussed.

3. PRIMORDIAL GERM CELLS AND PRIMORDIAL FOLLICLES

The primordial oocyte or germ cells (PGCs) in vertebrates arise outside gonads and reach to the developing gonad by active migration during the embryonic development. The establishment of germ line cells are in an extraembryonic site. Some important new findings about how these events occur in mammals have been recently obtained (see a recent review by De Felici *et al***)** (7). In general, each germ cell is enclosed by one layer of the somatic cells to form the primordial follicle in the genital ridge (1). Mammalian ovaries contain thousands of thousand primordial follicles which are the only source of gametes during the entire reproductive life.

3.1. Primordial germ cell migration

PGCs formation from proximal epiblast requires multiple steps, involving growth factor signaling, cell-cell interaction, cell movement and cell to extracellular matrix (ECM) adhesion. A number of adhesion, or putative adhesion molecules, have been identified in mammalian PGCs (7-9). However, their precise role and the associated signal transduction events remain unknown because of difficulties in accessing mammalian PGCs within the embryo and in defining and employing suitable *in vitro* experimental models as well as in applying molecular manipulation techniques to PGCs.

It was well defined that the PGCs arise from precursors located in the rim of the proximal epiblast in the mouse embryo (9, 10). At 6-7 days post coitus (dpc) the PGC precursors move from the epiblast to the posterior edge of the primitive streak where the germ cell lineage is established. PGCs then migrate into the endoderm of the developing hindgut and eventually into the gonadal ridges at 10.5-12.5 dpc. PGCs differentiate into oocytes in the ovary, and into prospermatogonia in the testis at 12-13 dpc(8-12).The adhesion-dependent allocation of the PGC precursors to a niche within the epiblast and the forming extraembryonic mesoderm during the pre-gastrulation period is crucial for their commitment. PGC migration and homing within the gonadal ridges require integrated signals involving contact of PGCs with ECM molecules and cellular substrates. Wylie and Anderson (13) have demonstrated that, PGC migration from the hindgut toward the gonadal ridges occurs over a period that does not exceed two days, involves roughly 400-500 cells. The surface of PGCs is equipped with an array of molecules allowing them to adapt themselves to the rapidly changing environment in which they develop. Adhesion molecules might once again turn out to be crucial in controlling not only the germ cell lineage and PGC migration but also the PGC differentiation fate itself (13, 14).

3.2. Interaction of primordial germ cells with somatic cells in gonadal ridges

It has been reported that mouse PGCs between 8 and 9 dpc enter by active migration into the embryonic endoderm that will give rise to the hindgut (14), there for about one day, PGCs remain as stationary cells within the hindgut epithelium, from 9.5-11.5 dpc they actively migrate toward the gonadal ridges, displaying motile features (15). Little information, however, is available about the molecular mechanisms and the related signaling. A balance between attractant and repellent molecules secreted by the surrounding cells result in mobilization of PGCs and direct them toward the gonadal ridges (7). It has been postulated that gonadal ridges produce chemoattractants for PGCs and some evidence for this has also been obtained in mouse (17). Th possible candidates for the chemoattractants suggested may be the transforming growth factor (TGF)(17-19), kit ligand (KL),(also known as stem cell factor SCF) (20), and stromal-cell derived factor-1 **(**SDF-1) (21,22). Besides cell motility, extracellular matrix (ECM) and cellular substrates may be also necessary for directional movement and to provide migratory cells with molecules needed for their survival and growth.

Migratory mouse PGCs have been found to establish transitory close contact with the somatic cells (7,13,23). N-cadherin and P-cadherin function might be related to the interactions of germ cells with somatic cells (24). In fact the former is expressed only by post-migratory PGCs in local contact with somatic cells and the latter is present in the somatic cells of sex-indifferent mouse gonads. PGCs isolated from the dorsal mesentery at 10.5 dpc and cultured on fibroblast monolayers are motile and contact each other through long thin processes, forming clusters of several cells (25). PGCs isolated from 12.5-13.5 dpc gonads do not show any features of motile cells *in vitro* (26).These studies raised the possibility that germ cell-germ cell contacts are necessary to link together PGCs during migration and have a role in switching off the migratory phenotype upon arrival within the gonadal ridges. More recent observations of living GFP-stained PGCs in the mouse embryo showed that some PGCs are capable of independently migrating towards the gonadal ridges (27). After PGCs reach the gonadal ridges, changes in E-

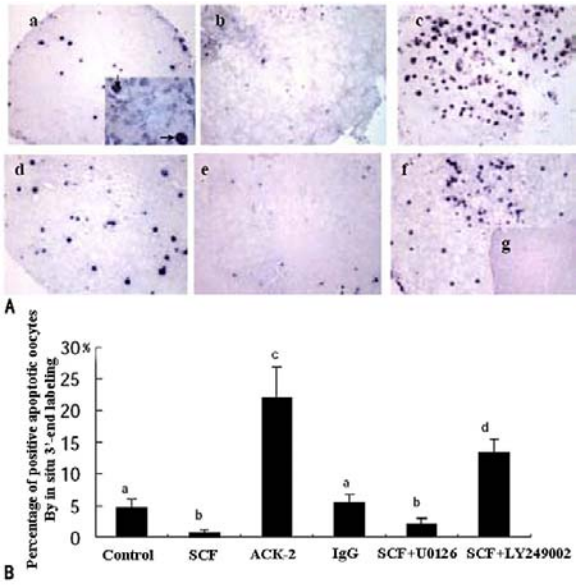

Figure 1. Anti-apoptotic action of SCF on oocytes in cultured ovaries and statistical analysis of apoptotic oocytes in ovaries of different treatment (Reproduced with permission from 31,32). A. Anti-apoptotic action of SCF on oocytes in cultured ovaries: Apoptotic nuclei are stained dark using the *in situ* 3'-end labeling technique. a) Cultured ovaries without any treatment. Inset is area of higher magnification showing apoptotic staining is localized in nuclei of oocytes (arrows); b) Ovaries treated with 100ng/ml SCF for 2 days. c) Ovaries treated with SCF plus c-kit antibody. d) Ovaries treated with SCF plus IgG; e). Ovaries treated with SCF plus MEK inhibitor U0126; f) Ovaries treated with SCF plus PI3K inhibitor LY294002; g) Ovaries without SCF treatment and incubated with normal serum IgG as a negative control. Magnification is × 400 ; Magnification of inset is x 1000; B. Statistical analysis of apoptotic oocytes in ovaries of different treatments. Vertical axis represents the percentage of apoptotic cells over total number of oocytes (mean ± SEM n=3); Statistical analysis was performed using ANOVA followed by the Student-New-Man-Keuls multirange test. Bars with different letters indicate statistically significant differences ($P<0.05$).

cadherin functionality might have a role in their settlement at the stationary stage (7,28). PGC migration from their site of origin to the gonadal ridges is regulated by a variety of interactions and molecules. PGC migration and homing within the gonadal ridges require integrated signals involving contact of PGCs with ECM molecules and cellular substrates and attraction by the developing gonads (7).

3.3. Initiation of primordial follicle growth

A primordial follicle is characterized by an undifferentiated oocyte surrounded by a single layer of squamous pregranulosa cells. During onset of primordial follicle growth, flattened pre-granulosa cells become cuboidal and begin to proliferate. The enclosed oocyte begins to grow at the same time (4-5,7). The onset of primordial follicle growth morphologically can be divided into two stages: the squamous pregranulosa cells become cuboidal and then begin to proliferate. Growth of GC in the follicle is a key process in initiation and development of primordial follicle. Control of primordial follicle growth initiation appears very complex, and probably involves a classic cell crosstalk of oocyte – pregranulosa cells.

Oocyte factors direct growth of primordial follicles. Evidence has been shown that oocytes isolated from secondary follicles at 12-day-old in rat ovary were co-cultured with somatic cells isolated from the primordial follicles of newborn ovaries could form re-aggregated ovaries. Within 9 days some recombined follicles developed into large antral-follicle stage (29,30), suggesting that the oocytes may produce factors to initiate primordial follicular growth by affecting the surrounding somatic cells. It has been reported that stem cell factor (SCF) may play an important role during this process (31). We have demonstrated that ovaries from neonatal rats were cultured with SCF for 8 days, SCF is capable of inducing primordial follicle development and initiating folliculogenesis by inhibiting oocyte apoptosis and up-regulating anti-apoptosis protein Bcl-2 and Bcl-Xl expression (31), as shown in Figure 1. PI-3K/AKT pathways may be involved in the regulatory process (32). We also demonstrated that the early developing oocytes expressed steroidogenic factor 1 (SF-1), steroidogenic acute regulatory protein (StAR) and cytochrome P450 aromatase (P450arom) (33).SCF up-regulated SF-1 and StAR expression and increased P450arom mRNA level, but suppressed GC FSHR mRNA expression (33).

It has been reported that granulosa cells of preantral follicles produce kit ligand (KL)/SCF under the stimulation of oocyte, while its receptor kit is expressed in the theca-interstitial cells and oocyte. Follicular development and oogenesis are abnormal in KL or kit null mice. The cultured follicles and oocytes have been identified with the specific targets for the Kit-Kit Ligand interaction (34). During early folliculogenesis, Kit together with Kit Ligand controls oocyte growth and theca cell differentiation, and protects preantral follicles from apoptosis. Formation of an antral cavity requires a functional Kit-Kit ligand system. In large antral follicles, the Kit-Kit ligand interaction modulates the ability of the oocyte to undergo cytoplasmic maturation and helps to maximize theca androgen output. Hence, many steps of oogenesis and folliculogenesis appear to be, at least in part, controlled by paracrine interactions between these two proteins. Evidence also showed that spontaneous primordial follicular development was completely blocked by a c-kit antibody (ACK-2) that could block KL actions (30). In postnatal ovaries, the initiation of follicular growth from the primordial pool and progression beyond the primary follicle stage appear to involve Kit-Kit Ligand interactions (34). In conclusion, interaction of granulosa cell-derived kit ligand with oocyte and theca cell-derived c-Kit is important for multiple aspects of oocyte and follicle development, though little is known about the specific roles of KL and c-Kit during human oogenesis (35).

In primordial follicles, oocyte apoptosis is likely responsible for follicular degeneration. FSH may be important for follicular development at early stage. However, FSH is unlikely to exert a direct action on primordial follicles because FSH receptors have not yet developed at this stage.

Therefore, the onset of primordial follicles is independent of the gonadotrophic hormones. Crosstalk and interaction between follicular cells and oocyte may be important for the onset of the primordial follicles. Several intracellular signaling pathways have been linked directly to promoting granulosa cell or oocyte survival, including pathways involving gonadotrophin- and vasoactive intestinal peptide (VIP)-induced cAMP formation (36,37),mitogen-activated protein kinase (MAPK) (39) and phosphoinositol-3-kinase-Akt (37,40). PI3K/Akt pathways play an important role in mediating the anti-apoptotic action of SCF in oocytes of primordial follicles. Jin *et al* (32) found that the anti-apoptotic effect of SCF on oocytes was significantly inhibited by the PI3K inhibitor. Moreover, PI3K inhibitor could also revere the effect of SCF on the expression of Bcl-xL and Bax**.**

Establishment of primordial germ cells within the ovary, primordial follicle activation, oocyte survival and growth, granulosa cell proliferation, theca cell recruitment and the maintenance of meiotic arrest depend on the paracrine signaling between oocyte and its surrounding somatic cells. The interaction and signal transduction between the germ cells and the somatic cells in the primordial and early developing follicles are fundamental to the processes of oogenesis and folliculogenesis in mammals.

4. FOLLICULOGENESIS AND INTERCELLULAR COMMUNICATION

Ovarian folliculogenesis is a complex process involving dramatic morphological and functional changes in granulosa and theca cells. Intercellular communication in the follicles is required.

4.1. Follicular development and differentiation

Follicular development and differentiation may be regulated in a follicle-stage dependent manner. In an early developing follicle, in addition to FSH and interactions between oocyte and somatic cells, gap junctional communication may be important for regulation of folliculogenesis.

The onset of primordial follicle growth and proliferation to form a primary follicle which is associated with expression of follistatin. A primary follicle contains an oocyte encircled by a single layer of cuboidal granulosa cells. The follicle cells proliferate and differentiate quickly and form a secondary follicle with a developing oocyte(4). Two to three layers of cuboidal granulosa cells are usually observed at this stage of follicular development. In the tertiary follicles multi-layers of granulosa cell can be clearly observed and a cavity appears. After this stage, the granulosa cells in the follicle differentiate into two cell species, the mural granulosa cells (mGC) that surround the follicle wall and the cumulus cells (CC) that circumfuse the OC (41).These cells are regulated by various hormones and growth factors. It was noted that these two type of granulosa cells have diferent features and response to the gonadotropins and IGF-1 regulation. CCs replicated ten times more than mGC. Similarly, progesterone secretion by CC was six times more than by mGC. The IGF-I had a positive effect on cell replication of mGC, but a negative effect on the cell replication of CC. With respect to progesterone secretion, IGF-I had a negative effect on CC but a positive effect on mGC (41). It is concluded that CC behaved differently from mGC in response to gonadotrophins and IGF-I.

Evidences have shown that ovarian follicular growth and dominance are controlled by series intraovarian events including a decrease in intra-follicular IGF-binding proteins (IGFBPs) -2, -4 and -5 levels. Proteolytic enzymes such as pregnancy-associated plasma protein-A (PAPP-A) degrade IGFBPs and increase bioavailability of IGF-I and -II during follicular development. Aad *et al* demonstrated that the regulation of PAPP-A mRNA abundance in granulosa and theca cells differs and estrogen (E2) may be part of an intraovarian negative feedback system which may reduce bioavailable IGFs in theca layer during growth and selection of follicles (42).

At preovulatory stage, both granulosa and theca cells in the follicle express LH Receptors. Both cell types are able to respond to impending LH surge. At this stage both FSH and LH suppress degree of apoptosis in the isolated preovulatory rat follicles, and interaction of granulosa and theca cells produces the highest estrogen in the follicle that may be important for preventing the selected dominate follicle atresia and going to ovulation (43,44).

We have examined signal pathways of FSH-induced rat GC proliferation and differentiation (45,46). Treatment of GC with FSH induced p38 MAPK phosphorylation rapidly. Such activation was protein kinase A-dependent. Inclusion of SB203580 in the media enhanced FSH-stimulated StAR mRNA production, but decreased the FSH-stimulated P450arom mRNA expression. FSH is also capable of up-regulating liver receptor homolog-1 (LRH-1) expression, a member of the orphan receptor family that activates P450arom activity in ovary. Inactivation of p38 MAPK down- regulated LRH-1 expression (45). Similarly, PKA inactivation by H89 also inhibited FSH-promoted PCNA expression and steroids synthesis (46). Further study by Park *et al.* showed that GC proliferation and differentiation in response to FSH with activin required both the FOXO1 release from the cyclin D2 promoter and the positive signaling from Smad2/3 (47).

Preovulatory LH surge triggers resumption of meiosis and cumulus expansion. Inhibition of MAPK activation could prevent LH-stimulated resumption of meiosis with a rise in Has2 and Ptgs2 expression. These two genes have been reported to be necessary for normal cumulus expansion (48). LH via its receptor on cumulus

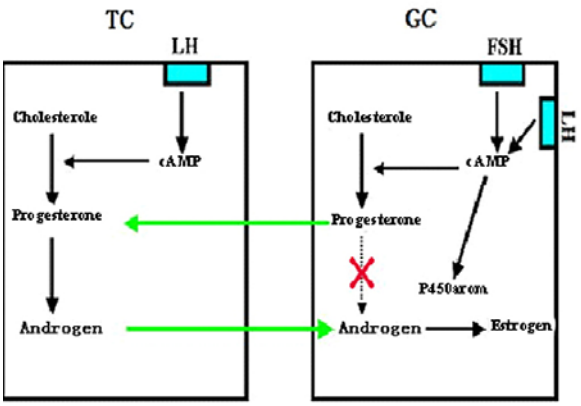

Figure 2. A representative diagram showing involvement of interaction between granulosa cell (GC) and theca cell (TC) in estrogen production in the follicle (Reproduced with permission from 43, 44). Under the regulation of LH, TC synthesizes androgen, but it cannot be converted into estrogen for lack of P450arom in TC, while GC under the action of FSH (also by LH at the late stage) synthesizes progesterone, the latter?, however cannot be converted further into androgen for short of the necessary converting enzymes in GC. Although GC has the P450arom, it cannot synthesize estrogen by itself. Progesterone produced by GC can be used by TC to convert androgen, while GC is capable of using the androgen produced by TC to convert into estrogen. The interaction of TC and GC in the follicle is a prerequisite for estrogen biosynthesis in the ovary.

cells stimulates MAPK activation and increase in phosphorylation of Cx43 and junctional communication (49).

Oocyte is surrounded and nursed by granulosa cells, but oocyte is not quiescent cell and may play key roles in folliculogenesis. It is widely accepted that oocyte directs granulosa cell differentiation and promotes follicle growth (50). Oocyte is capable of secreting soluble paracrine growth factors by acting on its neighboring granulosa cells, in turn to regulate oocyte self development. In preantral follicles, the oocyte directs granulosa cells to regulate oocyte growth. Oocyte-cumulus cell interaction has been reported to be capable of preventing cumulus cell luteinization and regulating steroidogenesis, inhibin synthesis and suppressing LH receptor expression (4,50,51). The identities of these oocyte-secreted growth factors regulating such key ovarian functions remain unknown. The nature of oocyte-somatic cell interactions at various stages of follicle development may be varied. Illustrating the nature of such regulation will have important implications for our understanding of factors regulating folliculogenesis, ovulation rate and fecundity.

In conclusion, mammalian oocyte development occurs through tight coordination and interaction between all ovarian structures. In fact, bi-directional communication between the oocyte and its companion GC in the ovarian follicle seems essential for GC proliferation, differentiation, and production of a functional female gamete.

4.2. Interaction and communication of granulosa cells with theca cells

After onset of primordial follicles, the molecules secreted by oocyte and granulosa cells or factors originating from blood plasma may play essential roles in regulating differentiation of the developing follicles.

It is well known that granulosa cells contain enzymes required for progesterone synthesis, and aromatases for androgen conversion into estrogens. However, GCs do not contain any enzyme for androgen production from progesterone. Therefore, GC itself can not produce any estrogen. On the other hand, theca-interstitial cells are capable of forming androgens from progesterone, but no aromatase is present in the cells. Neither granulosa nor theca-interstitial cells alone are capable of producing estrogens. A synergistic interaction between granulosa and theca cells is a prerequisite for estrogen biosynthesis (44, 52, 53). Our early experiments further demonstrated that granulosa cells obtained from preovulatory follicles under the action FSH and LH produce high level of progesterone, which can be used by the theca-interstitial cells to synthesize high level of androgens under the influence of LH (44), as shown in Figure 2, the coorperative interaction between these two cell types under the action of both FSH and LH in the preovulatory follicles may be responsible for increase in the estrogen production just prior to ovulation, which may be important for preventing the selected dominate follicle(s) from atresia and leading to ovulation.

Granulosa cells have been reported to produce kit ligand (KL), while its receptor kit is expressed in the theca-interstitial cells and oocyte. In KL or kit null mice folliculogenesis and oogenesis are abnormal (34). In large antral follicles, the interaction of Kit produced by theca-interstitial cells and Kit ligand sectrated by granulosa cells has been found to modulate ability of the oocyte to undergo cytoplasmic maturation and helps to maximize theca-interstitial cell androgen output (34). Evidence also showed that spontaneous primordial follicular development was completely blocked by a c-kit antibody (30). Interaction of granulosa cell-derived KL with theca cell/oocyte-derived c-Kit is important for multiple aspects of oocyte and follicle development. However, little is known about the specific roles of KL and c-Kit during oogenesis (35).

Increasing evidences have demonstrated that interaction of granulosa and theca-interstitial cells under the action of midcycle LH surge is necessary for follicular maturation and rupture. It has been demonstrated that granulosa cells mainly express tissue type plasminogen activator (tPA), while theca-interstitial cells express plasminogen activator inhibitor type-1 (PAI-1) under the action of gonadotropins, as shown in Figure 3, The LH-regulated coordinated expression of tPA and PAI-1 in the preovulatory follicles is responsible for a controlled and directed proteolysis in the follicle, leading to ovulation in the rat and monkey (54-57)

In recent years, significant progress has been made in identifying ovarian local factors in different cell types of follicles induced by various hormones. Nodal,

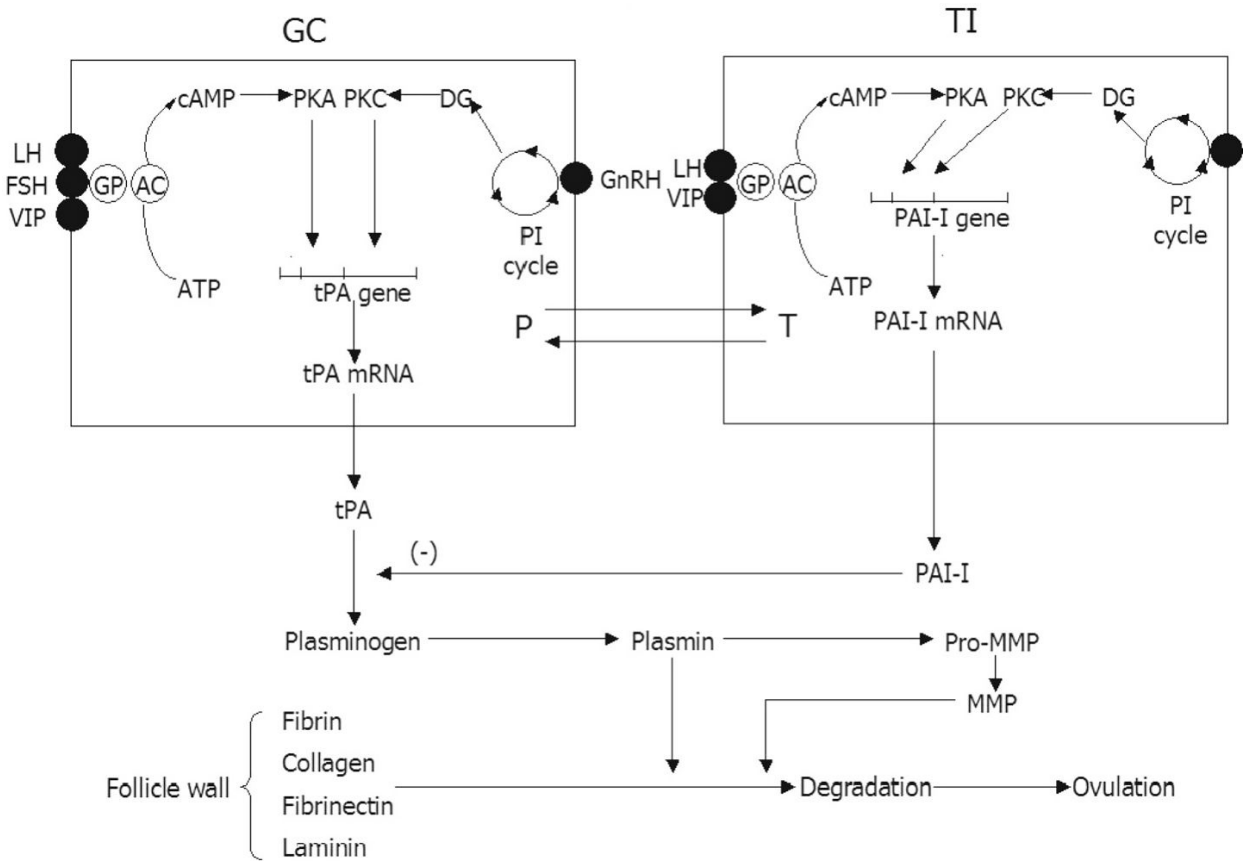

Figure. 3. Schematic representation of involvement of ovarian tPA produced mainly by GC and PAI-1 secreted by TC in the process of ovulation (Reproduced with permission from 54-57). P, Progesterone; T, testosterone; GP, G-protein; AC, adenylate cyclase; DG,1,2-diacylglycerol; PI, phospholipid; PKA, protein kinase A; PKC, protein kinase C. For the explanation of the other abbreviations, see the text.

which is secreted by the theca and promotes apoptosis of the differentiated granulosa cells through a mechanism involving Smad2 signaling and suppression of the PI3K/Akt pathway (58). Nodal is a physiological inducer of granulosa cell apoptosis (58,59). In healthy follicles, Nodal is found in the theca cell layer, while its type I receptor is expressed in the granulosa cells (59**).** However, in atretic follicles, Nodal and ALK7 are co-localized, which may allow Nodal to serve as a marker to identify follicles destined to undergo atresia. In healthy follicles, Nodal protein is detectable primarily in the theca cell layer, while ALK7 is detected in granulosa cells. However, the induction of follicular atresia via gonadotropin withdrawal results in the co-localization of Nodal and ALK7 to the granulosa cell layer. Nodal/ALK7 signalling pathway is involved in promoting follicular atresia (59).

4.3. Interaction and communication of granulosa cells with oocyte

Oocyte factors direct growth and differentiation of granulosa cells. Cross-talk between oocyte and somatic cells in the ovary plays an essential role in follicular development, differentiation and oocyte maturation. Mammalian oocyte development occurs through tight coordination and interaction between all ovarian structures (51). Gap junction between oocyte and granulosa cells appear to be important in cross-talk between the two cell types. Gap junctions are intercellular membrane channels composed of connexins (Cx), a family of integral membrane proteins, in which Cx43 and Cx37 may play the most important roles in ovarian folliculogenesis (60**).** The oocyte is an effective modulator of granulosa-theca interactions. Oocyte control of granulosa and theca cell function may be mediated by several growth factors via a local feedback loop(s) between these cell types.

The oocyte-secreted polypeptide growth factors, GDF9 and BMPs are crucial factors in follicular growth and development. Among the BMP ligands, BMP-7 which uses ActRII as their type II receptor, strongly binds to ALK-2 as their type I receptor. Evidence showed that FSH and E2 regulate expression of *ActRII* and *ALK-2* genes in bovine GCs and suggested that BMP7/ActRII/ALK-2 system may be critically involved in the process of selection of bovine follicles (61**).** The oocyte derived BMP15, also known as GDF9B, has also shown to be essential for ovarian follicular development. However, the action of BMP15 and GDF9 varied with respect to the species of origin and the stages of follicle development. Moreover, the effects of GDF9 and BMP15 together were

often co-operative and not always the same as those observed for these growth factors alone (62). Oocyte derived GDF9 stimulates rat granulosa cell proliferation, cumulus expansion, and preantral follicle growth *in vitro,* whereas down-regulation of GDF9 by intra-oocyte injection of a GDF9 antisense morpholino attenuates both the basal and FSH-induced follicle growth, while addition of recombinant GDF9 enhances the basal and FSH-induced follicular growth (63).

The FecB (Booroola) mutation occurred in the signaling domain of the BMP-1B receptor. FecB mutation increases the BMP response of both granulosa and theca cells when stimulated to differentiate by gonadotropins (64)**.**

BMP-15 stimulates stem cell factor (SCF, or Kit ligand) expression in granulosa cells, while SCF inhibits BMP-15 expression in oocyte, thus forming a negative feedback loop between the oocytes and granulosa cells (65). The SCF activation of the PI3 kinase pathway in mouse and rat oocytes has now been demonstrated (60,66,67).

Just recently we have provided the primary evidence to show that the early developing oocytes may also express steroidogenic factor 1 (SF-1), steroidogenic acute regulatory protein (StAR) and cytochrome P450 aromatase (P450arom). SCF stimalated SF-1, StAR and P450arom production and increased number of primordial and primary follicles, in contrast SCF suppressed GC FSHR mRNA expression (33). GDF-9 can promote GCs proliferation, while suppress their differentiation, FSH-induced cAMP production, as well as estrogen/progesterone production (33, 68).

We speculate that the oocyte in the developing follicles at the very early stage might be capable of producing estrogen which was regulated by SCF, and the oocyte-originated estrogen may play a major role for the early follicular development. However, the hypothesis for the OC-originated estrogen production should be further documented.

In addition to the function in GC, GDF-9 has been also specifically reported to promote COC formation and regulate cumulus expansion (69). BMP-15 suppresses FSHR mRNA expression, thereby decreasing expression of StAR, cholesterol side-chain cleavage cytochrome P450 (P450scc), and P450arom. BMP-15 may function through Smad1/5/8 pathway (70). BMP-15 and GDF-9 synergistically maintain COC integrity (71). Oocyte maturation, cumulus expansion, ovulation, and the preimplantation developmental competence of the fertilized egg are all abnormal in Gdf9+/_Bmp15_/_ null mutant mice (69).

BMP-15 and KL regulate GC mitosis via communication between COC and GC (72). In the adult ovary, pituitary FSH via interaction with its receptor (FSHR) is required for follicular maturation and granulosa cell development. Using human granulosa-like tumor cell line KGN, Miyoshi et al demonstrated that FSH administration upregulated BMP type IA (BMPR1A) and IB (BMPR1B) receptors, activin type II receptor (ACVR2) and BMP type II receptor (BMPR2). FSH-cAMP pathway and BMP system are reciprocally and functionally linked (73). Estrogen plays a major role in regulating ovarian FSHR mRNA expression in the early developing follicles of primate fetus, and the developmental increase in FSHR mRNA levels reflects the estrogen-dependent increase in the number of granulosa cells and oocytes (74).

Dominant follicular selection mainly depends on granulosa cell apoptosis. At least five cell death ligand-receptor systems have been reported in granulosa cells of porcine ovaries (75). They are fas (also called APO-1/CD95) ligand and receptors; tumor necrosis factor (TNF) alpha and receptors; TNF-related apoptosis-inducing ligand (TRAIL; also called APO-2) and receptors; APO-3 ligand and receptors and PFG-5 ligand and receptors. Intracellular inhibitor proteins were also detected in the granulosa cells, such as the cellular FLICE-like inhibitory protein short form (cFLIPS) and long form (cFLIPL), which may act as anti-apoptotic/survival factors.

Mihun et al have provided bovine dominant follicles (DF) as a unique model to identify novel genes potentially involved in survival and apoptosis of follicular cells, and to determine FSH-, estradiol- and LH-target genes regulating follicular growth and function (76). Their studies suggested that the dominant follicles experience a reduction in FSH-dependence but acquire an increased LH dependence as they grow during the low FSH milieu of follicular waves. Small ubiquitin-related modifier-1 (SUMO-1) is a member of a family of ubiquitin-related proteins. In the ovary, inhibition of SUMO-1 expression is regulated by LH receptor stimulation in granulosa cells concomitant with ovulation in the mouse ovary (77).

4.4. Granulosa cell factors direct oocyte function

We have comparatively examined correlation of inhibin with LHR expression in GC in relation to tPA activity in oocytes at the same section of the follicle. It is interesting to note that tPA mRNA in oocytes was detected at the early stages of follicles, but the oocyte tPA mRNA does not translate into its protein until the onset of meiosis maturation is triggered by the LH peck. High inhibin expression in GC observed in the same section of the early developing follicle may prevent the oocyte tPA mRNA translation. Once inhibin expression decreases in GC of the developing follicles an increasing oocyte tPA activity was observed, indicating that the translated oocyte tPA activity may induce its certain morphological changes similar to GVBD, leading to the oocyte apoptosis and the follicle atresia (78,79). Based on this finding, we have proposed a hypothesis of follicular atresia originating from oocyte apoptosis (Figure 4).

In the ovary angiogenesis occurs cyclically and involves interaction of numerous cytokines and growth factors. The angiogenic processes are regulated by a balance between pro- and anti-angiogenic factors. It has been reported that the anti-angiogenic thrombospondin

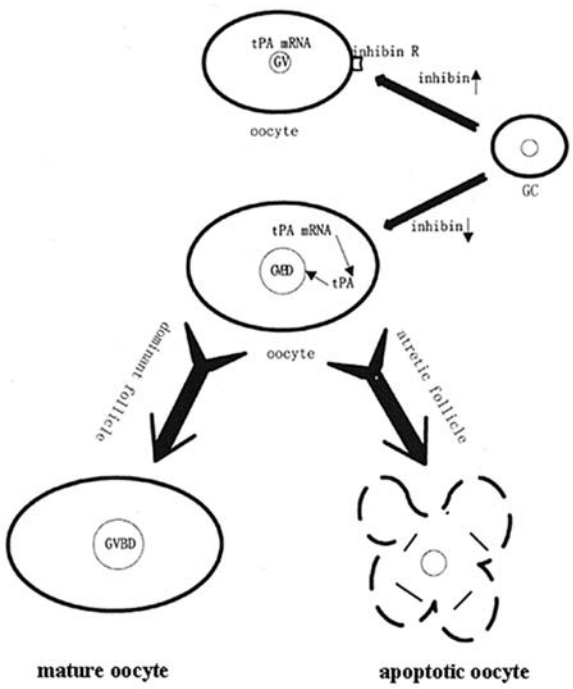

Figure 4. Effect of inhibin emanated from GC on tPA mRNA translation in oocyte (Reproduced with permission from 78.79). Inhibin originating from the GCs inhibits the oocyte maturation by inhibiting tPA mRNA translation in the oocyte. Once inhibin expression in GCs is decreased, the oocyte tPA mRNA starts to translate into its active protein, the subsequently increased tPA activity induces the oocyte GVBD in the dominant follicle leading to the oocyte maturation and ovulation; On the other hand, decreases in GC inhibin expression in the developing follicle, the oocyte tPA mRNA is triggered to translate tPA protein which is capable of inducing its certain morphological changes similar to GVBD in the developing follicle, subsequently leading to the oocyte and/or the follicle apoptosis.

(TSP) family (TSP-1 and -2) protein levels were significantly higher in small bovine follicles, while the pro-angiogenic vascular endothelial growth factor (VEGF) was inversely expressed. FSH, but not LH, stimulated TSP-1 and -2 coordinate expression in the extravascular compartment of the ovary during early follicle development. The regulated expression and localization of these proteins suggest their involvement in regulating growth and development of early developing follicles (80). Cohen *et al* have identified a cytoplasmic protein in human granulosa cells, 14-3-3 tau, a member of a family of homodimeric cytoplasmic adapter proteins.14-3-3 tau co-immunoprecipitated with FSH receptor (FSHR). Over-expression of 14-3-3tau resulted in a modest decrease of FSH-induced cAMP accumulation, suggesting 14-3-3tau regulates FSHR function.(81). FSH and IGF-I are both important determinants of follicle development and the process of cumulus cell-oocyte complex expansion. FSH stimulates the phosphorylation of Akt by mechanisms involving phosphatidylinositol 3-kinase (PI3-K), a pattern of response mimicking that of IGF-I. FSH has been reported to amplify IGF-I-mediated Crtl1 production, possibly via PI3-K-Akt signaling cascades in rat granulosa cells 82).

Connexin43 (Cx43) is a gap junction protein, strongly expressed in granulosa cells. Evidence has shown that in mice lacking Cx43, or in mice expressing a mutant form of Kit ligand which is also produced by granulosa cells, follicle growth is impaired. The impaired folliculogenesis in mice lacking Cx43 is due to reduced responsiveness of GCs to the oocyte-derived GDF9 (83). GC derived follistatin can form an inactive complex with oocyte derived BMP-15, through which follistatin inhibits BMP-15 bioactivities. Specifically, follistatin attenuated BMP-15 stimulation of granulosa cell proliferation and reversed BMP-15 inhibition of FSH receptor mRNA expression leading to the suppression of FSH-induced progesterone synthesis (84). Inhibin derived from granulosa cells is stimulated by FSH and several TGF beta family ligands, such as BMPs and activins. GDF-9 is a member of the TGF beta/activin family and stimulates ovarian inhibin-alpha content in the neonatal ovaries. Further experiments showed that GDF-9, alone or together with FSH, increased inhibin production, inhibin subunit mRNA expression, and inhibin-alpha promoter activity by rat granulosa cells (85). Therefore, a synergistic stimulation of inhibin secretion by GDF-9 and FSH could play an important role in the feedback regulation of FSH release, leading to the follicle maturation and ovulation.

Androgens stimulate porcine mGC proliferation *in vitro* by potentiating the growth-promoting effects of oocytes or GDF9, via a mechanism that involves the AR. These signaling pathways are likely to be important regulators of folliculogenesis *in vivo*, and may contribute to the excess follicle growth that is observed in androgen-treated female animals (86). At the late stage of the follicular development synthesis of epiregulin and amphiregulin, of the EGF-like growth factor family, is stimulated by LH in human granulosa cells, H89, a PKA inhibitor, attenuated the expression of these growth factors, suggesting PKA involvement in this signaling pathway (87). It has been demonstrated that PAIRBP1expression in granulosa and luteal cells was regulated by the gonadotropins and PAIRBP1 may play an important role in mediating P4 antiapoptotic action in these ovarian cell types (88).

4.5. Signaling for cumulus-oocyte communication and oocyte maturation

In response to the LH surge, the compact cumulus-oocyte complex (COC) undergoes expansion by synthesis of the mucopolysaccharide hyaluronan (HA) accompanying oocyte maturation. The LH surge caused a rapid 120-fold increase in *HAS2* mRNA expression, whereas a delayed 2-fold up-regulation of *HAS3* mRNA production. The *HAS1* transcripts were barely detected (89,90). Expression of *CD44* mRNA greatly increased during *in vitro* maturation of COCs, indicating its important role in the process. The authors suggested that HAS2 may be mainly responsible for rapid HA synthesis in COCs and

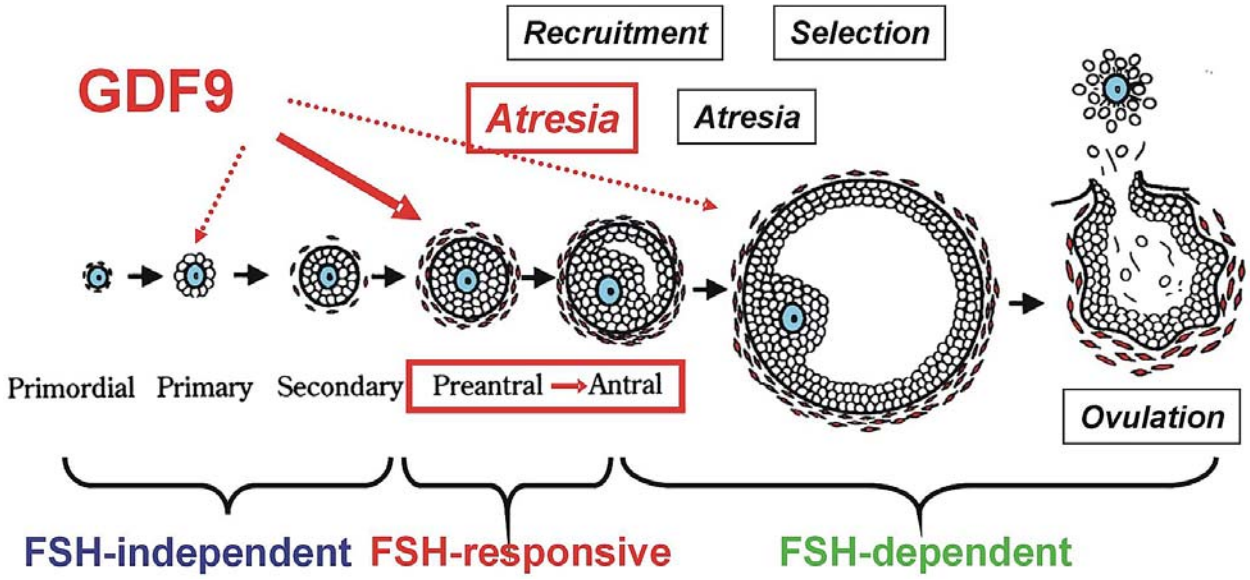

Figure. 5. A representative diagram for gonadotrophin-induced mammalian oocyte meiotic resumption (Reproduced with permission from 48). The gonadotrophins bind to the G protein-coupled receptors, resulting in activation of adenylyl cyclase and increased production of cAMP. The elevated cAMP in cumulus cells activate MAPK possibly by PKA, PKC/CaMII, MAS, P4, and/or EGF worknet, which in turn induces the release of a signal(s) that trigger meiotic resumption. The elevated cGMP could inhibit meiotic resumption by decreasing MAPK activity in cumulus cells and increasing cAMP level in oocyte, which may be blocked by LH. Oocyte has a positive effect on MAPK activity in cumulus cells.

GCs, and the transcriptional up-regulation of both *HAS2* and the receptor *CD4* in the COCs appear to be important prerequisites for initiating HA-mediated effects during final oocyte development and sperm-egg interaction**.**

Maturation of cumulus-oocyte complexes (COC) for both meiosis resumption in the oocyte and expansion of the cumulus oophorus are mainly controlled by LH. Both processes require activation of mitogen-activated protein kinase (MAPK) in granulosa cells (48,89,90). Inhibition of MAPK activation prevented LH-induced resumption of meiosis and increased i*Has2* and *Ptgs2* expression. It has demonstrated that i*Has2* and *Ptgs2* gene products are essential for normal cumulus expansion. However, inhibition of MAPK does not block LH-induced elevation of CC cAMP level, suggesting that MAPK activation required for inducing GVB, while cumulus expansion is needed for decrease in cAMP production. An interaction between oocyte and cumulus cells may be required for LH-induced maturation process in COC (48)

Gonadotropins, via activation of MAPK pathway in cumulus cells, induce meiotic resumption of mammalian oocyte. The function of cumulus cells is also regulated by oocytes. It is shown that oocytectomized mouse cumulus cell complexes do not produce hyaluronic acid and undergo expansion after FSH stimulation until addition of fully-grown oocytes (91,92). Further studies indicate that mouse oocytes secrete a cumulus expansion-enabling factor that promotes cumulus expansion in response to FSH (93). The paracrine factor may be GDF-9, since recombinant GDF-9 can promote *Has2* (a key gene for hyaluronic acid synthesis) expression and cumulus expansion. Moreover, mouse cumulus expansion requires gonadotropins-dependent increased expression of several key genes in cumulus cells, including Has2 and Ptgs2 (94,95). A study showed that denuded mouse oocytes significantly enhanced MAPK activity in cocultured cumulus cells after FSH stimulation, while MAPK activity is low in the absence of the oocytes (48). Evidence also showed that mouse oocytes control the intercellular metabolic cooperativity between cumulus cells and oocytes, which is needed for energy production by granulosa cells and for oocyte and follicular development (96), these results indicate that oocytes also play a key role in enabling or licensing maturation of oocytes and cumulus oophorus. It is suggested that the gonadotropins may induce elevation of cAMP levels in granulosa cells to promote the activation of MAPK, and then followed by the events leading to oocyte meiotic resumption and cumulus expansion. However, the activation of MAPK in cumulus cells may be also required for the paracrine factors secreted by the oocyte (48), indicating that MAPK activation alone is not sufficient to initiate COC maturation processes. As shown in Figure 5, the gonadotrophins binds to the G protein-coupled receptors, resulting in activation of adenylyl cyclase and increased production of cAMP. The elevated cAMP in cumulus cells activate MAPK possibly by PKA, PKC/CaMII, MAS, P4, and/or EGF worknet, which in turn induces the release of a signal(s) that trigger meiotic resumption. The elevated cGMP could inhibit meiotic resumption by decreasing MAPK activity in cumulus cells and increasing cAMP level in oocyte,

which may be blocked by LH. Oocyte has a positive effect on MAPK activity in cumulus cells.

FSH and IGF-I are both important determinants of follicle development and process of cumulus cell-oocyte complex expansion (81,82). Treatment with FSH or IGF-I increased Crtl1 production, the FSH- and IGF-I-dependent Crtl1 production were abrogated by PI3-K inhibitors, and Crtl1 production was partially inhibited by p38 mitogen-activated protein kinase inhibitor, suggesting FSH amplifies IGF-I-mediated Crtl1 production, possibly via PI3-K-Akt signaling cascades in rat granulosa cells.

It is generally accepted that cumulus cells during maturation period support IVM of oocytes to the metaphase-II stage and are involved in the cytoplasmic maturation.Without cumulus cells, porcine oocytes could not develop beyond the 4 cell stage (97)**.** Factors that facilitate cytoplasmic maturation may be transported from cumulus cells to the oocytes through GJC. Some molecular substrates such as ions, nucleotides, amino acids and hormones have been reported to traverse from cumulus cells to oocytes via GJC. Alternatively, cumulus cells might neutralize the harmful effect of ROS during IVM to protect the oocytes from oxidative stresses and to improve oocyte maturation and subsequent development after IVF. Tatemoto *et al.*reported that porcine DOs were highly susceptible to oxidative stress resulting in the oocyte degeneration, and that cumulus cells efficiently prevented oocytes from the cell damage (98).

4.6. Mitotic and meiotic checkpoint signaling in mammalian oocyte

Only a few checkpoint proteins have been reported to exist in mammalian meiotic cells (refer the recent review by Wang *et al*) (99). It appears at least three groups of proteins have been reported to participate in the spindle checkpoint signal pathway.

The first may be called transporting proteins, such as dynein, Zw10, Rod, and perhaps MAP kinase. These proteins are responsible for transporting checkpoint proteins between kinetochores and cytoplasm (including spindle microtubules and spindle poles). It has been suggested that Human Zw10 and Rod are mitotic checkpoint proteins (100), as cells lacking these proteins at kinetochores fail to arrest in mitosis when exposed to microtubule inhibitors. However, it appears that both Zw10 and Rod affect checkpoint pathway through dynein (101), which participates in the transport of some checkpoint proteins, but Bub3 loading to kinetochore needs neither Zw10 nor Rod3 (102), suggesting that Bub3 detachment from the kinetochores is not dependent upon dynein. However, the transportation of Mad2 and BubR1 to the spindle poles relies on dynein's presence, suggesting that Zw10 and Rod function as indirect checkpoint proteins (103).

The second are the binding proteins, such as Mps1, Bub1, BubR1 and CENP-E. These proteins bind to kinetochores at very early stages of the cell cycle and remain at kinetochores even when the cells are at the metaphase stage in which the spindle has formed and chromosomes have aligned at the spindle equator. Without these binding proteins checkpoint proteins cannot bind to kinetochores and the checkpoint cannot be activated (99,104).

The third are the real checkpoint proteins, such as Mad1 (perhaps partial) and Mad2, that perform checkpoint functions as the final signal transduction components (99,105).When spindle formation and chromosome alignment are completed, these proteins are immediately released from kinetochores, thus the checkpoint is deactivated or silenced, leading to anaphase onset. In contrast, once the connection between microtubules and chromosomes is affected, they are moved back to kinetochores and re-bind to kinetochores to re-activate the checkpoint, resulting in metaphase arrest. In mouse oocytes, Mad1 was observed around nuclei at the GV stage (106), on kinetochores at ProM-I stage, and moved to spindle poles at M-I, early A-I and M-II stages. These results suggest that differences for Mad1 localization are present between mitosis and meiosis.

These three groups of proteins work together and form a complicated checkpoint system (99). When any protein in the system is inhibited, affected or deleted, final checkpoint signal transduction and chromosome segregation will be affected, causing aneuploid formation. The information and evidence on the spindle checkpoint in mammalian meiosis are still very limited, further studies remain necessary to address the exact mechanism(s) by which aneuploid formation is prevented.

5. PERSPECTIVE

Establishment of primordial germ cells within the ovary, primordial follicle activation, oocyte survival and growth, granulosa cell proliferation, theca cell recruitment and the maintenance of meiotic arrest all depend on the paracrine signaling between oocyte and its surrounding somatic cells. After onset of primordial follicles, the factors secreted by oocyte and somatic cells or originating from blood plasma may play essential roles in regulating differentiation of the developing follicles in mammals.

Follicular growth, development and atresia are governed by a host of endocrine, paracrine and autocrine signals and balanced between these factors. Oocyte factors direct growth and differentiation of granulosa cells. Cross-talk between oocyte and somatic cell in the ovary plays an essential role in follicular development. The oocyte-secreted polypeptide growth factors, GDF9 and BMPs and the gap junction between oocyte and granulosa cells appear to be important in cross-talk between the two cell types, specifically, interaction of granulosa cell-derived kit ligand (KL) with oocyte and theca cell-derived c-Kit has been demonstrated to be essential for multiple aspects of oocyte and follicle development.

FSH may be important for follicular development, however, FSH is unlikely to exert a direct action on primordial follicles because FSH receptors have not yet developed at this stage. Therefore, the onset of primordial follicles and the early follicle development may be independent of the gonadotrophic hormones.

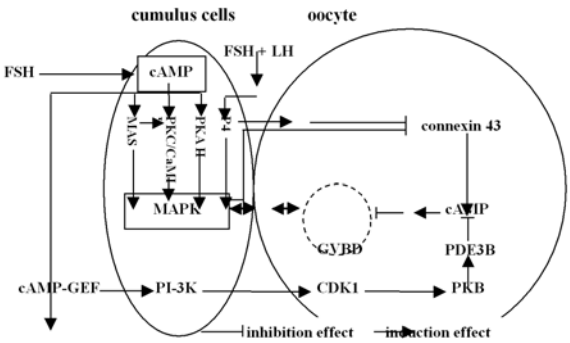

Figure. 6. A representative diagram to show the action of FSH and GDF9 on follicular development and atresia in a stage-dependent manner of follicular development **(**Reproduced with permission from 58). Growth of the follicle can be classified into distinct stages, each of which is influenced by a different subset of factors. From primordial to secondary follicle development FSH may be not needed. At this stage the follicular development may be termed gonadotropin-independent. Transition of the follicle from the preantral to early antral stage is primarily controlled by intraovarian regulators such as GDF9, the gonadotropin at this stage may be not required, termed as gonadotropin-responsive. The continual growth past antrum formation to the preovulatory stage may be FSH-dependent.

Intracellular interaction in primordial follicles and growth factors may be of importance. In addition to GDF-9 and BMP-15, growth factor EGF may also play a role in the process (107). Figure. 6 shows a representative diagram for the action of FSH and GDF9 on follicular development and atresia. From primordial to secondary follicle development FSH may be not needed. At this stage the follicular development may be termed gonadotropin-independent. Transition of the follicle from the preantral to early antral stage is primarily controlled by intraovarian regulators such as GDF9, the gonadotropin at this stage may be not required, termed as gonadotropin-responsive. The continual growth past antrum formation to the preovulatory stage may be FSH-dependent.

The SCF activation of PI3 kinase pathway in oocytes has now been suggested to be of importance in controlling oocyte growth during activation and early development of ovarian follicles. However, only limited evidence for the functional roles of the PI3 kinase pathway in oocyte growth has been obtained by *in vivo* approaches. Thus, a search for downstream molecules of the PI3 kinase pathway that directly control the growth of mammalian oocytes will be of great importance.

Several intracellular signaling pathways have been linked directly to promoting granulosa cell or oocyte survival, including pathways involving gonadotrophin- and vasoactive intestinal peptide (VIP)-induced cAMP formation. MAPK and PI3K/Akt pathways have been also suggested to play an important role in mediating the anti-apoptotic action of SCF in oocytes of primordial follicles. Gonadotropins induce mammal oocyte meiotic resumption mainly through cAMP/MAPK pathway. Recent studies suggest that MAS, gonad steroid hormones and EGF network induced by FSH and/or LH are also involved in meiotic resumption. Growing evidence indicates that cGMP-dependent signaling pathways exert a wide range of influences on gonadotropins-induced meiotic resumption. Further studies in this area may yield important new insights into the mechanisms regulating multiple aspects of oocyte maturation.

In the literature information regarding some very important physiological events for initiation and early growth of primordial follicles is very limited and remains no answer, such as: 1) Why and how some primordial follicles in mammal ovary are capable of starting to grow, while their neighbor sisters remain of quiescent? 2) What is the signal(s) responsible for selection of primordial follicle growth? 3) After mitosis occurred in the somatic cells in the early primordial follicle, why the germ cells immediately initiate undergoing the first meiotic division, what is the signal molecule (s)? 4) During both mitosis and meiosis within the primordial follicles, why large numbers of germ cells are culled from the ovary, resulting in less than one-third of the total number of potential germ cells being endowed in the ovary shortly after birth? 5) At birth, the number of germ cells in the primordial follicles has decreased markedly due to germ cell apoptosis occurring before formation of ovarian follicles, what are the molecular mechanisms and the signal pathways to induce the germ cell apoptosis? All these questions are remaining for the future investigation.

6. ACKNOWLEDGEMENT

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Abbreviations: PGCs: primordial oocyte or germ cells; ECM: extracellular matrix; dpc: days post coitus; TGF: transforming growth factor; GDF-9: growth and differentiation factor 9; BMPs: bone morphogenetic proteins; KL: kit ligand; SCF: stem cell factor;SDF-1: stromal-cell derived factor-1; VIP: vasoactive intestinal peptide; MAPK: mitogen-activated protein kinase; GC: granulosa cells; mGC: mural granulosa cells; CC: the cumulus cells; COC cumulus-oocyte complexes; TC: theca cells; IGFBPs: intra-follicular IGF-binding proteins; PAPP-A: pregnancy-associated plasma protein-A; LRH-1:liver receptor homolog-1; SF-1: steroidogenic factor 1; StAR: steroidogenic acute regulatory protein; TNF tumor necrosis factor; TRAIL: TNF-related apoptosis-inducing ligand;SUMO-1: small ubiquitin-related modifier-1; VEGF: vascular endothelial growth factor; tPA : tissue type plasminogen activator; PAI-1: plasminogen activator inhibitor type 1

Key Words: Primordial Follicle, Folliculogenesis, Granulosa Cell, Theca Cell, Oocyte, Intracellular Interaction, Signal Transduction, Review

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