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Follicular growth, differentiation and atresia

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Abstract **Only limited numbers of primordial follicles in mammalian ovary grow and differentiate to reach the stage of dominate follicles and ovulate. 99% of the follicles in the ovary undergo atresia at various stages of development. Regulation of follicular growth, development and atresia is a complex process and involves interactions between endocrine factors and intraovarian regulators. This review summarized: i) FSH may not be a survival factor in regulating slow-growing preantral follicles. Some locally produced growth factors, activin and orphan receptors might play a more important role at this stage. ii) Estrogen, activin/inhibin and follistatin coordinate with FSH to regulate and control follicle differentiation. iii) There are two types of follicular atresia induced by apoptosis which originates from GC or oocyte, respectively. Early translation of tPA mRNA into tPA protein in oocyte may be associated with oocyte apoptosis.**

Keywords: primordial follicle, oocyte, granulose cell, differentiation, atresia.

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The folliculogenesis is a complex process involving dramatic morphological and functional changes in granulosa and theca cells. This process is sequential and dictated by specifically, tightly regulated response to endocrine hormones and intraovarian regulators. They control follicular development by determining which of the growing follicles continue to develop and which become atretic. Follicle selection is the process wherein one follicle develops from a wave of growing follicles and becomes the only follicle with ovulatory capacity. Most follicles (> 99.9%) never reach ovulatory status, but undergo atresia at some point along this extended developmental pathway. Over the past two decades it has become apparent that FSH is a major survival factor to regulate follicular development. FSH receptor (FSHR) has been found in granulosa cells at the primary stage. It is reported, however, that preantral follicle development is regulated predominantly by factors produced locally within ovary or follicle[1]. Follicles in ovary from FSHR-null (–/–) mutant mice could develop up to the preantral stage[2], indicating that FSH is not a necessary survival factor in the early developing preantral follicles, some locally produced factors may play a more important role at the early stage.

1 Regulation of initiation of primordial follicle

Mammalian ovaries contain thousands of primordial follicles which are the only source of gametes during the entire reproductive life. Primordial follicle consists of an oocyte surrounded by a single layer of flattened pregranulosa cells. The primordial follicles may survive more than 50 years in woman ovary. Once a group of primordial follicles begin to grow, they will differentiate either into dominant follicle(s) and ovulate or undergo atresia at various stages of development. During onset of primordial follicle growth, flattened pre-granulosa cells become cuboidal and begin to proliferate and associate with expression of follistatin. The enclosed oocyte begins to grow at the same time[3,4]. It is interesting to note 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 known. Growth of GC in the follicle is a key process in initiation and development of primordial follicle. Using *in situ* hybridization, we have detected FSHR mRNA expression in the GC on D6 after birth. The receptor mRNA increased in a time-dependent manner following their further development.

Consistent with this observation, a significant GC proliferation was detected in the ovary on D7 after injection of FSH[5]. Several reports have shown that a variety of growth factors are capable of stimulating GC growth and proliferation *in vitro* in various species, leading to the concept that paracrine and autocrine mechanisms in the ovary are of primary importance in regulation of follicle growth. The architecture of the ovary shows that primordial follicles are located in the outermost part of ovarian cortex, an almost unvascular area, whereas growing follicles occupy the innermost part of ovarian cortex which has a rich vascular supply[6], suggesting that initiation of primordial follicle growth probably depends on some factors originated in blood. Treatment of 2-d-old rat ovaries in organ culture with NGF increased FSHR mRNA within 8-h exposure. This effect was cAMP-independ- ent, but had an additive increase in FSHR gene expression either with forskolin or vasoactive intestinal peptide. These results indicate that one of the functions of NGF in the developing ovary is to facilitate the differentiation process by which early growing follicles become gonadotropin-dependent during postnatal life, and that it does so by increasing the synthesis of FSHRs[7]. The ovaries from homozygote NGF-null (–/–) mutant mice analyzed after completion of ovarian histogenesis exhibited a markedly reduced population of primary and secondary follicles in the presence of normal serum gonadotropin levels, and an increased number of oocytes that failed to be incorporated into a follicular structure. These results suggest that the

delay in follicular growth observed in NGF(–/–) mice may be related to the loss of a proliferous signal provided by NGF to the nonneural endocrine component of the ovary[8]. Our experiment also demonstrated that epidermal growth factor (EGF) stimulates the early growth of the primordial follicles and GC proliferation, while no such influence of FSH during this very early stage could be found, suggesting that growth factor receptors like EGF receptor may differentiate earlier than FSH receptor in the GC in the primordial follicles during initiation of follicle growth[5]. The total number of oocyte in EGF+FSH+androgen (A) group was significantly higher than that in FSH+A group between D7 to D9 in the mouse fetal ovary culture period, a higher level of estradiol in FSH+A group was detected from 7 to 9 culture days, while at the same period only low concentration of estradiol existed in EGF+FSH+A group. These data suggest that the stimulatory effect of EGF on follicular development is independent of estrogen production[9].

In addition to growth factors, follicular development is also associated with other paracrine factors. Growth and development of primordial follicles to reach the ovulatory status is associated with marked proliferation, recruitment and differentiation of somatic cells and with changes in oocyte size and morphology reflecting both nuclear and cytoplasmic maturation. The mechanisms that regulate the ordered recruitment of quiescent primordial follicles into the growing pool have not been determined, but are likely to involve locally produced growth and differentiation factors, some of which emanate from the oocyte itself, such as growth and differentiation factor 9 (GDF-9) and bone morphogenetis protein 15 (BMP-15)[10,11], some of which emanate from the GC, SCF[12] for example. Meiosis activating sterols (MAS) is recently found to induce oocyte resumption from meiotic arrest, likely an important physiological substance which involves in the progress of germ cell maturation mediated by gonadotropins[13]. 4-d-old rat ovaries were cultured and the degree of primordial to primary follicle transition was measured. Insulin increased the primordial to the primary follicle transition by 30% over the control with a half maximal effective concentration (EC50) between 2.5 and 5 ng/mL. Insulin was shown to have an additive effect with KL and LIF[14]. Follicular development and oocyte maturation are related not only to growth factors, but also to activin and inhibin, as well as to orphan receptors. Their functions may be regulated by an autocrine/paracrine pathway and closely related to FSH and FSHR regulation[15]. Orphan receptor is a category of receptors whose cognate ligand are still unknown. It belongs to the nuclear receptor superfamily including steroid, thyroid and vitamin D3 receptor[16]. Large numbers of orphan receptor have been found. TR3 (also known as CGFIB, Nur77) was originally identified as an immediate-early gene product, rapidly induced by a variety of stimuli including growth factors. TR3 has been known to be a mediator of hormonal and neurological response in the hypothalamus-pituitary-adrencortical axis[17]. TR3 has also been found in testis and in some cell lines and expressed in sperms of rat[18], mouse[19] and monkey[20]. It can be induced quickly by growth factors, and involved in signal transduction *in vivo*[21]. TR3 mRNA expression was also identified and localized in the rat ovary, first appearing in the proliferating GC in early developmental follicules. The high expression was mainly in the proliferating cells, but not in the differentiated cells. It is suggested, therefore, that orphan receptor TR3 may play a role in regulating the initiation and differentiation of the follicular somatic cells in early follicular development. TR3 mRNA levels could be up-regulated by EGF, especially in the early stage of follicular development. In our previous studies, we reported that some growth factors such as EGF could stimulate the GC proliferation in smaller follicles. FSHR mRNA has been demonstrated to begin to differentiate from D6 after birth, and EGF stimulated the GC proliferation on D8, while markedly correlatively enhanced TR3 mRNA expression[15]. The physiological function of TR3 in reproduction is not completely understood.

2 Differention of follicular cells

Differentiation of GC may be affected by growth factors, such as EGF, TGF-α, IGF- I, secreted by ovarian stroma, and GDF-9, bFGF, secreted by oocyte. Once granulose cells have acquired functional FSH receptors, their proliferation and differentiation will be driven mainly by FSH and LH (at the preovulatory stage), and modulated also by other extrinsic and locally produced factors which may have both stimulatory and inhibitory action (Fig. 1).

Inhibin, activin and follistatin were first identified in ovarian follicular fluid and their ability was demonstrated to modulate secretion of FSH from the pituitary gonadotrophs in culture, such as inhibin and follistatin suppress FSH secretion, whereas activin enhances FSH secretion[22]. Inhibin and activin are disulphide-linked dimeric glycoprotein belonging to the TGF-β superfamily. Inhibin is dimmer of a unique α subunit linked to either a βA or βB subunit to generate inhibin A (α-βA) or inhibin B (β-βB). Dimerization of β subunits alone gives rise to three forms of activin referred to as activin A (βA-βA), activin AB (βA-βB) and activin B (βB-βB). Follistatin, a cysteine-rich monomeric glycoprotein encoded by a single gene, is structurally unrelated to the TGF-β superfamily. There are several different isoforms of follistatin due to alternative mRNA splicing and post-translational modification[23]. Activin-induced proliferation has been observed with

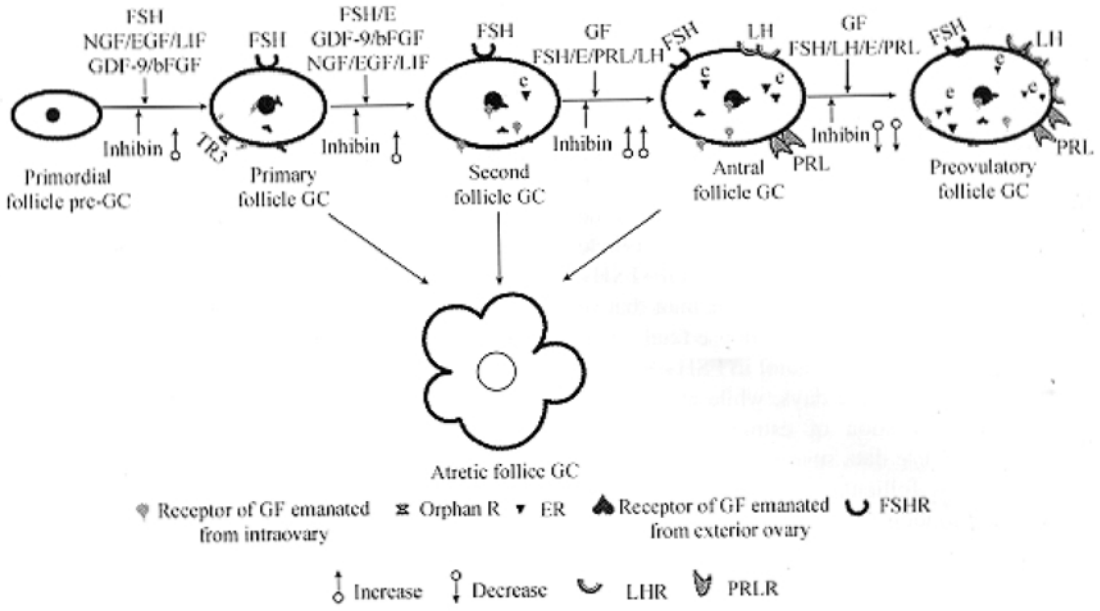

Fig. 1. Progress of GC growth, differentiation and atresia. From activation of primordial follicles to selection of secondary follicles, growth factors appear to exert important effects on the growth and differentiation of GC. Progression to the antral follicle stage is probably the most critical stage of follicle development *in vivo*. Granulosa cells at this stage have acquired functional FSH receptors, their proliferation and differentiation are driven mainly by FSH and LH (at the preovulatory stage), and modulated by other extrinsic and locally produced factors which may have both stimulatory and inhibitory action. Apoptosis occurs at each stage of follicular development.

cultured rat granulose cells from both small and large follicles and with human granulose lutein cells[24]. In contrast, in knockout mice lacking activin type ⅡB receptors, follicle development was arrested at an early antral stage, suggesting its key role in granulose cell proliferation and differentiation[25]. Activin stimulates cAMP production, aromatase activity and increases gonadotropin receptors[26]. Inhibin produced by dominant follicle may act as a paracrine factor inhibiting the growth of neighboring follicles, thus participating in the mechanism of follicular selection[27,28]. Granulosa cells in a dominant follicle produce a higher amount of inhibin and estrogen, which exert an inhibitory effect on FSH secretion from pituitary on one hand, and contribute to enhancement of LHR differentiation and increase in aromatase activity on the other hand. Those changes also help to increase the sensitivity of follicles in response to gonadotropin stimulation[29,30]. Other follicles without LHR differentiation will undergo atresia[31]. LHR in granulose cells is induced by FSH synergistically with estrogen, the LHR differentiation can also increase estrogen synthetic capacity by increasing aromatase activity, and therefore further support follicular development. Estrogen is likely the major candidate in stimulation of FSH secretion in the infantile female rat, and inhibin regulation of pituitary FSH secretion is through its negative feedback in the infantile female rat[31,32].

3 Follicular atresia

Atresia is the fate of most follicles. Follicular atresia induced by apoptosis may originate from granulose cells or oocytes[33—35]. Understanding how these physiological regulators participate in determining the destiny of the follicles is fundamental. Gonadotropins and some of locally produced growth and differentiation factors control follicular growth, differentiation and atresia. Active expression of NAIP mRNA was reported to be localized in granulose cells of developing follicles from primary to Graafian stage, not or weakly expressed in the follicles that was undergoing atresia. Gonadotropin-inducible NAIP may indirectly affect oocyte survival as a result of inhibition of GC apoptosis during folliculogenesis[36]. Some growth factorts alter the susceptibility of granulose cells to FasL-induced apoptosis, EGF or bFGF inhibit FasL-induced killing, whereas keratinocyte growth factor (KGF), transforming growth factor (TGF), platelet-derived growth factor (PGF), FSH and LH had no such effect[37]. The ovaries collected on D1 of birth and treated with TNFα decreased follicle and oocyte numbers during 3-d culture and we found that oocytes, interstitial cells, and granulose cells were undergoing apoptosis[38]. It is also reported that interaction between proto-oncogene Bcl-2 family and tumor-suppressive gene family may play an important role in determining the future of developing

follicles[39].

Oocyte apoptosis is considered to be one of the causes of follicle degeneration. tPA mRNA in oocytes has been detected at the early stages of follicular development, but translation of the tPA mRNA into the protein in the oocyte does not start until onset of meiosis maturation[40—42]. Increase of tPA activity in matured oocyte is accompanied with germinal vesicle breakdown (GVBD). Certain morphological changes similar to GVBD during meiosis maturation in normal oocyte could also be observed in atretic follicles. In our previous study we have demonstrated for the first time that tPA in oocyte is associated with follicular atresis. Follicles that failed to be recruited for further development could also express tPA[43]. This is possible because some regulatory factors produced by GC alter the programming of tPA translation in the oocyte. The tPA activity inside oocyte may play an important role in self-destruction or clearance of death cell fragment during atresia. We have traced the expression of inhibin-α mRNA during the follicular development of newborn rat ovary. Expression of inhibin-α mRNA in GC began from D5, increased with the follicular development and reached the peak level in the antral follicles, suggesting that inhibin may play a certain role in follicular development at the early stage[5]. Inhibin-α mRNA expression in rat GC was reversibly correlated with tPA activity in the oocytes. High expression of inhibin-α mRNA in GC was always correlated with no measurable amount of tPA activity in the oocyte, and no inhibin mRNA expression in GC was related to high tPA activity in the oocyte, suggesting that inhibin expression in GC may play an essential role in preventing tPA mRNA translation in oocyte[43], suggesting that inhibin in GC may play an essential regulatory role in deciding the fate of follicles via oocyte apoptosis. Inhibin is constantly expressed during normal follicular development and therefore the translation of tPA in the oocyte is suppressed. When ovulatory stage approaches, the expression of inhibin in granulose cells decreases, while the translation of tPA mRNA in oocyte begins, and its activity is increased significantly, that may be responsible for the cumulus cell expansion and dispersion, and oocyte maturation. Inhibin emanated from GC inhibits oocyte maturation by inhibiting tPA mRNA translation in oocyte, once inhibin production in GC decreases, the increased tPA activity in oocyte may induce certain morphological changes in oocyte similar to GVBD, conducing oocyte apoptosis (Fig. 2).

Whereas in normal follicle development progress, plasma ir-inhibin concentrations begin to increase before ovulation, they decrease when the LH surge is initiated, thereafter, a further sharp rise in circulating ir-inhibin concentrations occur during the process of ovulation, followed by an abrupt decline, then expression of tPA in oocyte increases, that may be important for the oocyte maturation and ovulation[43]. *In vitro* studies showed that Anti-Mullerian hormone (AMH) inhibits the recruitment of primordial follicles into the pool of growing follicles and the expression of inhibin-α[44], whether the effect is via tPA regulation has not been reported.

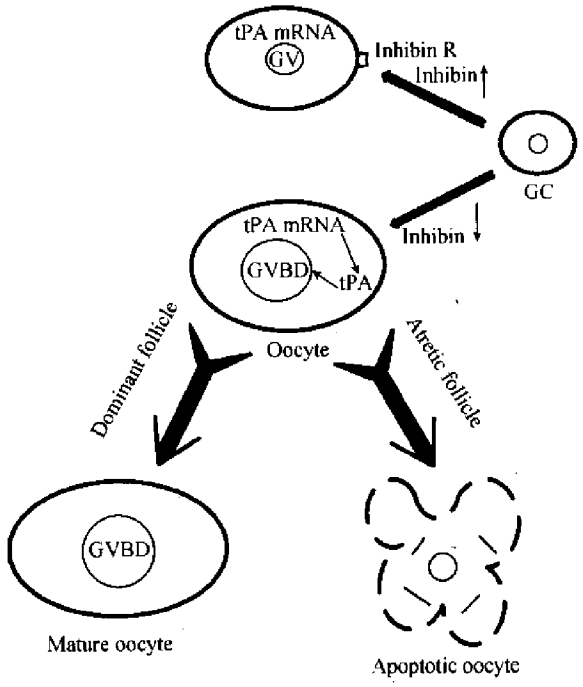

Fig. 2. Effect of inhibin emanated from GC on expression of tPA activity in oocyte. Inhibin emanated from GC inhibits oocyte maturing by inhibiting the expression of tPA activity in oocyte, once the expression of inhibin in GC decreased, tPA activity increases, which induces certain morphological changes in oocyte similar to GVBD. The matured oocyte ovulates, the unmatured oocyte undergos apoptosis.

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