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Actions of the TGF Superfamily Members in Ovarian Follicle Growth and Development

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난포성장 발달에 있어서 TGF-β Superfamily의 작용

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An ovarian follicle progresses through several stages during its lifetime: quiescence (primordial follicles), growth (preantral and antral follicles), ovulation, and atresia. The entrance of a primordial follicle into the growing pool, its selection as a dominant follicle committed to ovulation and luteinization, or its exit from the pathway through atresia are determined by the concerted actions of endocrine, paracrine, and autocrine factors.[1]

The members of the transforming growth factor-β (TGF-β) superfamily and their roles in the regulation of follicle growth and development have been reviewed in several recent articles.[2~4] TGF-β superfamily members are involved in every stage of follicle growth. TGF-β, activin, inhibin, growth differentiation factor-9 (GDF9), anti-Mullerian hormone (AMH, MIS), and several of the bone morphogeneic proteins (BMPs) are involved in stimulatory and inhibitory paracrine communication between the oocyte, granulosa cells, and theca cells and autocrine signaling within each cell type to ensure the timely maturation and growth of a single follicle. Because early follicle growth is gonadotropin-independent, the primordial to primary follicle transition and the development of the theca layer in early follicle development is directed by local communication between the oocyte, granulosa cells, and stroma. Therefore, their roles in early follicle growth have been of particular interest in the last 5 years, as well as TGF-β superfamily signaling is also essential for the acquisition of follicle-stimulating hormone (FSH) responsiveness by preantral follicles and for the modulation of gonadotropin-stimulated antral follicle growth and steroidogenesis.

The expression patterns and localization of the TGF-β superfamily ligands and signal transduction components in the mammalian ovary have been the subject of numerous reviews and will not be recounted in detail here.[3,5~9] Suffice to say; the ovary expresses TGF-β superfamily receptors and signaling components in every follicle stage, with primordial follicles containing intact TGF-β and BMP signal transduction pathways, and preantral and antral follicles capable of supporting activin, TGF-β, and BMP signals.

Understanding the role of the TGF-β superfamily ligands in ovarian follicle development is complicated

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by cross-talk that occurs at every level of their signaling pathways.[10] In addition, the actions of the TGF-β superfamily ligands in the ovary modulate and are in turn regulated by the gonadotropins, steroid hormones, various growth factors, and the interleukins. All of these factors act within and between discrete cellular compartments of the ovarian follicle in a stage-dependent manner to direct follicle recruitment, oocyte maturation, follicle cell proliferation and differentiation, atresia, steroidogenesis, ovulation, and luteinization.

This review will examine the roles of particular TGF-β superfamily members in directing ovarian follicle growth and development, followed by a discussion of future research directions for this field.

TGF-β

TGF-β is expressed in granulosa cells of primary follicle stage and in theca cells of large preantral follicles,[11,12] and the TGF-β type I and II receptors are found in granulosa, theca and interstitial compartment.[13] TGF-β is generally anti-proliferative, but its effects can vary based on cell type, follicle stage, and species. For example, a recent study showed that in immature rat granulosa cell cultures, TGF-β acts as an autocrine stimulator of DNA synthesis, an effect which is enhanced by FSH and estradiol.[14] TGF-β action may also be age-dependent in that it stimulates follicle growth in adult mice but not in immature mice.[15] In cultured theca cells, TGF-β inhibits androgen synthesis, likely by inhibiting StAR expression,[16] whereas in granulosa cells, TGF-β augments FSH-induced luteinizing hormone (LH) receptor expression and potentiates progesterone production.[17] TGF-β has also been shown to stimulate inhibin expression in rat ovarian cells, which may be necessary for initiation of inhibin production by early growing follicles.[18]

Activin

In order to progress past the small antral stage and bypass atresia, follicles must acquire FSH responsiveness; it is believed that activin is involved in the preantral to antral follicle transition by up-regulating FSH receptor expression in granulosa cells.[19,20] Thus, the follicle with the greatest capacity for activin signaling may also be more responsive to FSH, and better prepared to progress to the antral stage. Further, it has been suggested that activin inhibits the progression of non-dominant follicles; in one study, activin from secondary follicles suppressed the growth of primary follicles.[21] It also appears that activin actions may be age-dependent, though contrary to that of TGF-β: activin induces follicle growth in immature but not in mature mice.[15] Outside the granulosa cell, activin stimulates proliferation of theca-interstitial cells in culture,[22] suppresses LH-stimulated androgen production and premature luteinization in small to medium antral follicles,[23,24] and may affect oocyte maturation and competence.[25]

On the molecular level, a recent study demonstrated that Smad2 and Smad3 are highly expressed in the granulosa cells of preantral follicles, further supporting a role for activin in the acquisition of FSH responsiveness in these follicle classes.[26] Smad3 null mice were recently found to have a higher number of primordial follicles and lower number of large preantral and antral follicles than their wild-type littermates.[27] Perhaps the loss of activin signaling through Smad3 in these mice prevents the progression of preantral follicle growth and favors the anti-proliferative effects of TGF-β signaling through Smad2. Likewise, the activation of primordial follicles might be regulated thru Smad3 involved signaling pathway.

Inhibin

The activin and inhibin subunits are produced in the granulosa cells and act on all cellular compartments of the follicle. Intraovarian levels of Activin and inhibin through folliculogenesis dependent on the relative

abundance of α-subunit and β-subunits.[28,29] Production of the inhibin β-subunit increases as follicles grow, such that antral follicles primarily produce inhibin. In addition, the β_A-subunit is expressed in all follicle stages, whereas the β_B-subunit expression is limited to small antral follicles. Thus, antral follicles shift from inhibin A to inhibin B production as they grow. Though no independent inhibin receptor has been found to date, inhibin has been shown to have direct effects on the ovary. Prendergast et al. recently demonstrated that ovariectomy or immuno-neutralization of inhibin causes an increase in follistatin transcription that can not be rescued by estradiol replacement or by blocking gonadotropin releasing hormone (GnRH).[30] In addition, inhibin promotes LH-stimulated androgen production in the theca cell[23,31] and suppresses estradiol production by cultured bovine granulosa cells.[32] A recent in vivo study has suggested that inhibin produced by large antral follicles may determine dominant follicle selection through induction of apoptosis in non-dominant follicles (preantral and early antral follicles).[33]

BMPs

In antral follicles, BMP4 and BMP7 are produced in the theca cells, whereas BMP receptors are expressed in the oocyte and granulosa cells of most follicles in ovaries of normal cycling rats[34,35] and, to a lesser extent, the theca layer of antral follicles in sheep ovaries.[36] BMP4 and BMP7 appear to delay luteinization and atresia in antral follicles by modulating granulosa and theca cell responsiveness to gonadotropins. In vivo, BMP7 treatment of rat ovaries suppresses ovulation and luteinization.[37] Female BMP type IB receptor null mice are infertile, with decreased cumulus expansion and aromatase activity.[38,39] In granulosa cells, BMP4 and BMP7 augment FSH-stimulated estradiol synthesis and suppress progesterone secretion[35] through modulation of FSH-stimulated aromatase expression and down-regulation of StAR expression.[37] In cultured theca cells, BMP4 attenuates cAMP-stimulated androstenedione and progesterone production.[40]

BMP4 and BMP7 are also involved in promoting preantral follicle growth. BMP7 increases proliferation of granulosa cells in the presence of FSH,[37] and both BMP4 and BMP7 treatment of rat ovaries in culture results in a larger number of developing primary follicles and a smaller primordial follicle pool.[37,41] Furthermore, culture of ovaries with a BMP4 immuno-neutralizing antibody are smaller, have a progressive loss of primordial follicles, and show an increase in apoptotic activity.[41]

BMP6 is produced by the oocyte in the primary follicle stage.[42] Unlike BMP4 and BMP7, BMP6 has no effect on the proliferation of granulosa cells or estradiol production, but suppresses FSH-induced progesterone synthesis through down-regulation of adenylyl-cyclase activity.[42] BMP6 also stimulates inhibin B and β_B-subunit production in primary human granulosa cell cultures.[43,44] The precise role of BMP6 in early follicle growth is not completely understood. BMP6 null mice are viable and fertile and show no overt defects in tissues known to express BMP6 mRNA. Patterns of BMP2 and BMP6 expression in other tissue suggest the possibility of functional compensation,[45] because BMP2 also has been shown to increase estradiol and inhibin A production without affecting the proliferation of the cells by sheep granulosa cells.[36]

AMH

In the adult rat, AMH and the AMH type II receptor (AMHRII) are exclusively expressed in granulosa cells of preantral and small antral follicles, not in primordial follicles. The colocalization of AMH and AMHRII mRNAs in granulosa cells of specific follicle types suggests that actions of AMH via AMHRII are autocrine in nature.[46] In general, AMH inhibits primordial follicle recruitment and suppresses FSH-responsiveness of pre-

antral follicles.[47] As follicles grow, AMH levels decline. Mice null for either AMH or the AMH type II receptor are fertile,[39,48,49] however, AMH null mice have an increased number of growing follicles and decreased number of primordial follicles than their wild type litter mates.[49] The inhibitory role of AMH in small follicle growth is supported by recent findings in cultured mouse ovaries in which AMH suppressed recruitment of primordial follicles into the growing pool[50] and counteracted FSH-stimulated preantral follicle growth.[51] Expression pattern of AMH is located in GCs closest to oocyte. So, it should be autocrine or juxta-crine, rather than paracrine or endocrine. In vitro studies demonstrated that AMH signals are transduced through ALK2 and Smad1.[52]

GDF9 and BMP15/GDF9B

In the rat, GDF9 and BMP15 (also called GDF9B) are expressed exclusively in the oocyte and act in a paracrine manner on granulosa and theca cells.[53,54] Interestingly, primate granulosa cells also secrete and respond to GDF9, suggesting that it acts as an autocrine factor in primates.[55] GDF9 appears to be a general marker for oocyte and follicle health.

GDF9 deficient mice show arrest of follicular development at the primary one-layer follicle stage.[56] Recombinant GDF9 induces preantral rat follicle growth in vitro,[57] as well as promotes granulosa cell proliferation in early preantral follicle stages[58,59] and in larger follicles, increases progesterone synthesis in vitro and induces cumulus expansion.[60] Recent studies have demonstrated that GDF9 also stimulates Smad2 activation and inhibin production in rat and human granulosa cells.[61,62] GDF9 suppresses FSH-induced granulosa cell differentiation (steroidogenesis, cAMP production and LH-receptor expression) in vitro.[63]

More significantly, GDF9 indirectly acts on the theca cell to promote differentiation[58,64] by stimulating kit ligand production in granulosa cells, which in turn initiates theca cell recruitment from the stroma.[65,66] GDF9 enhanced both basal and stimulated androstenedione accumulation in the primary and transformed theca-interstitial cell (TIC) cultures.[67] The effects of GDF9 on steroidogenesis by preovulatory follicles were relatively modest. Likewise, it did not affect the maturation of follicle-enclosed oocytes. The effect of GDF9 on TIC androgen production suggests a regulatory role of the oocyte on theca cell function and hence on follicle development and differentiation.

In cultured human theca cells, however, GDF9 decreases cAMP-stimulated progesterone and StAR expression, lending support to the suggestion that this factor acts differently in primates and rats.[68]

Like GDF9, BMP15 stimulates cell proliferation, decreases FSH-stimulated progesterone production (by decreasing FSHR expression), and stimulates kit ligand expression in granulosa cells.[54,69,70] BMP15 expression is in turn down-regulated by kit ligand in the oocyte, and loss of this feedback loop reduces granulosa cell proliferation.[69] BMP15 actions are dose-sensitive, as sheep homozygote for a naturally occurring BMP15 mutation have reduced fertility, whereas the heterozygotes exhibit increased ovulation.[71] Recent work has demonstrated that GDF9 binds to the BMP-activated type II receptor (BMPRII), but, its downstream actions are mediated by the type I receptor, ALK5, and the Smad2 and Smad3 proteins. Because ALK5 is a known receptor for TGF-β, diverse members of the TGF-β family of ligands appear to interact with a limited number of receptors in a combinatorial manner to activate two downstream Smad pathways.[72] Unlike GDF9, BMP15 was found to interact with ALK6 and activates the downstream Smad1.[73] Thus, the actions of paralogous GDF9 and BMP15 are mediated by distinct receptors and intracellular pathways, suggesting these oocyte ligands could play unique roles in follicle development.

Follistatin and betaglycan

Follistatin and betaglycan are TGF-β superfamily signaling modulators; follistatin binds and neutralizes activin and the BMPs,[4] and betaglycan has been shown to modulate TGF-β, activin, and BMP signal transduction.[74~76] Follistatin is primarily expressed in granulosa cells of antral follicles and in luteinized granulosa cells, and betaglycan is expressed in granulosa and theca cells in antral and preovulatory follicles.[77] Thus, early follicles are activin-dominant; as they grow, follicles gradually lose their activin "tone" as follistatin, betaglycan, and inhibin expression increase. Follistatin counteracts activin and BMP actions in the ovary and thus generally pro-

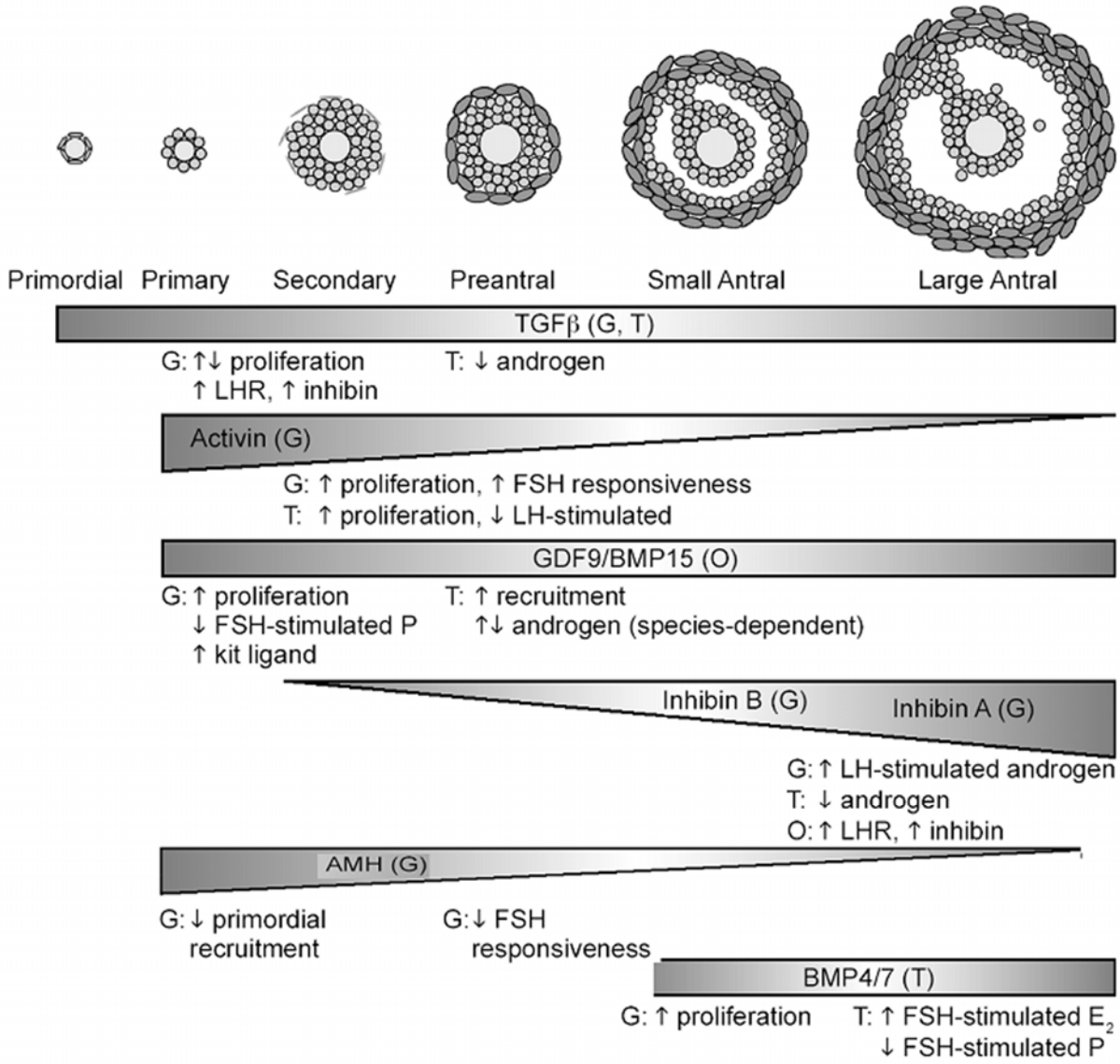

Figure 1. The members of the TGF-β superfamily act in a stage-specific manner to direct follicle recruitment, oocyte maturation, granulosa and theca cell proliferation and differentiation, and steroidogenesis. Cellular expression is indicated in parenthesis next to each TGF-β superfamily member, with the actions on follicle cell types listed below each member. G, granulosa; T, theca; O, oocyte; LH, luteinizing hormone; FSH, follicle-stimulating hormone; LHR, LH receptor; E_2, estradiol; P, progesterone.

motes luteinization and atresia. Follistatin also inhibits BMP15-stimulated granulosa cell proliferation and rescues FSH-stimulated progesterone secretion.[78] Whether betaglycan has similar inhibitory effects on activin and BMP actions in the ovary is yet to be discovered.

Future directions/Conclusions

Though our understanding of the role of the TGF-β superfamily in regulation of ovarian follicle growth and development continues to grow, recent studies have begun to address the mechanics of paracrine signaling within the ovary. In particular, new research is beginning to uncover the role of cell-cell interaction and cell-extracellular matrix (ECM) interactions in the directional movement and delivery of paracrine factors between follicle cell compartments and across the zona pellucida (ZP) and basal lamina. Recent work has identified transzonal projections that cross the ZP and deliver growth factors from the granulosa cell layer to the oocyte.[79] Paracrine signaling within the follicle also requires remodeling of barriers between cell layers and reorganization of the ECM, the components and structures of which constantly change as a follicle grows.[80] The ECM facilitates paracrine factor concentration at site of action and protection from degradation, with release requiring the actions of matrix metalloproteinases (MMPs).[81] In turn, members of the TGF-β superfamily have been shown to modulate the ECM; TGF-β1, activin, and GDF9 dose-dependently increase the expression of lysyl oxidase, an enzyme required for ECM cross-linking,[82] and the expression of connective tissue growth factor, which is thought to be involved in theca layer recruitment and deposition of the basement membrane.[83]

In conclusion, the members of the TGF-β superfamily act as stage- and cell-specific paracrine factors that, in conjunction with the steroid hormones, the gonadotropins, and other growth factors, orchestrate the timely growth and development of the follicle (summarized in Figure 1). These factors influence the differentiation and proliferation of granulosa cells, the recruitment of the theca cell layer, the maturation of the oocyte, and steroidogenesis. Research continues to clarify the roles of each of these factors as well as unravel their relationships with one another, with other factors, and with ovarian cell structures with the goal of understanding how a follicle is guided from a primordial follicle to a follicle competent for ovulation.

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