[Frontiers in Bioscience 11, 126-135, January 1, 2006]

Stem cell factor (SCF)-Kit mediated phosphatidylinositol 3 (PI3) kinase signaling during mammalian oocyte growth and early follicular development

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1. ABSTRACT

The bi-directional communication between mammalian oocytes and their surrounding granulosa cells has been shown to be crucial for ovarian follicular development. Studies on molecules derived from the oocytes, such as growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15), have attracted great interest during the past decade, and it is common knowledge nowadays that these molecules participate in the bi-directional dialogue between the oocytes and their surrounding granulosa cells as well as follicular development. However, signaling molecules and pathways inside mammalian oocytes that control oocyte growth and early development of ovarian follicles, which may be monitored by factors produced by granulosa cells, have not been studied extensively. Based on our own data as well as ovarian phenotypes observed in several gene modified mice strains that were generated for studies of signal transduction, immunology and cancer, the current review focuses on the key features of the activation of oocyte phosphatidylinositol 3 kinase (PI3 kinase) pathway and its possible roles during mammalian oocyte growth and follicular development. We propose that the cascade from the granulosa cell-produced stem cell factor (SCF) to the oocyte surface SCF receptor Kit, and to the oocyte PI3 kinase pathway, may play an important role in the regulation of growth rate of mammalian oocytes, as well as in the activation and development of ovarian follicles.

2. INTRODUCTION

The adult mammalian ovary is a heterogeneous organ, consisting of follicles and copora lutea at various stages of development. The primary function of the ovary is to produce fertilizable oocytes during the reproductive cycle. Before the first wave of follicular development, an ovary is populated predominantly by primordial follicles, each composed of a meiotically-arrested primary oocyte enclosed within several flattered pre-granulosa cells. In mammals, there is a continuous recruitment and development of miniature follicles during the repetitive estrous cycles. The development of follicles involves the initial recruitment of primordial follicles from the resting pool, the continued growth of the follicles, the cyclic recruitment of dominant follicles, ovulation, and luteinization. This continuously ongoing process requires dramatic cyclic changes in tissue architecture, which are controlled by the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), as well as other ovarian factors (for papers and reviews, see 1-6).

Each follicle contains an oocyte that is surrounded by granulosa cells. Activation of primordial follicles involves unrevealed mechanisms that are intrinsic to the ovary before FSH signaling (1, 7). The growth and meiotic regulation of oocytes are both dependent on granulosa cells (1-3, 8). Previous studies have suggested

that oocytes are not quiescent cells that are solely nursed by the surrounding granulose cells, but that they play key roles in folliculogenesis. The bi-directional communication between the oocyte and somatic cells is essential for the proliferation and differentiation of the granulosa cells which maintain female reproduction (for reviews, see 3, 5, 9-12). The roles of oocyte-derived factors such as growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15) in follicular development have been demonstrated clearly by the use of gene deficient mice from Dr. Martin Matzuk's laboratory (13-15), and the mechanisms whereby they influence granulosa cells, ovulation rate and litter size continue to be one of the most active research areas in reproductive biology today (for reviews and papers, see 12, 16-21). On the other hand, granulosa cells also regulate the secretion and function of oocytes. For example, it has been shown that BMP-15 stimulates stem cell factor (SCF, or Kit ligand) expression in granulosa cells, and SCF also inhibits BMP-15 expression in oocytes, thus forming a negative feedback loop between the oocytes and granulosa cells (16). In addition, it has also been shown recently that the correct concentration of FSH is crucial for appropriate modulation of SCF and BMP-15, which promote oocyte growth (22).

3. SCF, KIT AND THE PI3 KINASE PATHWAY

3.1. Kit and Kit ligand SCF

Kit (CD 117), or SCF receptor, is a receptor protein tyrosine kinase (RPTK) encoded by the proto-oncogene c-kit. The ligand for Kit is SCF, which is also known as steel factor (SF) or mast cell growth factor (MGF). SCF is synthesized from two alternatively spliced mRNAs as transmembrane proteins that can either be proteolytically cleaved to a soluble form of SCF, or can function as cell surface-bound factor. In mice, the Kit receptor tyrosine kinase and SCF are encoded at the white spotting (W) and steel (Sl) loci, respectively (23).

Kit is a 976-amino acid protein and belongs to the platelet-derived growth factor (PDGF) receptor family. The binding of SCF to Kit leads to the auto-phosphorylation and activation of Kit, which subsequently leads to the phosphorylation and activation of various downstream substrates, thereby activating distinct signaling cascades. Molecules known to associate with the Kit receptor *in vivo* include the p85 subunit (p85α and p85β) of class IA phosphatidylinositol 3 kinase (PI3 kinase), phospholipase Cγ-1 (PLCγ), Grb2 adaptor protein, Src, and tyrosine phosphatases SHP1 and SHP2. The association of Kit receptor with substrates through which the downstream signals are relayed is known to induce cell proliferation, differentiation, migration and survival in many types of cells or tissues (24-26). Kit is known to play roles in melanogenesis, hematopoiesis and gametogenesis, and mutations at both the W and the Sl loci cause deficiencies in these processes (25, 26). Kit is also expressed in a number of cancers, including melanoma, glioblastoma, germ cell cancer, small cell lung cancer, and ovarian cancers – implying that this molecule may have a role in tumorigenesis (24, 26, 27).

3.2. PI3 kinase

The PI3 kinases are a family of lipid kinases that phosphorylate the 3'-OH group of the inositol ring in inositol phospholipids. Phosphorylated lipids are produced at cellular membranes during signaling events, and contribute to the recruitment and activation of various signaling components. PI3 kinases catalyze the production of phosphatidylinositol-3, 4, 5-triphosphate ($PtdIns(3,4,5)P_3$) by phosphorylating phosphatidylinositol-4, 5-bisphosphate ($PtdIns(4,5)P_2$), which process is essential for cell survival, regulation of gene expression, cell metabolism, and rearrangements of the cytoskeleton (24). The class I PI3 kinases are heterodimers consisting of a 110-kDa (p110) catalytic subunit and a regulatory subunit. To date, for the catalytic p110 subunit, three isoforms, p110α, p110β and p110δ have been identified; and for the regulatory subunit, seven adaptor proteins generated by alternative splicing of three genes p85α, p85 β and p55γ have been reported (24, 28).

PI3 kinases can be activated in several ways. First of all, growth factors and hormones can trigger the activation of PI3 kinase (28). Activation of the RPTK, such as epidermal growth factor (EGF) receptor and Kit, can recruit PI3 kinase to the cell membrane area via the binding of one or several Src homology 2 (SH2) domains of the regulatory subunit of PI3 kinase to the phosphorylated tyrosines of the RPTK. For example, as illustrated in Figure 1, in mouse Kit, PI3 kinase binds to the phosphorylated Tyrosine 719 (Y719-P) of the receptor (25), and this binding leads to the activation of the catalytic domain (p110) of PI3 kinase. Secondly, activated Ras (Ras-GTP) can bind directly to the N-terminal region in p110, leading to the activation of PI3 kinase. As also illustrated in Figure 1, the p110 phosphorylated $PtdIns(3,4,5)P_3$ in cells can then recruit from cytoplasm proteins with two lipid-binding domains, the FYVE or the pleckstrin-homology (PH) domain (29). Proteins containing the PH domain are known to be key mediators of PI3 kinase signaling, which include the serine/threonine kinases 3'-phosphoinositide-dependent kinase-1 (PDK-1) and Akt (also sometimes referred to as protein kinase B, PKB). Both PDK1 and Akt are essential molecules for relaying signals from the RPTK-activated PI3 kinase (24).

3.3. Akt and its activation

Akt is a cellular homolog of the transforming viral oncogene v-Akt, which has significant homology to protein kinase A (PKA) and protein kinase C (PKC) (30). There are three mammalian isoforms, α, β and γ, which all contain an N-terminal PH domain, a central kinase domain (with a Threonine 308 phosphorylation site), and a conserved regulatory serine phosphorylation site (Serine 473) near the C terminus (24, 31). The C-terminal PH domain of PDK-1 binds phospholipids with 10-fold higher affinity than the Akt PH domain, which results in a constant localization of PDK-1 at the plasma membrane. As shown in Figure 1, with the mouse Kit receptor as an example, SCF binding to Kit leads to activation of the Kit molecule and the above-mentioned recruitment and activation of PI3 kinase, which leads to the production of $PtdIns(3,4)P_2$ and $PtdIns(3,4,5)P_3$ at the inner side of the membrane. This recruits Akt by interacting with its PH domain from the cytoplasm and leads to its translocation to the inner membrane. Akt is subsequently phosphorylated

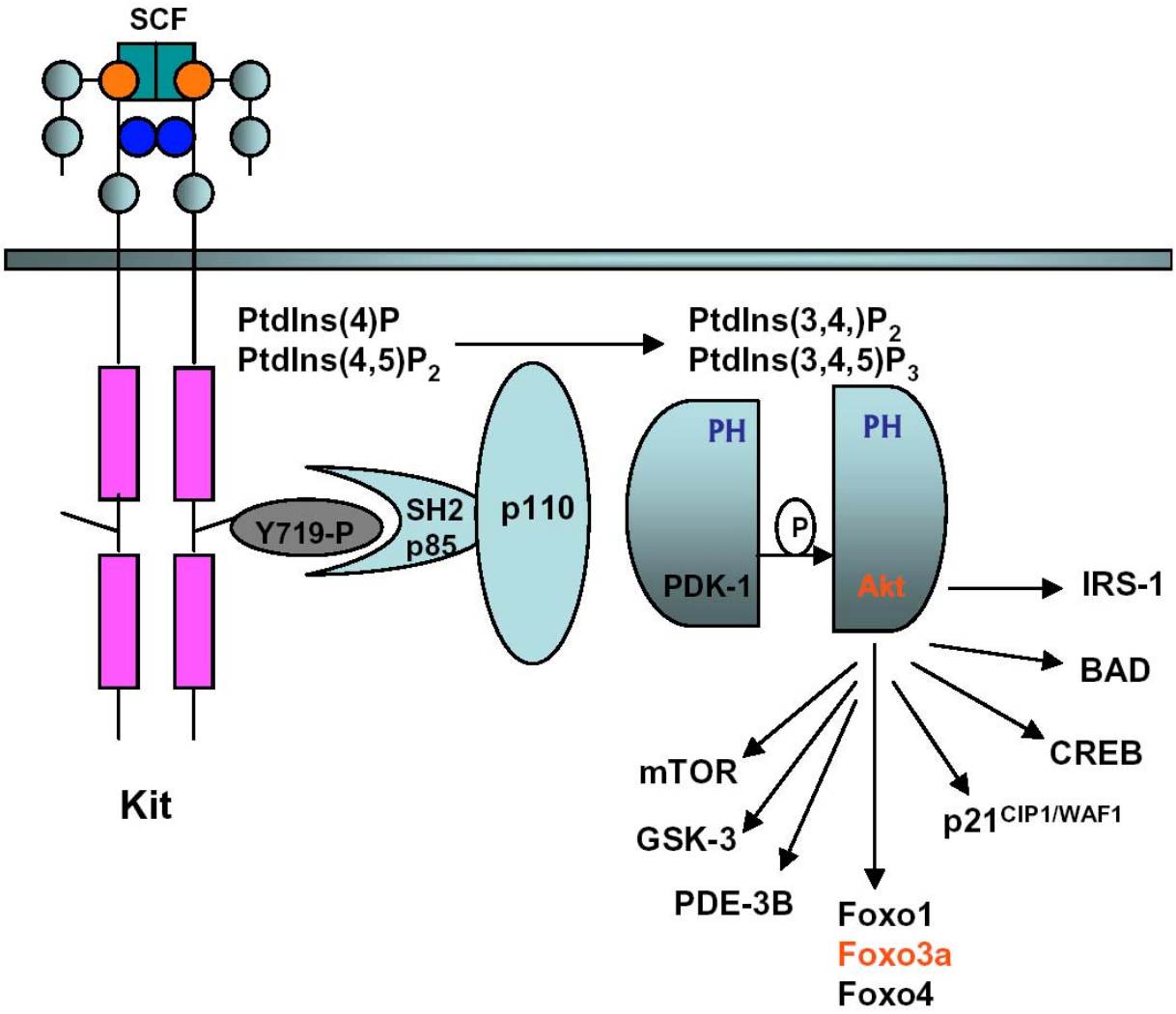

Figure 1. SCF and Kit receptor induced signaling cascade via the activation of PI3 kinase pathway. The figure illustrates signaling via the mouse Kit. Green boxes represent two SCF molecules that are bound to dimers of Kit molecules. Orange filled circles represent the ligand binding domains of Kit, and blue filled circles represent the dimerization domains of Kit. Pink filled boxes represent the kinase domains of Kit. The binding of SH2 domain of the p85 subunit of PI3 kinase to phosphorylated tyrosine 719 of mouse Kit, and the subsequent activation of PDK-1 and Akt, as well as Akt downstream molecules, are shown (see text for details). The focused molecules of this review, Akt and Foxo3a, are highlighted in red.

by PDK1 at the Threonine 308 which stabilizes Akt in its activation conformation. Phosphorylation of Akt at Serine 473 is also required for Akt activation (32). In addition, activated Akt can also be translocated to the nucleus, where several of its substrates reside (24, 33).

3.4. The FOXO subclass of forkhead box transcription factors

To date, at least 13 substrates of Akt have been identified in mammalian cells (24). These substrates have been classified into two major groups: apoptosis regulators and cell growth regulators. As illustrated in Figure 1, the Akt substrates involved in protein synthesis, glycogen metabolism and cell-cycle regulation include glycogen synthase kinase-3 (GSK-3), phosphodiesterase-3B (PDE-3B), mammalian target of rapamycin (mTOR), insulin receptor substrate-1 (IRS-1), the cyclin-dependent kinase inhibitor $p27^{kip1}$ and $p21^{CIP1/WAF1}$ (24, 34). The substrates of Akt involved in apoptosis include the FOXO subclass of forkhead box transcription factors, the pro-apoptotic Bcl-2 family member Bad, and the cyclic AMP response element-binding protein (CREB) (24, 35). In this review, Akt and Foxo3a are given special attention due to their possible roles in controlling oocyte growth (see below).

In the FOXO subclass of the forkhead box transcription factors, three of the four members, Foxo1 (FKHR), Foxo3a (FKHRL1) and Foxo4 (AFX) are

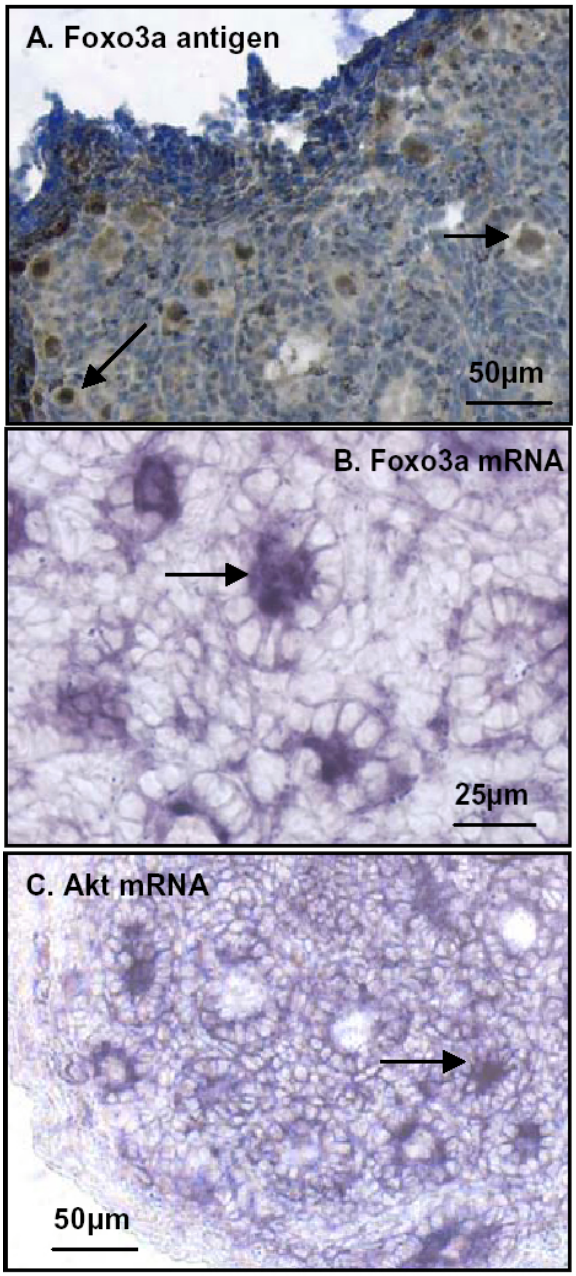

Figure 2. Expression of Foxo3a and Akt in growing mouse oocyte. The figures show the expression of Foxo3a antigen (A), Foxo3a mRNA (B) and Akt mRNA (C) in growing mouse oocytes (arrows) obtained from postnatal 8-day-old C57BL/6 mice. Modified from Reddy *et al.*, 2005 (60).

substrates of Akt (35). Foxo6 lacks some phosphorylation sites and exhibits a unique pattern of subcellular localization (36). The FOXO subclass of the forkhead box transcription factors is evolutionarily conserved (35). The *Caenorhabditis elegans* ortholog of Foxo is DAF-16, which modulates the lifespan, metabolism and fertility of the worm (37-39). In mammals, Akt can directly phosphorylate three conserved residues of serine/threonine in Foxo1, Foxo3a and Foxo4, which leads to their nuclear export and subsequent inhibition of their transcriptional activities (35, 36, 40-43). It has been reported that each member of the Foxo subclass of the forkhead box transcription factors has a unique pattern of expression in tissues and exhibits a distinct response under a variety of conditions (44), suggesting that their *in vivo* physiological roles may be different. Several *in vitro* over-expression studies have suggested that FOXO genes have important roles in various biological processes such as control of cell cycle, apoptosis, and stress response. Some shared downstream transcriptional targets have been identified (45-46).

4. KIT-PI3 KINASE SIGNALING IN MAMMALIAN OOCYTES

4.1. Kit and SCF in the mammalian ovary

In gametogenesis, Kit is expressed in primordial germ cells, spermatogonia, and primordial and growing oocytes, implying that it may have a role at three distinct stages of gametogenesis (23, 25). Kit is well known to be present in oocytes at all stages of follicular development in postnatal ovaries of mouse, rat and humans (47-50). In contrast to Kit, the Kit ligand SCF is produced by the surrounding granulosa cells (51-54). The interaction and communication between oocytes and granulosa cells through the function of Kit and SCF in follicular development have been under investigation for decades (for reviews and papers, see 3, 5, 9-11, 16, 23). Several lines of evidence have suggested that Kit may play an essential role in oocyte growth and follicular development (55-57). For example, *in vitro* or *in vivo* administration of Kit blocking antibodies ACK2 in mice disturbed postnatal follicular growth, indicating that ovarian follicle growth is dependent on Kit at a time when functional FSH receptors are not yet expressed in mouse ovary (58, 59).

4.2. Activation of the oocyte PI3 kinase pathway in mouse and rat oocytes by SCF

The indispensable roles of SCF-Kit in oocyte growth and follicular development processes have been well-established using both *in vitro* and *in vivo* approaches, including the usage of naturally occurred or induced mutant mice (see Section 5) (3, 9, 10, 23, 25). However, the downstream signaling pathways of Kit in mammalian oocytes remain largely unknown. Recently, by using isolated oocytes from postnatal mice and rats, it has been demonstrated in our laboratory that components of the PI3 kinase pathway, including Akt and Foxo3a, are expressed in growing mouse oocytes (Figure 2). By using an *in vitro* oocyte culture system, we found that oocyte-derived Akt and Foxo3a are regulated by SCF (60). Treatment of cultured oocytes with SCF not only leads to rapid phosphorylation and activation of Akt, but also leads to phosphorylation and functional *suppression* of Foxo3a at the same time (Figure 3). This occurs through the action of PI3 kinase, as pre-treatment of the cultured oocytes with a specific PI3 kinase inhibitor (LY 294002) can abolish the phosphorylation of Akt and Foxo3a completely (Figure 3). Thus, we assume that in mammalian oocytes, granulosa cell derived SCF can lead to activation of Akt and inhibition of Foxo3a activities in oocytes. We therefore suggest that the PI3 kinase pathway in oocytes is regulated by the surrounding granulosa cells (60).

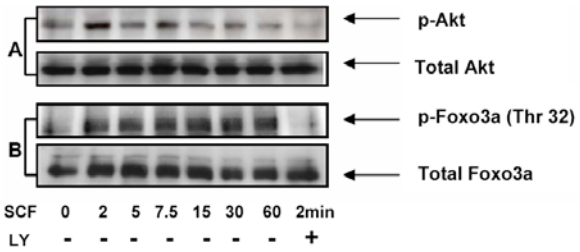

Figure 3. SCF regulated phosphorylation of Akt and Foxo3a in growing mouse oocytes. Oocytes were isolated from 8-day-old mice, starved, and stimulated with 150 ng / ml SCF for 2, 5, 7.5, 15, 30 and 60 min, or pre-treated with the specific PI3 kinase inhibitor LY 294002 (LY) for 1 hour before treatment with SCF for 2 min. A. Levels of phosphorylated Akt (Ser 473) and total Akt; B. Levels of phosphorylated Foxo3a (Thr 32) and total Foxo3a. Modified from Reddy *et al.*, 2005 (60).

Akt is a signaling molecule that enhances cell proliferation, survival, and also glycogen and protein synthesis (24). Thus, activation of Akt in mammalian oocytes may directly enhance the growth, and probably also the secretion ability, of the oocytes. Foxo3a is a substrate of Akt, and it is known as a transcription factor that leads to apoptosis and cell cycle arrest. Upon phosphorylation, Foxo3a is excluded from the nuclei and is suppressed from functioning as a transcription factor for apoptosis and cell cycle arrest (36). As the activation of individual follicles involves unknown triggering mechanisms intrinsic to the ovary before the action of FSH comes into play (1, 7), our data suggest that the action of SCF on primordial to primary follicle transition and subsequent follicle development may involve activation of Akt and inhibition of Foxo3a activities in oocytes. We hypothesize that the role of Akt in oocytes may be to enhance follicular development and that the role of Foxo3a in oocytes may be to inhibit follicular development. Our finding that SCF is capable of regulating the "positive molecule" Akt and the "negative molecule" Foxo3a at the same time in oocytes suggests that the *SCF-Kit-PI3 kinase-Akt-Foxo3a* cascade integrated by oocyte-granulosa cell communication may be an important intrinsic ovarian mechanism for follicular activation. As this activation cascade was present constantly in growing oocytes of primordial and primary follicles, as well as in partially grown oocytes (60), we suggest that the Kit downstream PI3 kinase pathway in oocytes is of great importance for oocyte growth and secretion of factors that influence granulosa cell proliferation and differentiation. It is our hypothesis that the activation of the oocyte PI3 kinase pathway is of importance for the bi-directional dialogue between oocytes and granulosa cells, which in turn is crucial for follicular activation and development.

A notable event that influences primordial follicle endowment is the extensive oocyte apoptosis occurring during the late fetal and early neonatal stages, and after which, mammalian ovaries are endowed with a fixed number of non-growing follicles that will be gradually recruited into a growing pool during reproductive life (61, 62), indicating a slowing-down of the apoptotic process of oocytes. Updated understanding of the function of SCF has implied that SCF may play an essential role to prevent oocytes from apoptosis, and that this action is achieved through the activation of Akt (63). In the report of Jin *et al.*, SCF treatment of cultured newborn rat ovaries could up-regulate the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL, and could also decrease the expression of the pro-apoptotic factor Bax, thus protecting the rat oocytes from apoptosis (63). Moreover, the PI3 kinase inhibitor LY 294002 reversed the regulatory effect of SCF on Bcl-xL and Bax. Thus, it seems that SCF initiated an anti-apoptotic signal through its membrane receptor Kit and the PI3 kinase pathway, and relayed the survival/growth signals to Bcl-2 family members (63).

5. MUTANT MOUSE MODELS FOR THE STUDY OF KIT-PI3 KINASE PATHWAY IN OOCYTES

5.1. Spontaneously occurred Kit or SCF mutant mice

Almost all spontaneously occurred mutations in mice involving Kit and SCF at the W and the Sl loci cause deficiencies in gametogenesis, melanogenesis and hematopoiesis, with different degrees of severity (23). Most of these mouse strains are now available to the research community from the Jackson Laboratory. The shown absence of primordial germ cells indicates the importance of the SCF-Kit system in primordial germ cell survival. For example, Steel Panda (Sl^{pan}) is an SCF expression mutation in which transcription levels of SCF RNA are reduced in most tissues (23). In female Sl^{pan} / Sl^{pan} mice, ovarian follicle development is arrested at the one-layered cuboidal stage as a result of reduced SCF expression in follicle cells, indicating that Kit has a role in oocyte and follicular growth (23). More recently, the degeneration and absence of primordial germ cells in the Kit-deficient W^v / W^v mutant mice have been shown to be mediated by the death receptor Fas (64). Nevertheless, the fact that most of these Kit and SCF mutant mouse strains showed an absence of germ cells limited their usage in studies of oocyte growth, as well as in research on follicular recruitment and early development.

Published reports supporting the role of a Kit-activated PI3 kinase in oocyte growth and follicular development come from sporadic studies using gene-modified mice developed for other purposes, such as signal transduction, cancer or immunology studies. Although investigations on the roles of Kit-PI3 kinase in mouse oocytes were mostly not primarily planned by research groups who generated the gene-modified mice, when phenotypes from several gene-deficient mice models are reviewed jointly, they do reveal and support a role for the PI3 kinase pathway in mammalian oocyte growth and follicular development. The related gene-modified mouse strains are reviewed in the following section.

5.2. Kit ^{Y719F} mutant mice

Using knock-in approaches, studies from two independent research groups reported the targeted mutation of a tyrosine to alanine in the mouse Kit molecule at amino acid 719, (Kit^{Y719F}) (25, 26). Tyrosine 719 in the mouse Kit is a key site which can bind the p85 subunit of the PI3

kinase when phosphorylated, thereby recruiting the PI3 kinase to the cell membrane area and activating the PI3 kinase pathway (Figure 1) (24).

In reports from both Kissel *et al.* (25) and Blume-Jensen *et al.* (26), Kit^{Y719F}/Kit^{Y719F} males were found to be sterile due to a block at the premeiotic stages of spermatogenesis. As to the females, in the report by Kissel et al., the postnatal follicular development at days 7, 10 and 17 appears to be delayed and greater numbers of small follicles in the cortex of the ovary were observed than in the wild-type controls. Most of these follicles are types 2, 3a and 3b with a single layer of follicle cells, and only very few follicles containing two or more layers of follicle cells (types 4, 5a and 5b) are present in the ovarian medulla of the mutants. The authors also reported that as a result of limited recruitment of follicles for growth, the number of pre-antral and antral follicles and the size of the whole ovary are greatly reduced (25). According to the authors, the impaired follicular development is the reason for reduced fertility. However, compared to the complete arrest in spermatogonial development in mutant male mice, ovarian follicle development is not completely blocked – as superovulation with adult females could produce low numbers of ova, suggesting the notion that later stages of oogenesis are not affected by the mutation (25). The Kit^{Y719F}/Kit^{Y719F} female mice were also found to develop ovarian cysts and ovarian tubular hyperplasia (25).

On the other hand, in the report by Blume-Jensen *et al.* (26), the Kit^{Y719F}/Kit^{Y719F} female mice were reported to be fully fertile, as concluded solely from the normal litter size, and no thorough analysis of the ovaries was performed. This substantial difference in female fertility of Kit^{Y719F}/Kit^{Y719F} female mice from two independent laboratories could be caused by different genomic backgrounds of the mice strains. However, the Kit^{Y719F}/Kit^{Y719F} female mice generated by Blume-Jensen *et al.* (26) were later on found to be less fertile, and their fertility may be quite sensitive to stress (personal communication with Dr. Simon Gibbons), indicating that there may also be defects in the ovaries of these mice which remain to be investigated.

5.3. Foxo3a deficient mice

The Akt deficient mice are fertile (personal communication with Drs. Han Cho and Morris Birnbaum). However, whether or not the Akt deficient females suffer from a possible reduced fertility has not been fully investigated (65). However, mice lacking the Akt substrate Foxo3a have shown interesting phenotypes in the ovaries (44, 66, 67). As compared to phenotypes of mice lacking other members of the FOXO subclass of forkhead transcription factors (Foxo1 and Foxo4) (44), only Foxo3a seems to be the key molecule which may have a role in regulating ovarian function. From 3 independent research groups studying cancers or immunology, Foxo3a deficient mice were reported to exhibit female infertility in adult life, excessive activation from primordial to primary follicles, and enlarged primary oocytes (44, 66, 67). Foxo3a has been reported to be expressed in the corpus luteum of adult rats (68), which cannot be the reason for the phenotypes seen in postnatal mouse ovaries in Foxo3a deficient mice.

Data from our laboratory have indicated that in mouse primordial and primary follicles, Foxo3a is mainly expressed in oocytes. Foxo3a protein was found to be expressed mostly in the nuclei of mouse oocytes from primordial and primary follicles (Figure 2) (60). Based on our current understanding of Foxo3a, the nuclear localization implies an active state of this pro-apoptosis and cell cycle arrest transcription factor. Our results therefore indicate that Foxo3a may be the negative regulator of oocyte growth. Thus, we propose that the excessive activation from primordial to primary follicles observed by Castrillon *et al.* (66) was mostly caused by the absence of Foxo3a in oocytes. Our data thus reinforce the theory that oocytes carry signals that are of prime importance for follicular activation and development (3, 5, 9-12).

5.4. GDF-9 deficient mice

GDF-9 is a member of the transforming growth factor-β (TGF-β) superfamily. Studies on the roles of GDF-9 in the bi-directional communication between oocytes and granulosa cells during follicular development have been one of the most active areas of reproductive research (5, 18). The GDF-9 deficient mice have provided a useful model for demonstrating that female mice lacking GDF-9 exhibit primary infertility. Despite the well-documented phenotype that the GDF-9 deficient mice showed a blocked follicular development at type 3b (13, 69), the oocytes of GDF-9 deficient females also grew consistently more rapidly than wild-type oocytes (69), and they showed an approximately 27% increase in average oocyte diameters (73.5 μm in GDF-9 deficient mice vs. 60 μm in wild type mice) in follicles of about 110 μm (as calculated by the author of this review, based on the report by Carabatsos *et al.*) (69).

Moreover, GDF-9 deficient mice were found to show a 32-fold increase in the expression level of SCF in granulosa cells (14). Regardless of how the deficiency in GDF-9 upregulates SCF expression in granulosa cells, it seems to us that the substantially elevated level of SCF may constantly activate the PI3 kinase pathway in the oocytes, leading to the activation of Akt and suppression of Foxo3a, which may enhance oocyte growth. Taken together with the report from our lab (60), the current available data lead us to suggest that the cascade from granulosa cell SCF to oocyte Akt and Foxo3a may control the growth rate of oocytes.

In spite of the enlarged sizes, oocytes from GDF-9 deficient mice were also found to exhibit perinuclear organelle aggregation, unusual peripheral Golgi complexes, and a failure to form cortical granules as well as modified interconnections between granulosa cells and oocytes by Dr. David Albertini's laboratory (69). They also showed that these modifications included a decrement in the number of actin-based transzonal processes and modifications of microtubule-based projections that over time gave rise to invasion of the perivitelline space with eventual loss of oocyte viability (69).

6. PERSPECTIVES

In summary, the SCF activation of the PI3 kinase pathway in mouse and rat oocytes has now been

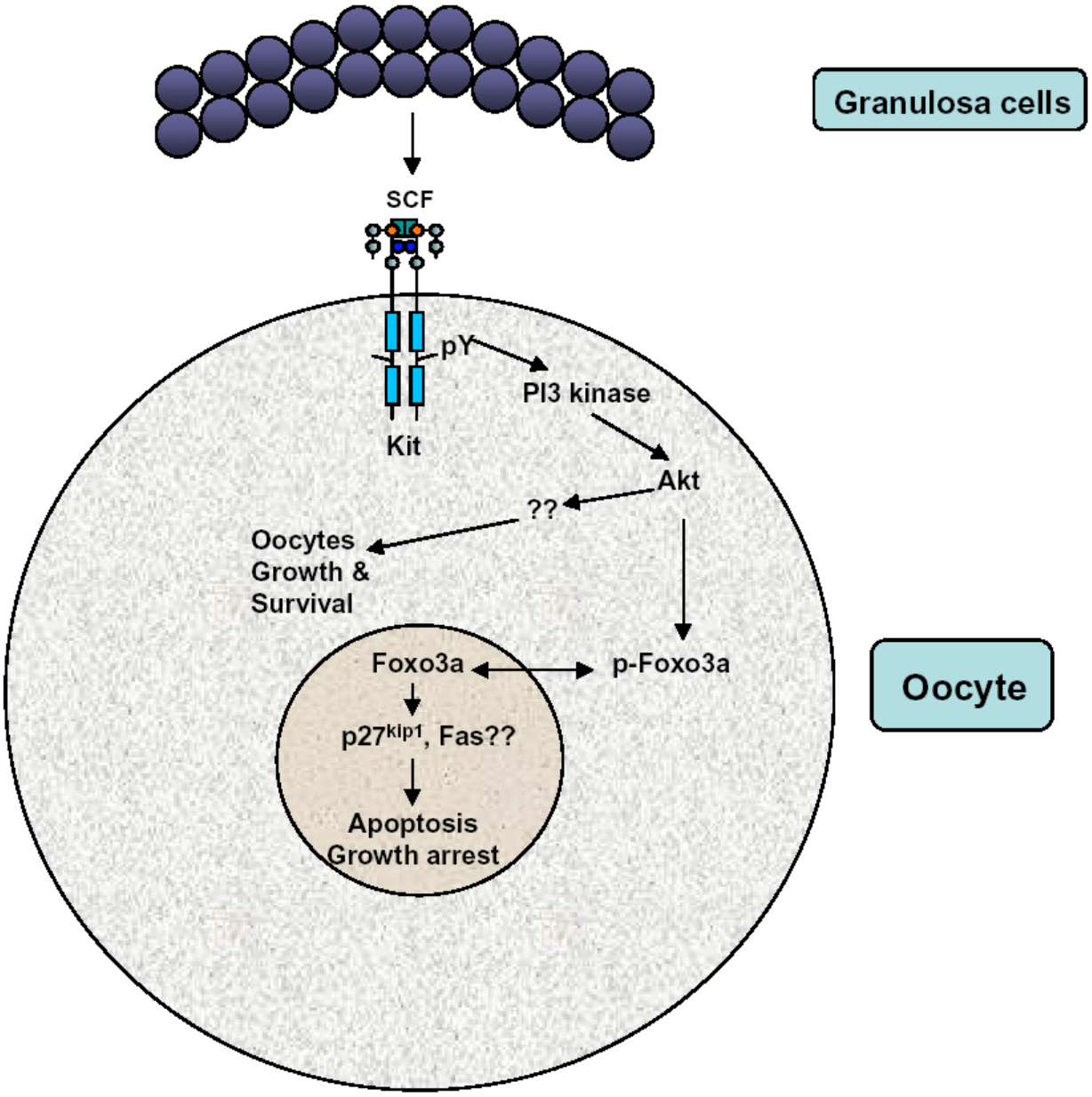

Figure 4. Hypothesized SCF-Kit activated PI3 kinase pathway in regulation of oocyte growth. SCF molecules produced by the surrounding granulosa cells bind to and activate oocyte surface Kit, and the phosphorylated tyrosine (pY) in Kit recruits and activates PI3 kinase, which subsequently activates Akt. Akt will enhance oocyte growth and survival, and at the same while, Akt also phosphorylates Foxo3a, which leads to the nuclear exclusion of phosphorylated Foxo3a, thereby prevents the molecule from functioning as a transcription factor for apoptosis and cell cycle arrest. The non-phosphorylated form of Foxo3a in the nuclei can induce apoptosis and growth arrest via hypothetically the action of $p27^{kip1}$ and Fas, and other molecules.

demonstrated (60). Together with information accumulated from gene deficient mouse models, we hypothesize that this pathway is of importance in controlling oocyte growth during activation and early development of ovarian follicles, which is regulated by factors such as SCF from the granulosa cells, as illustrated in Figure 4. However, to date, downstream effectors of Akt and Foxo3a in mammalian oocytes have not been identified. 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 – using gene deficient or transgenic mice, or

direct introduction of exogenous genes into oocytes – will be of great importance.

7. ACKNOWLEDGEMENT

This work was supported by the Swedish Cancer Foundation (Project Nr. 4988-B04-01XAB), Swedish Medical Research Council (Project Nr. K2005-72X-15379-01A), Swedish Cancer Research Foundation in Norrland (LP 03-1574; LP 04-1593; LP-04-1620), and Medical Faculty of Umeå University, Sweden.

8. REFERENCES

1. Elvin J, A & M. M. Matzuk: Mouse models of ovarian failure. *Rev Reprod* 3, 183-95 (1998)

2. McGee E, A & A. J. Hsueh: Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 21, 200-214 (2000)

3. Vanderhyden B C., Molecular basis of ovarian development and function. Front. Biosci. 7, 2006-2022 (2002)

4. Richards J. S, D. L. Russell, S. Ochsner, M. Hsieh, K. H. Doyle, A. E. Falender, Y. K. Lo & S. C. Sharma: Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. *Recent Prog Horm Res*57, 195-220 (2002)

5. Matzuk M, M, K. H. Burns, M. M. Viveiros & J. J. Eppig: Intercellular communication in the mammalian ovary: oocytes carry the conversation. *Science* 296, 2178-2180 (2002)

6. Ny T, P. Wahlberg, I. Brandstrom: Matrix remodeling in the ovary: regulation and functional role of the plasminogen activator and matrix metalloproteinase systems. Mol. Cell. Endocrinol. 187, 29-38 (2002)

7. Peters H, A. G. Byskov, R. Himelstein-Braw & M. Faber: Follicular growth: the basic event in the mouse and human ovary. *J Reprod Fertil.* 45, 559-66 (1975)

8. P M Wassarman & D. F. Albertini: The mammalian ovum**.** In: The Physiology of Reproduction. Eds: Knobil E, Neill J D, Raven Press, NY (1994)

9. Eppig J. J: Oocyte control of ovarian follicular development and function in mammals. *Reproduction* 122, 829-838 (2001)

10. Nilsson E & M. K. Skinner: Cellular interactions that control primordial follicle development and folliculogenesis. *J Soc Gynecol Investig* 8 , S17-20 (2001)

11. Albertini D. F. & S. L. Barrett: Oocyte-somatic cell communication. *Reprod Suppl* 61, 49-54 (2003)

12. Shimasaki S, R. K. Moore, F. Otsuka & G. F. Erickson: The bone morphogenetic protein system in mammalian reproduction. *Endocr Rev.* 25, 72-101 (2004)

13. Dong J, D.F. Albertini, K. Nishimori, T. R. Kumar, N. Lu & M. M. Matzuk: Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 383, 531-535 (1996)

14. Elvin J, A, C. Yan, P. Wang, K. Nishimori & M. M. Matzuk: Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. *Mol. Endocrinol* 13, 1018-34 (1999)

15. Yan C., P. Wang, J. DeMayo, F J. DeMayo, J A. Elvin, C. Carino, S V. Prasad, S S. Skinner, B S. Dunbar, J L. Dube, A J. Celeste, M M. Matzuk: Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Mol Endocrinol15, 854-66 (2001)

16. Otsuka F & S. Shimasaki: A negative feedback system between oocyte bone morphogenetic protein 15 and granulosa cell kit ligand: its role in regulating granulosa cell mitosis. *Proc Natl Acad Sci* 99, 8060-8065 (2002)

17. Erickson GF & Shimasaki S: The physiology of folliculogenesis: the role of novel growth factors. *Fertil Steril*76, 943-9 (2001)

18. Mazerbourg S & A. J. Hsueh: Growth differentiation factor-9 signaling in the ovary. *Mol Cell Endocrinol.* 2021, 31-6 (2003)

19. Moore R. K, F. Otsuka & S. Shimasaki: Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. *J Biol Chem* 278, 304-10 (2003)

20. Moore R. K, Erickson GF & S. Shimasaki: Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? *Trends Endocrinol Metab* 15, 356-61 (2004)

21. Shimasaki S, RK Moore, GF Erickson & F. Otsuka: The role of bone morphogenetic proteins in ovarian function. *Reprod Suppl*61:323-37 (2003)

22. Thomas F.H, JF Ethier, S. Shimasaki & BC Vanderhyden: Follicle-stimulating hormone regulates oocyte growth by modulation of expression of oocyte and granulosa cell factors. *Endocrinology* 146,941-9 (2005)

23. Besmer P, K. Manova, R. Duttlinger, E. J. Huang, A. Packer, C. Gyssler & R. F. Bachvarova: The kit-ligand (steel factor) and its receptor c-kit/W: pleiotropic roles in gametogenesis and melanogenesis. *Development Suppl* 125-137 (1993)

24. Blume-Jensen P & T. Hunter: Oncogenic kinase signalling. *Nature* 411, 355-65 (2001)

25. Kissel H, I. Timokhina, M. P. Hardy, G. Rothschild, Y. Tajima, V. Soares, M. Angeles, S. R. Whitlow, K. Manova & P. Besmer: Point mutation in kit receptor tyrosine kinase reveals essential roles for kit signaling in spermatogenesis and oogenesis without affecting other kit responses. *EMBO J* 19, 1312-1326 (2000)

26. Blume-Jensen P, G. Jiang, R. Hyman, K. F. Lee, S. O'Gorman & T. Hunter: Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3'-kinase is essential for male fertility. *Nat Genet* 24, 157-62 (2000)

27. Shaw T.J, E J. Keszthelyi, A M. Tonary, M. Cada , B C. Vanderhyden: Cyclic AMP in ovarian cancer cells both inhibits proliferation and increases c-KIT expression. *Exp Cell Res* 273, 95-106 (2002)

28. Cantley L. C, The phosphoinositide 3-kinase pathway. *Science* 296, 1655-7 (2002)

29. Pawson T & P. Nash: Protein-protein interactions define specificity in signal transduction. *Genes Dev* 14, 1027–1047 (2000)

30. Bellacosa A, T. F. Franke, M. E. Gonzalez-Portal, K. Datta, T. Taguchi, J. Gardner, J. Q. Cheng, J. R. Testa & P. N. Tsichlis: Structure, expression and chromosomal mapping of c-akt: relationship to v-akt and its implications. *Oncogene* 8, 745–754 (1993)

31. Burgering B. M & P. J. Coffer: Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 376, 599-602 (1995)

32. Toker A & A. C. Newton: Cellular signaling: pivoting around PDK-1. *Cell* 103, 185–188 (2000)

33. Meier R & B. A. Hemmings: Regulation of protein kinase *B J Recept Signal Transduct Res* 19, 121–128 (1999)

34. Viglietto G, ML Motti, P. Bruni, RM Melillo, A. D'Alessio, D. Califano, F. Vinci, G. Chiappetta, P. Tsichlis, A. Bellacosa, A. Fusco & M. Santoro. Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27(Kip1) by PKB/Akt-mediated phosphorylation in breast cancer. *Nat Med* 8, 1136-44 (2002)

35. Arden K. C. & W. H. Biggs, III: Regulation of the FoxO family of transcription factors by phosphatidylinositol-3 kinase-activated signaling. *Arch Biochem Biophys* 403, 292-298 (2002)

36. Accili D & K. C. Arden: FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* 117, 421-426 (2004)

37. Lin K, J. B. Dorman, A. Rodan & C. Kenyon: daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. *Science* 278, 1319-1322 (1997)

38. Ogg S, S. Paradis, S. Gottlieb, G. I. Patterson, L. Lee, H. A. Tissenbaum & G. Ruvkun: The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature* 389, 994-999 (1997)

39. Tissenbaum H. A & G. Ruvkun: An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans. *Genetics* 148, 703-717 (1998)

40. Biggs W.H., III, J. Meisenhelder, T. Hunter, W. K. Cavenee & K. C Arden: Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci USA* 96, 7421-7426 (1999)

41. Brunet A, A. Bonni, M. J. Zigmond, M. Z. Lin, P. Juo, L. S. Hu, M. J. Anderson, K. C. Arden, J. Blenis & M. E. Greenberg: Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96, 857-868 (1999)

42. Kops G. J, N. D. de Ruiter, A. M. De Vries-Smits, D. R. Powell, J. L Bos & B. M. Burgering: Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 398, 630-634 (1999)

43. Nakae J, B. C. Park & D. Accili: Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. *J Biol Chem* 274, 15982-15985 (1999)

44. Hosaka T, W.H. Biggs 3rd, D. Tieu, A. D. Boyer, N. M. Varki, W. K. Cavenee & K. C. Arden: Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. *Proc Natl Acad Sci*101, 2975-2980 (2004)

45. Burgering B. M & R. H. Medema: Decisions on life and death: FOXO Forkhead transcription factors are in command when PKB/Akt is off duty. *J. Leukocyte Biol* 73, 689-701 (2003)

46. Tran H, A. Brunet, E. C. Griffith & M. E. Greenberg: 2003. The many forks in FOXO's road. Sci STKE RE5 (2003)

47. Orr-Urtreger A, A. Avivi, Y. Zimmer, D. Givol, Y. Yarden & P. Lonai: Developmental expression of c-kit, a proto-oncogene encoded by the W locus. *Development* 109, 911-923 (1990)

48. Manova K, K. Nocka, P. Besmer & R. F. Bachvarova: Gonadal expression of c-kit encoded at the W locus of the mouse. *Development* 110, 1057-1069 (1990)

49. Horie K, K. Takakura, S. Taii, K. Narimoto, Y. Noda, S. Nishikawa, H. Nakayama, J. Fujita & T. Mori: The expression of c-kit protein during oogenesis and early embryonic development. *Biol Reprod* 45, 547-552 (1991)

50. Horie K, J. Fujita, K. Takakura, H. Kanzaki, H. Suginami, M. Iwai, H. Nakayama & T. Mori: The expression of c-kit protein in human adult and fetal tissues. *Human Reprod* 8, 1955-1962 (1993)

51. Motro B & A. Bernstein: Dynamic changes in ovarian c-kit and Steel expression during the estrous reproductive cycle. *Dev Dynamics* 197, 69-79 (1993)

52. Manova K, E. J. Huang, M. Angeles, V. De Leon, S. Sanchez, S. M. Pronovost, P. Besmer & R. F. Bachvarova: The expression pattern of the c-kit ligand in gonads of mice supports a role for the c-kit receptor in oocyte growth and in proliferation of spermatogonia. *Dev Biol* 157, 85-99 (1993)

53. Laitinen M, E. M. Rutanen & O. Ritvos: Expression of c-kit ligand messenger ribonucleic acids in human ovaries and regulation of their steady state levels by gonadotropins in cultured granulosa-luteal cells. *Endocrinology* 136, 4407-4414 (1995)

54. Ismail R, J. N. Okawara, J. N. Fryer & B. C. Vanderhyden: Hormonal regulation of the ligand for c-kit in the ovary and its effects on spontaneous oocyte meiotic maturation. *Mol Reprod Dev* 43, 458-469 (1996)

55. Kuroda H, N. Terada, H. Nakayama, K. Matsumoto, Y. Kitamura: Infertility due to growth arrest of ovarian follicles in Sl/Slt mice. *Dev Biol* 126, 71-79 (1988)

56. Huang E. J., K. Manova, A. Packer, S. Sanchez, R F. Bachvarova, P. Besmer: The murine steel panda mutation affects kit ligand expression and growth of early ovarian follicles. *Dev Biol* 157, 100-109 (1993)

57. Packer A. I, Y. C. Hsu, P. Besmer & R. F. Bachvarova: The ligand of the c-kit receptor promotes oocyte growth. *Dev Biol* 161, 194-205 (1994)

58. Yoshida H, N. Takakura, H. Kataoka, T. Kunisada, H. Okamura & S. I. Nishikawa: Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. *Dev Biol*184, 122-137 (1997)

59. Parrott J. A & M. K. Skinner: Kit-ligand/stem cell factor induces primordial follicle development and initiates folliculogenesis. *Endocrinology* 140, 4262-4271 (1999)

60. Reddy P, L. J. Shen, C. Ren, K. Boman, E. Lundin, U. Ottander, P. Lindgren, Y. X. Liu, Q. Y. Sun & K. Liu: Activation of Akt (PKB) and Suppression of FKHRL1 in Mouse and Rat Oocytes by Stem Cell Factor during Follicular Activation and Development**.** *Dev Biol* 281, 160-170 (2005)

61. Tilly J. L, H. Bilig, I. Furuta & A. J. W. Hsueh: Biochemical and *in situ* analysis of apoptosis during neonatal and prepubertal ovarian development in the rat. In: Program of the 75th Annal Meeting of the Endocrine Society Las Vegas, NV. Abstract B248 (1993)

62. Chun S.Y. & A. J. W. Hsueh AJW: Paracrine mechanisms of ovarian follicle apoptosis. *J Reprod Immunol* 39, 63-75 (1998)

63. Jin X, C. S. Han, F. Q. Yu, P. Wei, Z. Y. Hu & Y. X. Liu: Anti-apoptotic action of stem cell factor on oocytes in primordial follicles and its signal transduction. *Mol Reprod Dev* 70, 82-90 (2005)

64. Sakata S, K. Sakamaki, K. Watanabe, N. Nakamura, S. Toyokuni, Y. Nishimune, C. Mori & S. Yonehara: Involvement of death receptor Fas in germ cell degeneration in gonads of Kit-deficient Wv/Wv mutant mice. *Cell Death Differ* 10, 676-86 (2003)

65. Cho H, J L. Thorvaldsen JL, Q. Chu , F. Feng & M J. Birnbaum: Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J Biol Chem* 276, 38349-52 (2001)

66. Castrillon D. H, L. Miao, R. Kollipara, J. W. Horner & R. A. DePinho: Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 301, 215-218 (2003)

67. Lin L., J D. Hron, & S L. Peng: Regulation of NF-kappaB, Th activation, and autoinflammation by the forkhead transcription factor Foxo3a. *Immunity* 21, 203-213 (2004)

68. Richards J. S., S C. Sharma, A E. Falender, Y H. Lo: Expression of FKHR, FKHRL1, and AFX genes in the rodent ovary: evidence for regulation by IGF-I, estrogen, and the gonadotropins. Mol Endocrinol 16, 580-99 (2002)

69. Carabatsos M. J, J Elvin, M M Matzuk, D F Albertini: Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. *Dev Biol* 204, 373-84 (1998)

Key Words: Kit, SCF, PI3 Kinase, Mammalian Oocytes.

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