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Review

Driving folliculogenesis by the oocyte-somatic cell dialog: Lessons from genetic models

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A B S T R A C T

This review focuses on the role of the dialog between the oocyte and its companion somatic cells in driving folliculogenesis from the primordial to the preovulatory follicle stage. Mouse and sheep genetic models have brought complementary evidence of these cell interactions and their consequences for ovarian function. In mouse, the deletion of genes encoding connexins has shown that functional gap junction channels between oocytes and granulosa cells and between granulosa cells themselves maintain the follicle in a functionally integrated state. Targeted deletions in oocytes or granulosa cells have revealed the cell- and stage-specific role of ubiquist factors belonging to the phosphatidylinositol 3 kinase signaling pathway in primordial follicle activation, oocyte growth and follicle survival. Various models of transgenic mice and sheep carrying natural loss-of-function mutations associated with sterility have established that the oocyte-derived factors, bone morphogenetic protein (BMP) 15 and growth differentiation factor 9 orchestrate follicle development, support cumulus metabolism and maturation and participate in oocyte meiosis arrest. Unexpectedly in sheep, mutations resulting in the attenuation of BMP signaling lead to enhanced ovulation rate, likely resulting from a lowered follicular atresia rate and the enhancement of FSH-regulated follicular maturation. Both the activation level of BMP signaling and an adequate equilibrium between BMP15 and growth differentiation factor 9 determine follicle survival, maturation, and development toward ovulation. The physiological approaches which were implemented on genetic animal models during the last 20 years have opened up new perspectives for female fertility by identifying the main signaling pathways of the oocyte-somatic cell dialog.

1. Introduction

Follicular development, namely folliculogenesis, is a multistage process involving growth and functional maturation [1]. In mammals, it begins with the recruitment of primordial follicles from a resting pool formed early in life and ends with either ovulation or follicular death by atresia. Each primordial follicle is composed of a germ cell named an oocyte, in which meiosis is arrested at the diplotene stage, surrounded by a single layer of flattened resting somatic cells named granulosa cells. The initiation of growth is characterized by a change in the shape of granulosa cells from flattened to cuboidal, an increase in the number of granulosa cells which begin to proliferate, and enlargement of the oocyte. Thereafter, multiple layers of proliferating granulosa cells develop around the oocyte while the oocyte further enlarges. At this stage, a vascularized theca differentiates and surrounds the granulosa tissue, whereas small cavities filled with follicular fluid form then merge in the

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granulosa, giving rise to a central cavity called the antrum. Antrum formation divides the population of granulosa cells into two main groups: cumulus cells associated with the oocyte and mural granulosa cells lining the follicular wall. During terminal follicular development, the oocyte acquires competence to resume meiosis and to be fertilized, and the mural granulosa cells undergo final maturation and become highly estrogenic and LH responsive.

The existence of a privileged dialog between the oocyte and its surrounding follicular cells coordinating the different phases of follicular development has been hypothesized by pioneer studies showing that removal of the oocyte from an antral follicle promotes both oocyte meiotic resumption [2] and spontaneous granulosa cell luteinization [3]. From our current knowledge, this dialog operates through two types of mechanisms (Fig. 1). First, the presence of gap junctions permits molecular exchanges by the transit of small molecules (ions, cAMP, cGMP, amino acids, and metabolites) between the oocyte and granulosa/cumulus cells [4]. Second, cytokines and growth factors produced by one cell type can bind specific receptors present on the other cell type and activate signaling pathways. Particularly, oocyte growth rate is known to be enhanced by KITLG (KIT ligand), expressed as both diffusible (KITLG1) and membrane-bound (KITLG2) isoforms by the granulosa/cumulus cells, and acting on oocyte KIT receptors; conversely granulosa/cumulus cell proliferation and differentiation are regulated by GDF9 (growth differentiation factor 9) and BMP15 (bone morphogenetic protein 15), both secreted by the oocyte and acting on BMP receptors on granulosa cells [5,6]. It can be noted that the mural

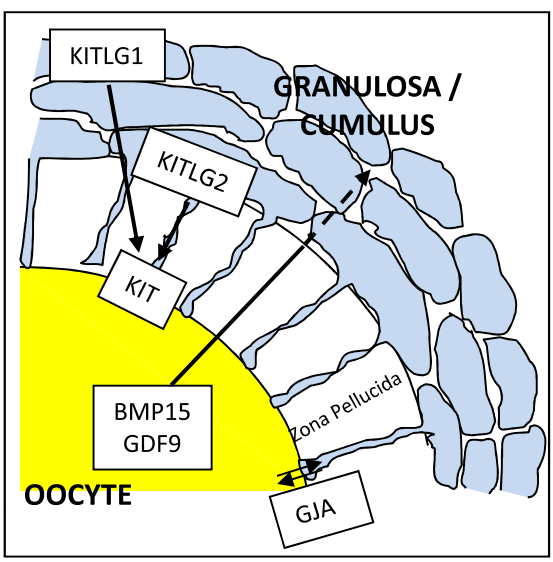

Fig. 1. Cell interactions supporting the dialog between the oocyte and its surrounding granulosa or cumulus cells in follicles. Intercellular channels consisting of gap junctions (GJA) located at the tips of granulosa/cumulus cell transzonal cytoplasmic projections permit molecular exchanges with the oocyte. In another type of interaction mechanism, granulosa/cumulus cells produce cytokines such as KIT ligand (KITLG) able to act on the oocyte, and conversely, the oocyte secretes specific growth factors such as bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9), able to act on its companion granulosa/cumulus cells. KITLG1, diffusible isoform of KITLG; KITLG2, membrane-bound isoform of KITLG.

follicular cells produce many other growth factors, for instance inhibin and activin, anti-Müllerian hormone (AMH), epidermal growth factor (EGF), insulin-like growth factors, and fibroblast growth factors, all able to modulate the dialog between the oocyte and its surrounding granulosa/cumulus cells [7–9].

This review focuses on the importance of the dialog between oocyte and somatic cells in driving folliculogenesis from the primordial to the preovulatory follicle stage, as reported using different murine and ovine genetic models. This perspective has been chosen because studying the *in vivo* effects of mutations is likely the best way to establish the role of a specific protein in a physiological context and highlight the redundancy or compensation effects between regulatory factors. Moreover, historically the main discoveries of the role of specific factors involved in the oocyte-somatic cell dialog have been on the basis of the experimental invalidation or overexpression of specific genes by transgenesis or the identification of natural mutations associated with an alteration of ovarian function.

2. Activation of primordial follicles

Phenotypes of mice carrying *Kit* and *Kitlg* mutations have long ago suggested roles for KIT signaling in melanogenesis, hematopoiesis, and gametogenesis. However, as most mutations in KIT signaling affect the survival, migration and proliferation of the primordial germ cells, compromising the reserve of primordial follicles [10], the specific role of the KITLG/KIT system in follicular growth activation could not be evidenced from the study of mutant mice.

Initial finding of the mechanisms controlling the activation of primordial follicles was provided by the knock out of the gene encoding the transcriptional factor FOXO3A (forkhead box O3), a downstream effector of KIT and other receptors containing a tyrosine kinase domain; indeed, in the ovaries of *Foxo3a*−/− mice the primordial follicle reserve depletes early, due to widespread follicular activation [11]. Later, targeted invalidations (driven by the promoters of *Vasa* or *Gdf9* oocyte-specific genes) of *Foxo3a* and *Pten* (phosphatase and tensin homolog) in mouse oocytes have found that FOXO3A is a phosphatidylinositol 3 kinase (PI3K)–dependent molecular switch for controlling the initiation of oocyte growth [12,13]. Further targeted invalidations of other effectors of the PI3K pathway (Fig. 2A) have shown that CDKN1B (cyclin-dependent kinase inhibitor 1B) can suppress follicle activation [14], whereas PDPK1 (3-phosphoinositide-dependent protein kinase 1) and RPS6 (ribosomal protein 6) maintain follicle survival [15]. Finally, it was hypothesized that the dormancy and activation of primordial follicles is regulated by mTORC1 (mammalian target of rapamycin complex 1), a protein complex with kinase activity, known to activate RPS6 (promoting protein translation) and regulate cell growth, proliferation, and metabolism [16]; indeed, in mutant mice with elevated mTORC1 activity in oocytes, the entire pool of primordial follicles is activated prematurely [17,18].

The respective role of the oocyte and its surrounding granulosa cells in triggering primordial follicle activation was not understood until recently. In mice lacking the gene encoding the transcriptional factor FOXL2 (forkhead box