REVIEW ARTICLE

Role of oocyte-derived paracrine factors in follicular development

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ABSTRACT

Mammalian oocytes secrete transforming growth factor β (TGF-β) superfamily proteins, such as growth differentiation factor 9 (GDF9), bone morphogenetic protein 6 (BMP6) and BMP15, and fibroblast growth factors (FGFs). These oocyte-derived paracrine factors (ODPFs) play essential roles in regulating the differentiation and function of somatic granulosa cells as well as the development of ovarian follicles. In addition to the importance of individual ODPFs, emerging evidence suggests that the interaction of ODPF signals with other intra-follicular signals, such as estrogen, is critical for folliculogenesis. In this review, we will discuss the current understanding of the role of ODPFs in follicular development with an emphasis on their interaction with estrogen signaling in regulation of the differentiation and function of granulosa cells.

Key words: *cumulus cell, estrogen, follicle, granulosa cell, oocyte.*

INTRODUCTION

Although many intra- and extra-ovarian factors, including follicle stimulating hormone (FSH), luteinizing hormone (LH) and estrogen, play important roles in the development of follicles, paracrine signals derived from oocytes seem to be one of the predominant determinants of the developmental state of follicles. This was evidenced, for example, by a study of follicles in which the developmental stages of oocytes and follicular somatic cells were mismatched (Eppig *et al*. 2002). In that study, when growing oocytes from 12-day-old mice were combined with the somatic cells from neonatal ovaries, the developmental stage of the follicles caught up to that of oocytes rather than that of somatic cells. Therefore, oocytes play a critical role in determining the fate of ovarian somatic granulosa cells and ultimately the rate of development of follicles.

The mechanism by which oocytes coordinate the development of follicles has been studied actively for decades, and the emerging evidence suggests that cooperation of the oocyte-derive paracrine signal with other intra-follicular signals, such as estrogen signals, is critical for the development and function of follicles. This mini-review will focus on the current state of our understanding of the regulation of follicular development by oocyte-derived paracrine factors (ODPFs) with an emphasis on their interaction with other intra-follicular signals.

OVERVIEW OF FOLLICULAR DEVELOPMENT

Ovarian follicular development starts from the generation of primordial follicles in which squamous somatic cells, often called pre-granulosa cells, encircle a primary oocyte arrested at the first meiotic prophase (Fig. 1). An oocyte-specific transcription factor, folliculogenesis specific basic helix-loop-helix (FIGLA), is required for the formation of primordial follicles, since the ovaries of *Figla*-deficient mice have no primordial follicles (Soyal *et al*. 2000). Therefore, oocytes are required from the very beginning of the follicular development.

When primordial follicles develop into primary follicles, the oocytes begin to grow and the shape of the granulosa cells becomes cuboidal. Then, as the granulosa cells proliferate, two or more layers of
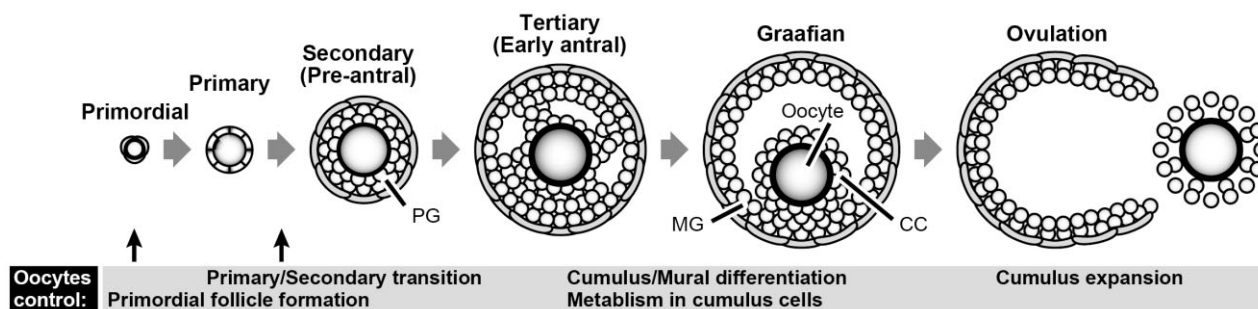
Figure 1 Role of oocytes in each step of follicular development. PG, pre-antral granulosa cells; MG, mural granulosa cells; CC, cumulus cells.

granulosa cells encircle the oocytes and the follicles become covered with theca cells. At this stage, the follicles are called secondary follicles. Female mice deficient in growth differentiation factor 9 (GDF9, see below), one of the ODPFs, are infertile due to a block of folliculogenesis at the primary stage, indicating that oocyte-produced GDF9 is required for the transition of primary to secondary follicles (Dong et al. 1996). Interestingly, the expression levels of transcripts encoding inhibin alpha (Inha) are significantly up-regulated in the Gdf9-deficient ovaries (Elvin et al. 1999), and the block of folliculogenesis at the primary stage was not observed in Gdf9/Inha double knockout mice (Wu et al. 2004). This suggests that aberrant expression of Inha is the main cause of the block of follicular development observed in Gdf9-deficient ovaries.

When a secondary follicle develops and becomes a tertiary follicle, a fluid-filled antrum is formed between the granulosa cell layers. The follicles before and after antrum formation are called pre-antral and antral follicles, respectively. The transition of pre-antral to antral follicles is accompanied by the differentiation of granulosa cells of pre-antral follicles (pre-antral granulosa cells) to cumulus cells, which encircle oocytes and play an essential role in oocyte development, and mural granulosa cells, which line the follicular wall and serve a primary endocrine function (Fig. 1). The opposing gradients of extra-follicular FSH and intra-follicular ODPF signals are critical for determining the fate of the granulosa cell differentiation (Diaz et al. 2007a). Whereas FSH signal promotes pre-antral granulosa cells to differentiate into mural granulosa cells, ODPFs promote cumulus cell differentiation. In the following section, the requirement of ODPFs in determining granulosa cell differentiation as well as follicular development during the transition of pre-antral to antral follicles is reviewed.

OOCYTE-DERIVED PARACRINE FACTORS (ODPFs)

Transforming growth factor β (TGF-β) superfamily proteins are the most characterized ODPFs. Mamma-lian oocytes secrete several ligands of the TGF-β super-family, including GDF9 and bone morphogenetic proteins (BMPs) such as BMP15 and BMP6. The expression of proteins or transcripts encoding these ligands is detected in oocytes of many mammalian species, including mice (Lyons et al. 1989; McGrath et al. 1995; Dong et al. 1996; Dube et al. 1998; Elvin et al. 2000), rats (Hayashi et al. 1999; Jaatinen et al. 1999; Erickson & Shimasaki 2003), cattle (Bodensteiner et al. 1999), sheep (Bodensteiner et al. 1999; Galloway et al. 2000), goats (Silva et al. 2005), pigs (Prochazka et al. 2004; Brankin et al. 2005), rhesus monkeys (Duffy 2003) and humans (Sidis et al. 1998; Aaltonen et al. 1999). In some species, including primates, goats and pigs, the expression of these ligands is also detected in granulosa cells (Sidis et al. 1998; Duffy 2003; Prochazka et al. 2004; Brankin et al. 2005; Silva et al. 2005).

The critical roles of these TGF-β superfamily members in normal follicular development and female fertility have mainly been revealed through the investigation of animals that are deficient in these proteins. For example, ewes which have a homozygous mutation in the BMP15 gene are infertile due to the abnormal development of follicles after the primary stage (Galloway et al. 2000). Similar infertile phenotypes have been reported in ewes with many other natural mutations of GDF9 or BMP15 genes (Hanrahan et al. 2004; Bodin et al. 2007; Martinez-Royo et al. 2008; Monteagudo et al. 2009). Injecting a GDF9 gene fragment into the ovaries of prepubertal gilts results in an increase in the numbers of primary follicles, whereas it induces a decrease in the number of primordial follicles (Shimizu et al. 2004). In addition, abnormal follicular development with impaired fertility has been reported in sheep and cattle actively immunized against BMP15 and GDF9 (Juengel et al. 2002, 2009). Therefore, GDF9 and BMP15 play a critical role in regulating follicular development in these mammalian species.

In contrast, female mice with homozygous mutation in Bmp15 and/or Bmp6 do not exhibit an aberrant phenotype in their ovaries (Yan et al. 2001; Sugiura

et al. 2010a). However, female mice deficient in genes encoding BMP signal mediators, SMAD1/5/8, or BMP receptors, BMPR1A and/or BMPR1B, in granulosa cells exhibit in impaired ovarian function and subsequent infertility (Yi *et al.* 2001; Pangas *et al.* 2008; Middlebrook *et al.* 2009; Edson *et al.* 2010), indicating that BMP signals are also required for normal development and function of the ovaries in mice. It seems likely that the requirement of oocyte-derived BMP signals varies among species and, in mice, the BMP signals produced by somatic cells may sufficiently compensate for the loss of oocyte-derived BMP signals in the *Bmp15/6* mutant mice.

Synergistic effects of GDF9 and BMP15 on granulosa cell development and function, as well as on follicular development, were first reported in mice. *Bmp15* null mice exhibit a relatively mild phenotype, whereas additional deletion of one allele of the *Gdf9* gene (i.e. *Bmp15*$^{-/-}$/*Gdf9*$^{+/-}$ mice) results in severe infertility (Yan *et al.* 2001; Su *et al.* 2004). A similar genetic interaction between *BMP15* and *GDF9* genes was also reported in sheep (Hanrahan *et al.* 2004). At the protein level, many studies have shown the existence of this synergism using recombinant proteins (McNatty *et al.* 2005a,b; Mottershead *et al.* 2011). Although the mechanisms underlying the synergistic interaction of BMP15 and GDF9 signaling are not fully resolved, a recent study has suggested involvement of the BMP15/GDF9 heterodimer in this interaction (Peng *et al.* 2013a). This study showed that the BMP15/GDF9 heterodimer is 10- to 3000-fold more biopotent than the homodimers of BMP15 or GDF9.

The other well-known factors derived from oocytes are fibroblast growth factors (FGFs). The production of FGFs by oocytes has long been recognized in mice (Valve *et al.* 1997) and cattle (Buratini *et al.* 2005a, b, 2007). However, the function of FGF8 during follicular development was not understood until more recently, when FGF8 and BMP15 were shown to promote the expression of genes encoding glycolytic enzymes in mouse cumulus cells *in vitro* (Sugiura *et al.* 2005, 2007). In addition, FGF8 promoted the suppressive effect of recombinant BMPs on FSH-induced cyclic adenosine monophosphate (cAMP) production and the BMP-stimulated SMAD1/5/8 phosphorylation in diethylstilbestrol-primed rat preantral granulosa cells (Miyoshi *et al.* 2010). Therefore, a cooperative interaction between FGF and BMP signals may be critical in the regulation of granulosa cell development and function. However, since human recombinant BMP proteins were used in these studies, the question of whether endogenous mouse/rat BMPs undergo the same interaction with FGFs may require further investigation. Importantly, the mouse BMP15 homodimer appears to exhibit less activity than the human BMP15 homodimer (Peng *et al.* 2013a).

CROSSTALK BETWEEN THE ODPF SIGNAL AND THE OTHER INTRAFOLLICULAR SIGNALS

Although paracrine signals derived from oocytes seem to be one of the predominant determinants of granulosa cell differentiation, other follicular signals, such as FSH, LH and steroids, are also important. Obviously these follicular signals affect each other, and the interaction between these signals is critical for the proper regulation of granulosa cell development. Recent studies revealed the importance of the interaction between oocyte-derived paracrine signals and estrogen signals for regulation of the development and function of granulosa cells. The following section summarizes the current state of our understanding of the interaction between signals of ODPFs and estrogen.

Estrogen signals within the follicles are mainly mediated by estrogen receptor 2 (ESR2; also known as estrogen receptor β). *Esr2*-deficient mice are subfertile because of their attenuated follicular development (Krege *et al.* 1998; Cheng *et al.* 2002; Emmen *et al.* 2005) and reduced ovulation rate (Couse *et al.* 2005). Moreover, estrogen promotes proliferation (Rao *et al.* 1978), suppresses apoptosis of granulosa cells (Billig *et al.* 1993) and augments the effects of FSH on granulosa cell differentiation and function (Adashi & Hsueh 1982; Zhuang *et al.* 1982). Therefore, estrogen itself plays important roles in regulating the development and function of granulosa cells as well as the development of follicles.

The cooperative action of ODPFs and estrogen was first reported in a study using rat primary cultured granulosa cells (Otsuka *et al.* 2005). In the presence of oocytes, estrogen promoted the FSH-stimulated expression of several transcripts, including *Cyp19a1*, *Fshr* and *Lhcgr*, and the production of cAMP by rat granulosa cells; however, in the absence of oocytes, estrogen had no effect. Therefore, ODPFs are required for the action of estrogen on FSH signaling in rat granulosa cells.

Cumulus expansion or mucification is an essential process for normal ovulation (Chen *et al.* 1993). Normal expansion requires the expression of several transcripts encoding HAS2, PTGS2, PTX3 and TNFAIP6 (Davis *et al.* 1999; Varani *et al.* 2002; Fulop *et al.* 2003; Ochsner *et al.* 2003; Sugiura *et al.* 2009). Cumulus expansion is induced by an LH surge the signal of which within the follicles is mediated by epidermal growth factor (EGF)-like peptides produced by granulosa cells (Park *et al.* 2004; Shimada *et al.* 2006). Cumulus cells of *Bmp15* null or *Bmp15*$^{-/-}$/*Gdf9*$^{+/-}$ mice are less able to undergo expansion and to express *Has2* and *Ptgs2* transcripts (Yan *et al.* 2001; Su *et al.* 2004) and ODPFs are required for preantral granulosa cells to acquire the ability to undergo expansion *in vitro* (Diaz *et al.* 2007b). Therefore, oocyte-produced GDF9 and

BMP15 are required for cumulus cells to become competent to undergo expansion. In addition to the ODPFs, estrogen appears to be critical for the cumulus cells to become competent to undergo the expansion process, since the cumulus cells of estrogen-signal-deficient mice are not competent for full expansion and expression of the *Ptgs2* transcript (Dupont *et al.* 2000; Couse *et al.* 2005; Emmen *et al.* 2005). Our recent study also showed that the cooperative interaction of estrogen and ODPFs, especially BMP15 and GDF9, is required for maintaining cumulus cell-competence to undergo the expansion process (Sugiura *et al.* 2010b). Therefore, both ODPFs and estrogen are required for the cumulus cells to become competent and to maintain their competence.

Another example of the ODPF/estrogen interaction was recently reported in the context of regulation of the meiotic arrest of oocytes in Graafian follicles. Natriuretic peptide type C (NPPC) (also known as C-type natriuretic peptide, CNP) is expressed by mural granulosa cells, whereas its receptor, natriuretic peptide receptor 2 (NPR2), is mainly expressed by cumulus cells (Zhang *et al.* 2010). The expression of *Npr2* in cumulus cells is cooperatively controlled by signals of ODPFs and estrogen (Zhang *et al.* 2010, 2011; Lee *et al.* 2013). Treating cumulus-oocyte complexes (COCs) with NPPC was shown to prevent the meiotic resumption of mouse oocytes *in vitro*. Moreover, mutant mice for *Nppc* or *Npr2* exhibited precocious resumption of oocyte meiosis in Graafian follicles (Zhang *et al.* 2010; Kiyosu *et al.* 2012; Tsuji *et al.* 2012). The importance of the NPPC/NPR2 system for the meiotic arrest of oocytes has also been demonstrated in other mammalian species, including goats (Peng *et al.* 2013b), pigs (Hiradate *et al.* 2013) and humans (Kawamura *et al.* 2011). Therefore, the NPPC/NPR2 system appears to be a common mechanism for maintenance of oocyte meiotic arrest in mammals.

To understand the underlying mechanism of the ODPF/estrogen signal cooperation in more detail, we recently conducted microarray comparisons in which the effects of ODPFs and estrogen on the cumulus cell transcriptome were examined (Emori *et al.* 2013). For this purpose, we cultured isolated cumulus cell complexes (oocytectomized (OOX) cumulus cells) with or without the presence of ODPFs and/or estrogen. Then, the transcriptomes of the cumulus cells were analyzed with microarray analyses. The biological processes regulated by ODPFs in cumulus cells are largely unaffected by the presence of estrogen, whereas those regulated by estrogen are significantly affected by ODPFs. For example, in the presence of ODPFs, estrogen significantly promoted cumulus cell biological processes related to phosphorylation-mediated signal transduction, including the signaling pathways of EGF, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF). The signaling pathways of EGF (Park *et al.* 2004), VEGF (Shimizu *et al.* 2003) and PDGF (May *et al.* 1992; Duleba *et al.* 1999; Nilsson *et al.* 2006; Sleer & Taylor 2007; Schmahl *et al.* 2008) have been implicated as critical regulators of follicular development. Therefore, the cooperative interaction between ODPFs and estrogen is critical for regulating follicular development.

The underlying mechanism governing the cooperative interaction of ODPFs and estrogen is yet to be determined. Generally, signals of estrogen are affected by multiple co-factors which bind with receptors of estrogen (McKenna *et al.* 1999). We previously reported that the expression of one of the ESR-binding proteins, nuclear receptor interacting protein 1 (*Nrip1*, also known as RIP140), in cumulus cells is regulated by ODPFs (Sugiura *et al.* 2010b). In addition, the expressions of several ESR-binding proteins, including *Foxl2* and *Ncoa3*, in cumulus cells are regulated by ODPFs (Emori *et al.* 2013; unpublished data). Therefore, regulation of the expression of these ESR co-factors by ODPFs may be the critical mechanism in the cooperative interaction of ODPFs and estrogen.

Conclusion

Many extra- and intra-follicular factors, including gonadotropins, steroids and growth factors produced within follicles, have been identified as essential components of a signal network that governs follicular development. The signals of these factors affect each other, and the coordination of these signals is critical for production of functional oocytes. Accumulating evidences suggests that the ODPF signal, interacting with other follicular signals, plays an active role in determining the state of differentiation and function of granulosa cells as well as the development of follicles. Ongoing research into the signal interactions will provide a new perspective on our understanding of follicular development.

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