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Stage-dependent role of growth differentiation factor-9 in ovarian follicle development☆

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Abstract

GDF-9 was shown to be essential for follicle progression and is the only factor secreted by the oocyte shown to increase the number of primordial and primary follicles in vivo. Furthermore, GDF-9 is a major growth factor involved in the oocyte control of granulosa cell differentiation. A concentration gradient of the paracrine factor GDF-9 established by the oocyte could provide the basis to explain the stratification of granulosa cells in antral and preovulatory follicles. The stimulatory effects of GDF-9 on early follicle development provide a basis for the use of GDF-9 in the treatment of infertility. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: Ovary; Follicle; GDF-9; Granulosa; Theca; Oocyte factor

1. Introduction

Growth differentiation factor-9 (GDF-9) belongs to the transforming growth factor-beta (TGF-beta) superfamily and was discovered together with GDF-3 by McPherron and Lee (1993) using polymerase chain reaction employing degenerate oligonucleotide primers corresponding to conserved regions among known TGF-beta family members. While GDF-3 was found to be expressed in multiple tissues, GDF-9 transcripts were detected only in the ovary. Furthermore, GDF-9 was shown to be expressed exclusively in the oocyte (McGrath et al., 1995). In 1996, the essential role of GDF-9 in folliculogenesis was shown by targeted deletion of the GDF-9 gene in mice (Dong et al., 1996). The GDF-9 null mice are incapable of ovulation due to an arrest of follicle development at the primary stage. Thus, GDF-9 was the first oocyte-derived growth factor shown to be required for ovarian somatic cell function. The exclusive localization of GDF-9 mRNA and protein in the oocyte were confirmed in diverse species (Dong et al., 1996; Hayashi et al., 1999; Bodensteiner et al., 1999; Aaltonen et al., 1999). GDF-9 protein was found in oocytes throughout the entire period of follicle development even after ovulation. The exclusive expression of GDF-9 in the oocyte is unique among known growth-factor-like molecules and suggested that the oocyte could, at least partially, control follicle development by secreting this putative paracrine factor. The availability of bioactive recombinant GDF-9 allowed the analysis of GDF-9 effects in vitro. Studies indicated that GDF-9 induces preantral follicle growth (Hayashi et al., 1999) and cell proliferation but inhibits gonadotropin-induced cell differentiation in vitro (Vitt et al., 2000a). These studies together with the phenotype of GDF-9 null mice, demonstrated that GDF-9 affects follicle development in a stage dependent manner.

2. The role of the oocyte in follicle development

2.1. Follicle development

Development of the mammalian ovary is characterized by the initial endowment of a fixed number of

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primordial follicles. The pool of primordial follicles is gradually depleted during reproductive life. The follicles develop through primordial, primary and preantral stages before acquiring an antral cavity (Hirshfield, 1991; Adashi, 1994; McGee and Hsueh, 2000) (see Fig. 1). Some follicles begin to grow as soon as they are formed but most enter a state of suspended animation (Cran and Moor, 1980). The primary follicle has cuboidal-shaped granulosa cells which proliferate during the formation of preantral follicles concomitant with an increase in oocyte size (Anderson, 1974). Once follicles reach the small antral stage, most of them undergo atresia unless rescued by follicle stimulating hormone (FSH) (Hsueh et al., 1994; Chun and Hsueh, 1998). Under the influence of gonadotropins, the antrum is formed and the selected antral follicles further increase in size until they reach the preovulatory stage.

During follicular development, the granulosa cells surrounding the centrally located oocyte display a balance of cell proliferation and differentiation which leads to the formation of two distinct granulosa cell subpopulations. In antral follicles, the mural granulosa cells closest to the basal lamina show a differentiation mainly characterized by their prominent steroidogenic

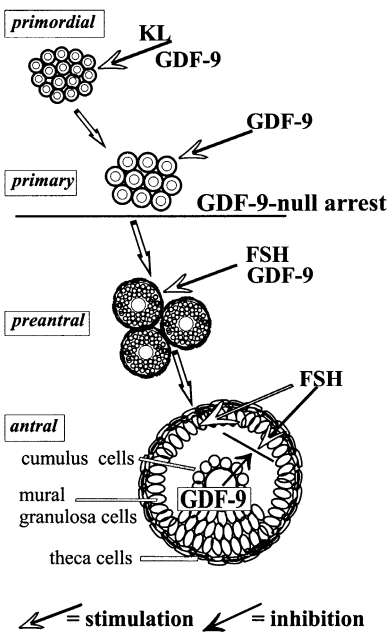

Fig. 1. Schematic presentation of follicle development and the effects of GDF-9. GDF-9 promoted both primordial and primary follicle growth in vivo. The former is also known to be promoted by kit ligand (KL). In GDF-9 null mice, follicle development is arrested at the primary stage as indicated by the horizontal line. During preantral follicular growth, both GDF-9 and FSH promote follicle growth. In antral follicles, FSH induces granulosa cell differentiation which is partly blocked by GDF-9, thus explaining the under-differentiated phenotype of the cumulus cells surrounding the oocyte. White arrows, stimulatory effects; filled arrows, inhibitory effects.

Table 1
Effects of GDF-9 on different functional parameters of granulosa cells and similar effects of oocyte factor(s)

	GDU-9	Oocyte factor
Granulosa cell proliferation	↑	↑
Cumulus:		
Expansion	↑	↑
Hyaluronic acid synthesis	?	↑
HAS 2 mRNA	↑	?
Luteinization	?	↓
FSH-induced differentiation:		
Steroid production	↓	↓
LH/CGR	↓	↓
cAMP	↓	?
CYP17	↑	?
StAR	↑	?
COX2	↑	?

potential and high luteinizing hormone receptor content. In contrast, the granulosa-cumulus cells surrounding the oocyte are characterized by a lower steroid production and LH receptor levels and a higher production of hyaluronic acid (Amsterdam et al., 1975; Lawrence et al., 1980).

In addition to granulosa cell development, folliculogenesis is characterized by recruitment and growth of theca cell layers. Theca cells are derived from the mesenchymal tissue surrounding the follicles (Hirshfield, 1991). Although a clear theca cell layer cannot be distinguished in early follicle stages, the secondary follicle is surrounded by theca cells which proliferate during follicle progression. Theca and granulosa cells communicate via different paracrine factors. In vitro studies suggest that these two somatic cell types modulate each other's proliferation, differentiation and responsiveness to gonadotropins in a reciprocal manner (Kotsuji and Tominaga, 1994). Theca cells are characterized by expression of specific markers such as 17-alpha-hydroxylase (CYP17) and c-kit receptors. Signaling of c-kit receptors is modulated by granulosa cell-derived kit ligand. On the other hand, theca cells produce keratinocyte growth factor and hepatocyte growth factor that influence granulosa cell physiology (Parrott and Skinner, 1998; McGee et al., 1999). Furthermore, following stimulation by LH, theca cells secrete androgens to serve as substrates for the estrogen-producing granulosa cells (Hsueh et al., 1984; Hedin et al., 1987).

2.2. Oocyte control of follicle development

It is well accepted that follicular growth and differentiation are controlled by pituitary gonadotropins (Hsueh et al., 1984; Fagbohun et al., 1990) as well as follicular paracrine factors of granulosa and theca cell origin (Adashi, 1994; Eppig, 1994). In addition, several studies (summarized in Table 1, column 3) indicate that

the oocyte contributes to follicular development as well. In early studies it was shown that the oocyte prevents luteinization of ovarian granulosa cells (Nekola and Nalbandov, 1971). The development of oocytectomy, a procedure to retrieve cumulus cell complexes devoid of the oocyte, and analysis using oocyte-conditioned media, revealed a role of oocyte factors in the control of granulosa cell physiology. While oocytectomy lead to an increase in progesterone production by the remaining cumulus cells, oocyte conditioned media reduced steroidogenesis in cultured cumulus and mural granulosa cells (Coskun et al., 1995; Vanderhyden and Tonary, 1995). Furthermore, mouse oocytes secrete a cumulus expansion-enabling factor (Vanderhyden et al., 1990; Eppig et al., 1993) and enhance hyaluronic acid synthesis by granulosa cells (Salustri et al., 1990). In addition, mouse oocytes were shown to promote proliferation of granulosa cells in vitro (Vanderhyden et al., 1992; Buccione et al., 1990) and to reduce FSH-induced LH receptor mRNA content in cultured granulosa cells (Eppig et al., 1997). Therefore, the oocyte controls granulosa cell growth and influences FSH function on granulosa cells and it is conceivable that the undifferentiated status of cumulus cells surrounding the oocyte is due to paracrine factors produced by the oocyte. The influence of the oocyte on granulosa cells appears to decrease with increasing distance because the cumulus cells closest to the oocyte show a phenotype different from the distally located mural granulosa cells. This indicates that a gradient of the factor produced by the centrally located oocyte might be responsible for the formation of two distinct subpopulations of granulosa cells (Eppig, 1991).

3. GDF-9 control of follicle development

3.1. Primordial and primary follicle growth

Application of GDF-9 in vivo led to an increase in the number of primary and preantral follicles and a decrease in the number of primordial follicles (Vitt et al., 2000b) (Fig. 2). The expression of GDF-9 mRNA and protein was confined to oocytes of primary and larger follicles in rats (Hayashi et al., 1999; Jaatinen et al., 1999), mice (McGrath et al., 1995; Dong et al., 1996) and humans (Aaltonen et al., 1999). Of interest, in ovine and bovine ovaries, GDF-9 mRNA was found in primordial follicles as well (Bodensteiner et al., 1999). The present findings suggest that once GDF-9 is produced by the oocyte of a given primordial follicle, this follicle could start to grow. Because follicles can progress to the primary stage in GDF-9 null mice (Dong et al., 1996), it is possible that GDF-9 is not absolutely required for the transition from primordial to primary follicles. Furthermore, the enhancement of primary follicle progression could lead to an increase in the number of primordial follicles entering the growing pool (Hirshfield, 1994). Therefore, one cannot exclude the possibility that the effect observed here of GDF-9 on primordial follicles is secondary to its stimulation of primary follicle progression to small preantral follicles.

3.2. Preantral follicular growth

In addition to stimulating the progression of the primary to the small preantral follicle, GDF-9 also enhanced the number of growing preantral follicles

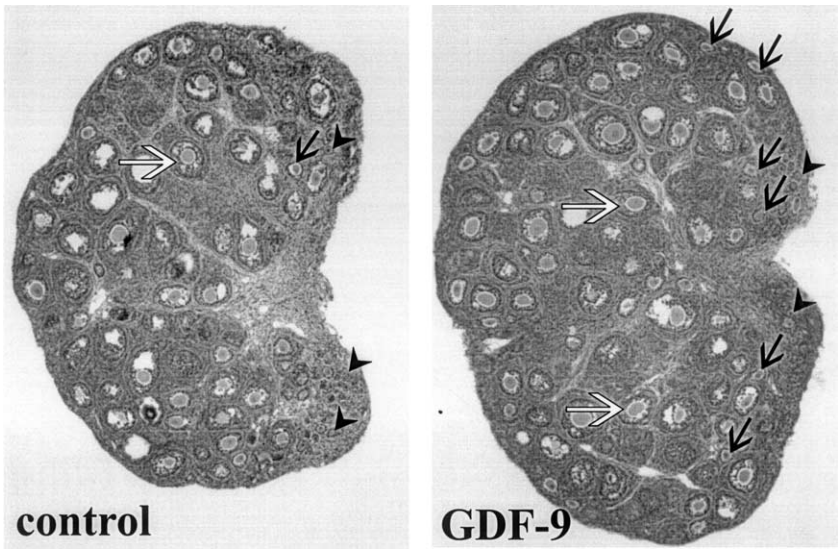

Fig. 2. In vivo application of GDF-9 induced an increased number of primary (black arrows) and preantral (white arrows) follicles as compared with the control group. Furthermore, the number of primordial follicles (arrowheads) was reduced. Note also the differences in size between the treatment groups which are reflected by an increase in ovarian weight induced by GDF-9. Sections are shown at a magnification of 50.

(Vitt et al., 2000b) (Fig. 2). In corroboration of the above, GDF-9 was shown to induce preantral follicle growth in vitro and to have an additive effect on FSH-induced follicular growth (Hayashi et al., 1999). Furthermore, GDF-9 was shown to increase the inhibin-alpha content of ovarian explants cultured in vitro. Although GDF-9 is not essential for the production of inhibin-alpha because follicles of GDF-9-deficient-mice demonstrate an up-regulation of inhibin-alpha (Elvin et al., 1999b), it could be in part responsible for preantral follicular differentiation. Therefore, the oocyte, via the expression of GDF-9, could contribute continuously to the growth and differentiation of follicles from the primary, up to and throughout, the preantral stages.

3.3. Granulosa cell differentiation of early antral and preovulatory follicles

Studies using granulosa cells derived from early antral and preovulatory follicles revealed, that GDF-9 promotes granulosa cell proliferation but inhibits FSH-induced steroidogenesis and LH receptor expression (Vitt et al., 2000a). These functions of GDF-9 correspond to some of the effects described for oocyte-conditioned media. A comparison of the effects of GDF-9 and the oocyte is summarized in Table 1. The inhibition of FSH-induced differentiation by GDF-9 could explain the inhibitory role of the oocyte on FSH-induced steroidogenesis and LH receptor expression (Vanderhyden and Tonary, 1995; Eppig et al., 1997). Furthermore, the induction of granulosa cell proliferation by GDF-9 is consistent with the growth-promoting effect seen with oocyte-conditioned media in vitro (Vanderhyden et al., 1992). In addition, treatment with GDF-9 enhances FSH-induced cumulus expansion (Elvin et al., 1999a), substantiating the presence of a cumulus expansion-enabling factor (Vanderhyden et al., 1990; Eppig et al., 1993; Salustri et al., 1990; Buccione et al., 1990) in oocyte-conditioned media. Thus, the diverse effects of GDF-9 studied so far could explain most of the divergent regulatory effects of the oocyte on granulosa/cumulus cells and could be responsible for the formation of the two distinct subpopulations of granulosa cells.

In addition to the inhibition on FSH-induced steroidogenesis, GDF-9 treatment alone was shown to enhance cyclooxygenase 2 and steroidogenic acute regulator protein mRNA (Elvin et al., 1999a) as well as to enhance progesterone production in cultured granulosa cells (Vitt et al., 2000a; Elvin et al., 1999a) via a prostaglandin E2/EP2 receptor pathway (Elvin et al., 2000). It remains unclear which role this stimulatory effect of GDF-9 has in vivo where high levels of FSH are commonly present.

3.4. Theca cell differentiation

In GDF-9 null mice, follicles lack selective theca cell markers such as 17-alpha-hydroxylase (CYP17), LH receptors and c-kit receptors (Huang et al., 1993). Furthermore, in vivo application of GDF-9 led to an increase in ovarian CYP17 content (Vitt et al., 2000b). It is not possible to determine whether GDF-9 directly induces these factors or if their absence is primarily due to the lack in follicle development. C-kit receptor and its ligand, kit ligand, play an essential role on ovarian development (Huang et al., 1993 Godin et al., 1991). Kit ligand, which is produced by granulosa cells, was shown to induce oocyte growth and theca cell proliferation (Packer et al., 1994; Parrott and Skinner, 1997, 2000). Therefore, defective theca cell formation in GDF-9-deficient animals could be due to the lack of c-kit receptor expression. Based on antibody neutralization experiments, the initiation of primordial follicle growth has been shown to be regulated by c-kit receptor signaling (Yoshida et al., 1997). In addition, it was shown that immature oocytes reduce kit ligand expression by granulosa cells in vitro (Joyce et al., 1999). Therefore, early follicle progression could be coordinated through combined actions of oocyte-derived GDF-9 and granulosa cell-derived kit ligand.

In support of the hypothesis that GDF-9 might regulate theca cell differentiation, theca cells were found to be responsive to GDF-9 treatment. Androstenedione production was increased by GDF-9 in primary cultures of theca cells (Solovyeva et al., 2000). These results indicate that GDF-9 influences both granulosa and theca cell differentiation. Future studies are necessary to assess whether GDF-9 production by the centrally located oocyte affects theca cell function in vivo.

4. Implications for GDF-9 in clinical trials

Current ovarian stimulation protocols for infertility treatment mainly influence preantral and antral follicle growth using gonadotropins (Diedrich and Felberbaum, 1998). However, a subset of patients have been found to be poor responders to gonadotropin stimulation (Scott, 1996). Although several factors have been considered to be associated with ovarian failure (Bannatyne et al., 1990; Kater and Biglieri, 1994), most cases remain idiopathic. Furthermore, in cases of premature ovarian failure, different approaches with current infertility protocols have not resulted in enhanced ovulation rates and oocyte donation is the only therapy for such patients who desire pregnancy (Kalantaridou et al., 1998). For poor responders to gonadotropins, GDF-9 treatment represents an alternative approach as it stimulates primary and secondary follicle develop-

ment and increases the number of gonadotropin-responsive preantral follicles in vivo. Because most women with premature ovarian failure still contain primordial follicles in their ovaries (Olivar, 1996), treatment with GDF-9 could initiate the development of these follicles, thus allowing pregnancy using the patient's own oocytes.

5. Future studies

In order to assess potential effects of GDF-9 treatment in vivo, it is necessary to investigate the existence of GDF-9 binding sites in diverse tissues. To date the GDF-9 receptor and signaling pathway have not been characterized, and the formulation of current hypotheses relies on comparisons between GDF-9 and related proteins. Members of the TGF-beta superfamily bind to type II serine/threonine kinase receptors which complex with type I receptors to initiate signal transduction (Wrana et al., 1994). Different type I and type II receptors are used by several TGF-beta family members with preferential binding affinities to different type I and II receptors (Solloway and Robertson, 1999). In rat granulosa cells, several type I and type II receptors have been described (Shimasaki et al., 1999). Even though it was shown that granulosa cells are responsive to GDF-9, the localization and characterization of GDF-9 receptors remains a subject of future studies.

Future studies are also necessary to assess the potential interaction of GDF-9 with other oocyte factors. GDF-9B/BMP-15 is closely related to GDF-9 and was discovered to be coexpressed with GDF-9 in oocytes (Aaltonen et al., 1999; Dube et al., 1998; Laitinen et al., 1998). The essential role of GDF-9B/BMP-15 in fertility was shown by the block of folliculogenesis in Inverdale sheep which is caused by a mutation of the GDF-9B/BMP-15 gene (Galloway et al., 2000).

Other members of the TGF-beta superfamily form covalent hetero- or homodimers. Both GDF-9 and GDF-9B lack the cysteine residue necessary for covalent dimer formation but, due to their structural similarity to other family members, they may form non-covalent dimers. Heterodimerization could lead to changes in the bioactivity of the resulting molecule. Of interest, heterodimers formed between other members of the TGF-beta superfamily have been shown to elicit functions which are different from that of the respective homodimers (Nishimatsu and Thomsen, 1998). Furthermore, inhibins, which form heterodimers of alpha and beta subunits are functional antagonists to activins, which are homo- or heterodimers of different beta subunits (Vale et al., 1986; Ling et al., 1986; Robertson et al., 1992; Mathews, 1994). Therefore, future studies are necessary to reveal whether GDF-9 interacts with GDF-9B to modulate follicle development.

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