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Oocyte–somatic cell interactions in the human ovary—novel role of bone morphogenetic proteins and growth differentiation factors

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BACKGROUND: Initially identified for their capability to induce heterotopic bone formation, bone morphogenetic proteins (BMPs) are multifunctional growth factors that belong to the transforming growth factor β superfamily. Using cellular and molecular genetic approaches, recent studies have implicated intra-ovarian BMPs as potent regulators of ovarian follicular function. The bi-directional communication of oocytes and the surrounding somatic cells is mandatory for normal follicle development and oocyte maturation. This review summarizes the current knowledge on the physiological role and molecular determinants of these ovarian regulatory factors within the human germline-somatic regulatory loop.

OBJECTIVE AND RATIONALE: The regulation of ovarian function remains poorly characterized in humans because, while the fundamental process of follicular development and oocyte maturation is highly similar across species, most information on the regulation of ovarian function is obtained from studies using rodent models. Thus, this review focuses on the studies that used human biological materials to gain knowledge about human ovarian biology and disorders and to develop strategies for preventing, diagnosing and treating these abnormalities.

SEARCH METHODS: Relevant English-language publications describing the roles of BMPs or growth differentiation factors (GDFs) in human ovarian biology and phenotypes were comprehensively searched using PubMed and the Google Scholar database. The publications included those published since the initial identification of BMPs in the mammalian ovary in 1999 through July 2016.

OUTCOMES: Studies using human biological materials have revealed the expression of BMPs, GDFs and their putative receptors as well as their molecular signaling in the fundamental cells (oocyte, cumulus/granulosa cells (GCs) and theca/stroma cells) of the ovarian follicles throughout follicle development. With the availability of recombinant human BMPs/GDFs and the development of immortalized human cell lines, functional studies have demonstrated the physiological role of intra-ovarian BMPs/GDFs in all aspects of ovarian functions, from follicle development to steroidogenesis, cell–cell communication, oocyte maturation, ovulation and luteal function. Furthermore, there is crosstalk between these potent ovarian regulators and the endocrine signaling system. Dysregulation or naturally occurring mutations within the BMP system may lead to several female reproductive diseases. The latest development of recombinant BMPs, synthetic BMP inhibitors, gene therapy and tools for BMP-ligand sequestration has made the BMP pathway a potential therapeutic target in certain human fertility disorders; however, further clinical trials are needed. Recent studies have indicated that GDF8 is an intra-ovarian factor that may play a novel role in regulating ovarian functions in the human ovary.

WIDER IMPLICATIONS: Intra-ovarian BMPs/GDFs are critical regulators of folliculogenesis and human ovarian functions. Any dysregulation or variations in these ligands or their receptors may affect the related intracellular signaling and influence ovarian functions, which accounts for several reproductive pathologies and infertility. Understanding the normal and pathological roles of intra-ovarian BMPs/GDFs, especially as related to GC functions and follicular fluid levels, will inform innovative approaches to fertility regulation and improve the diagnosis and treatment of ovarian disorders.

Key words: bone morphogenetic proteins / growth differentiation factors / activin receptor like-kinase / human ovary / human granulosa cells / human granulosa cell line / female infertility

Introduction

In humans, the ovarian follicle is the core functional unit of the female reproductive system, which may correspond to different developmental stages in the ovary. During the female reproductive cycle, these follicles progress through a highly coordinated regulatory process that involves several neural, neuroendocrine, endocrine and paracrine/autocrine control systems to achieve full ovulatory and steroidogenic capability (Gougeon, 1996). The primary roles of the pituitary gonadotrophin hormones, FSH and LH, GnRH and the gonadal hormones (estrogen, androgen and progesterone) in the female reproductive system are well established. However, normal ovarian function and follicular development also depend on a variety of locally produced growth factors and cytokines that exert their effects in a paracrine/autocrine fashion (Knight and Glister, 2006). In the last few decades, studies on a variety of species have demonstrated that members of the transforming growth factor-β (TGF-β) superfamily, which include bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), TGF-βs, activins and inhibins, and anti-Müllerian hormone (AMH), are expressed in the ovary and play essential roles in the regulation of folliculogenesis, oogenesis and ovarian functions (Gougeon, 1996; Juengel and McNatty, 2005; Knight and Glister, 2006). With over 20 members, BMPs/GDFs constitute the largest subfamily of the TGF-β superfamily and were initially identified as osteoinductive cytokines that may promote bone and cartilage formation (Wang *et al.*, 1988; Wagner *et al.*, 2010). Several publications have documented that locally produced BMPs are implicated in the formation and function of mammalian germ cells and that they participate in the regulation of ovarian functions (Yi *et al.*, 2001; Shimasaki *et al.*, 2004; Khan *et al.*, 2016; Rossi *et al.*, 2016). Moreover, naturally occurring gene mutations of BMP ligands and dysregulated BMP signaling are associated with prominent pathologies of human reproduction (Persani *et al.*, 2014; Qin *et al.*, 2015).

While the fundamental process of follicular development is highly similar across species, most information on the regulation of ovarian function is obtained from studies in rodents. Our understanding of the development and function of the human ovary has advanced only recently due to a combination of technologies including recombinant human BMPs/GDFs, immortalized human granulosa cell (GC) lines, tissue microarrays and pharmaceutical development. Thus a significant portion of this review is devoted to the human ovarian physiologies and human reproductive disorders associated with BMP/GDF signaling.

Methods

A comprehensive literature review was conducted using two databases, PubMed and Google Scholar to identify and retrieve information relevant to the field of BMP/GDF biology in the human ovary, since its initial identification in the mammalian ovary in 1999 (Shimasaki *et al.*, 1999) through July 2016. A systematic review of English-language publications was carried out using the following keywords: BMP, GDF, human ovary, human GCs, human GC line, female infertility and therapeutic targets. The goal of this review is to provide an overview of the current knowledge of the physiological roles of BMPs and GDFs in human female reproduction and their potential therapeutic application for regulation of fertility.

BMPs and BMP receptors

BMP ligands

BMPs consist of an extensive subfamily of phylogenetically conserved growth factors in the TGF-β superfamily with critical physiological functions in organogenesis and morphogenesis in both invertebrates and vertebrates (Hogan, 1996; Bier and De Robertis, 2015). In the modern nomenclature, BMPs are regarded as encompassing growth factors that are either BMPs or GDFs due to the overlap between the two classification systems without significant separate lineages (Rider and Mulloy, 2010) (Fig. 1). Similar to other members of the TGF-β superfamily, BMPs are initially translated as a similar structure consisting of three components: a signal peptide, a prodomain and a mature peptide (mature domain). The signal peptide (20–30 amino acids long) is present at the N-terminal end of the newly synthesized large preproproteins, and it directs these ligands for proper secretion out of the cells (Veitia and Caburet, 2009). The BMPs share a conserved structure: the seven characteristically spaced cysteine residues indicative of the cysteine-knot motif of the TGF-β superfamily (Shi and Massague, 2003). Six of these cysteine residues can form intramolecular disulfide bonds known as cysteine knots, and the seventh cysteine is involved in the dimerization with another monomer via a disulfide bond to form homodimers or heterodimers (Sun and Davies, 1995). However, BMP15 and GDF9 are two of the few TGF-β members with only six cysteine residues in their cysteine-knot domain, each lacking the one cysteine residue responsible for covalent dimer formation (McIntosh *et al.*, 2008). The inactive BMP precursors are proteolytically cleaved by a proprotein convertase family, proprotein convertase Subtilisin/Kexin, which is composed of nine members named PCSK1–PCSK9 (Seidah and Prat, 2012). At least one member of this family, PCSK3 (furin), recognizes the amino acid motif -Arg-X-Arg/Lys-Arg- at the linker site between the prodomain and the mature domain and activates the mature BMP homodimers or heterodimers (Harrison *et al.*, 2011). Several BMP heterodimers, such as BMP2/BMP7, BMP2/BMP6, BMP4/BMP7 and BMP15/GDF9, have been suggested to exist and function both *in vivo* and *in vitro* (Israel *et al.*, 1996; Little and Mullins, 2009; Valera *et al.*, 2010; Peng *et al.*, 2013). Most of these BMP heterodimers are more biologically potent than their respective homodimers in inducing cellular functions (Israel *et al.*, 1996; Valera *et al.*, 2010; Peng *et al.*, 2013). The most likely explanation for these differential effects is that the heterodimer activity could be mediated by a different or an additional receptor subtype.

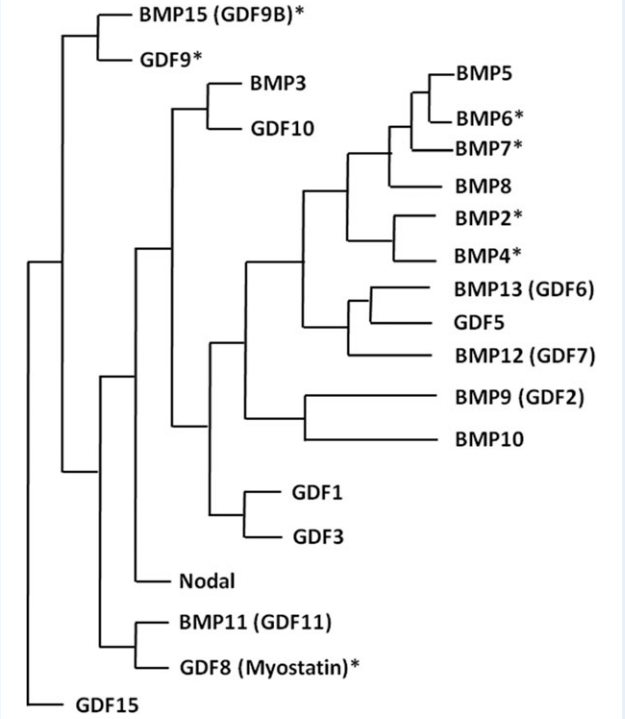

Figure 1 Phylogenetic relationship of the BMP/GDF subfamily members. The alignment of 21 BMP/GDF-related ligands was based on published literature: (Ducy and Karsenty, 2000; Mazerbourg and Hsueh, 2006; Rider and Mulloy, 2010). Ligands with an asterisk are expressed in the human ovary. BMP/GDF, bone morphogenetic protein/growth differentiation factor.

It has been widely argued that the biological action of oocyte-derived BMP15 and GDF9 is redundant or synergistic. Evidence from a study of *Bmp15* and *Gdf9* double knockout mice demonstrated that there is a biological cooperation between these two growth factors (Yan *et al.*, 2001). There is a great debate as to whether such cooperation results from the synergistic interaction of BMP15 and GDF9 homodimers or from the existence of a biologically active BMP15/GDF9 heterodimer (Mottershead *et al.*, 2013; Peng *et al.*, 2013; Persani *et al.*, 2014). Interestingly, the BMP15/GDF9 heterodimer can be produced and secreted in 293 T cells (a specific cell line originally derived from human embryonic kidney cells) when two constructs are co-expressed (Liao *et al.*, 2004). Indeed, the human BMP15/GDF9 heterodimer is approximately 1000- to 3000-fold more potent than the human BMP15 homodimer (Peng *et al.*, 2013).

BMP receptors

TGF-β superfamily members signal through distinct sets of transmembrane serine/threonine kinase receptors (type I and type II receptors), which further regulate downstream gene expression by phosphorylating the Sma- and Mad-related protein (SMAD) transcription factors (Shi and Massague, 2003). To date, five distinct type II receptors (BMPR2, ACVR2A, ACVR2B, TβR2 and AMHR2) and seven type I receptors (also known as activin receptor like-kinase; ALK1–7) have been identified in mammals (Massague, 1998; Miyazono *et al.*, 2001).

All of these receptors share a very similar structure consisting of an extracellular domain (N-terminal), a transmembrane domain and an intracellular domain with Ser/Thr kinase activity (C-terminal). Each ligand of the TGF-β superfamily members can bind to the N-terminal extracellular domains of different combinations of type II and type I receptors and trigger downstream signaling complexes (Massague, 1998; Miyazono *et al.*, 2001). Of the five type I receptors, three (BMPR2, ACVR2A and ACVR2B) are employed by one or more BMPs. Likewise, six (ALK2-7) of the seven type I receptors have been implicated in BMP-induced intracellular signaling (Macias-Silva *et al.*, 1998; Kaivo-Oja *et al.*, 2003; Moore *et al.*, 2003; Mazerbourg and Hsueh, 2006; Miyagi *et al.*, 2012). Receptors and signaling pathways utilized for various intra-ovarian BMPs/GDFs in human cells are listed in Table I.

BMP signaling

Members of the TGF-β superfamily exert their functions by binding to type I and type II receptors, which further regulate downstream target gene expression by phosphorylating the receptor-regulatory SMAD (R-SMAD) transcription factors, SMAD1–8. Generally, two distinct models of the ligand–receptor interaction exist. BMPs, AMH and some GDFs activate SMAD1/5/8 via ALK2, ALK3 and/or ALK6; whereas, TGF-βs, activins and nodal activate SMAD2/3 via ALK4, ALK5 and/or ALK7 (Drummond, 2005; Mueller and Nickel, 2012). Upon phosphorylation of type I receptors, BMPs activate the canonical SMAD1/5/8 group, which then associate with the common SMAD (SMAD4), and this complex translocates to the nucleus to exert functions that regulate gene expression in most tissues.

Quite recently, novel non-canonical SMAD2/3 signaling (commonly induced by TGF-βs and activins) activated by BMPs to regulate cancer progression and hormone production has been given considerable attention (Holtzhausen *et al.*, 2014; Wang *et al.*, 2014). In human granulosa tumor cells and mouse GCs, recombinant human BMP15, but not mouse BMP15, mildly phosphorylated SMAD2/3 and stimulated mouse cumulus–oocyte complex (COC) expansion (Peng *et al.*, 2013). In line with these reports challenging the standard guideline that BMPs exert cellular functions solitarily through canonical SMAD1/5/8 signaling, our most recent studies showed that BMPs-induced *hyaluronan synthase type 2* (*HAS2*) expression is mediated by noncanonical SMAD2/3 signaling through binding to ALK4/5/7 in immortalized human GCs (Zhang *et al.*, 2016). Differences in receptor expression levels and receptor binding properties between different BMPs (homodimers or heterodimers) most likely contribute to the differential activation of noncanonical SMAD2/3 signaling in certain cell types.

In addition to the SMAD signaling, several SMAD-independent (non-SMAD) signaling pathways for BMPs have been identified in specific tissues. It is widely believed that the mitogen-activated protein kinase (MAPK) signal pathway is the major SMAD-independent pathway induced by BMPs. BMP4 has been found to phosphorylate TGF-β activated kinase 1, a serine/threonine kinase of the MAPKKK family (Yamaguchi *et al.*, 1995; Derynck and Zhang, 2003). Previous studies indicate that BMPs are also able to activate several signaling pathways, including phosphoinositide 3-kinase, extracellular signal-regulated kinase (ERK), protein kinase (PK) A, PKC and PKD (PKCμ) (Yamaguchi *et al.*, 1995; Nakamura *et al.*, 1999; von Bubnoff and Cho, 2001). Interestingly, our recent study showed that the BMP subfamily member GDF8 decreases steroidogenic acute regulatory protein (StAR) expression by activating both the SMAD3 and ERK signaling pathways (Fang *et al.*, 2015). Upon ligand–receptor binding and interaction, the subsequent pathway activated by BMPs is most likely dependent on other cellular activity, the extracellular environment and crosstalk with other signaling pathways.

Intra-ovarian BMPs and oocyte–somatic cell interactions

Human GC lines

To clearly understand the underlying mechanisms by which hormones or growth factors regulate folliculogenesis and ovarian

Table I Putative receptors and signaling pathways of BMPs/GDFs in the human ovary.

Ligand	Type II receptor	Type I receptor	SMAD	References
BMP2	BMPR2, ACVR2A	ALK3/6	SMAD1/5/8	Mazerbourg and Hsueh (2006)
BMP4	BMPR2, ACVR2A	ALK3/6	SMAD1/5/8	Miyagi *et al.* (2012) Chang *et al.* (2015a, 2015c)
		ALK3/4/5	SMAD2/3	Zhang *et al.* (2016)
BMP6	BMPR2, ACVR2A	ALK2/3/6	SMAD1/5/8	Mazerbourg and Hsueh (2006)
BMP7	BMPR2, ACVR2A	ALK2/3/6	SMAD1/5/8	Macias-Silva *et al.* (1998) Chang *et al.* (2015a, 2015b, 2015c)
BMP15	BMPR2	ALK3/6	SMAD1/5/8	Moore *et al.* (2003) Chang *et al.* (2013a)
GDF8	ACVR2A, ACVR2B	ALK5	SMAD2/3	Chang *et al.* (2015d) Chang *et al.* (2016a, 2016b, 2016c)
GDF9	BMPR2	ALK5	SMAD2/3	Mazerbourg and Hsueh (2006) Kaivo-Oja *et al.* (2003)

ACVR, activin receptor; ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; GDF, growth differentiation factor; SMAD, Sma- and Mad-related protein.

steroidogenesis, available cell models that are suitable for *in vitro* studies are warranted. GC-based *in vitro* cell model systems have provided valuable tools for studying ovarian biology. The major source of human GCs for *in vitro* studies is usually from infertile patients undergoing IVF. However, these cells are obtainable only in small numbers, which make it difficult to conduct extensive experiments related to detailed molecular analysis. In addition, the clinically obtained GCs are generally luteinized because of their extensive stimulation with FSH/LH and hCG prior to cell isolation. Therefore, these GCs have a limited life span with a slow proliferation rate, and they do not survive *in vitro* for many passages (Breckwoldt *et al.*, 1996). Furthermore, primary GCs derived from different patients may exhibit wide variability, which adds to the difficulty of obtaining reproducible results. Because of these obstacles, a substitute human GC line has become an attractive option. In the last few years, there has been a growing interest in developing several cell lines from human GCs (Table II). These cell lines were generated through various techniques, including oncogenic transformation (Rainey *et al.*, 1994; Lie *et al.*, 1996; Hosokawa *et al.*, 1998; Nitta *et al.*, 2001; Tajima *et al.*, 2002; Okamura *et al.*, 2003), transfection with site-directed mutagenesis (Bayasula *et al.*, 2012) and explants of human tumors (Ishiwata *et al.*, 1984; van den Berg-Bakker *et al.*, 1993; Nishi *et al.*, 2001). Notably, each cell line may have different cell properties with regard to steroidogenic functions, gonadotrophin (FSH and LH) responsiveness, cAMP responsiveness, BMP responsiveness, and mitogenic and differential capabilities (Havelock *et al.*, 2004) (Table II). Therefore, it is essential to choose the appropriate cell line for individual studies. For instance, the SVOG cell line was developed by transfection of human granulosa-lutein (hGL) cells using SV40 large T antigen (Lie *et al.*, 1996) (Table II). Primary hGL cells were used to generate the immortalized cells and the two display similar biological responses to many different treatments, such as LH, hCG, cAMP and various growth factors (Lie *et al.*, 1996). As demonstrated in numerous previous publications from our laboratory as well as those of other investigators, these cells are a widely accepted model to study the ovarian response during the periovulatory and early luteal phases (Havelock *et al.*, 2004; Chang *et al.*, 2015a, 2015c, 2016c, 2016a; Fang *et al.*, 2016a). COV434 and KGN are two widely used GC lines originating from ovarian GC tumors (van den Berg-Bakker *et al.*, 1993; Zhang *et al.*, 2000; Nishi *et al.*, 2001). Unfortunately, the development of an androgen-producing thecal cell line has faced a tough challenge with limited success (Magoffin and Erickson, 1988; McAllister *et al.*, 1989).

Expression of BMPs and BMP receptors in the human ovary

A number of studies performed over the past decade have provided important information about the expression of the BMP system in the mammalian ovary, with the most comprehensive study using adult cycling rats (Erickson and Shimasaki, 2003; Shimasaki *et al.*, 2004). However, the expression pattern of BMPs in the rat ovary may not necessarily apply to other species. For instance, BMP4 and BMP7 are primarily expressed by theca and stroma cells in rats (Erickson and Shimasaki, 2003); however, both BMP4 and BMP7 have been detected in human oocytes (Abir *et al.*, 2008). GDF9

Table II The cellular properties of human ovarian GC lines.

Cell line	Origin	Transfection	Progesterone production	Aromatase activity	Steroidogenic enzyme	FSH response	LH/hCG response	cAMP response	BMP response	References
SVOG	Luteinized GC	SV40 T	(+)	(−)	(+)	(−)	(+)	(+)	(+)	Lie *et al.* (1996)
HGL5	Luteinized GC	HPV16E6/E7	(+)	(+)	(+)	(−)	(−)	(+)	(+)	Rainey *et al.* (1994)
HO-23	Luteinized GC	SV40 T, Ha-ras p53	(+)	ND	(+)	ND	(−)	(+)	ND	Hosokawa *et al.* (1998)
HGP53	Luteinized GC	Ha-ras & p53val135	(+)	ND	(+)	(+)	ND	(+)	ND	Takebayashi *et al.* (2000)
GCI a	Non-luteinized GC	Mouse SF-I	(−)	(−)	(−)	(−)	(−)	ND	ND	Nitta *et al.* (2001) Okamura *et al.* (2003)
HGrCI	Non-luteinized GC	HPV16E6/E7, CDK4R24C, cyclin D1	(+)	(+)	(+)	(+)	(+)	ND	(+)	Bayasula *et al.* (2012)
COV434	Metastatic GC tumor		(+)	(+)	(−)	(+)	(−)	(+)	(+)	van den Berg-Bakker *et al.* (1993)
KGN	GC carcinoma		(+)	(+)	(−)	(+)	(−)	(+)	(+)	Nishi *et al.* (2001)
HTOG	Granulosa-theca cell tumor		(+)	(+)	ND	ND	ND	ND	ND	Ishiwata *et al.* (1984)

GC, granulosa cell; ND, Not documented.

has been identified in hGL cells and oocytes (Aaltonen et al., 1999; Teixeira Filho et al., 2002; Huang et al., 2009; Shi et al., 2009), but GDF9 is exclusively expressed in the oocytes of ovine and bovine ovaries (Bodensteiner et al., 1999; Hayashi et al., 1999). Likewise, BMP15, which was previously thought to be oocyte-derived (Aaltonen et al., 1999), has been detected in human cumulus cells (Li et al., 2014), GCs and stroma cells in girls and adults (Margulis et al., 2009). The development and physiological functions of the human reproductive system are mostly influenced by the tissue-specific and time-dependent expression of BMP subfamily members (Shimasaki et al., 2004), even though the spatiotemporal expression pattern of BMPs in the human ovary remains largely unknown. The expression of BMP/GDF ligands and their receptors in human ovarian tissues is listed in Table III. In the human ovary, BMP2, BMP4, BMP5, BMP6, BMP7 and BMP8A are expressed in the GCs from normally cycling and polycystic ovary syndrome (PCOS) women (Khalaf et al., 2013). In concert with this study, a study using isolated human pre-antral follicles that compared the expression levels of BMPs in five size-matched populations demonstrated that BMP6 and BMP15 are the most abundantly expressed ligands (Kristensen et al., 2014). In the human corpus luteum, BMP2, BMP4 and BMP6 are highly expressed in the granulosa-lutein and theca-lutein cells and are involved in the process of luteolysis (Nio-Kobayashi et al., 2015).

During the antral follicular stage, the growth and maturation of the oocyte are dependent on several intra-ovarian factors present in the follicular fluid (Hsieh et al., 2009). The concentrations of some of the follicular fluid growth factors are correlated with the serum levels of these factors (Qiao and Feng, 2011). The imbalance of any of these intra-ovarian factors may lead to abnormal follicular development and dysfunction of oocyte maturation in humans (Franks et al., 2008; Qiao and Feng, 2011). Recent studies have shown that BMP2, BMP4, BMP7, BMP15, GDF8 and GDF9 are detectable in the follicular fluid (Table III) (Sugiyama et al., 2010; Wu et al., 2012; Chang et al., 2015a; Chang et al., 2015d). Among these follicular fluid BMPs, the results from clinical data suggested that BMP2 and BMP15 may potentially be used as indicators of oocyte fertilization and/or oocyte maturation (Wu et al., 2007; Sugiyama et al., 2010).

Previous research has documented the expression of the mRNA and protein of BMP receptors in various follicular compartments of pre-antral and antral follicles of the human ovary (Khalaf et al., 2013; Kristensen et al., 2014). Among these receptors, BMPR2 is the most abundantly expressed type II receptor (Kristensen et al., 2014). In comparison with the type I receptors ALK1, ALK2 and ALK7, four type I receptors ALK3, ALK4, ALK5 and ALK6 are expressed at modest levels in the human pre-antral follicles (Kristensen et al., 2014). A recent study showed that the dysregulation of ALK6 in human GCs is associated with reduced ovarian reserve and the age-related decline in fertility (Regan et al., 2016).

BMPs and primordial germ cell development

Primordial germ cells (PGCs), the precursors of the sperm and egg in the adult, are a group of germline stem cells that develop only during the early embryonic stage (McLaren, 2003). These stem cells migrate to the primitive gonadal fold and mitotically proliferate to increase cell numbers, which subsequently differentiate into primordial follicles (oocytes associate with the surrounding somatic cells) (Pepling and Spradling, 2001). Members of the BMP subfamily play critical roles in regulating ovarian development and function (Shimasaki et al., 2004; Knight and Glister, 2006). However, the developmental processes are differentially regulated in rodents and humans with respect to the spatiotemporal organization (Lawson et al., 1999; Childs et al., 2010). In mouse embryos, the extraembryonic ectoderm-derived BMP4 and BMP8B collaboratively induce PGC formation (Ying et al., 2002). In human fetal ovaries, BMP4 and BMP signaling modulates post-migratory PGC numbers by promoting apoptosis (Childs et al., 2010). During oogenesis in humans, GDF9 is transiently secreted by oocytes before follicle formation, which is accompanied by activin βA signaling from somatic cells to determine selective germ cell survival (Bayne et al., 2015).

BMPs and intra-ovarian cell–cell communication

The ovarian follicle is the principal functional unit of the female reproductive system. A coordinated interplay within this biological compartment between the oocyte and the follicular cells (cumulus cells/GCs and theca/stroma cells) depends heavily on functional gap junctions (Granot and Dekel, 2002). These connexin-coupled cell junctions directly mediate cell–cell communication by allowing the passage of small molecules (ions, metabolites, nutrition and small signaling molecules) between two adjacent cells (Caspar et al., 1977; Makowski et al., 1977). In the growing follicles in humans, connexin 43 (Cx43) primarily contributes to the formation of gap junctions between cumulus cells/GCs, whereas gap junctions that connect the oocyte to the surrounding cumulus cells are mainly composed of connexin 37 (Cx37) (Furger et al., 1996; Tsai et al., 2003; Gershon et al., 2008). Studies in knockout mice demonstrated that mice lacking *Cx43* exhibit a phenotype of reduced germ cell numbers in the fetal gonads, retarded growth of oocytes and fertilization failure (Ackert et al., 2001). In addition, the ablation of *Cx37* leads to an abolition of intercellular coupling between oocytes and cumulus cells, disruption of follicle development at the antral stage, incompetent oocytes and ovulatory dysfunction (Simon et al., 1997).

Cx43 is abundantly expressed in the GCs throughout all follicular stages and its expression is required for GC proliferation (Ackert et al., 2001; Gittens et al., 2005). The cyclic expression of Cx43 in the GCs is developmentally and hormonally regulated by gonadotrophins (FSH and LH) and steroid hormones (estrogen, progesterone and androgen) in many species, including humans (Petrocelli and Lye, 1993; Granot and Dekel, 2002; Wu et al., 2010). Our recent studies have shown that three intra-ovarian BMPs (BMP4, BMP7 and BMP15) and TGF-β1 may act in a paracrine/autocrine manner to modulate *Cx43* expression and intercellular communication in human GCs (Fig. 2) (Chang et al., 2013b, 2014b, 2015). Specifically, BMP-mediated signaling suppresses *Cx43* expression, whereas TGF-β1 increases *Cx43* expression, indicating competing regulatory roles for these paracrine/autocrine factors. This functional discrepancy

Table III Localization of BMPs/GDFs and receptors in the human ovary.

Ligands	Localization	Expression	Detection method	References
BMP2	Luteinized GC	mRNA	RT-qPCR	Khalaf *et al.* (2013)
	CL	mRNA	RT-qPCR	Nio-Kobayashi *et al.* (2015)
	FF (1–115 ng/ml)	Protein	ELISA	Sugiyama *et al.* (2010)
BMP4	Luteinized GC	mRNA & Protein	RT-qPCR, IHC	Khalaf *et al.* (2013)
	Oocyte[a]	Protein	IHC	Abir *et al.* (2008)
	Theca/stroma cells[a]	Protein	IHC	Abir *et al.* (2008)
	GC[a]	Protein	IHC	Abir *et al.* (2008)
	CL	mRNA & Protein	RT-qPCR, IHC	Nio-Kobayashi *et al.* (2015)
	FF (1–20 pg/ml)	Protein	ELISA	Chang *et al.* (2015a)
BMP5	Luteinized GC	mRNA	RT-qPCR	Khalaf *et al.* (2013)
BMP6	Luteinized GC	mRNA & Protein	RT-qPCR, IHC	Khalaf *et al.* (2013)
	CL	mRNA & Protein	RT-qPCR, IHC	Nio-Kobayashi *et al.* (2015)
BMP7	Luteinized GC	mRNA & Protein	RT-qPCR, IHC	Khalaf *et al.* (2013)
	Oocyte	Protein	IHC	Abir *et al.* (2008)
	Theca/stroma cells	Protein	IHC	Abir *et al.* (2008)
	GC	Protein	IHC	Abir *et al.* (2008)
	FF (50–130 ng/ml)	Protein	ELISA	Sugiyama *et al.* (2010)
BMP8A	Luteinized GC	mRNA	RT-qPCR	Khalaf *et al.* (2013)
BMP15	Pre-antral follicles (>75 µm)	mRNA	Tissue microarray	Kristensen *et al.* (2014)
	Cumulus cells	mRNA	RT-qPCR	Li *et al.* (2014)
	Oocyte	mRNA & Protein	*In situ* hybridization, RT-qPCR, IHC	Margulis *et al.* (2009) Aaltonen *et al.* (1999) Teixeira Filho *et al.* (2002)
	Theca/stroma cells	mRNA & Protein	*In situ* hybridization, IHC	Margulis *et al.* (2009)
	GC	mRNA & Protein	*In situ* hybridization, IHC	Margulis *et al.* (2009)
	FF	Protein	Western blot	Wu *et al.* (2012)
GDF8	Luteinized GC	mRNA	RT-qPCR	Chang *et al.* (2015d)
	FF (2.01–4.17 ng/ml)	Protein	ELISA	Chang *et al.* (2015d)
GDF9	Pre-antral follicles	mRNA	Tissue microarray	Kristensen *et al.* (2014)
	Cumulus cells	mRNA	RT-qPCR	Li *et al.* (2014)
	Oocyte	mRNA & Protein	*In situ* hybridization, IHC	Aaltonen *et al.* (1999) Teixeira Filho *et al.* (2002)
	Luteinized GC	mRNA & Protein	RT-PCR, Western blot	Huang *et al.* (2009)
	FF (0.83–53.9 ng/ml)	Protein	ELISA	
ALK2	Luteinized GC	mRNA	RT-qPCR	Khalaf *et al.* (2013)
ALK3	Luteinized GC	mRNA & Protein	RT-qPCR, IHC	Khalaf *et al.* (2013)
	GC	Protein	IHC	Abir *et al.* (2008)
	Oocyte	Protein	IHC	Abir *et al.* (2008)
	CL	mRNA & Protein	RT-qPCR, IHC	Nio-Kobayashi *et al.* (2015)
ALK5	Luteinized GC	mRNA	RT-qPCR	Khalaf *et al.* (2013)
ALK6	Luteinized GC	mRNA & Protein	RT-qPCR, IHC	Khalaf *et al.* (2013)
	Oocyte	Protein	IHC	Abir *et al.* (2008)
	Stroma cells	Protein	IHC	Abir *et al.* (2008)
	GC	Protein	IHC	Abir *et al.* (2008)
	CL	mRNA & Protein	RT-qPCR, IHC	Nio-Kobayashi *et al.* (2015)
BMPR2	Luteinized GC	mRNA & Protein	RT-qPCR, IHC	Khalaf *et al.* (2013)
	Oocyte	Protein	IHC	Abir *et al.* (2008)
	Stroma cells	Protein	IHC	Abir *et al.* (2008)
	GC	Protein	IHC	Abir *et al.* (2008)
	CL	mRNA & Protein	RT-qPCR, IHC	Nio-Kobayashi *et al.* (2015)

BMR, bone morphogenetic proteins; GDF, growth differentiation factors; CL, corpora lutea; ELISA, enzyme-linked immunosorbent assay; FF, follicular fluid; IHC, immunohistochemistry; RT-qPCR, quantitative real-time PCR.
[a]Expressed only in fetal ovary.

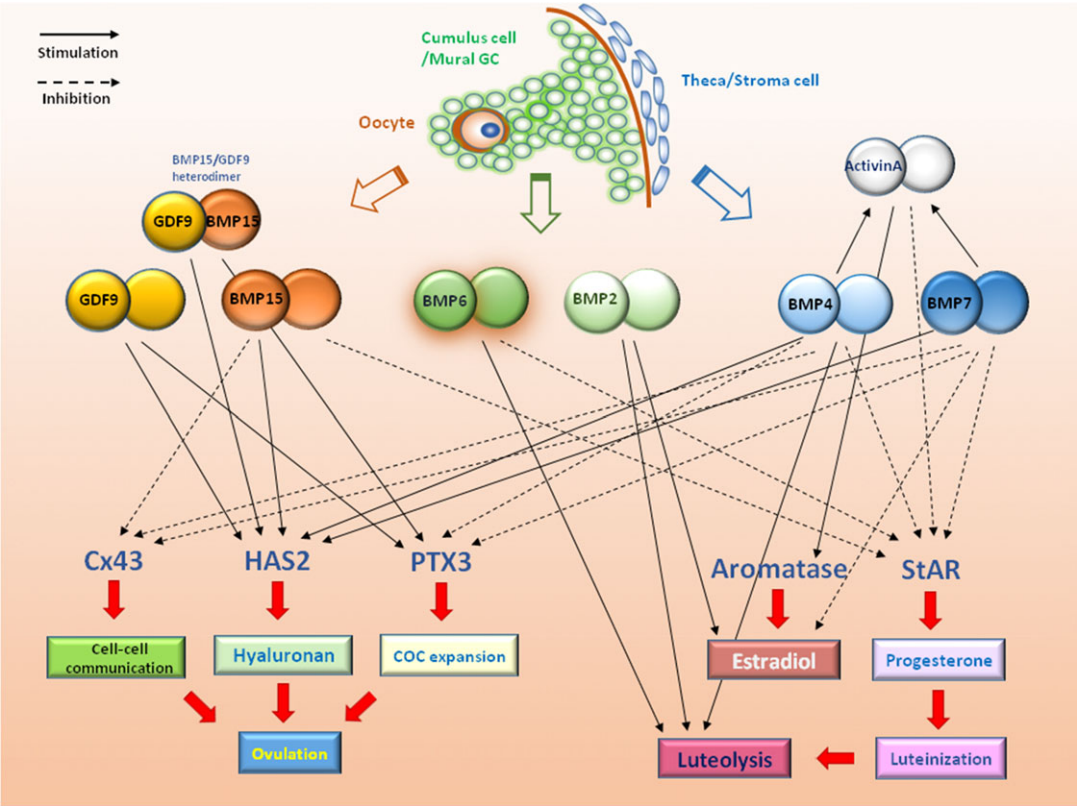

Figure 2 Schematic diagram summarizing functional roles of BMPs and GDF9 in the human ovary. The potential physiological roles of intra-ovarian BMPs in regulating human ovarian functions, including steroidogenesis, activin production, cumulus–oophorus complex formation and expansion, cell–cell communication, ovulation and luteolysis are shown. BMP, bone morphogenetic protein; COC, cumulus–oophorus complex; Cx43, connexin 43; GC, granulosa cell; HAS2, hyaluronan synthase type 2; PTX3, pentraxin 3; StAR, steroidogenic acute regulatory protein.

between the growth factors belonging to the TGF-β superfamily of differential signaling molecules (TGF-βs and BMPs) may influence a broad range of cellular actions.

Oocyte-somatic cell interactions

In the mammalian ovary, oocytes do not merely reside inside the follicles and passively receive the developmental signals from the surrounding cumulus cells/GCs, but they actively govern and modulate follicular development and ovulation. Within the follicular microenvironment, oocytes and the supporting somatic cells coordinately control the development and maturation of the follicle as well as the acquisition of a meiotically competent oocyte (Eppig, 2001). The results obtained from animal studies indicated that oocytes might promote follicular development and cell differentiation by releasing some growth factors that act in a paracrine manner to affect the neighboring cumulus cells/GCs. These paracrine effects, in turn, modulate oocyte development and maturation (Eppig, 2001). Studies in sheep and mice have shown that the experimental ablation (removal, absence or destruction) of the oocytes results in impaired folliculogenesis, indicating a critical role of the oocyte factors or oocyte-induced paracrine signaling in follicular development (Nekola and Nalbandov, 1971; Gilchrist *et al.*, 2004). An *in vivo* study has revealed that ovectomy can lead to the spontaneous transformation of the Graafian follicle into the corresponding corpus luteum (el-Fouly *et al.*, 1970). *In vitro* experiments have demonstrated that oocyte-secreted factors may potently promote DNA synthesis and cell proliferation in the surrounding cumulus/mural GCs (Vanderhyden *et al.*, 1992; Gilchrist *et al.*, 2001). In addition, these oocyte-derived potent mitogens can also augment the effects of several GC regulators (FSH, insulin-like growth factor-I and androgen) on cell growth activities (Armstrong *et al.*, 1996; Fraidenraich *et al.*, 1998; Li *et al.*, 2000; Hickey *et al.*, 2004).

Aside from the mitogenic effects, oocytes are potent modulators of cumulus cell/GC differentiation in various species, including humans (Eppig, 2001). In particular, oocytes modulate the FSH-stimulated estradiol and progesterone production by GCs (Vanderhyden, 1993; Coskun *et al.*, 1995; Vanderhyden and Tonary, 1995), whereas they suppress the expression of LH receptor (LHR) induced by FSH in GCs (Eppig *et al.*, 1997). Apart from the gonadotrophins secreted by the pituitary gland, oocyte-derived BMP15 and GDF9 also participate in the modulation of certain target genes related to ovulation and luteinization (Pangas and Matzuk, 2005; Diaz *et al.*, 2007). In human GCs, oocyte-derived BMP15 decreases progesterone production by down-regulating the expression of *StAR* (Chang *et al.*, 2014b). Collectively, these results support a previously proposed hypothesis that the oocyte is capable of inhibiting follicular luteinization (Fig. 2) (el-Fouly *et al.*, 1970).

Data collected from the cumulus cell transcriptome revealed that bi-directional communication between the human oocyte and cumulus cells is essential for the production of a competent oocyte (Huang and Wells, 2010). In this regard, the oocyte acts as a central regulator of neighboring follicular cell function by secreting various growth factors and cytokines to modulate cell proliferation, cell differentiation, apoptosis and luteinization (Gilchris *et al.*, 2008). At ovulation, cumulus expansion is a complicated process that is required for the optimal extrusion of the cumulus–oocyte cell mass from the follicle (Russell and Robker, 2007). The cumulus expansion process and oocyte maturation are highly dependent on the interactions of two signals induced by epidermal growth factor (EGF)-like peptides (triggered by LH or hCG) and an oocyte-derived paracrine factor (Hsieh *et al.*, 2009; Fang *et al.*, 2013). Specifically, the oocyte-derived factor capacitates the response of cumulus cells to three EGF-like peptides, which induces the expression of several target genes [*HAS2*, *tumor necrosis factor alpha-induced protein 6 (TNFAIP6)*, pentraxin 3 *(PTX3)* and *prostaglandin-endoperoxide synthase 2 (PTGS2)*] related to extracellular matrix formation and stability (Diaz *et al.*, 2007). Indeed, the *in vitro* studies have shown that oocyte factors, GDF9 and, to a lesser extent, BMP4 and BMP7 induce *HAS2* expression and hyaluronan synthesis as well as prostaglandin E_2 production, which is essential for normal ovulation (Elvin *et al.*, 1999; Zhang *et al.*, 2016). The knowledge obtained from cumulus–oocyte interactions during the periovulatory stage will enable clinicians to design optimal procedures for ART, especially the IVM protocol.

BMPs prevent premature luteinization

In humans, the LH surge stimulates multiple intra-follicular activities and triggers ovulation (Russell and Robker, 2007). At the time of ovulation, a series of morphological transitions and tissue remodeling cause the ruptured ovarian follicle to develop into the corpus luteum, a temporary endocrine structure that secretes progesterone (Oon and Johnson, 2000). The release of progesterone targets and prepares the reproductive tract for initiation of fertilization and maintenance of early pregnancy (Niswender *et al.*, 2000). Premature luteinization refers to an elevation of serum progesterone levels on (or before) the day of hCG administration in patients undergoing controlled ovarian stimulation (Al-Azemi *et al.*, 2012). The premature rise of progesterone can shift the implantation window (synchronization between embryonic development and endometrial receptivity), which may hamper embryo implantation and decrease the pregnancy rate (Achache and Revel, 2006). During the antral follicle stage, one of the most important physiological functions is the prevention of premature luteinization, which maintains follicular growth and somatic cell proliferation (Baerwald *et al.*, 2012).

Evidence from animal studies has shown that the regulation of steroid hormones by BMPs is species- and stage-specific in the pre-antral and antral follicular stages. In humans, some inconsistent results have been published regarding the regulatory effects of BMPs on ovarian steroid hormone synthesis. In a human granulosa tumor cell line (KGN), BMP4 and BMP7 suppressed forskolin- or cAMP-induced progesterone production without affecting forskolin- or cAMP-induced estradiol production (Miyoshi *et al.*, 2006). In contrast, a cohort study using GCs from normal healthy women and PCOS women showed that BMP7, but not BMP4, suppressed basal estradiol production, and both BMP4 and BMP7 had no effect on FSH-induced estradiol production in the normal women (Khalaf *et al.*, 2013). Studies using primary GCs obtained from IVF patients showed that BMP2, BMP4 and BMP6 suppressed *StAR* expression, whereas BMP2 induced aromatase expression (Shi *et al.*, 2011; Nio-Kobayashi *et al.*, 2015). Consistent with some of the previous studies, our recent results showed that BMP4, BMP7 and BMP15 all decreased progesterone production by down-regulating ALK3-mediated *StAR* expression in immortalized GCs (SVOG cells), but not in KGN cells (Chang *et al.*, 2013; Zhang *et al.*, 2015). Moreover, BMP4 and BMP7 increased the synthesis of bioactive activin A by up-regulating the production and proteolytical processing of the inhibin βA subunit in human immortalized GCs (Chang *et al.*, 2015c). Activin A is another potent luteinization inhibitor because of its ability to decrease the basal and FSH- or LH-induced progesterone production in hGL cells (Chang *et al.*, 2014a). GDF9, a close relative of BMP15, has divergent roles in the regulation of human steroid production. In human GCs and theca cells, GDF9 suppresses 8-bromo-cAMP-induced StAR expression and progesterone production without affecting basal StAR protein levels or progesterone production (Yamamoto *et al.*, 2002; Shi *et al.*, 2010).

Taken together, all of these studies in the human follicle cells suggest that the secretion of BMPs from oocytes, GCs or theca/stroma cells inhibits StAR expression and, in turn, decreases progesterone production. On the other hand, oocyte-derived GDF9 interacts with pituitary gonadotrophins to further reduce progesterone production (Fig. 2). After ovulation, the dramatic decrease in GDF9 and BMP15 levels in the corpus luteum leads to elevated StAR expression and a subsequent increase in progesterone production.

BMPs modulate COC formation and expansion

During the antral follicle stage (characterized by the formation of a fluid-filled antrum), the original GCs that surround the oocyte differentiate into two functionally and anatomically distinct sublineages, the cumulus cells and mural GCs. The cumulus cells intimately interact with the oocyte to form an elaborate structure called the COC, while mural GCs are layered against the follicle wall and close to the basement membrane and theca/stroma cells (Albertini *et al.*, 2001). During the periovulatory stage, these two types of GC exhibit highly divergent characteristics. In general, cumulus cells are enriched for transcripts involved in cell proliferation and metabolism, whereas mural GCs are enriched for transcripts associated with cell differentiation and signaling (Wigglesworth *et al.*, 2015). In contrast to mural GCs, cumulus cells display a higher cell proliferation rate, a higher AMH expression level, a lower steroidogenic capacity, a lower LHR expression level and have the ability to secrete hyaluronic acid for COC expansion (cumulus expansion) (Armstrong *et al.*, 1996; Eppig *et al.*, 1997; Li *et al.*, 2000; Grondahl *et al.*, 2011). The cumulus cells associate with the oocyte and the extended viscoelastic extracellular matrix to form a unique structure, the hyaluronan-rich COC matrix (Russell and Robker, 2007). Hyaluronan is the structural backbone of this matrix, which is further stabilized by a complex network of binding proteins, including versican, tumor necrosis factor-stimulated gene 6 protein (TSG-6), inter-α trypsin inhibitor and PTX3 (Russell and Salustri, 2006; Baranova *et al.*, 2014).

At mid-cycle, the LH surge initiates the ovulatory process by decreasing cell–cell communication, resuming oocyte meiotic maturation, generating tissue remodeling and inducing the expansion of the COC (Russell and Robker, 2007). The cumulus expansion, characterized by a process of morphological change involved in the proliferation and dispersion of cumulus cells, is initiated under the control of several endocrine, paracrine and oocyte-derived factors (Eppig, 1980; Varani *et al.*, 2002; Russell and Robker, 2007; Fang *et al.*, 2013). *In vitro* studies have demonstrated the requirement of either the oocyte or its conditioned medium for the response of cumulus cells to EGF, FSH or cAMP in the synthesis of hyaluronan (Buccione *et al.*, 1990; Salustri *et al.*, 1990). In cumulus cells, the key enzyme responsible for the polymerization and elongation of the hyaluronan chains to localize into the intercellular space is a transmembrane protein, HAS2 (Weigel *et al.*, 1997). Indeed, the human BMP15 homodimer and mouse GDF9 are able to upregulate the cumulus expansion-related genes (*Has2*, *Ptx3* and *Ptgs2*) in mouse GCs and promote cumulus expansion *in vitro* (Peng *et al.*, 2013).

In the structure of the hyaluronan-based extracellular matrix, PTX3 plays a critical role in the assembly process. In this stable and sustainable hyaluronan network, PTX3 acts as an aggregating reagent to link to a molecule, TSG-6, which is further bound to the distinct hyaluronan strand (Baranova *et al.*, 2014). The knockout mouse model has been used to highlight the essential roles of PTX3 and cumulus expansion in the processes of oocyte maturation and ovulation, efficient transportation of the oocyte through the oviducts and *in vivo* fertilization (Vanderhyden and Armstrong, 1989). Mice lacking *Ptx3* display subfertility, structural defects of the COC and failed *in vivo* fertilization (Varani *et al.*, 2002). Our recent study showed that BMP4 and BMP7 down-regulated the expression and protein production of PTX3 in human GCs, indicating that BMPs may participate in extracellular matrix formation and tissue remodeling. Collectively, we can speculate that oocyte-secreted factors (mainly BMP15 and GDF9) maintain the cumulus cell phenotype by promoting cumulus expansion, whereas theca/stroma-derived BMPs (mainly BMP4 and BMP7) decompose the structure of the extracellular matrix in the neighboring mural GCs to facilitate the separation of COC and mural GCs in the human ovary (Fig. 2).

BMPs and luteal function

In the absence of pregnancy, the corpus luteum begins to regress at the end of the luteal phase, a degradation process called luteolysis (Hussein, 2005). Luteolysis is a complicated process involving a loss of the structural and functional integrity of the corpus luteum accompanied by a decrease in progesterone production (Hussein, 2005). Studies using human ovarian tissues demonstrated that the expression levels of BMP2, BMP4 and BMP6 are increased during luteal regression and they are differentially regulated by hCG, suggesting that these BMPs may be involved in the process of luteolysis (Nio-Kobayashi *et al.*, 2015) (Fig. 2).

Role of BMPs in female reproductive pathology

Any abnormality in the intra-ovarian BMPs or BMP signaling may negatively affect oocyte–somatic cell interactions, steroidogenesis, GC proliferation, oocyte maturation, cumulus expansion, ovulation, embryonic quality and luteal function, leading to female infertility and reproductive pathology. Knocking out of *Bmp4* or the depletion of *Alk2* in mouse embryos results in a lack of PGC formation (Lawson *et al.*, 1999). Genetic depletion of the *Bmp6* gene in female mice causes a decrease in number of ovulated eggs and reduced litter size (Sugiura *et al.*, 2010). Oocyte-derived BMP15 and GDF9 are the prominent BMP/GDF associated with various ovarian functions and ovulation rate (Persani *et al.*, 2014). Abnormal expression of these two factors may be related to female infertility (Gilchrist *et al.*, 2008). The results from clinical data have suggested that BMP15 levels in the follicular fluid may be used as an indicator of oocyte quality and subsequent fertilization ability (Wu *et al.*, 2007). In addition, a couple of BMP receptors have been shown to be involved in implantation and maternal–fetal interactions during human pregnancy. Disruption of BMPR2-mediated signaling in the uterine decidua leads to placental abruption, fetal demise and female sterility (Nagashima *et al.*, 2013). During mouse pregnancy, mice carrying a conditional ablation of *Alk2* in the uterus (*Alk2* cKO mice) exhibit delayed embryo invasion into the uterine stroma, failed uterine decidualization and sterility. Likewise, ablation of *ALK2* using siRNA targeted to *ALK2* in human uterine stromal cells may alter uterine decidualization and embryo implantation, leading to female sterility (Clementi *et al.*, 2013).

Polycystic ovary syndrome

Using immunohistochemical staining, the expression levels of BMP15 and GDF9 proteins in the oocyte and GCs of follicles in PCOS ovaries are reduced and delayed during the early follicular stage (Wei *et al.*, 2014). Compared with the control group, the expression levels of BMP15 and GDF9 proteins in the oocytes tended to be higher in women with PCOS, which could be the result of PCOS follicular dysplasia (Zhao *et al.*, 2010). However, the expression levels of GDF9 protein in cumulus cells are lower in PCOS women, which may result in premature luteinization, poor oocyte competence and luteal dysfunction, leading to higher miscarriage rates in PCOS patients (Zhao *et al.*, 2010). These results provide evidence of two valuable intra-ovarian growth factors in the regulation of oocyte maturation in the follicular microenvironment and will help in the design of a potential technique to improve IVM protocols for oocytes from women with PCOS (Qiao and Feng, 2011). Most serum BMP protein levels are either undetectable or at the lower limit of detection in PCOS women, and therefore provide limited information about this disease (van Houten *et al.*, 2013). The data generated from genome-wide association studies indicate that the involvement of *BMP15* or *GDF9* genetic variations in the etiology of PCOS remains controversial (Takebayashi *et al.*, 2000; Gonzalez *et al.*, 2008; Liu *et al.*, 2011; Persani *et al.*, 2014).

Primary ovarian insufficiency

Several *BMP15* gene mutations have been reported in primary ovarian insufficiency (POI) patients with primary or secondary amenorrhea (Takebayashi *et al.*, 2000; Di Pasquale *et al.*, 2004, 2006; Chand *et al.*, 2006; Dixit *et al.*, 2006; Persani *et al.*, 2010; Tiotiu *et al.*, 2010; Auclair *et al.*, 2013; Ferrarini *et al.*, 2013). The first missense mutation in the *BMP15* gene was identified in two Italian sisters with familial ovarian

dysgenesis and primary amenorrhea (Di Pasquale *et al.*, 2004). Compared to the normal human BMP15 protein, a recombinant BMP15 developed from this mutant gene fails to stimulate GC growth activity and is unable to inhibit progesterone production in primary human GCs (Auclair *et al.*, 2013). Like *BMP15*, several cohort studies involving different populations with POI have been conducted to screen for *GDF9* gene mutations (Dixit *et al.*, 2005; Laissue *et al.*, 2006; Kovanci *et al.*, 2007; Wang *et al.*, 2013; Simpson *et al.*, 2014). However, various missense mutations encoding different GDF9 mutant proteins may display contrasting results in functional studies. Some variants significantly decrease mature GDF9 protein production and diminish the ability of GDF9 to stimulate GC proliferation (Wang *et al.*, 2013). In contrast, other variants may encode a mutant GDF9 that significantly increases GC proliferation (Simpson *et al.*, 2014).

Endometriosis

Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity, which affects 10% of reproductive-aged women (Vigano *et al.*, 2004). Although many theories have been proposed, the precise pathogenesis and molecular mechanism of this common disease remain unknown (Bulun *et al.*, 2015). Immunohistochemical staining of endometriotic lesions obtained from 85 patients revealed that BMP6 is strongly expressed in both the epithelial and stromal cells (Athanasios *et al.*, 2012). A genome-wide profiling analysis comparing the gene expression of ectopic and eutopic endometrial tissues showed that BMP4 and its antagonist, GREM1, are dysregulated in endometriosis (Crispi *et al.*, 2013). Future studies aimed at addressing the functional roles of BMPs in the development of endometriosis will be of great interest.

Novel role of GDF8 in the human ovary

Originally identified in the musculoskeletal system, GDF8 is one of the members of the TGF-β superfamily (McPherron *et al.*, 1997). GDF8 and BMP11 (GDF11) are two closely related growth factors that share a similar structure and function (Gamer *et al.*, 1999). Also known as myostatin, GDF8 is a potent inhibitor of skeletal muscle growth and development (McPherron *et al.*, 1997). Mice carrying a targeted mutation of the *Gdf8* gene have an ~25–30% increase in muscle mass because of muscle fiber hypertrophy and hyperplasia (McPherron *et al.*, 1997). Naturally occurring *GDF8* gene mutations leading to increased muscle mass have been reported in several species, including cattle, sheep, dogs and humans (McPherron and Lee, 1997; Schuelke *et al.*, 2004; Clop *et al.*, 2006; Mosher *et al.*, 2007). In the last few years, there has been a growing interest in the investigation of GDF8's functional roles outside of the musculoskeletal systems. Notably, the expression and potential functions of GDF8 have recently been investigated in several reproductive organs, including the uterus and placenta (Islam *et al.*, 2014; Peiris *et al.*, 2014). Furthermore, data from clinical samples have highlighted the possible involvement of GDF8 in the pathogenesis of certain reproductive disorders, such as uterine myoma, pre-eclampsia and PCOS (Chen *et al.*, 2012; Guo *et al.*, 2012; Islam *et al.*, 2014).

GDF8 and ovarian steroidogenesis

In chicken embryos, GDF8 is extensively expressed in various tissues, including the gonads (testis and ovary) (Kubota *et al.*, 2007). In bovine ovaries, microarray analysis revealed the expression of *GDF8* in the GCs of different sizes of antral follicles (Skinner *et al.*, 2008). Our most recent immunohistochemistry analysis revealed that GDF8 and its putative functional receptors (ACVR2A, ACVR2B and ALK5) are expressed in the GCs of growing follicles in the human ovary (unpublished data). Moreover, the mature GDF8 protein is detectable in the follicular fluid obtained from IVF patients (Chang *et al.*, 2015d). In addition, recent studies have identified a novel role for GDF8 in the regulation of human GC steroidogenesis. Specifically, GDF8 regulates steroid production by increasing aromatase/estradiol production while decreasing StAR/progesterone production in primary hGL cells (Chang *et al.*, 2016b). Analysis of the data from clinical samples supports these findings, as there is a negative correlation between GDF8 concentrations and progesterone concentrations in the serum and follicular fluid (Fang *et al.*, 2015, 2016b). Moreover, GDF8 may enhance the FSH-induced aromatase/estradiol production, whereas GDF8 suppresses the LH-induced StAR/progesterone production in hGL cells (Chang *et al.*, 2016b). In this regard, GDF8 exerts a regulatory function by modulating GC responsiveness to gonadotrophins. Notably, GDF8 enhances GC responsiveness to FSH by up-regulating FSH receptor (FSHR) expression while suppressing GC responsiveness to LH by down-regulating LHR expression (Chang *et al.*, 2016b). In line with these results, our previous studies showed that activins (activin A, B and AB) may act in a similar manner to modulate steroidogenesis in the same cell model (Chang *et al.*, 2015b). The downstream signaling pathway may account for the similar cellular effects, as both GDF8 and activins act through SMAD2/3-SMAD4-mediated target gene regulation in human GCs (Fang *et al.*, 2015; Chang *et al.*, 2015b).

GDF8 and GC proliferation

During follicular development, GC proliferation and GC terminal differentiation are the key processes essential for oocyte maturation, ovulation and luteinization (Baerwald *et al.*, 2012). At the periovulatory stage, various regulatory endocrine and paracrine factors meticulously modulate the functional switch of GCs from a highly proliferative status to a non-proliferative, terminally differentiated status (McGee and Hsueh, 2000). Beyond its role in regulating steroid production, an *in vitro* study showed that GDF8 suppresses human GC proliferation (Chang *et al.*, 2016). This negative regulatory effect is mediated by another growth factor, connective tissue growth factor (CTGF), indicating that GDF8 and CTGF may be involved in the control of highly proliferative and non-proliferative events during human follicular development (Chang *et al.*, 2016).

GDF8 and extracellular matrix formation

Lysyl oxidase (LOX) is the key enzyme for the final assembly and stabilization of the extracellular matrix that is essential for follicle and oocyte maturation (Kagan and Trackman, 1991; Woodruff and Shea, 2007). In the rat ovary, the LOX expression level is positively correlated with oocyte quality (Jiang *et al.*, 2010). In many organs, CTGF is a principle mediator of extracellular matrix-related tissue remodeling

(Lipson et al., 2012). Both CTGF and LOX are expressed in human GCs, and CTGF mediates the GDF8-induced increase in LOX activity and LOX protein expression (Chang et al., 2016c). Interestingly, the GDF8-induced up-regulation of *CTGF* expression in human GCs occurs through the ALK5-mediated SMAD2/3-SMAD4 signaling pathway (Chang et al., 2016c). This finding is inconsistent with a previous study demonstrating that GDF8 signaling is mainly dependent on ALK4, but not ALK5, in mouse C2C12 myoblasts, indicating that the type I receptor-mediated GDF8 downstream signaling is cell-type specific (Kemaladewi et al., 2012). Specifically, GDF8 acts through ALK4 in myoblast cells, while ALK5 mediates the GDF8-induced cellular action in non-myogenic cells.

GDF8 and COC expansion

The occurrence of COC and the extent of cumulus expansion have been linked to oocyte competence and are potentially useful as indicators for oocyte selection in the IVF program (Ball et al., 1983; Foote, 1987). The combined results of the *in vitro* studies and the clinical data also revealed a positive correlation between *PTX3* expression levels in cumulus cells and their corresponding oocyte quality and subsequent fertilization rates (Zhang et al., 2005; Huang et al., 2013). Consistent with the effects of BMP4 and BMP7, GDF8 might modulate cumulus expansion by down-regulating the key linking protein, PTX3, in human GCs (Chang et al., 2015d).

Taken together, GDF8 is expressed in human GCs, and its mature proteins are detectable in follicular fluid. A series of functional studies have demonstrated the roles of GDF8 in regulating steroidogenesis, gonadotrophin responsiveness, cell proliferation, *LOX* expression, LOX activity and *PTX3* expression in human GCs (Fig. 3). These findings suggest that this unique TGF-β superfamily member might play a critical role in modulation of the final differentiation processes in the growing follicles, most likely by acting as both a maturation stimulator and a luteinization inhibitor.

Perspectives and therapeutic potential

A more comprehensive understanding of the physiological roles of BMPs/GDFs in the human ovary will provide significant insights into ovarian pathology and lead to new methods of approaching fertility regulation, whether the goal is to develop alternative forms of contraception, to diagnose and treat human infertility, or to develop safer and reliable protocols of inducing ovulation in ART. The involvement of BMP/GDF signaling pathways in a wide range of developmental and pathophysiological processes of reproductive biology makes targeting these pathways a potential therapeutic approach to overcome female infertility. At present, several recombinant human BMPs/GDFs and their inhibitors or antagonists have undergone clinical trials or have received US Food and Drug Administration (FDA) approval in human skeletal and muscular systems (Gautschi et al., 2007; Kinouchi et al., 2008; Lissenberg-Thunnissen et al., 2011; Smith and Lin, 2013; Lee and Wikesjo, 2014; Schmidt-Bleek et al., 2016). Furthermore, the latest development of gene therapy and BMP-ligand trapping approaches are currently attracting more attention (Kinouchi et al., 2008; Hawinkels et al., 2013; Sherman et al., 2013; Spiekerkoetter et al., 2013). All of the above information clearly reflects the availability of

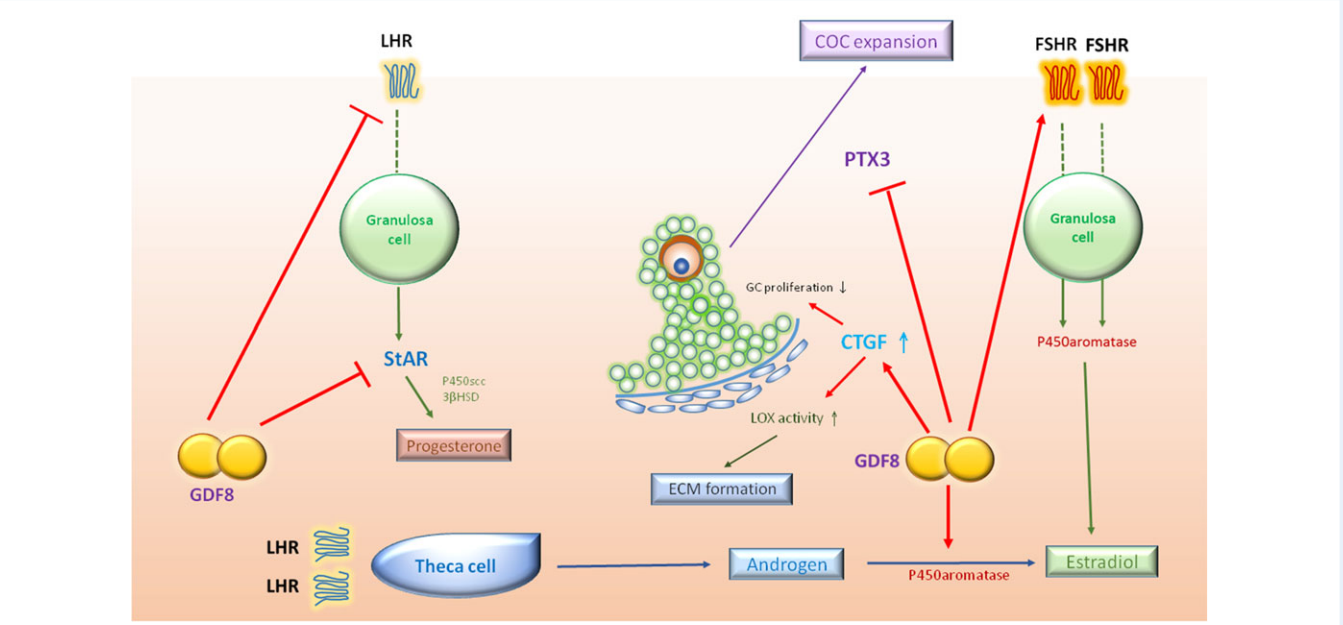

Figure 3 Schematic diagram summarizing potential roles of GDF8 in a human growing follicle. In this follicular microenvironment, the locally produced GDF8 may promote aromatase/estradiol and FSHR expression, suppress StAR/progesterone and LHR expression and down-regulate PTX3 expression. In addition, GDF8 induces the expression of CTGF, which contributes to the suppression of GC proliferation and the increase in LOX activity. 3βHSD, 3β-hydroxysteroid dehydrogenase; CTGF, connective tissue growth factor; ECM, extracellular matrix; LOX, lysyl oxidase; P450scc, P450 side-chain cleavage enzyme; PTX3, pentraxin 3; FSHR, FSH receptor; LHR, LH receptor.

potential new medications acting on the BMP/GDF pathways for the treatment of a variety of human diseases, including female reproductive pathologies. However, further human clinical trials are warranted to investigate the efficacy, safety and administration routes of these pharmaceutical applications.

Conclusion

In the last decade, research regarding locally produced growth factors and intra-follicular signaling between the oocyte and the follicle cells has been of considerable interest. The accumulated data indicate that the intra-ovarian BMP/GDF system is of great importance in controlling female reproduction, including PGC development, cell–cell communication, steroidogenesis, COC formation and expansion, oocyte maturation, ovulation and luteolysis. Thus, the relationship between the oocyte and its supporting cells (GCs and theca/stroma cells) should be regarded as a synchronous partnership. During follicular development, all of these cell types play essential, yet sometimes distinct, roles in regulating ovarian functions to generate a competent oocyte for embryo development. A comprehensive understanding of the expression, actions and underlying molecular mechanisms of the BMP/GDF system in the human ovary is critical to the development of clinical approaches (diagnosis and/or treatment) for women suffering from infertility and ovulation dysfunction.

Authors' roles

H.-M.C. collected the information, designed the pictures and wrote the manuscript. J.Q. collected the information and critically revised the manuscript. P.C.K.L. collected the information, designed the pictures and critically revised the manuscript. All the authors have seen and approved the final version.

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Conflict of interest

We declare that Hsun-Ming Chang, Jie Qiao and Peter C.K. Leung have no conflict of interest with the contents of this manuscript.

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