SMAD7 antagonizes key TGFβ superfamily signaling in mouse granulosa cells in vitro

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Abstract

Transforming growth factor β (TGFβ) superfamily signaling is essential for female reproduction. Dysregulation of the TGFβ signaling pathway can cause reproductive diseases. SMA and MAD (mothers against decapentaplegic) (SMAD) proteins are downstream signaling transducers of the TGFβ superfamily. SMAD7 is an inhibitory SMAD that regulates TGFβ signaling in vitro. However, the function of SMAD7 in the ovary remains poorly defined. To determine the signaling preference and potential role of SMAD7 in the ovary, we herein examined the expression, regulation, and function of SMAD7 in mouse granulosa cells. We showed that SMAD7 was expressed in granulosa cells and subject to regulation by intraovarian growth factors from the TGFβ superfamily. TGFβ1 (TGFβ1), bone morphogenetic protein 4, and oocyte-derived growth differentiation factor 9 (GDF9) were capable of inducing Smad7 expression, suggesting a modulatory role of SMAD7 in a negative feedback loop. Using a small interfering RNA approach, we further demonstrated that SMAD7 was a negative regulator of TGFβ1. Moreover, we revealed a link between SMAD7 and GDF9-mediated oocyte paracrine signaling, an essential component of oocyte–granulosa cell communication and folliculogenesis. Collectively, our results suggest that SMAD7 may function during follicular development via preferentially antagonizing and/or fine-tuning essential TGFβ superfamily signaling, which is involved in the regulation of oocyte–somatic cell interaction and granulosa cell function.

Reproduction (2013) 146 1–11

Introduction


To initiate signal transduction and elicit cellular responses, TGFβ ligands bind to their type 2 and type 1 receptors and activate intracellular SMA and MAD (mothers against decapentaplegic) SMAD proteins. Activation of SMAD2/3 or SMAD1/5/8 is generally associated with the respective TGFβ/activin signaling and bone morphogenetic protein (BMP) signaling (Chang et al. 2002). SMAD proteins are intracellular components of the TGFβ signaling pathway. In mammalian species, eight SMAD proteins have been identified and are classified into receptor-regulated SMADs (SMAD1, 2, 3, 5, and 8), the common SMAD (SMAD4), and inhibitory SMADs (SMAD6 and SMAD7).

Expression of SMADs in the ovary has been documented. In rats, SMAD2 and SMAD3 are strongly localized to the granulosa cells of preantral and small antral follicles. However, expression of SMAD2 and SMAD3 is low in large antral follicles (Xu et al. 2002). Phospho-SMAD2 (pSMAD2) is present in mouse granulosa cells of small and medium preantral follicles. Expression of pSMAD2, pSMAD3, and pSMAD1/5/8 is more uniform or stronger in the cumulus cells than in the mural granulosa cells (Tian et al. 2010), which suggests the involvement of oocyte paracrine factors in the regulation of SMADs. A recent study showed that SMAD6 is mainly localized to the oocyte, while SMAD7 is expressed in oocytes and granulosa cells from preantral and antral follicles (Quezada et al. 2012). In addition, a number of SMADs, including SMAD4, are expressed in the oocyte (Xu et al. 2002, Pangas et al. 2006, Tian et al. 2010, Quezada et al. 2012). The aforementioned studies point to a likely role of SMADs in folliculogenesis and/or oocyte development. However, the specific functions of these SMADs in the ovary remains elusive until cell-specific knockout mice for
SMADs were generated. Conditional ablation of Smad1 and Smad5 in ovarian granulosa cells leads to the development of metastatic granulosa cell tumors, which resemble human juvenile granulosa cell tumors (Pangas et al. 2008, Middlebrook et al. 2009). Thus, SMAD1 and SMAD5 act as tumor suppressors in the ovary. Mechanistically, tumor development is at least partially associated with the upregulation of platelet-derived growth factor alpha (PDGFA), which is controlled by SMAD1/5 and the trans-acting transcription factor 1 (SP1) (Tripurani et al. 2012). In contrast to SMAD1 and SMAD5, conditional deletion of Smad2 and Smad3 in granulosa cells causes fertility disorders including reduced ovulation and cumulus cell expansion. These defects are partially caused by the disruption of oocyte paracrine signaling that is mediated by growth differentiation factor 9 (GDF9) and its target genes associated with cumulus expansion (Li et al. 2008b). SMAD3 was reported to be required for normal follicle growth and ovarian cell differentiation and response to FSH when a different targeting construct was utilized (Tomic et al. 2002, Gong & McGee 2009). Interestingly, disruption of the signaling of the common SMAD, SMAD4, in ovarian granulosa cells results in subfertility and premature luteinization of granulosa cells, which is accompanied by defects in cumulus cell expansion and ovulation (Pangas et al. 2006). However, oocyte-specific knockout of Smad4 only causes a slight reduction in litter size, challenging a major role for TGFβ signaling in female germ cells (Li et al. 2012).

Despite the well-characterized functions of receptor-regulated SMADs and the common SMAD in the ovary, the role of inhibitory SMADs in female reproduction remains poorly defined. It has been suggested that SMAD6 preferentially inhibits BMP signaling (Hata et al. 1998, Ishisaki et al. 1999), whereas SMAD7 is capable of targeting TGFβ or both TGFβ and BMP pathways (Nakao et al. 1997, Souchelnytskyi et al. 1998, Ishisaki et al. 1999). The functions of inhibitory SMADs are being revealed by knockout mouse models. The Smad6 mutant mice showed partial lethality due to cardiovascular abnormalities (Galvin et al. 2000). In contrast, Smad7 knockout mice demonstrated variable phenotypes ranging from defective B cell response to renal fibrosis (Li et al. 2006, Chung et al. 2009, Chen et al. 2011), impaired cardiac functions (Chen et al. 2009), growth retardation, and a reduction in viability (Tojo et al. 2012).

As an initial effort to probe the reproductive function of SMAD7, we examined the expression and regulation of SMAD7 by intraovarian growth factors. Furthermore, we explored the role of SMAD7 in regulating TGFβ superfamily signaling in ovarian granulosa cells using a small interfering RNA (siRNA) approach. These studies identify SMAD7 as a negative regulator of key TGFβ superfamily signaling in mouse granulosa cells.

Materials and methods

Animals

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University (protocol number 2011-0007). Mice were maintained on C57BL/6/129S6/SvEv genetic background and were allowed to feed and drink ad libitum. All necessary procedures were taken to minimize the discomfort, distress, and pain to the mice.

Gonadotropin treatment

Mice (21 day) received injections of 5 IU PMSG for 48 h (n = 4) or both PMSG (48 h) and hCG (5 IU) (48 h; n = 3). Ovaries from age-matched untreated mice (n = 4 for each group) were used as controls. Collection of ovaries was performed under a stereomicroscope. The ovaries were used for immunohistochemical and RNA analyses as detailed below.

Granulosa cell isolation and culture

Primary mouse granulosa cell culture was described previously (Pangas et al. 2007, Li et al. 2009, 2011). Briefly, granulosa cells were collected in collection medium (DMEM containing 0.1% BSA (Sigma), 100 U/ml penicillin–streptomycin, and 10 mM HEPES) by puncturing large antral follicles using 26 gauge needles under a stereomicroscope. The cell suspension was filtered through a 40 μm nylon cell strainer (BD) to remove oocytes and debris. The cell pellet was then washed, resuspended, and cultured for the following experiments: i) recombinant protein treatment: granulosa cells were treated with control buffer, BMP4 (50 ng/ml; R&D), TGFβ1 (10 ng/ml; R&D, Minneapolis, MN, USA), and GDF9 (50 ng/ml) to examine Smad7 regulation and target gene induction. The production and purification of recombinant GDF9 were carried out as described previously (Li et al. 2009, 2011). For TGFβ1 treatment, granulosa cells were cultured overnight and then serum-starved for 24 h before addition of recombinant TGFβ1. The serum starvation procedure was included to eliminate the potential basal activity of TGFβ in the serum. ii) Small-molecule inhibitor assay: granulosa cells were pre-treated for 1 h with ALK2/3/6 inhibitor dorsomorphin (4 μM; Sigma) or ALK4/5/7 inhibitor SB-505124 (1 μM; Sigma). The cells were then incubated with recombinant BMP4 or GDF9 and collected after 5 h. Selection of the doses of the growth factors and incubation time was based on our pilot dose–response and time-course experiments and/or the published literature (Matsubara et al. 2000, Dahlqvist et al. 2003, Li et al. 2008b, 2009). Total RNA was isolated and quantitative PCR was performed to analyze Smad7 mRNA expression. iii) siRNA knockdown experiments, described below. All culture experiments were repeated at least three times.

Reproduction (2013) 146 1–11
RNA isolation and RT

Total RNA was extracted from the ovaries using RNA isolation reagent (TRI reagent; Sigma) or from mouse granulosa cells using an RNeasy Micro Kit (Qiagen) according to the manufacturer’s instructions (Li et al. 2009). RNA concentration was measured using a NanoDrop Spectrophotometer ND 1000 (NanoDrop Technologies, Wilmington, DE, USA). DNase I (Invitrogen) treatment was performed when using TRI reagent-derived RNA. On-column DNase (Qiagen) digestion was included during RNA isolation when using the RNeasy Micro Kit. For RT, 1 μg or 200 ng of the respective ovary and granulosa cell total RNA, random hexamers/oligo dT primers (Invitrogen), and superscript III (Invitrogen) reverse transcriptase were used. As negative controls, reactions where superscript III was substituted with water were included to monitor potential genomic DNA contamination.

Conventional PCR and quantitative real-time PCR

Smad7 was amplified from ovary cDNA by conventional RT-PCR using Jumpstart Taq polymerase (Sigma) and gene-specific primers (forward: AAGATGGTCCCTGG-TTCTCCAT CAAAGC; reverse: CTACCGGCTGTTGAA- gene-specific primers (forward: AAAGTGTTCCCTGG- and gene-specific primers (Table 1) or Taqman gene expression assays (Livak & Schmittgen 2001).

Immunohistochemistry

Immunohistochemistry was performed using an ABC kit (Vector Labs, Burlingame, CA, USA) as described elsewhere (Li et al. 2011). In brief, sections (5 μm) were prepared from paraffin-embedded tissue blocks and then deparaffinized in xylene and rehydrated in graded alcohol. To expose the antigen, antigen retrieval was carried out by boiling the sections in 10 mM citrate buffer (pH 6.0) for 20 min. The slides were treated with 0.3% (v/v) hydrogen peroxide to eliminate endogenous peroxidase activity. The sections were then blocked with 3% goat serum for 30 min and incubated with rabbit anti-SMAD7 antibody (Imgenex; 1:300) (Reynolds et al. 2008) at 4 °C overnight. After primary antibody incubation, the sections were washed and sequentially incubated with biotinylated anti-rabbit IgG (1 h) and ABC reagent (1 h; Vector Labs) at room temperature. Subsequently, the immunoreactive signals were developed using a DAB substrate kit (Vector Labs), followed by counterstaining with hematoxylin and mounted with Permount (Fisher, Fair Lawn, NJ, USA).

Small interfering RNA

Freshly prepared mouse granulosa cells were seeded onto 24-well plates (∼1×10^5 cells/well) in growth medium containing 5% fetal bovine serum (PAA Laboratories, Dartmouth, MA, USA) and insulin–transferrin–selenite (Sigma). The cells were cultured overnight before siRNA/vehicle transfection. Gapdh siRNA (Ambion, Carlsbad, CA, USA) was included as a positive control whereas an siRNA that has no significant sequence similarity to the mouse gene was used as a negative control (Ambion). A substantial knockdown of Smad7 was achieved using X-tremeGENE siRNA Transfection Reagent (Roche) and Smad7 silencer siRNA (73 nM; Ambion) in our granulosa cell culture system. Transfection of siRNA was conducted according to the manufacture’s protocol. After 48 h of transfection, granulosa cells were serum-starved for 24 h before adding TGFβ1. After 5 h treatment, the cells were collected in RNA lysis buffer, RNA was isolated using a Qiagen RNeasy Micro Kit, and gene expression was analyzed by qPCR.

Table 1 Primers for SYBR green-based real-time PCR.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smad7</td>
<td>Forward: GGGCTTTCAGATCCCACTTT</td>
<td>NA</td>
</tr>
<tr>
<td>Tgfb1</td>
<td>Forward: CGCAAGTCACTCCTCACAGC</td>
<td>PrimerBank</td>
</tr>
<tr>
<td>Pigs2</td>
<td>Forward: TGGAGAATCTTCCACACGAC</td>
<td>PrimerBank</td>
</tr>
<tr>
<td>Ptx3</td>
<td>Forward: CTTCGCCCTCTGCTTTG</td>
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<tr>
<td>Id1</td>
<td>Forward: ACAGACATACACGCGGTC</td>
<td>Birkenkamp et al. (2007)</td>
</tr>
<tr>
<td>Id2</td>
<td>Forward: ATGAAAGCTTCAGCCGGTG</td>
<td>PrimerBank</td>
</tr>
<tr>
<td>Id3</td>
<td>Forward: CTGCACATCTCCGCGGTG</td>
<td>RTPrimerDB</td>
</tr>
<tr>
<td>Gapdh</td>
<td>Forward: CATTGTCCTCGTGCACT</td>
<td>Li et al. (2011)</td>
</tr>
</tbody>
</table>
Statistical analyses

One-way ANOVA was performed to determine the difference among groups. When a significant difference was detected by ANOVA, the difference between means was further assessed by a post hoc Tukey’s HSD test. Comparison of means between two groups was made by $t$-test. Data are shown as mean $\pm$ S.E.M. Statistical significance was defined at $P<0.05$.

Results

**SMAD7 is expressed in mouse granulosa cells**

As a first step to define the potential ovarian function of SMAD7, we examined the expression of SMAD7 in mouse granulosa cells. RT-PCR analysis revealed that mouse granulosa cells expressed *Smad7* transcripts (Fig. 1A). In agreement with the RT-PCR result (Fig. 1A), SMAD7 was detected in primary, secondary, and antral follicles by immunohistochemistry (Fig. 1B). Furthermore, ovarian *Smad7* mRNA abundance was reduced after 48 h of pregnant mare’s serum gonadotropin (PMSG) injection and 48 h of PMSG plus 48 h of human chorionic gonadotropin (hCG) injection compared with age-matched non-treated controls (Fig. 1F). Immunoreactive SMAD7 staining remained detectable in granulosa cells after gonadotropin injection compared with those in the follicles (Fig. 1D and E).

**TGFβ superfamily ligands induce Smad7 in mouse granulosa cells**

Real-time PCR demonstrated that TGFβ1 (TGFβ1) (10 ng/ml) induced *Smad7* mRNA expression in mouse granulosa cells within 5 h after treatment (Fig. 2A). BMP4 (50 ng/ml), a thecal cell-secreted protein, increased *Smad7* transcript levels more than fivefold in granulosa cells compared with vehicle-treated controls (Fig. 2B). Incubation of mouse granulosa cells with 50 ng/ml of GDF9, an oocyte-derived factor, resulted in significantly higher *Smad7* mRNA levels than controls (Fig. 2C). To gain further mechanistic understanding of *Smad7* induction by the aforementioned TGFβ family ligands, we performed small-molecule inhibitor assays. The small-molecule compound 2-(5-benzo[1,3]dioxol-5-yl-2-tert-butyl-3H-imidazol-4-yl)-6-methylpyridine hydrochloride (SB-505124) selectively inhibits activin-like kinase (ALK)4, ALK5, and ALK7 activity and blocks the activation of SMAD2/3 (DaCosta Byfield et al. 2004). Dorsomorphin is a selective inhibitor of BMP type I receptors ALK2, ALK3, and ALK6 and suppresses BMP-induced SMAD1/5/8 phosphorylation (Yu et al. 2008). Using these small-molecule inhibitors, we demonstrated that SB505124 (1 μM) suppressed GDF9-induced *Smad7* expression (Fig. 2D). Similarly, dorsomorphin (4 μM) significantly reduced BMP4-promoted *Smad7* transcript expression (Fig. 2E).

**Knockdown of Smad7 did not affect BMP4-induced inhibitor of DNA binding (Id) gene and inhibin beta-B (Inhbb) expression**

TGFβ1 significantly increased pentraxin 3 (*Ptx3*), prostaglandin-endoperoxide synthase 2 (*Ptgs2*), and tumor necrosis factor alpha-induced protein 6 (*Tnfaip6*) mRNA transcript expression in mouse granulosa cells (Fig. 3A). Upregulation of *Id1*, *Id2*, and *Id3* mRNA by BMP4 in mouse granulosa cells was demonstrated (Fig. 3B). Moreover, *Ptx3* (Li et al. 2011), *Tnfaip6*, and *Ptgs2* were upregulated by purified GDF9 recombinant

Figure 1 Expression and localization of SMAD7 in mouse ovary. (A) *Smad7* mRNA was expressed in mouse granulosa cells. RT Neg, no reverse transcriptase. (B, C, D, and E) Immunostaining of SMAD7 using ovaries from untreated immature mice (B) and PMSG (48 h) + hCG (48 h)-treated mice (D) and controls (E). Negative control without primary antibody is depicted in (C). Note that omission of the primary antibody abolished the SMAD7 signals. GC, granulosa cell; CL, corpus luteum. Arrows indicate follicles. Scale bar = 50 μm (B and C) and 100 μm (D and E). (F) Gonadotropin administration reduced *Smad7* mRNA levels in the ovary. Total RNA was isolated from ovaries of age-matched untreated controls (CTRL; n = 4/group), PMSG (48 h)-treated (n = 4), and PMSG (48 h)/hCG (48 h)-treated mice (n = 3). Gene expression was analyzed by qPCR using the ΔΔCT method. *Smad7* mRNA levels in the treatment group were expressed as fold change of the corresponding age-matched controls. *P<0.05 vs the corresponding CTRL.
Role of SMAD7 in ovarian granulosa cells

In contrast to the effect of Smad7 knockdown on BMP4 signaling, we found that stimulation of Smad7 siRNA-treated granulosa cells with TGFβ1 enhanced TGFβ1-induced gene expression (Fig. 5). Figure 5A and B depicts the experimental protocol utilized and the validation of Smad7 knockdown respectively. As expected, granulosa cells treated with TGFβ1 and negative siRNA showed increased Ptx3, Ptgs2, and Tnaiap6 mRNA levels (Fig. 5C, D, and E). Interestingly, a further increase in Ptx3, Ptgs2, and Tnaiap6 transcript levels was found in TGFβ1-treated granulosa cells that were incubated with Smad7 siRNA (P<0.05; Fig. 5C, D, and E). However, a similar effect of Smad7 siRNA on TGFβ1-induced Serpine1/plasminogen activator inhibitor-1 (PAI1) was not detected (data not shown).

SMAD7 negatively influences GDF9-mediated oocyte paracrine signaling

To test the hypothesis that SMAD7 antagonizes GDF9 signaling in mouse granulosa cells, we performed siRNA assays using mouse primary granulosa cell culture (Fig. 5A). As anticipated, Ptx3 was induced by GDF9 in the presence of negative siRNA (Fig. 5G). We demonstrated that knockdown of Smad7 (Fig. 5F) caused a significant increase in GDF9-induced Ptx3 mRNA expression compared with controls (P<0.05; Fig. 5G). A similar effect of Smad7 knockdown on GDF9-induced Ptgs2 (Fig. 5H) and Tnaiap6 (Fig. 5I) expression was also found. A role of SMAD7 in modulating growth factor signaling in granulosa cells is proposed in Fig. 6.

Discussion

TGFβ ligands signal through type 2 and type 1 receptors and downstream SMAD proteins, which regulate gene transcription in concert with co-activators and co-repressors (Massague 2000). TGFβ signaling is regulated at multiple levels including ligand traps (e.g. Chordin and Noggin), inhibitory SMADs (i.e. SMAD6 and SMAD7), and interactive pathways such as the MAP kinase (MAPK) pathway (Massague 2000).
SMAD7 is an inhibitory SMAD that antagonizes TGFβ and BMP signaling in vitro (Nakao et al. 1997, Yanagisawa et al. 2001). SMAD7 is functionally distinct from SMAD6 (Yan et al. 2009, Yan & Chen 2011). We demonstrated the expression of SMAD7 in mouse granulosa cells, which confirmed findings by Quezada et al. (2012). As gonadotropins are key regulatory signals for follicular development, we examined the effect of exogenous gonadotropin administration on Smad7 gene expression. We showed that Smad7 mRNA was downregulated in the ovary by gonadotropin administration. It is plausible that the reduced Smad7 expression by gonadotropin exposure could potentially facilitate follicular development and/or luteal formation. However, regulation of Smad7 by FSH was not observed in mouse granulosa cells cultured in vitro (Quezada et al. 2012). Thus, the physiological significance of this finding remains to be identified.

SMAD7 is induced by TGFβ signaling in several cell types (Nakao et al. 1997, Afrakhte et al. 1998, Stopa et al. 2000). As the ovary is a rich source of a variety of TGFβ ligands that are produced from distinct cellular compartments and are essential regulators of ovarian function, we sought to determine whether Smad7 is induced by these growth factors in ovarian granulosa cells. Smad7 is known to be induced by TGFβ ligands (Afrakhte et al. 1998), and we were able to confirm that Smad7 is a target of TGFβ1 in mouse granulosa cells (Quezada et al. 2012). We also showed that Smad7 was induced by BMP4 in mouse granulosa cells. Moreover, Smad7 mRNA levels were rapidly increased by GDF9, which is an oocyte-secreted TGFβ family protein and a key regulator of granulosa cell function (Dong et al. 1996, Yan et al. 2001, Otsuka et al. 2011). These data suggest that SMAD7 may modulate GDF9-mediated oocyte paracrine signaling essential for coordinating the cross talk between ovarian somatic cells and germ cells. It has been shown that the ALK4/5/7 inhibitor SB431542 attenuates TGFβ1-induced Smad7 mRNA expression in ovaries.
mouse granulosa cells (Quezada et al. 2012). We further demonstrated that TGFβ and BMP type 1 receptors were involved in the induction of Smad7 by GDF9 and BMP4 respectively. In contrast to the aforementioned findings, Smad6 mRNA was not affected by GDF9 treatment (data not shown), suggesting a distinct role of the two inhibitory SMADs in GDF9-mediated signaling. As further support, Smad6 is induced by BMP15 but not TGFB1 in granulosa cells (Li et al. 2009, Quezada et al. 2012). Collectively, these results suggest that SMAD7 may act as a negative regulator of TGFβ superfamily signaling in mouse granulosa cells. Thus, the preference of SMAD7 in antagonizing TGFβ superfamily signaling was further exploited in this study.

Although major functions of the BMP and TGFβ signaling pathways in mouse granulosa cells have been identified (Li et al. 2008b, Pangas et al. 2008), the role of SMAD6 in each signaling cascade remains elusive. Id genes are direct targets of BMPs (Hollnagel et al. 1999, Korchynskyi & ten Dijke 2002). Although BMP4 potently induced Smad7 expression, we did not find a significant effect of Smad7 knockdown on BMP4-stimulated Id gene expression in the current study, which suggests that SMAD7 may not act as a direct antagonist for BMP4 signaling in mouse granulosa cells. However, the role of SMAD6 in regulating BMP4 signaling in mouse granulosa cells remains to be elucidated. In contrast to the findings from the BMP4 studies, we found that TGFB1-induced expression of the Ptx3, Ptg2, and Tnlaip6 genes was potentiated in Smad7 siRNA-treated granulosa cells, which implies an inhibitory role of SMAD7 in TGFβ signaling in mouse granulosa cells. Based on the fact that SMAD7 forms a complex with activated type 1 receptors to inhibit receptor-regulated SMAD (R-SMAD) phosphorylation (Hayashi et al. 1997, Nakao et al. 1997, Mochizuki et al. 2004), it is plausible that reduction of SMAD7 may enhance TGFβ type 1 receptor-SMAD2/3 signaling activity in granulosa cells. Intriguingly, a similar effect of Smad7 knockdown on TGFB1-induced Serpine1/PAI1, a known TGFβ target gene, was not detected. However, a p38 inhibitor significantly reduced TGFB1-induced PAI1 expression (Y Gao and Q Li 2012, unpublished observations), suggesting that the p38 MAPK pathway is involved in TGFB1-induced PAI1 expression in mouse granulosa cells. Involvement of p38 MAPK in TGFβ-induced PAI1 expression has been
observed in other cell types (Liao et al. 2001, Vayalil et al. 2007). Collectively, these results suggest a possible role for SMAD7 in controlling TGFβ type 1 receptor-
SMAD2/3 signaling activity in mouse granulosa cells.

Of particular interest is the finding that GDF9 signaling is modulated by SMAD7 in granulosa cells, because oocyte-derived TGFβ superfamily ligands (e.g. BMP15 and GDF9) are pivotal mediators of oocyte paracrine signaling essential for folliculogenesis and oocyte developmental competence (Hussein et al. 2006, Li et al. 2008a, Yeo et al. 2008). Within a follicle, bidirectional intercellular communications between the oocyte and companion somatic cells are established via gap junctions and paracrine signaling and are indispensable to normal ovarian function (Eppig et al. 1997, Eppig 2001, Gilchrist et al. 2008, Li et al. 2008a). GDF9 and BMP15 are oocyte paracrine factors whose functions in follicular development have been well elaborated (Dong et al. 1996, Vitt et al. 2000, Otsuka et al. 2001, Yan et al. 2001, Otsuka & Shimasaki 2002, Shimasaki et al. 2004, McNatty et al. 2005, Gilchrist et al. 2006, Yoshino et al. 2006, Sugiuira et al. 2007, Edwards et al. 2008, Su et al. 2008). GDF9 is expressed in oocytes in mice and humans (McGrath et al. 1995, Aaltonen et al. 1999) and plays a key role in normal follicular development (Elvin et al. 1999, Orisaka et al. 2006).

Interestingly, our data indicated that knockdown of
Smad7 in mouse granulosa cells significantly enhanced GDF9-induced expression of Ptgs2, which is a cumulus expansion-related transcript as well as a potential granulosa cell marker of oocyte competence (Zhang et al. 2005). In addition, induction of Tnaiap6 and Ptgs2 by GDF9 was potentiated in Smad7 siRNA-treated granulosa cells, suggesting increased GDF9 signaling activity in the absence of SMAD7. As enhanced oocyte paracrine signaling in ovarian somatic cells could promote oocyte competence during in vitro maturation (IVM; Hussein et al. 2006), it is tempting to speculate that targeting Smad7 in a temporal and spatial manner could be potentially utilized to benefit oocyte development in assisted reproductive technology clinics.

Despite the above findings, the functional requirement for Smad7 in mouse granulosa cells warrants further investigation using Smad7 mutant mouse models. Currently, there are conflicting reports on the phenotype of Smad7 knockout mice, which is potentially due to incomplete Smad7 loss-of-function in some mutant mice. Four Smad7 mutant mouse lines have been established (Li et al. 2006, Chen et al. 2009, Kleiter et al. 2010, Tojo et al. 2012). Deletion of exon 1 of Smad7 gene (Li et al. 2006) results in a fertile phenotype. Another Smad7 knockout model was generated by deletion of exon 4 encoding the entire MH2 domain (Chen et al. 2009). Unlike the exon 1 knockout mice, the majority of Smad7 exon 4 knockouts died in utero because of defective cardiovascular development (Chen et al. 2009). Two of the Smad7 mutant mouse lines were recently described (Kleiter et al. 2010, Tojo et al. 2012). In one report, the promoter region and exon 1 of the Smad7 gene were flanked by LoxP sites and could be deleted upon Cre-mediated recombination. In contrast to the previous reports, Smad7 homozygous mice generated by this targeting strategy display complete embryonic mortality, which suggests that deletion of the promoter region and exon 1 of the Smad7 gene probably creates a null mutation (Kleiter et al. 2010). In the other report, disruption of both the MH2 domain and poly(A) signal sequence of Smad7 in mice causes either early postnatal mortality or growth retardation depending on the genetic background of the mice (Tojo et al. 2012). Despite the phenotypic variability of Smad7 mutant mouse models, the overall results point to a role for SMAD7 in the essential processes of growth and development. Results from the current study provide a rationale for further functional analysis, which would allow for an unequivocal assessment of the role of Smad7 and its functional redundancy with Smad6, if any, in ovarian somatic cells.

In summary, our findings, together with a recent report that Smad7 mediates TGFβ-induced apoptosis in ovarian granulosa cells (Quezada et al. 2012), highlight Smad7 as a potential regulator of ovarian function. Results of this study suggest that Smad7 may function during follicular development through antagonizing and/or fine-tuning essential TGFβ superfamily signaling, which is involved in the regulation of oocyte–somatic cell interaction and granulosa cell function. Therefore, targeting inhibitory SMAD signaling may represent a strategic approach to manipulate the TGFβ signaling pathway in female reproduction.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Reproduction (2013) 146 1–11

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Role of SMAD7 in ovarian granulosa cells 11

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Reproduction (2013) 146 1–11

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