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Progesterone decreases bone morphogenetic protein (BMP) 7 expression and BMP7 inhibits decidualization and proliferation in endometrial stromal cells

Ako Kodama, Osamu Yoshino, Yutaka Osuga[1], Miyuki Harada, Akiko Hasegawa, Kahori Hamasaki, Masashi Takamura, Kaori Koga, Yasushi Hirota, Tetsuya Hirata, Yuri Takemura, Tetsu Yano, and Yuji Taketani

Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

[1]Correspondence address. Tel: +81-3-3815-5411; Fax: +81-3-3816-2017; E-mail: yutakaos-tky@umin.ac.jp

BACKGROUND: Regulation of decidualization is decisive for proper implantation and the establishment of pregnancy. Recent studies have suggested that several bone morphogenetic proteins (BMPs) play physiological roles in reproduction. In the present study, we examined the expression of BMP7 in the endometrium and the effect of BMP7 on decidualization and proliferation of endometrial stromal cells (ESC).

METHODS: The gene expression of BMP7 in endometrial tissues collected from women with regular menstrual cycles was determined and the effect of ovarian steroid hormones on BMP7 gene expression was investigated in cultured ESC. The effect of BMP7 on the decidualization of ESC was determined by measuring the gene expression and protein secretion of insulin-like growth factor binding protein 1 (IGFBP1), a marker of decidualization. The effect of BMP7 on the proliferation of ESC was examined by the bromodeoxyuridine (BrdU) incorporation assay.

RESULTS: The gene expression of BMP7 in endometrial tissues was low at and after the mid-secretory phase of the menstrual cycle. Progesterone suppressed the gene expression of BMP7 in cultured ESC. Treatment with progesterone and estradiol for 12 days achieved decidualization of ESC, increasing the gene expression and protein secretion of IGFBP1. Addition of BMP7 protein to the culture almost completely inhibited these increases. BMP7 suppressed BrdU incorporation in ESC, which indicated an antiproliferative effect of BMP7 on ESC.

CONCLUSIONS: Progesterone-induced suppression of BMP7 and BMP7-induced inhibition of decidualization and proliferation of ESC suggest an elaborate regulatory mechanism for decidualization through BMP7 in the endometrium.

Key words: BMP7 / IGFBP1 / progesterone / decidualization / proliferation

Introduction

The endometrium undergoes dynamic changes during the menstrual cycle. Proper endometrial changes are essential for successful implantation, and aberrant endometrial status may lead to implantation failure. In addition to ovarian steroids, which have a central role in the regulation of morphological and functional changes to the endometrium, there are many local factors that modulate endometrial status (Kayisli *et al*., 2004; Dimitriadis *et al*., 2005).

Bone morphogenetic proteins (BMPs), together with growth differentiation factors (GDFs), comprise a subfamily of the transforming growth factor-β superfamily. BMPs and GDFs are multifunctional growth factors and their effects have been reported mainly in bone, cartilage, ligament and tendon formation (Francis-West *et al*., 1999). However, BMPs and GDFs have also been demonstrated to control cellular proliferation, differentiation and apoptosis in reproductive tissues (Shimasaki *et al*., 2004).

Gene expression of BMP2 (Ying and Zhao, 2000), BMP4 (Ying and Zhao, 2000), BMP6 (Lyons et al., 1989), BMP7 (Ozkaynak et al., 1997; Paria et al., 2001), GDF9 (Fitzpatrick et al., 1998) and GDF10 (Zhao et al., 1999) has been reported in the mouse uterus. These BMPs are expressed in a different spatiotemporal pattern and are thus speculated to have specific functions in the uterus. Mice deficient in ALK6, the receptor for these BMPs, have an abnormal endometrium and are infertile (Yi et al., 2001). A recent study has further demonstrated the presence of BMP2, BMP4, BMP7, GDF5, GDF8 and GDF11 in the human endometrium (Stoikos et al., 2008). BMP7 is unique among these BMPs in that its mRNA is lost from the uterine epithelium shortly after implantation in mice (Ozkaynak et al., 1997). In the human, gene expression of BMP7 has been reported in cultured endometrial stromal cells (ESC), with the expression level not being changed by cAMP-induced decidualization (Stoikos et al., 2008). In addition, immunostaining of human biopsied specimens have shown that BMP7 can be detected in highly decidualized cells with a vesicle staining pattern but not in first trimester deciduas (Stoikos et al., 2008).

Although these findings imply a functional role for BMP7 in endometrial physiology, to date there have been no studies examining the effects of BMP7 on the endometrium. To determine the possible roles of BMP7 in the human endometrium, in the present study, we first examined the gene expression of BMP7 in the endometrium. We then studied the effects of BMP7 on decidualization of ESC, measuring insulin-like growth factor binding protein 1 (IGFBP1) as a marker of decidualization (Harada et al., 2006). We also examined the effects of BMP7 on proliferation of ESC.

Materials and Methods

Patients and samples

Endometrial tissue was obtained from 39 women, either by curettage under sterile conditions or from women undergoing hysterectomy for benign gynecologic disease. The mean ($\pm$SD) age of the women was 37.8 $\pm$ 8.2 years. All women had regular menstrual cycles and none had received hormonal treatment within the 6 months prior to surgery. The specimens were dated according to the women's menstrual history. In order to avoid contamination with trophoblast cells, decidual tissues were collected from five women with ectopic pregnancy but without uterine bleeding, by dilation and curettage according to previous studies (Koga et al., 2001; Hirota et al., 2005). The experimental procedures were approved by the institutional review board of the University of Tokyo, and all women provided written informed consent for the use of their endometrial tissue.

Isolation and culture of human ESC

ESC were isolated and cultured as described previously (Koga et al., 2001; Yoshino et al., 2003). Fresh endometrial biopsy specimens collected in sterile medium were rinsed to remove blood cells. Tissues were minced into small pieces and incubated in DMEM/F-12 containing type I collagenase (0.25%; Sigma, St Louis, MO, USA) and deoxynuclease I (15 U/ml; Takara, Tokyo, Japan) for 60 min at 37°C. The resulting dispersed endometrial cells were separated by filtration through a 40-μm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ, USA). Any intact endometrial epithelial glands that remained were retained by the strainer, whereas dispersed ESC passed through the strainer into the filtrate. ESC in the filtrate were collected by centrifugation at 250*g* and resuspended in phenol red-free DMEM/F-12 containing 5% charcoal-stripped fetal bovine serum (FBS), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 0.25 μg/ml amphotericin B. The ESC were seeded in a 100-mm culture plate and kept at 37°C in a humidified atmosphere of 5%CO_2−95% air. At the first passage, cells were plated at a density of 1×10^5 cells/ml into 12- or 96-well culture plates (Becton Dickinson) and used for further treatments.

Treatment of ESC

To determine the effects of estrogen and progesterone on the gene expression of BMP7 in ESC, ESC were treated with 2.5% charcoal/dextran-treated (stripped) FBS (HyClone, Logan, UT, USA) in the presence of estradiol (10 ng/ml) or progesterone (100 ng/ml) for 6, 12 and 24 h. To examine the effect of BMP7 on decidualization, *in vitro* decidualization was achieved as described previously (Koga et al., 2001). Briefly, after cells had reached 70% confluence in 12-well culture plates, they were rinsed and treated with 2.5% charcoal/dextran-treated (stripped) FBS in the presence of estradiol (10 ng/ml) plus progesterone (100 ng/ml) or 0.1% ethanol vehicle (control) for 12 days. BMP7 (0, 10 or 100 ng/ml; R&D Systems, Minneapolis, MN, USA) was also added to the culture medium. Culture media were collected and replenished every 3 days.

RNA extraction, reverse transcription and real-time quantitative PCR

Total RNA was extracted from endometrial tissues and ESC using an RNeasy Mini Kit (Qiagen, Hilden, Germany). After reverse transcription, real-time quantitative PCR and data analysis were performed using a LightCycler (Roche Diagnostic, Mannheim, Germany), as reported previously (Harada et al., 2006). Expression of BMP7 and IGFBP1 mRNA was normalized for RNA loading for each sample using human glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Toyobo) mRNA as an internal standard. The BMP7 prrimers chosen (sense: 5′-GCCTACTACTGTGA GGGGGAG -3′; antisense: 5′-GAAGTAGAGGACGGAGATGGC-3′) amplified a 163-bp fragment. The IGFBP1 primers chosen (sense: 5′-GA GAGCACGGAGATAACTGAGG-3′; antisense: 5′-TTGGTGACATGGA GAGCCTTCG-3′) amplified a 131-bp fragment. The PCR conditions were as follows: for BMP7, 40 cycles of: 95°C for 10 s, 64°C for 10 s and 72°C for 4 s; for IGFBP1, 40 cycles of: 95°C for 10 s, 67°C for 10 s and 72°C for 5 s; for GAPDH, 30 cycles of: 95°C for 10 s, 64°C for 10 s, 72°C for 18 s. All PCR conditions were followed by melting curve analysis.

Measurement of IGFBP1 protein

Concentrations of IGFBP1 in the conditioned media were determined using a specific ELISA kit (R&D Systems, Minneapolis, MN, USA). The limit of sensitivity of the kit was 31.3 pg/ml. The concentrations measured were normalized against the total protein of cell lysates from each well of the culture plates.

5-Bromo-2′-deoxyuridine proliferation assay

The bromodeoxyuridine (BrdU) proliferation assay was performed as described previously (OuYang et al., 2008) using the Biotrak Cell Proliferation ELISA System (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. Briefly, after incubation of ESC in serum-free medium for 24 h in 96-well plates, cells were treated for a further 24 h with serum-free medium containing either BMP7 (0, 10, 100 ng/ml) or 20% charcoal-stripped FBS as a positive control. After the 24 h incubation, 100 μl BrdU solution was added and cells were incubated at 37°C for an additional 2 h.

Statistical analysis

Expression of BMP7 mRNA in endometrial tissues was analyzed by the Kruskal–Wallis test, whereas other data were analyzed by ANOVA. Both tests were followed by *post hoc* analysis for multiple comparisons. $P < 0.05$ was considered significant.

Results

Expression of BMP7 mRNA in endometrial tissue throughout the menstrual cycle and in progesterone- and estradiol-treated ESC

As shown in Fig. 1, expression of BMP7 mRNA in endometrial tissues was significantly lower in the mid- and late secretory phases and in the decidua compared with expression in the mid-proliferative phase. In cultured ESC, treatment with progesterone, but not estradiol, decreased BMP7 mRNA expression at 12 and 24 h, compared with 0 h, in a time-dependent manner (Fig. 2A). Long-term culture of ESC in the presence of progesterone and estradiol remarkably decreased BMP7 mRNA expression on Day 3 and later, and distinctly induced IGFBP1 mRNA expression on Day 12 (Fig. 2B).

Effect of BMP7 on gene expression and secretion of IGFBP1 from ESC

Treatment with estradiol and progesterone for 12 days induced IGFBP1 mRNA expression in ESC. However, the addition of 10 and 100 ng/ml BMP7 to the culture medium markedly decreased the expression of IGFBP1 mRNA induced by the hormonal treatment in ESC (Fig. 3A). Figure 3B shows secretion of IGFBP1 protein from ESC, which was induced by estradiol and progesterone treatment on Day 9 and was increased to higher levels on Day 12. The addition of BMP7 to the culture medium markedly reduced IGFBP1 protein secretion, to almost undetectable levels in the presence of 100 ng/ml BMP7.

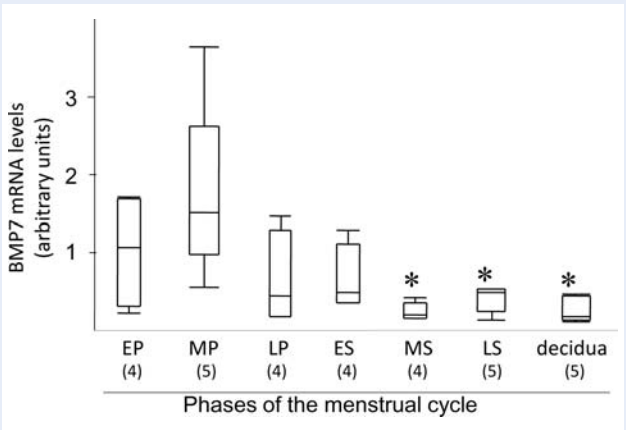

Figure 1 Expression of BMP7 mRNA in human endometrial tissues throughout the menstrual cycle and in early pregnant decidua.

Total RNA extracted from endometrial tissues and decidual tissues of ectopic pregnancies was reverse transcribed and then amplified by real-time PCR using primers for BMP7. Values were calculated by subtracting data for signal threshold cycles (Ct) of the internal standard (GAPDH) from Ct values for BMP7. The boxes represent the 25th and 75th percentiles. The median is denoted by the line that bisects the boxes. The whiskers indicate the extent of the data on the 1.5× interquartile range. *$P < 0.05$ compared with the MP. EP, early proliferative phase; MP, mid-proliferative phase; LP, late proliferative phase; ES, early secretory phase; MS, mid-secretory phase; LS, late secretory phase. The number of samples is shown in parentheses.

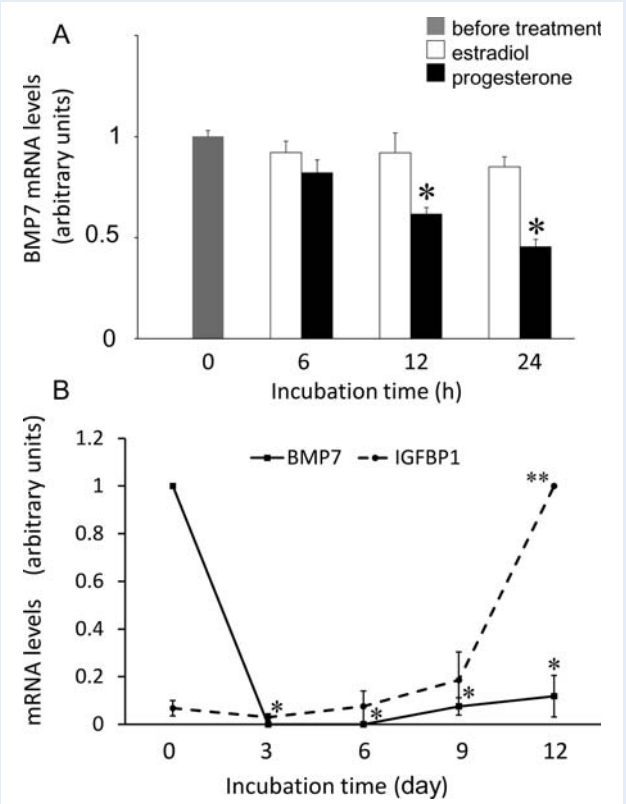

Figure 2 (**A**) Expression of BMP7 mRNA in ESC treated with estrogen (10 ng/ml) or progesterone (100 ng/ml) for 24 h. Data are the mean ± SEM of combined data from three independent experiments using different ESC from three patients. (**B**) Expression of BMP7 and IGFBP1 mRNA in ESC. *In vitro* decidualization of ESC was achieved by culturing ESC in the presence of estrogen (10 ng/ml) and progesterone (100 ng/ml) for 12 days. Data are the mean ± SEM of combined data from three independent experiments using different ESC from three patients. Total RNA isolated from ESC was reverse transcribed and then amplified by real-time PCR using primers for BMP7, IGFBP1 and GAPDH. Values were calculated by subtracting data for signal threshold cycles (Ct) of the internal standard (GAPDH) from Ct values for BMP7 or IGFBP1. (A) *$P < 0.05$ compared with 0 h. (B) *$P < 0.05$ compared with Day 0 (BMP7); **$P < 0.05$ compared with Day 0 (IGFBP1).

Effect of BMP7 on ESC proliferation

BMP7 at 10 and 100 ng/ml decreased BrdU incorporation in ESC by 20.5 ± 4.1 and 29.9 ± 4.2% (mean ± SEM of six replicate cultures) of the untreated controls, respectively (both $P < 0.05$ compared with the control), although 20% charcoal-stripped FBS increased BrdU incorporation by 134.8 ± 11.2%.

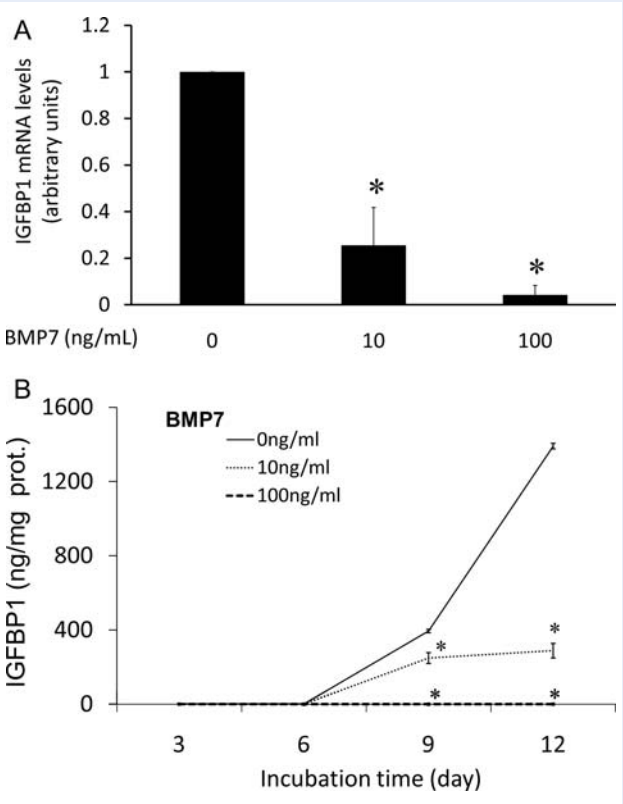

Figure 3 Effects of BMP7 on gene expression and protein secretion of IGFBP1 from ESC. (**A**) Effects of 10 and 100 ng/ml BMP7 on IGFBP1 mRNA expression in ESC treated with a combination of 10 ng/ml estradiol plus 100 ng/ml progesterone (EP) for 12 days. Total RNA isolated from ESC was reverse transcribed and then amplified by real-time PCR using primers for IGFBP1. Values were calculated by subtracting data for signal threshold cycles (Ct) of the internal standard (GAPDH) from Ct values for IGFBP1. Values are the mean ± SEM of four independent experiments using samples from four different patients. *$P < 0.05$ versus 0 ng/ml. (**B**) IGFBP1 concentrations in culture media of ESC treated with EP, with or without BMP7 (10 and 100 ng/ml), for 3, 6, 9 and 12 days. IGFBP1 concentrations were determined using a specific ELISA and normalized against the total protein of cell lysates from each well. Data are the mean ± SEM of duplicate cultures. *$P < 0.05$ compared with the respective control on each day. The result is representative of three separate experiments using samples from three different patients.

Discussion

In the present study, we demonstrated that gene expression of BMP7 in the endometrium was lower in the mid- and late secretory phases and in early pregnancy than in the mid-proliferative phase. Progesterone, but not estradiol, decreased BMP7 gene expression in ESC, which was significant after 12 h. Long-term incubation with progesterone and estradiol induced IGFBP1 protein secretion from ESC, which was inhibited by BMP7. BMP7 also decreased ESC proliferation.

In parallel with dynamic changes in the endometrium, the expression of many molecules in the endometrium changes spatiotemporally. Because embryos are accepted by the endometrium only during the 'implantation window', which corresponds to the mid-secretory phase, those substances for which levels in the endometrium change during the mid-secretory phase may have a role in preparing the receptive endometrium. In this context, the decrease in the gene expression of BMP7 in the mid-secretory phase may contribute to the development of the receptive endometrium.

Decidualization is a process in which remarkable structural and functional changes occur in ESC to prepare an appropriate environment for embryo implantation and maintenance of pregnancy. Decidualization is regulated by the ovarian steroid hormones estradiol and progesterone. In addition, the importance of other factors in the induction of decidualization has been demonstrated recently. For example, we found that mechanical stretch augments decidualization (Harada *et al*., 2006), and others have found that paracrine factors are involved in decidualization (Tang *et al*., 1994; Fazleabas and Strakova, 2002). The results of the present study, showing that BMP7 suppresses secretion of IGFBP1 protein from decidualizing ESC, suggest that BMP7 may act as an antidecidualization factor in the endometrium.

The antidecidualization activity of BMP7 is in marked contrast with the actions of BMP2, which increases the secretion of IGFBP1 and prolactin, another marker of decidualization, in decidualized ESC (Li *et al*., 2007; Stoikos *et al*., 2008). The expression patterns of BMP2 and BMP7 in the endometrium also appear to be different because *in-vitro* decidualization increases the expression of BMP2 in ESC (Li *et al*., 2007). Thus, as a result of their different spatiotemporal expression, it is possible that the opposing actions of these two BMPs support decidualization and the subsequent establishment of pregnancy. From a therapeutic perspective, therapies targeted for BMP7 and BMP2 could be applicable for the treatment of implantation failure caused by impaired decidualization. Interestingly, the opposing functions of BMP7 and BMP2 have been demonstrated recently in adipogenesis, with BMP7 contributing to the development of brown adipocytes and BMP2 contributing to the development of white adipocytes (Tseng *et al*., 2008).

The decrease in BMP7 expression in the decidualized endometrium may also be important for the successful development of the placenta. It has been shown that BMP7 suppresses the production of human chorionic gonadotrophin and progesterone from the trophoblast (Martinovic *et al*., 1996). Because these hormones are tremendously important for the maintenance of pregnancy, the presence of BMP7 in the endometrium would be problematic for invading trophoblasts. Therefore, reduced BMP7 expression may be necessary not only for the development of a receptive endometrium, but also for the invading trophoblasts to establish pregnancy.

Progesterone inhibited BMP7 gene expression in ESC. This suggests that the decreased expression of BMP7 in the endometrium from the mid-secretory phase is due to the effects of progesterone. Notably, the inhibition of BMP7 gene expression by progesterone was clearly observed as early as 12 h. In addition, the decrease in BMP7 expression evidently preceded the increase in IGFBP1 expression during decidualization with progesterone and estradiol. This result, however, appears to be inconsistent with the findings by Stoikos *et al*. (2008) which showed that BMP7 gene expression was not altered by *in vitro* decidualization with cAMP . This difference may indicate that progesterone is prerequisite for down-regulation of BMP7 expression in the process of decidualization. Collectively,

progesterone may suppress BMP7 gene expression in the early stage to facilitate subsequent decidualization. Another apparently inconsistent finding of Stoikos *et al*. (2008) was the vesicular staining for BMP7 in decidual cells in mid–late secretory endometrium although staining patterns were not shown in other phases of the menstrual cycle. The decrease in BMP7 gene expression by progesterone might be involved in the change, if any, of intracellular localization of BMP7. Another possible explanation for the inconsistency may be any cross-reactivity of the antibody used in that study.

BMP7 appears to stimulate or inhibit proliferation depending on the cell type; for example, BMP7 stimulates proliferation of ovarian granulosa cells (Lee *et al*., 2001) and Sertoli cells (Puglisi *et al*., 2004), but inhibits proliferation of aortic smooth muscle cells (Dorai *et al*., 2000), renal mesangial cells (Otani *et al*., 2007) and prostate cancer cells (Miyazaki *et al*., 2004). In the present study, BMP7 inhibited the proliferation of ESC. Thus, the decrease in BMP7 expression in the decidualized endometrium may contribute to the proliferation of decidual cells during pregnancy.

The present study has some limitations. First, the decidual tissues of ectopic pregnancies used in this study have advantages in that they are free from contamination with trophoblast cells, but they may have different characteristics from deciduas of normal pregnancies. Second, we measured mRNA levels but not protein levels of BMP7. Although cellular protein levels shown by immunostaining or immunoblotting are not necessarily proportional to their functional activities, knowledge about them would help our understanding of BMP7 in the endometrium. A further study is warranted regarding this point.

In summary, the results of the present study suggest that progesterone decreases BMP7 expression in the endometrium. The decrease in BMP7 expression may facilitate decidulalization of the endometrium, thus aiding the establishment of pregnancy.

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References

Dimitriadis E, White CA, Jones RL, Salamonsen LA. Cytokines, chemokines and growth factors in endometrium related to implantation. *Hum Reprod Update* 2005;**11**:613–630.

Dorai H, Vukicevic S, Sampath TK. Bone morphogenetic protein-7 (osteogenic protein-1) inhibits smooth muscle cell proliferation and stimulates the expression of markers that are characteristic of SMC phenotype *in vitro*. *J Cell Physiol* 2000;**184**:37–45.

Fazleabas AT, Strakova Z. Endometrial function: cell specific changes in the uterine environment. *Mol Cell Endocrinol* 2002;**186**:143–147.

Fitzpatrick SL, Sindoni DM, Shughrue PJ, Lane MV, Merchenthaler IJ, Frail DE. Expression of growth differentiation factor-9 messenger ribonucleic acid in ovarian and nonovarian rodent and human tissues. *Endocrinology* 1998;**139**:2571–2578.

Francis-West PH, Parish J, Lee K, Archer CW. BMP/GDF-signalling interactions during synovial joint development. *Cell Tissue Res* 1999;**296**:111–119.

Harada M, Osuga Y, Takemura Y, Yoshino O, Koga K, Hirota Y, Hirata T, Morimoto C, Yano T, Taketani Y. Mechanical stretch upregulates IGFBP-1 secretion from decidualized endometrial stromal cells. *Am J Physiol Endocrinol Metab* 2006;**290**:E268–E272.

Hirota Y, Osuga Y, Hirata T, Koga K, Yoshino O, Harada M, Morimoto C, Nose E, Yano T, Tsutsumi O *et al*. Evidence for the presence of protease-activated receptor 2 and its possible implication in remodeling of human endometrium. *J Clin Endocrinol Metab* 2005;**90**:1662–1669.

Kayisli UA, Guzeloglu-Kayisli O, Arici A. Endocrine-immune interactions in human endometrium. *Ann N Y Acad Sci* 2004;**1034**:50–63.

Koga K, Osuga Y, Tsutsumi O, Yano T, Yoshino O, Takai Y, Matsumi H, Hiroi H, Kugu K, Momoeda M *et al*. Demonstration of angiogenin in human endometrium and its enhanced expression in endometrial tissues in the secretory phase and the decidua. *J Clin Endocrinol Metab* 2001;**86**:5609–5614.

Lee WS, Otsuka F, Moore RK, Shimasaki S. Effect of bone morphogenetic protein-7 on folliculogenesis and ovulation in the rat. *Biol Reprod* 2001;**65**:994–999.

Li Q, Kannan A, Wang W, Demayo FJ, Taylor RN, Bagchi MK, Bagchi IC. Bone morphogenetic protein 2 functions via a conserved signaling pathway involving Wnt4 to regulate uterine decidualization in the mouse and the human. *J Biol Chem* 2007;**282**:31725–31732.

Lyons K, Graycar JL, Lee A, Hashmi S, Lindquist PB, Chen EY, Hogan BL, Derynck R. Vgr-1, a mammalian gene related to Xenopus Vg-1, is a member of the transforming growth factor beta gene superfamily. *Proc Natl Acad Sci USA* 1989;**86**:4554–4558.

Martinovic S, Latin V, Suchanek E, Stavljenic-Rukavina A, Sampath KI, Vukicevic S. Osteogenic protein-1 is produced by human fetal trophoblasts *in vivo* and regulates the synthesis of chorionic gonadotropin and progesterone by trophoblasts *in vitro*. *Eur J Clin Chem Clin Biochem* 1996;**34**:103–109.

Miyazaki H, Watabe T, Kitamura T, Miyazono K. BMP signals inhibit proliferation and *in vivo* tumor growth of androgen-insensitive prostate carcinoma cells. *Oncogene* 2004;**23**:9326–9335.

Otani H, Otsuka F, Inagaki K, Takeda M, Miyoshi T, Suzuki J, Mukai T, Ogura T, Makino H. Antagonistic effects of bone morphogenetic protein-4 and -7 on renal mesangial cell proliferation induced by aldosterone through MAPK activation. *Am J Physiol Renal Physiol* 2007;**292**:F1513–F1525.

OuYang Z, Hirota Y, Osuga Y, Hamasaki K, Hasegawa A, Tajima T, Hirata T, Koga K, Yoshino O, Harada M *et al*. Interleukin-4 stimulates proliferation of endometriotic stromal cells. *Am J Pathol* 2008;**173**:463–469.

Ozkaynak E, Jin DF, Jelic M, Vukicevic S, Oppermann H. Osteogenic protein-1 mRNA in the uterine endometrium. *Biochem Biophys Res Commun* 1997;**234**:242–246.

Paria BC, Ma W, Tan J, Raja S, Das SK, Dey SK, Hogan BL. Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. *Proc Natl Acad Sci USA* 2001;**98**:1047–1052.

Puglisi R, Montanari M, Chiarella P, Stefanini M, Boitani C. Regulatory role of BMP2 and BMP7 in spermatogonia and Sertoli cell proliferation in the immature mouse. *Eur J Endocrinol* 2004;**151**:511–520.

Shimasaki S, Moore RK, Otsuka F, Erickson GF. The bone morphogenetic protein system in mammalian reproduction. *Endocr Rev* 2004;**25**:72–101.

Stoikos CJ, Harrison CA, Salamonsen LA, Dimitriadis E. A distinct cohort of the TGFbeta superfamily members expressed in human endometrium regulate decidualization. *Hum Reprod* 2008;**23**:1447–1456.

Tang B, Guller S, Gurpide E. Mechanism of human endometrial stromal cells decidualization. *Ann N Y Acad Sci* 1994;**734**:19–25.

Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y *et al*. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008;**454**:1000–1004.

Yi SE, LaPolt PS, Yoon BS, Chen JY, Lu JK, Lyons KM. The type I BMP receptor BmprIB is essential for female reproductive function. *Proc Natl Acad Sci USA* 2001;**98**:7994–7999.

Ying Y, Zhao GQ. Detection of multiple bone morphogenetic protein messenger ribonucleic acids and their signal transducer, Smad1, during mouse decidualization. *Biol Reprod* 2000;**63**:1781–1786.

Yoshino O, Osuga Y, Hirota Y, Koga K, Hirata T, Yano T, Ayabe T, Tsutsumi O, Taketani Y. Endometrial stromal cells undergoing decidualization down-regulate their properties to produce proinflammatory cytokines in response to interleukin-1 beta via reduced p38 mitogen-activated protein kinase phosphorylation. *J Clin Endocrinol Metab* 2003;**88**:2236–2241.

Zhao R, Lawler AM, Lee SJ. Characterization of GDF-10 expression patterns and null mice. *Dev Biol* 1999;**212**:68–79.

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