Bone Morphogenetic Protein 2 Functions via a Conserved Signaling Pathway Involving Wnt4 to Regulate Uterine Decidualization in the Mouse and the Human*

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A critical role of progesterone (P) during early pregnancy is to induce differentiation of the endometrial stromal cells into specialized decidual cells that support the development of the implanting embryo. The P-induced signaling pathways that participate in the formation and function of the decidual cells remain poorly understood. We report here that the expression of the bone morphogenetic protein 2 (BMP2), a morphogen belonging to the TGFβ superfamily, is induced downstream of P action in the mouse uterine stroma during decidualization. To determine the function of BMP2 during this differentiation process, we employed a primary culture system in which undifferentiated stromal cells isolated from pregnant mouse uterus undergo decidualization. When recombinant BMP2 was added to these stromal cultures, it markedly advanced the differentiation program. We also found that siRNA-mediated silencing of BMP2 expression in these cells efficiently blocked the differentiation process. Gene expression profiling experiments identified Wnt4 as a downstream target of BMP2 regulation in stromal cells undergoing decidualization. Attenuation of Wnt4 expression by siRNAs greatly reduced stromal differentiation in vitro, indicating that it is a key mediator of BMP2-induced decidualization. We also observed a remarkable induction in the expression of BMP2 in human endometrial stromal cells during decidualization in vitro in response to steroids and cAMP. Addition of BMP2 to these cultures led to a robust enhancement of Wnt4 expression and stimulated the differentiation process. Collectively, our studies uncovered a unique conserved pathway involving BMP2 and Wnt4 that mediates P-induced stromal decidualization in the mouse and the human.

The steroid hormones progesterone (P)2 and estrogen (E) act in concert to control uterine competency for embryo implantation (1). A primary role of P is to induce differentiation of the endometrial stromal cells into morphologically distinct decidual cells (2, 3). In mice, this differentiation process, termed decidualization, is a prerequisite for successful implantation and is initiated at the time of blastocyst attachment to the uterine epithelium on day 4.5 of pregnancy. The attachment reaction is followed by the proliferation and differentiation of the stromal cells surrounding the implanting blastocyst to form the decidual bed (2, 3). The decidual cells are thought to produce hormones and cytokines that are critical for embryo development, secrete factors that control trophoblast invasion and serve an immunoregulatory function during pregnancy (4, 5). However, P-induced signaling molecules that participate in the formation and function of this unique tissue remain poorly understood.

It is well established that the majority of the physiological effects of P are mediated via the intracellular progesterone receptors (PRs) (6). Strong evidence in favor of an essential role of PR in P-mediated responses in the uterus came from a mouse model carrying a null mutation of this gene (7). Ablation of PR in mice rendered the uterus incapable of mounting a decidual response and unable to support embryo implantation. To understand how PR regulates decidualization, it is essential to identify the network of genes that are regulated by this ligand-inducible transcription factor during the differentiation process. To achieve this goal, we employed RU486, a well-characterized antagonist, which counteracts PR-dependent pathways by binding to the receptor and impairing its gene regulatory function (8). We used oligonucleotide microarrays to identify the genes whose expression in the uterus was repressed by this drug at the time of decidualization, indicating that these genes are potentially under P regulation (9). Interestingly, one of these repressed mRNAs was bone morphogenetic protein-2 (BMP2), a member of the TGFβ superfamily of morphogens. We observed that BMP2 and its receptor are expressed in the uterine stroma in a decidual stage-specific manner during early pregnancy, raising the possibility that BMP2 is a candidate...
mediator of the P-dependent changes underlying stromal differentiation during decidualization. To address the function of BMP2 in decidualization, we utilized a primary culture system in which undifferentiated stromal cells isolated from pregnant mouse uterus undergo decidualization. Using this in vitro system, we provide strong evidence that the BMP2 signaling pathway plays a critical role in decidualization.

Although evolutionarily very different, humans and rodents have a similar hemochorial type of placenta and exhibit similarities in terms of hormonal regulation of gene expression in the uterus. In preparation for implantation, the human endometrial stromal cells also undergo a differentiation process, known as “predecidualization,” during the P-dominated secretory phase of the menstrual cycle (10, 11). However, only a handful of P-regulated genes such as IGFBP1 (insulin-like growth factor-binding protein 1), tissue factor, and HOXA10, have been identified in human stromal cells (12–15). The precise functions of these P-regulated molecules in stromal differentiation remain largely unknown. In this study, we show that BMP2 is strongly induced in human endometrial stromal cells during decidualization. Furthermore, our results indicated that BMP2 plays a critical role in decidualization.

**EXPERIMENTAL PROCEDURES**

**Materials**—Recombinant human BMP2 was purchased from R&D systems (Minneapolis, MN).

**Animals and Tissue Collection**—All experiments involving animals were conducted in accordance with the National Institutes of Health standards for the use and care of animals. The animal protocols were approved by the University of Illinois Institutional Animal Care and Use Committee. Female mice (CD-1) were killed at various stages of gestation and the uteri collected.

Our studies involving human endometrial cell cultures and endometrial biopsies adhere to the regulations set forth for the protection of human subjects participating in clinical research and are approved by the IRBs of Emory University and the University of Illinois. Endometrium samples from early proliferative stage of the menstrual cycle were obtained at the Emory University Medical Center from fertile volunteers by Pipelle biopsy.

**Immunohistochemistry and Immunocytochemistry**—Paraflin-embedded endometrial sections were subjected to immunohistochemistry as described previously (36). Polyclonal antibodies against BMP2 and type II BMP receptor were obtained from Santa Cruz Biotechnology, Santa Cruz, CA. Mouse and human stromal cell cultures were fixed and stained with antibodies specific for BMP2 and its receptor in mouse uterus during early pregnancy. Uterine sections from days 5–7 (panels a–c) pregnant mice were subjected to immunohistochemical analysis using antibodies specific for BMP2 (upper panel) or BMP2 receptor (lower panel). E denotes Embryo.

*FIGURE 1. PR regulates BMP2 expression in mouse uterus during decidualization. B, mice on day 5 of pregnancy were injected with either vehicle (sesame oil), or RU486 (8 mg/kg body weight) and killed on day 6. Uterine RNA (20 mg) was analyzed by Northern blotting followed by hybridization with 32P-labeled cDNA probes for BMP2 and 36B4. Lanes 1 and 2 represent RNA from animals treated without and with RU486, respectively. B, spatial expression profiles of BMP2 and its receptor in mouse uterus during early pregnancy. Uterine sections from days 5–7 (panels a–c) pregnant mice were subjected to in situ hybridization using antibodies specific for BMP2 (upper panel) or BMP2 receptor (lower panel). E denotes Embryo.*

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**Isolation of Stromal Cells and Induction of Decidualization in Vitro**—Uterine horns of pregnant (day 4) mice were dissected longitudinally to expose the uterine lumen and cut into 3–5 mm pieces. After washing with Hank’s balanced salt solution (HBSS), uterine tissues were placed in HBSS containing 6 g/liter dispase (Invitrogen), 25g/liter pancreatin, and 100 units/liter penicillin, 0.1 g/liter streptomycin, 1.25 mg/liter fungizone (Invitrogen) for 1 h on ice followed by 1 h at room temperature and then 10 min at 37°C. The tubes were gently mixed, and the supernatant was discarded to remove the endometrial epithelial clumps. The partially digested tissues were then washed twice in HBSS and then placed in HBSS containing 0.5 g/liter collagenase. After incubation for 45 min at 37°C, the tubes were vortexed for 10–12 s until the supernatant became turbid with dispersed endometrial stromal cells. The contents of the tube were then passed through a 70-µm gauze filter (Millipore). Cells were resuspended in Dulbecco’s modified Eagle’s Medium-F12 medium (DMEM-F12; with 100 unit/liter penicillin, 0.1 g/liter streptomycin, 1.25 mg/liter fungizone) containing 2% heat-inactivated fetal calf serum and live cells were assessed by trypsin blue staining using a hemocytometer. 4 × 10⁶ cells were seeded in 6-well cell culture plates. The unattached cells were removed by washing several times with HBSS, and cell culture was continued after addition of fresh medium supplemented with P (1 µM) and E (10 nM).

**siRNA Transfections—siRNAs corresponding to mouse BMP2 (ACACAGGGTTAGTGAAATCATT), Wnt4 (ACT-CAAAGGCCTGATCCAG) and firefly luciferase mRNAs were synthesized and annealed by QIAGENE RNAi service center (QIAGENE). The annealed siRNA duplexes were transfected in the primary uterine stromal cells following the protocol of siPORT™ NeoFX™ (Ambion). Briefly, 4 µl of siPORT NeoFX transfection reagent was mixed with 100 nmol of SiRNA duplexes to form complexes and dispersed into 6-well cell culture plate.**

**RESULTS**

BMP2 Is a Downstream Target of PR Regulation in the Decidual Uterus—To identify the gene networks underlying PR function during decidualization, we examined the alterations in uterine mRNA expression profiles in response to the PR antagonist RU486 (9). Briefly, mice on day 5 of pregnancy were...
BMP2 Regulates Uterine Stromal Cell Differentiation

**FIGURE 2. Primary stromal culture from mouse uterus undergo in vitro decidualization.** Stromal cells isolated from preimplantation day 4 pregnant mouse uteri were cultured in the presence of P and E. A, cells were plated and allowed to attach for 2 h. After attachment, cells were collected at 24-h intervals, and their morphology was examined under a microscope. B, quantitative measurement of ALP activity in lysates of stromal cells cultured for 24, 48, and 72 h. The values represent average ± S.D. of three separate samples. C, qPCR analysis to monitor PRP mRNA expression in the stromal cells cultured up to 96 h. The relative fold induction of PRP mRNA expression at each time point compared with that of 24 h sample is shown. D, cells were fixed and subjected to immunofluorescence using anti-BMP2 antibody as described previously.

We verified the results of the microarray analysis by performing Northern blotting (Fig. 1A). Total RNA was obtained from the uteri of day 6 pregnant mice treated with either vehicle (sesame oil) or RU486, and uteri were collected on day 6 during the decidualization phase. The uterine transcripts were then hybridized to Affymetrix murine GeneChip 430A 2.0 Array, which interrogated over 14,000 well-characterized mouse genes. We identified several mRNAs whose expression was altered significantly (>2-fold) in the decidual uterus in response to RU486 (9). One of the mRNAs whose level was markedly down-regulated encoded bone morphogenetic protein-2 (BMP2), a well-studied morphogen that regulates a wide variety of biological processes, such as cell proliferation, differentiation, migration, adhesion, and apoptosis (16, 17).

We verified the results of the microarray analysis by performing Northern blotting (Fig. 1A). Total RNA was obtained from the uteri of day 6 pregnant mice treated with vehicle or RU486 and probed with a 32P-labeled BMP2 cDNA. A strong signal corresponding to BMP2 mRNA appeared in the RNA sample obtained from vehicle-treated mice on day 6 of pregnancy (lane 1). No such signal was observed in the uterine sample from RU486-treated mice (lane 2). These results confirmed that the gene encoding BMP2 is a downstream target of PR regulation in the decidual uterus.

We next examined the expression profile of BMP2 protein in mouse uterus during early pregnancy by employing immunohistochemistry (Fig. 1B, upper panel). BMP2 expression was undetectable in uterine sections obtained from day 4 pregnant mice in the period preceding implantation (data not shown). Distinct BMP2 immunostaining was visible in the stromal cells surrounding the implanted embryo in the uterine sections of mice on day 5 of pregnancy (panel a). On day 6, the BMP2 signal decreased in the stromal cells in the immediate vicinity of the implanted embryo but was still observed in the proliferating pre-decidual cells of the outer stroma (panel b). As pregnancy progressed to day 7, BMP2 expression was detected predominantly in the stromal cells at the mesometrial side of the implantation site (panel c).

BMPs exert their cellular effects via concerted actions of type I (BRI) and type II (BRII) receptors (16, 17). Previous studies have shown that both receptor types are required for high affinity ligand binding and BMP2 signaling (16, 17). We monitored the expression of BRII in pregnant uterus using immunohistochemistry. As shown in Fig. 1B, lower panel, BRII immunostaining was observed in the stromal cells on day 5 of gestation (panel a). On days 6 and 7 of pregnancy, the BRII expression was seen in the secondary decidual zone (panels b and c). The staining was initially seen predominantly in the antimesometrial area, but gradually spread to the mesometrial region as gestation proceeded. These results indicated that BMP2 ligand and its receptor are expressed in the uterine stroma during decidualization.

**BMP2 Promotes Differentiation of Endometrial Stromal Cells Cultured in Vitro**—To investigate the role of BMP signaling in uterine function, we established a mouse primary culture system in which undifferentiated stromal cells isolated from preimplantation pregnant uteri undergo decidualization in vitro. This system was adapted from an earlier protocol of rat uterine primary stromal cultures developed by Glasser and co-workers (18, 19). Briefly, uteri were isolated from mice on day 4 of pregnancy and digested with proteases. The epithelial layer was removed carefully and the remaining tissue was digested further with collagenase to release the stromal cells. The stromal cells were purified by fractionation through a sieve and plated in the presence of the steroid hormones E and P. Within 24–48 h of plating, the cells started to display distinct morphological characteristics of decidual cells (Fig. 2A). Starting with a typical fibroblastic appearance, they gradually became larger and multinucleated. We validated the in vitro decidualization biochemically by monitoring the expression of alkaline phosphatase (ALP) and decidual prolactin-related protein (PRP), two well-known markers of uterine stromal differentiation (20, 21). As the time of stromal cell culture progressed from 24 to 72 h,
BMP2 Regulates Uterine Stromal Cell Differentiation

we noted a steady enhancement in the expression of both ALP and PRP (Fig. 2, B and C). The decidualization process was therefore recapitulated morphologically and biochemically in a primary culture of pre-implantation uterine stromal cells in vitro. When we examined the BMP2 expression in these primary stromal cultures by immunocytochemistry, we observed an intense expression of BMP2 protein by 24 h of culture (Fig. 2D). The BMP2 expression was also observed at 48–72 h, although at a somewhat reduced level.

To investigate the functional consequences of BMP2 signaling during stromal decidualization, we added exogenous BMP2 to stromal cultures and examined its effects on the differentiation process. Increasing doses of recombinant BMP2 were added to these cultures at various times and ALP activity was measured by histochemical staining. We found that addition of BMP2 significantly enhanced the intensity of ALP staining, as well as the number of cells stained with ALP (Fig. 3A, top panel). A colorimetric assay showed that the overall activity of ALP enzyme increased in a time- and dose-dependent manner following BMP2 treatment, indicating that this morphogen promotes stromal cell differentiation (Fig. 3A, bottom panel). We also noted a marked enhancement in the expression of PRP in stromal cells in response to increasing amounts of BMP2. The BMP2-treated stromal cells exhibited significantly higher levels of PRP mRNA expression compared with untreated cells (Fig. 3B). Collectively, these results indicated that BMP2 promotes stromal cell differentiation.

In the canonical BMP signaling pathway, the activated receptor complex phosphorylates Smad proteins 1, 5, or 8, which in turn set in motion the signaling pathway leading to gene activation or repression (16,17). However, in addition to the Smad pathway, BMP2 is known to signal through other pathways involving MAP kinases or protein kinase C (22–24). As shown in Fig. 3C, addition of exogenous BMP2 significantly enhanced the phosphorylation of Smad 1/5/8, indicating that BMP2 operates via Smad signaling in stromal cells during decidualization.

BMP2 Is Essential for Decidualization of Endometrial Stromal Cells—To further ascertain the role of BMP2 on stromal differentiation, we employed RNA interference-mediated knockdown of its gene expression. Primary stromal cells were isolated from uteri of day 4 pregnant mice as described previously and transfected with siRNA targeted specifically to the BMP2 mRNA. In control experiments, cells were transfected with a siRNA against firefly luciferase (Luc). We observed that the cells transfected with BMP2 siRNA exhibited more than 50% reduction in BMP2 mRNA expression compared with cells transfected with Luc siRNA at 24 h. At 48-h post-transfection, there was ~80% reduction in BMP2 mRNA expression compared with cells treated with Luc siRNA (Fig. 4A). Immunocytochemical analyses revealed that the cells transfected with BMP2 siRNA exhibited a drastic decrease in BMP2 immunostaining, whereas transfection with luciferase siRNA did not affect this gene expression (Fig. 4B).

We next investigated the functional consequences of this blockade of BMP2 expression during stromal differentiation. As shown in Fig. 4C, siRNA-mediated down-regulation of BMP2 in the stromal cells led to a significant reduction in expression of differentiation markers such as ALP and PRP. In contrast, the level of PR remained unaltered in cells treated with either BMP2 or Luc siRNA. These results indicated that BMP2 expression is critical for successful progression of the stromal differentiation program during decidualization.

Wnt4 Is a Downstream Target of BMP2 during Decidualization—To identify the downstream target(s) of BMP2 signaling during decidualization, we performed gene expression profiling of uterine stromal cells treated with or without recombinant BMP2 using Affymetrix murine GeneChip arrays. We observed that the expression of several genes was altered within 24 h of BMP2 addition.3 Interestingly, we identified Wnt4 as one of the genes whose expression was markedly

3 Q. Li and I. Bagchi, unpublished results.
BMP2 Regulates Uterine Stromal Cell Differentiation

**FIGURE 4. siRNA-mediated down-regulation of BMP2 inhibits decidualization.** Mouse stromal cells (from day 4 pregnant uterus) were transfected with siRNA (100 nm) targeted against firefly luciferase (control) or BMP2 at the time of plating. **A**, qPCR analysis of BMP2 mRNA using RNA collected from stromal cells transfected with Luc-siRNA and BMP2 siRNA. **B**, BMP2 protein was monitored at 30 h after transfection using immunocytochemistry. **C**, qPCR analysis of ALP, PRP, and PR using RNA collected from stromal cells 72 h after transfection with Luc-siRNA and BMP2 siRNA.

elevated in response to exogenous BMP2. To validate the microarray analysis, uterine stromal cells collected from pre-implantation day 4 pregnant uteri were treated with BMP2, and the expression of Wnt4 as well as several other Wnt molecules such as Wnt3a, Wnt6, Wnt7a, and Wnt10a was determined by q-PCR using total RNA isolated from these cells (Fig. 5A). Consistent with microarray analysis, we observed a marked induction of Wnt4 mRNA expression in response to BMP2 treatment during stromal differentiation in vitro. In contrast, the expression of Wnt3a, Wnt6, Wnt7a, and Wnt10a did not alter significantly in response to BMP2. A time course analysis of Wnt4 expression in response to BMP2 showed a ∼2.5-fold induction in the level of Wnt4 mRNA within 24 h of addition of BMP2 to the stromal cultures (Fig. 5B). At 48 h, the level of Wnt4 induction increased to >7-fold. Taken together, these results revealed that Wnt4 as a downstream target of BMP2 signaling in uterine stromal cells.

**Down-regulation of Wnt4 Blocks BMP2-mediated Stromal Decidualization in Vitro**—We next examined whether siRNA-mediated attenuation of Wnt4 gene expression impacts on BMP2-mediated stromal differentiation. As shown in Fig. 5C, primary stromal cells treated with siRNA targeted to Wnt4 mRNA efficiently suppressed the level of this mRNA but did not significantly affect the level of PR mRNA. This specific down-regulation of Wnt4 expression was associated with a marked reduction in the expression of differentiation markers such as ALP and PRP compared with control siRNA-treated stromal cells in response to the hormone mixture as early as 4 days of initiating the culture (Fig. 6A). The level of BMP2 mRNA increased further by day 8. The expression of BMP2 protein followed a similar temporal pattern of expression (Fig. 6B). Interestingly, we noted that Wnt4 mRNA is also expressed during human stromal decidualization and its expression profile closely followed that of BMP2 (Fig. 6A). These results indicated that the expression of BMP2 and Wnt4, which are induced during stromal decidualization in the mouse, is conserved in the human stromal cells undergoing decidualization.

**Exogenous BMP2 Enhances Wnt4 Expression and Promotes Differentiation of Human Endometrial Stromal Cells Cultured in Vitro**—We next investigated the functional role of BMP2 in human stromal differentiation by adding exogenous BMP2 to primary endometrial stromal cells that were subjected to decidualization in the presence of progesterone, estrogen, and 8-bromo-cAMP (Fig. 6C). As expected, addition of the hormone mixture alone led to a marked induction of PRL and IGFBP1 during the in vitro decidualization process (panel EPC). However, the levels of PRL and IGFBP-1 mRNAs were further accelerated upon addition of exogenous BMP2 along with the hormone mixture to these cultures (panel EPCB). Whereas the expression of PRL mRNA increased more than 5-fold, that of IGFBP1 mRNA increased 10-fold upon addition of BMP2. Most interestingly, we observed a robust induction of Wnt4 (>3-fold) in response to recombinant BMP2 during human stromal differentiation (Fig.
BMP2 Regulates Uterine Stromal Cell Differentiation

These results revealed that (i) BMP2, a critical regulator of decidualization in the mouse, also promotes decidualization in the human, and (ii) it acts via a conserved signaling pathway involving its downstream target Wnt4.

DISCUSSION

During early pregnancy, P acting via PR controls the transformation of endometrial stromal cells into decidual cells, a process that is essential for successful implantation. To identify the PR-regulated signaling pathways involved in the creation of the decidual state of the uterus, we employed gene expression profiling to identify the genes whose expression is altered in pregnant mouse uterus in response to RU486, a potent PR antagonist. Using this strategy, we uncovered a novel signaling pathway, involving BMP2 and Wnt4, which functions downstream of PR signaling to critically regulate stromal differentiation. The most important finding of this report is that this pathway is conserved during mouse and human endometrial decidualization.

BMPs constitute a large family of morphogens belonging to the TGFβ superfamily of growth modulators. They were initially identified by their ability to induce ectopic formation of cartilage and bone, but were subsequently shown to influence a broad spectrum of physiological functions, including cell proliferation, differentiation, apoptosis, cell migration, and adhesion in a variety of cell types during embryonic development (16, 17). Previous studies employing in situ hybridization have shown that transcripts corresponding to several BMP family members are expressed in mouse uterus during the decidual phase of gestation (27, 28). While BMP4 is predominantly expressed in the vascular endothelial cells of mesometrial decidua, BMP5 expression is observed in the stroma close to the myometrium. BMP2 is expressed in the stromal cells surrounding the implanted embryo. BMP7 and BMP8a are reportedly expressed in the mesometrial decidual cells (27, 28). However, little is known about the roles of these BMPs in regulating uterine function. Studies by Dey and co-workers (28) demonstrated that placement of beads coated with the growth factor HB-EGF in pseudopregnant uterus induced decidualization, accompanied by BMP2 expression. These studies, however, did not provide any functional evidence that BMP2 expression is required for implantation or decidualization (28).

Analysis of our microarray data indicated that BMP2 is the sole member of the BMP family whose expression is under P regulation in the pregnant uterus. Overlapping expression of BMP2 and its receptor in the differentiating stromal cells during early pregnancy suggested that this morphogen is a critical mediator of decidualization. The use of mouse primary stromal culture system helped us establish a key role of BMP2 during the decidual process. We observed that addition of recombinant BMP2 to the undifferentiated stromal cells significantly elevated Smad signaling, which in turn markedly advanced the kinetics of the differentiation program as indicated by the increased and accelerated expression of the differentiation hallmarks, ALP and PRP, in these cells. So, our studies identified BMP2 as a critical factor promoting stromal decidualization.

We employed an in vitro decidualization system and a RNA knockdown strategy to evaluate the function of BMP2 in stromal differentiation. When a siRNA targeted to BMP2 mRNA was transfected into undifferentiated stromal cells, it efficiently...
suppressed BMP2 mRNA and protein levels. This siRNA-mediated loss of BMP2 expression and Smad signaling was accompanied by an inhibition of stromal differentiation as indicated by the drastically reduced expression of biochemical markers of the decidual state. These *in vitro* studies firmly established that BMP2-mediated canonical Smad signaling plays a critical role in stromal cell differentiation in the pregnant uterus. These findings are strongly supported by recent *in vivo* studies, which showed overlapping expression of BMP2 and Wnt4 in the uterine stromal compartment during early pregnancy (37).

Our studies revealed an important link between BMP2 and Wnt4 in pregnant uterus. Like BMPs, the Wnt family of signaling proteins participates in multiple developmental events during embryogenesis and has also been implicated in adult tissue homeostasis (29, 30). Wnt signals are pleiotropic, with effects that include mitogenic stimulation, cell fate specification, and differentiation. Previous studies involving a variety of differentiation systems indicated the existence of a cross-talk between BMP and Wnt signaling pathways (31–33). A recent study showed that BMP2 and Wnt signaling pathways share common target genes, such as Ids and follistatin in human embryonic carcinoma cells (34). Previous studies by Dey and co-workers (28) showed overlapping expression of BMP2 and Wnt4 *in vivo* in the uterine stromal compartment during early pregnancy. Using microarray analysis, we identified Wnt4 as a downstream
target of BMP2 signaling in mouse uterine stromal cells. We then utilized the in vitro stromal culture system to demonstrate that Wnt4 is indeed induced in response to BMP2 addition to these cultures. Most importantly, using a siRNA strategy, we further established that the BMP2-Wnt4 pathway critically controls stromal decidualization.

Another important aspect of this study is its exploration of the role of BMP2 signaling during decidualization of human endometrial stromal cells. In the human, a marked rise in the level of P along with a moderate increase in the level of E occurs during the post-ovulatory secretory phase of the menstrual cycle. In response to these steroids, the fibroblastic stromal cells start to differentiate into decidual cells during the mid-late secretory phase of the cycle. This differentiation of stromal cells into decidual cells, known as predecidualization, is a prerequisite for successful implantation and establishment of pregnancy. Predecidualization is followed by a more intense decidual response if pregnancy ensues.

The availability of a well-established culture system in which undifferentiated human stromal cells undergo decidualization in response to steroid hormones and cAMP allowed us to analyze the expression and function of BMP2 during this differentiation process. No expression of BMP2 was observed in undifferentiated stromal cells isolated from endometrial biopsies during uterine stromal cell differentiation in mice and humans. We further demonstrated that addition of exogenous BMP2 accelerates the differentiation program in the human endometrial stromal culture. The expression of stromal differentiation markers such as IGFBP1 and PRL was induced precociously, by at least 2 days in response to this morphogen. Most interestingly, we noted that BMP2-mediated advancement of the human stromal differentiation program was accompanied by an elevation in Wnt4 mRNA level in these cells. These results indicated that a conserved molecular pathway involving BMP2 and Wnt4 operates during uterine stromal cell differentiation in mice and humans.

It is of interest to note that a recent study described the unique expression patterns of several Wnt family members, including Wnt4, and their inhibitors in human endometrium during the menstrual cycle (35). However, the functional significance of the interplay of these molecules in human endometrium is unclear. Our future studies will address in detail the molecular mechanisms by which the BMP2-Wnt4 pathway controls the process of decidualization.

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