Differential expression of secreted frizzled-related protein 4 in decidual cells during pregnancy

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Abstract

During pregnancy, the uterus shows marked morphological and physiological changes under the regulation of ovarian steroid. To elucidate the molecular cues of these changes, we tried to identify the transcripts differentially expressed in the pregnant rat uterus by using the suppression subtractive hybridization method. Seven independent clones were isolated and one of the up-regulated genes was secreted frizzled-related protein 4 (sFRP4). sFRP4 contains a Wnt-binding domain and belongs to the secreted frizzled protein family whose members are assumed to function as modulators of the Wnt signal. The expression level of sFRP4 mRNA reached a peak in the pregnant uterus on day 12, when uterine decidualization was almost complete in the rat. *In situ* hybridization histochemistry revealed that sFRP4 transcripts were observed in the decidual cells. In addition, proliferating cell nuclear antigen (PCNA)-positive cells were shown to be overlapped in decidua, suggesting that sFRP4 mRNA expression was accompanied by the late phase of decidual cell proliferation. Moreover, sFRP4 and estrogen receptor- α transcripts were co-localized. Furthermore, we analyzed the regulation of sFRP4 by estrogen using 17 β -estradiol-treated ovariectomized rats. sFRP4 mRNA was detected in the uterus at 48 h after estrogen treatment, especially in endometrial stroma where PCNA-positive cells were also observed. The results in this study led us to the notion that sFRP4 mRNA may be up-regulated after estrogen treatment in the late phase of uterine cell proliferation.

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Introduction

The uterus undergoes morphological and physiological changes during gestation to accommodate and protect the developing conceptus. Both estrogen and progesterone play important roles in this process, maintaining uterine quiescence during gestation and determining the onset of parturition (Fu *et al.* 1994, Chibbar *et al.* 1995, Hansen 1998, de Ziegler *et al.* 1998). To elucidate the molecular basis of these mechanisms, it is important to identify the genes and factors involved in the

pregnant uterus. For example, the expression of collagen type I and III and fibronectin is increased in the myometrium of the pregnant uterus (Stewart *et al.* 1995), suggesting their physiological roles in the growth of the uterus during pregnancy. Several cytokines are also expressed in the pregnant uterus (Lim *et al.* 1998, Hatthachote & Gillespie 1999). Leukemia inhibitory factor, required for successful implantation, is secreted in the uterine endometrial gland (Charnock *et al.* 1994, Vogiagis & Salamonsen 1999). Macrophage-colony stimulating factor is widely distributed in the endometrium,

decidua and placenta, suggesting its role in placental morphogenesis and trophoblast differentiation (Saito *et al.* 1994). It is well recognized that regulation of these factors provides a framework for understanding gestational changes of the uterus. Especially, the up-regulated genes during implantation and parturition are investigated because they are important for establishment of pregnancy and the onset of labor. However, relatively few factors up-regulated during the mid and late phase of pregnancy have been identified so far. During the later phase of pregnancy, the decidualization is completed and then decidual cell regression occurs (Ogle *et al.* 1999). In addition, myometrium is undergoing hypertrophy (Macphee & Lye 2000) accompanied by a significant increase of fetal mass (Dowell & Kauer 1997). To investigate the up-regulated genes in this period is important for understanding the precise mechanism of these changes.

In the present study, we tried to isolate the genes regulated in the later phase of the pregnant rat uterus, including downstream targets regulated by ovarian hormone, by using suppression subtractive hybridization (SSH) (Diatchenko *et al.* 1996). Here, we identified a predominant expression of the secreted frizzled-related protein 4 (sFRP4), a member of the secreted frizzled family, whose expression gradually elevated until mid-pregnancy. The distribution of sFRP4 transcripts was also examined by *in situ* hybridization histochemistry. To explore the possible role of sFRP4 expression in cell proliferation, co-localization of sFRP4 mRNA with proliferating cell nuclear antigen (PCNA) (Kelman & Hurwitz 1998, Tsurimoto 1998) in the uterus was analyzed. Then, we examined the hormonal regulation of sFRP4 expression in ovariectomized rats treated with 17 β -estradiol.

Materials and methods

Animals and tissue preparation

Female 9-week-old Wistar rats with or without pregnancy were purchased from Charles River Japan (Tokyo, Japan). For pregnant rats, the day a vaginal plug was found was designated day 0, and rats on days 6, 9, 12 and 15 were used. The tissue preparation for Northern blot analysis was performed as follows. After surgical removal of the uteri under anesthesia, they were rapidly opened,

and the pups and placenta were removed. The tissues were frozen in liquid nitrogen and stored at -80°C until RNA isolation. For *in situ* hybridization and immunohistochemistry, tissues were fixed with 4% paraformaldehyde in PBS and embedded in paraffin. The serial sections were cut and mounted on slides and stored at 4°C . For estradiol treatment study, rats were ovariectomized. Two weeks after surgery, the rats received an s.c. injection of additional estradiol (10 $\mu\text{g}/\text{kg}$ body weight) (Sigma, St Louis, MO, USA) suspended in olive oil and then killed 0, 2, 6, 24 and 48 h later. All animal studies followed the National Institutes of Health (NIH) guidelines for the Care and Use of Experimental Animals.

Total RNA and poly A+ RNA isolation

Total RNA was extracted from frozen non-pregnant and pregnant uteri using Isogen reagent (Nippon Gene, Tokyo, Japan). Poly A+ RNA was extracted from 250 μg total RNA using an Oligotex-dT30 mRNA purification kit (Takara Shuzo, Tokyo, Japan).

SSH

The subtraction cDNA library was made using a PCR-Select cDNA subtraction kit, according to the manufacturer's instruction (Clontech, Palo Alto, CA, USA). Briefly, poly A+ RNA obtained from day 15 pregnant uteri and non-pregnant uteri were designated as a tester and a driver respectively. The double-stranded cDNAs were synthesized from 2 μg of each poly A+ RNA. The synthesized double-stranded cDNAs were then digested with *RsaI* and ligated to adapters. Subtractive hybridizations were performed between the tester and the driver and the hybridization products were amplified by PCR (Diatchenko *et al.* 1999).

Sequences analysis

The amplified cDNA fragments from the secondary PCR were ligated into a pCRII vector using a T/A cloning kit (Invitrogen, Madison, WI, USA). Fifty clones were isolated and sequenced by an ALF auto sequencer (Pharmacia Biotech, Tokyo, Japan) with an Autoreading sequencing kit (Pharmacia Biotech). The retrieved sequences were aligned with cDNA sequences in the GenBank

nucleotide database at the National Center for Biotechnology Information (NIH, Bethesda, MD, USA) using the Blastn program to search for sequence matches (<http://www.ncbi.nlm.nih.gov/blast/>).

Northern blot analysis

Total RNA was prepared from non-pregnant uteri and pregnant uteri on days 6, 9, 12 and 15 as described above. Total RNA (20 µg/lane) was separated in 1% agarose gel. Northern blot analysis was performed as described previously (Ogawa *et al.* 1998). The sFRP4 cDNA fragment obtained by SSH and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragment (Orimo *et al.* 1995) were labeled with [α -³²P]dCTP using a BcaBEST Labeling kit (Takara Shuzo) and used as probes. Autoradiography was carried out at -80 °C with an intensifying screen for 48 h. The intensity of the hybridization band was measured using BioMax 1D image analysis software (Kodak, Rochester, NY, USA).

In situ hybridization histochemistry

Digoxigenin (DIG)-labeled single-stranded RNA probes were prepared using a DIG RNA labeling kit (Roche Diagnostics, Rotkreutz, Switzerland). The sFRP4 riboprobe was generated using a 474 bp cDNA fragment obtained by SSH. The estrogen receptor- α (ER α) riboprobe was generated from a 1803 bp full-length fragment containing the entire open reading frame of the receptor (Koike *et al.* 1987). *In situ* hybridization histochemistry was performed as described previously (Ishikawa *et al.* 1999). The sections were deparaffinized, rehydrated, incubated with 20 µg/ml proteinase K for 30 min at room temperature, treated with 0.2 M HCl for 10 min, and then acetylated. Hybridization was carried out at 55 °C for 18 h in a solution containing 25% formamide, 1.25 × SSC, 0.1% SDS, 50 µg/ml heparin, 50 µg/ml yeast RNA, and 1 µg/ml RNA probe. After washing twice with solution I (50% formamide, 5 × SSC, 1% SDS) at 55 °C for 30 min and three times with solution II (0.5 M NaCl, 10 mM Tris-HCl, 0.1% Tween 20) at room temperature for 10 min, the sections were treated with 25 µg/ml RNase A to remove non-specific binding of the probe. The sections were reacted with blocking solution containing

casein (Roche Diagnostics) for 1 h, and incubated with alkaline phosphatase-labeled anti-DIG antibody for 18 h. The sections were washed and incubated with the chromogenic substrate solution containing 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate (Roche Diagnostics) until adequate staining was observed.

Immunohistochemistry

PCNA was detected using immunohistochemical staining. After sections were hydrated, non-specific binding was blocked with PBS containing 10% fetal bovine serum (FBS) for 1 h. The sections were incubated with anti-PCNA mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or mouse IgG as a negative control diluted 1:500 in PBS containing 10% FBS for 18 h at 4 °C, followed by biotinylation with secondary antibody (anti-mouse rabbit immunoglobulins including IgG, IgA and IgM) for 1 h at room temperature. Sections were incubated with strept-avidin conjugated with horseradish peroxidase and visualized by using peroxidase substrate 3,3'-diaminobenzidine (Histofine SAB-PO kit, DAB substrate kit; Nichirei Inc., Tokyo, Japan).

Results

Analysis of SSH PCR products

We selected fifty cDNA clones from the SSH cDNA library constructed between pregnant uteri on day 15 and non-pregnant uteri. The sequences were analyzed for similarity against all non-redundant database sequences using the gapped BLAST search. The isolated genes were summarized in Table 1. Some of the isolated clones corresponded to expressed sequence tags (ESTs). Seven differentially expressed clones were isolated from 50 clones and six out of seven clones were previously known genes that up-regulate during pregnancy, namely; α -2 macroglobulin (Fletcher *et al.* 1988, Thomas 1993), placental lactogen II (Faria *et al.* 1990), calcyclin (Thordarson *et al.* 1991, Waterhouse *et al.* 1992), plasma glutathione peroxidase (Takahashi *et al.* 1990), amiloride-binding protein (Lingueglia *et al.* 1993), and pregnancy-specific glycoprotein rnCGM (Chen *et al.* 1994). Interestingly, the last clone out of the

Table 1 Up-regulated genes in pregnant rat uterus isolated by suppression subtractive hybridization (SSH)

	cDNA	Localization	Day	Species	Reference
Novel	sFRP4	n.d.	n.d.	Rat	—
Known	α -2 Macroglobulin	Decidua, fetal liver	6–21	Rat	Fletcher <i>et al.</i> (1988) Thomas (1993)
	Placental lactogen II	Decidua, placenta, trophoblast giant cell	8–13	Mouse, rat	Faria <i>et al.</i> (1990)
	Calcyclin	Uterus, decidua, placenta	5–18	Mouse	Thordarson <i>et al.</i> (1991) Waterhouse <i>et al.</i> (1992)
	Plasma glutathione peroxidase	Placenta	n.d.	Human	Takahasshi <i>et al.</i> (1990)
	Amiloride-binding protein	Placenta	n.d.	Human, rat	Lingueglia <i>et al.</i> (1993)
	Pregnancy specific glycoprotein rnCGM3	Placenta	n.d.	Rat	Chen <i>et al.</i> (1994)
EST	EST 207450 (AI012999)	n.d.	n.d.	Rat	—
	EST 212820 (AI103531)	n.d.	n.d.	Rat	—

EST, expressed sequence tags; n.d., not determined.

seven isolates was identical to sFRP4, which was identified as a novel up-regulated gene during pregnancy. sFRP4 is a member of the secreted frizzled-related protein family, and previously reported to be expressed in mammary gland, ovary and prostate (Wolf *et al.* 1997).

Since up-regulation of sFRP4 during pregnancy is a novel finding, we focused on sFRP4 for further study. sFRP4 contains a cysteine-rich domain (CRD) that functions as a Wnt-binding domain in frizzled proteins (Wolf *et al.* 1997). While frizzled proteins have a seven-pass transmembrane domain (Bhanot *et al.* 1996), secreted frizzled-related proteins including sFRP4 lack this domain.

The size of sFRP4 cDNA was reported to be 1910 nucleotides containing a 1044 bp open reading frame that encodes 348 amino acids (Wolf *et al.* 1997). The fragment size of our isolated fragment was 474 bp and corresponded to the fragment of nucleotides 661–1134 of sFRP4 cRNA (Wolf *et al.* 1997) (Fig. 1).

Expression of sFRP4 during pregnancy

To determine the expression pattern of sFRP4 mRNA during pregnancy, Northern blot analysis was performed for non-pregnant uteri and pregnant uteri on days 6, 9, 12 and 15. As shown in Fig. 2A, variable amounts of 2.0 kb and 2.9 kb sFRP4 mRNAs were detected in the pregnant uteri on

days 9, 12 and 15. In contrast, sFRP4 mRNA was not detected in the pregnant uteri on day 6 and in non-pregnant uteri. The intensity of the hybridization bands was measured by a phosphor imager, and sFRP4 signals were normalized utilizing the GAPDH signals for each time point. The intensity of sFRP4 signal exhibited its peak on day 12 and declined gradually thereafter (Fig. 2B).

We further investigated the cell type-specific expression of sFRP4 in the pregnant uteri on day 12. *In situ* hybridization histochemistry revealed specific distributions of the sFRP4 transcripts, especially in the decidua and weakly in the myometrium (Fig. 3A). Control sections treated with sFRP4 sense probe showed no specific signals (Fig. 3B). In addition, the expression of ER α mRNA was investigated using an ER α -specific antisense probe. Although ER α mRNA was detected in the serosa, myometrium and decidua, strong signals were observed especially in the

Figure 1 Isolated cDNA fragment of sFRP4. The size of the cDNA fragment was 474 bp and corresponded to the fragment of nucleotides 661–1134 of sFRP4 (Wolf *et al.* 1997). Both ends of the fragment contain RsaI sites because the synthesized double-stranded cDNA was digested with this restriction enzyme.

A

B

Figure 2 Expression of sFRP4 mRNA in rat uterus during pregnancy. (A) Northern blots containing total RNA (20 µg) extracted from non-pregnant (Non-preg) uteri and pregnant uteri on days 6, 9, 12 and 15 were hybridized with the sFRP4 cDNA probe or the GAPDH probe as an internal control. Variable amounts of 2.0 kb and 2.9 kb sFRP4 transcripts were detected in pregnant uteri on days 9, 12 and 15. In contrast, sFRP4 mRNA was not detected in non-pregnant uteri and pregnant uteri on day 6. (B) Semiquantitative analysis of the ratio of sFRP4 transcripts levels against GAPDH transcripts levels in pregnant uteri on days 9, 12, and 15 ($n=3$ for each group). Values are means $\pm$ S.D.

decidua (Fig. 3C) where the sFRP4 transcripts were also detected (Fig. 3A). Control sections treated with ER α sense probe showed no specific signals (Fig. 3D). We further investigated whether expression of sFRP4 was involved in proliferation of deciduae using serial sections. As shown in Fig. 4A, a strong signal of sFRP4 was observed in the decidua. Distribution of PCNA-positive cells was overlapped in the decidua (Fig. 4C), suggesting that sFRP4 expression was observed in proliferating deciduae. There was no staining observed when the PCNA antibody was replaced by the mouse IgG (Fig. 4D).

Expression of sFRP4 by estrogen treatment

To investigate the possibility that sFRP4 is regulated by estrogen, the estrogen-treated ovariectomized rats were used as a model. Northern hybridization analysis revealed that the sFRP4 signal was detected in the uteri at 48 h after estrogen treatment. In contrast, sFRP4 mRNA was not detected in uteri at 0, 2, 6 and 24 h after estrogen treatment (Fig. 5A). No signal was observed at 0, 2, 6, 24 and 48 h after oil treatment (Fig. 5B). *In situ* hybridization histochemistry showed that sFRP4 mRNA was expressed predominantly in the endometrial stroma and less abundantly in the myometrium, whereas it was not detected in either luminal or glandular epithelium (Fig. 6A). The expression of sFRP4 in the endometrial stroma was observed more clearly by higher magnification (Fig. 6B). PCNA-positive cells were detected in the endometrial stroma, luminal epithelium and glandular epithelium (Fig. 6C).

Discussion

In the present study, using SSH we identified seven independent transcripts that up-regulated in the pregnant rat uterus. Six out of seven clones were previously known genes that up-regulate during pregnancy. One differentially expressed clone out of seven was found to be sFRP4 and this is the first report that sFRP4 is up-regulated during pregnancy. Amino acid sequences of sFRP4 proteins are well conserved among species. Rat sFRP4 was originally isolated from rat apoptotic tissues such as ovarian corpus luteum around parturition, involuted mammary gland and ventral prostate (Wolf *et al.* 1997, Guo *et al.* 1998). A human homologue of sFRP4 was identified as an up-regulated gene in endometrial carcinoma compared with normal endometrium (Abu-Jawdeh *et al.* 1999). A mouse homologue of sFRP4 was expressed in the developing teeth, eye and salivary gland of the embryo (Leimeister *et al.* 1998).

sFRP4 has a CRD, which functions as a Wnt-binding domain (Wolf *et al.* 1997). The Wnt family appears to induce multiple biological functions including cell growth, differentiation and survival (McMahon & Bradley 1990, Thomas & Capecchi 1990, Danielian & McMahon 1996). Wnt signal is transduced inside the cell by frizzled

Figure 3 Distribution of sFRP4 mRNA and ER α mRNA in pregnant rat uteri on day 12. *In situ* hybridization histochemistry of sFRP4 (A, B) and ER α (C, D) and hematoxylin and eosin staining (E) were performed using pregnant uteri on day 12. (A) sFRP4 mRNA was detected in the decidua (dc) as indicated by the arrow when sFRP4-specific antisense RNA probe was used. (B) No signal was detected by sFRP4 sense probe. (C) ER α mRNA was detected especially in the decidua (dc) as indicated by the arrow when the ER α -specific antisense RNA probe was used. ER α mRNA was also observed in the myometrium (m) and serosa (s). (D) No signal was detected by the ER α sense probe. All experiments were performed three times. Bars=200 μ m.

Figure 4 Proliferating cell nuclear antigen (PCNA)-positive cells were overlapped in the deciduae where sFRP4 mRNA was detected. *In situ* hybridization histochemistry of sFRP4 (A, B) and immunostaining of PCNA (C, D) were performed using serial sections of pregnant uteri on day 12. (A) Strong signal of sFRP4 mRNA was observed in the decidua. One of the positive signals of sFRP4 is marked with an arrow. (B) No signal was detected by sFRP4 sense probe. (C) PCNA-positive cells were also detected in the decidua. One of the positive signals for PCNA is marked with an arrow. (D) No signal was detected when mouse IgG was utilized. All experiments were performed three times. Bars=50 μ m.

proteins, which contain a CRD and a seven-pass transmembrane domain (Bhanot *et al.* 1996). In contrast, secreted frizzled-related proteins, which possess a conserved CRD, lack the transmembrane domain. Although the biological activities of secreted frizzled proteins remain to be studied, they are assumed to function as modulators of the Wnt–frizzled signaling pathway. For example, it is reported that human frpHE antagonized the

Wnt-8-mediated dorsal axis duplication in the *Xenopus* embryo (Abu-Jawdeh *et al.* 1999), suggesting that sFRP4 antagonizes Wnt signaling. In the rodent uterus, it is known that some Wnt genes such as *Wnt-4*, *-5a* and *-7a* are expressed (Miller *et al.* 1998). *Wnt-4* and *-5a* are expressed in the mouse uterine epithelium and stroma. *Wnt-7a* is expressed within the luminal epithelium. The expression levels of those Wnt genes in the uterus vary during the

Figure 5 sFRP4 mRNA was regulated by estrogen in the uterus. Northern blots containing total RNA (20 µg) extracted from the ovariectomized rat uteri at 0, 2, 6, 24 and 48 h after estradiol treatment (10 µg/kg) or olive oil treatment were hybridized with the sFRP4 cDNA probe or the GAPDH probe as an internal control. (A) sFRP4 mRNA was detected only in the uterus at 48 h after the treatment. (B) No signal was detected after olive oil injection. All experiments were performed three times.

estrous cycle (Miller *et al.* 1998), suggesting that those expressions may be regulated by estrogen and play key roles in adult uterine function including the morphological changes which occur in response to circulating hormone levels (Miller & Sassoon 1998). It was also reported that the uteri of *Wnt-7a*-null mutant mice were smaller than that of wild type, absence of uterine glands and reduction in the mesenchymally derived stroma suggesting that *Wnt-7a* is required in cytodifferentiation in the uterus (Parr & McMahon 1998). During pregnancy, the expression of *Wnt-4* observed in the stroma surrounding the embryo at the onset of implantation and then the expression increased in the decidua (Paria *et al.* 2001). From the structural aspects of sFRP4, it is tempting to speculate that sFRP4 modulates the Wnt signal pathway via binding to some Wnt genes such as *Wnt-4*, *-5a* and *-7a*.

Northern blot analysis revealed that the expression of sFRP4 mRNA was highest in the pregnant uteri on day 12. *In situ* hybridization histochemistry demonstrated that sFRP4 mRNA expression was restricted to the decidual cells. Moreover, PCNA-positive cells were mainly found in the decidual cells, where sFRP4 mRNA was detected. In the pregnant rat, endometrial stromal cells begin to proliferate and differentiate to form decidual tissue

after implantation on day 6 (Abrahamsohn & Zorn 1993), and proliferation continues until day 12 when decidualization completes (Ogle *et al.* 1998). Thus, it is possible that the marked increase of sFRP4 transcripts in the decidua on day 12 was accompanied by the late phase of decidual cell proliferation. Alternatively, it is known that internucleosomal DNA fragmentation in the decidua basalis begins in mid-pregnancy (Ogle *et al.* 1999). From day 14, the decidual tissue begins to regress to the end of pregnancy (Ogle *et al.* 1990, Gu *et al.* 1994). The controlled cell death of large numbers of decidual cells allows remodeling of the implantation chamber without disrupting the growth and development of the embryo or the integrity of the uterus (Welsh & Enders 1985). Another study indicated that DNA breakdown was first detected at day 10 and that it increased time-dependently (Gu *et al.* 1994, Moulton 1994). Taken together, it is possible that sFRP4 might be involved in the initiation of decidual apoptosis by modulating some Wnt signals.

ERα mRNA was detected in the decidua as well as sFRP4 mRNA. It is known that the steroid hormones such as estrogen and progesterone modulate the structure and function of the uterus during pregnancy through their nuclear receptors

Figure 6 Distribution of sFRP4 mRNA and PCNA-positive cells in rat uteri at 48 h after estrogen treatment. *In situ* hybridization histochemistry of sFRP4 (A, B) and immunostaining of PCNA (C, D) were performed using ovariectomized rat uteri at 48 h after estrogen treatment. (A) sFRP4 mRNA was expressed abundantly in the endometrial stroma (e) whereas there was little in the myometrium (m) when hybridized with sFRP4-specific antisense RNA probe. No sFRP4 mRNA expression was detected in either the luminal epithelium (l) or glandular epithelium (g). (B) Higher magnification of (A). (C) PCNA-positive cells were detected in the stroma, luminal epithelium and glandular epithelium. (D) Higher magnification of (C). All experiments were performed three times. Bars represent (A, C) 200 μ m and (B, D) 50 μ m.

(Pepe & Albrecht 1995). Thus, we speculate the regulation of sFRP4 expression by estrogen in the uterus. To investigate this possibility, we used ovariectomized rats treated with estrogen. sFRP4 mRNA expression was detected 48 h after estrogen treatment. Unlike during pregnancy, the 2.0 kb sFRP4 transcript was observed whereas the 2.9 kb transcript was not. It is possible that several splice variants of sFRP4 may exist and only the 2.0 kb transcript can be especially up-regulated by estrogen. Alternatively, other transcripts were not

detected in our Northern analysis because the expression levels were lower. In general, direct estrogen-responsive genes are up-regulated within several hours of estrogen treatment. For example, the level of progesterone receptor mRNA was elevated within 2 h after a single injection of 17 β -estradiol (Shughrue *et al.* 1997). It was rather late that sFRP4 mRNA was detected in the uterus, at 48 h after estrogen injection, suggesting that up-regulation of sFRP4 may be an indirect effect of estrogen.

The expression of sFRP4 mRNA was observed in the proliferating endometrial stroma. It was reported that the maximal number of PCNA-positive cells was found after 36 h in the epithelium and stroma (Gunin 1997). Thus, the expression of sFRP4 mRNA might be accompanied by the late phase of proliferation after estrogen treatment.

In summary, we have identified sFRP4 as an up-regulated gene in the uterus during pregnancy. Our present data suggest that the expression of sFRP4 mRNA was accompanied by a late phase of proliferation in the decidual cells. It is possible that sFRP4 modulates signals of Wnt genes in the pregnant uterus.

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