Cellular localization of human relaxin-like factor in the cyclic endometrium and placenta

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We have studied the cellular localization of the relaxin-like factor (RLF) in the histologically normal cyclic endometrium collected from days 3–26 of the menstrual cycle. RLF transcripts and protein were detected in the luminal and glandular epithelium and in stromal cells at all stages of the cyclic endometrium. Increased expression of RLF was observed in endometrial tissues in the proliferative as compared to the secretory phase, suggesting that oestrogens affect RLF gene activity in the human endometrium. The cellular localization of RLF transcripts and protein was also determined in first trimester placental tissues obtained from normal and ectopic tubal implantation sites and in third trimester placentae of normal and pre-eclamptic pregnancies. In first trimester placenta, weaker expression of RLF was observed in the syncytiotrophoblast as compared to the underlying cytotrophoblast. Extravillous trophoblast cells constitutively expressed RLF. Trophoblast cells were the main source of RLF in the human placenta and trophoblastic RLF gene activity was unaffected by either the site of implantation or the invasive properties of the cytotrophoblast as demonstrated by samples from patients with tubal implantation and pre-eclampsia respectively. Decidual cells weakly expressed RLF. The presence of unprocessed and cleaved immunoreactive RLF in term placenta was determined by Western analysis. The above results suggest a functional role for both RLF isoforms within normal placental tissue.

Key words: endometrium/placenta/relaxin-like factor/uterus

Introduction

The relaxin-like factor (RLF), also known as Leydig-insulin-like peptide (Ley-IL) or insulin-like factor 3 (INSL-3), is structurally closely related to relaxin, and belongs to the family of insulin-like hormones (Adham et al., 1993; Büllersbach and Schwabe, 1995; Pusch et al., 1996; Ivell, 1997). Within the testis, RLF is a suitable marker of testicular Leydig cells in a number of species (Adham et al., 1993; Burkhardt et al., 1994; Bathgate et al., 1996; Pusch et al., 1996; Ivell et al., 1997; Spiess et al., 1999; Hombach-Klonisch et al., 1999, 2000a). Mice with a deleted RLF gene fail to form a gubernaculum testis and display cryptorchidism and male infertility (Nef and Parada, 1999). In the fallow deer, the endometrium and the syngnathoschial placenta have been identified as a source of RLF. The placental binucleate trophoblast cells display differential RLF gene expression (Hombach-Klonisch et al., 2000a). Information on the endometrial expression of RLF in normal cycling women is lacking and despite the detection of RLF mRNA in human placental extracts (Tashima et al., 1995), the cellular localization of RLF transcripts within the haemochorial human placenta has not yet been shown.

In this study, we demonstrate the expression and cellular localization of RLF in the endometrium of normal cycling women and identify the cellular source of RLF within the placenta in the first and third trimester of pregnancy.

Materials and methods

Tissues and RNA isolation

Human endometrial tissue samples from days 3–26 of the menstrual cycle (n = 86; Table I) were obtained from women who were
25–45 years of age (25–31 years: n = 15; 32–38 years: n = 35; 39–45 years: n = 35) and who had at least three regular cycles and were not treated with hormonal contraceptives. All tissue specimens had been histologically stained with haematoxylin and eosin and the cycle stage (Table I) and adequate tissue transformation had been confirmed (Küchenhoff et al., 1999). Upon elective Caesarean section at term of normal pregnancies, biopsies were taken from each placenta of the villous placental part, the decidua basalis and the decidua parietalis (n = 12). Similar biopsies were obtained from placentae of three patients with pre-eclampsia and clinically indicated premature termination of pregnancy by Caesarean section. Tissues were fixed in Bouins solution and embedded in paraffin wax. Placental tissues of normal pregnancies at the first trimester from week 8 to 13 (n = 10) and ectopic tubal implantation sites (n = 10) between weeks 6 and 12 of pregnancy were collected and fixed in 10% formalin. The study was approved by the ethical committee of the Martin-Luther-University Halle-Wittenberg and the informed consent of the patients was obtained prior to tissue collection. For the extraction of protein and total RNA, term placental tissue was frozen in liquid nitrogen and stored at −80°C until used. The human choriocarcinoma cell lines JEG-3, JAR and BeWo (ATCC, Manassas, Virginia, USA) were cultured in Ham’s F-12/Dulbecco’s modified Eagle’s medium (Life Technologies, Eggenstein, Germany) plus 10% fetal calf serum. Total RNA was extracted with Trizol reagent (Life Technologies). The amount of RNA isolated was determined spectrophotometrically at 260 and 280 nm (Sambrook et al., 1989).

cDNA synthesis and RT-PCR

Approximately 2 µg of total RNA of normal human term placental tissue of two patients and of human postpubertal testicular tissue were used for first strand cDNA synthesis employing the Superscript reverse transcriptase kit and 500 ng/ml of oligo d(T) primer (both Life Technologies). PCR reactions were carried out in 50 µl solution containing 1 µl of these cDNA preparations or a cDNA library of the human placenta (Clontech, Heidelberg, Germany), 5 µl of 10× Advantage cDNA polymerase mix buffer, 100 µmol/l of dNTP, 10 pmol of each primer (forward primer 5’CAGAGATGCGTGAGACGGTGCC3’, reverse primer 5’TCAATAGGGAGAGAGGTCAGC3’) and 2.5 U Advantage cDNA mix polymerase (Clontech). The PCR cycles consisted of an initial denaturation for 3 min at 95°C, followed by 40 cycles of denaturation at 95°C and annealing at 68°C, both for 1 min each, and elongation for 2 min at 72°C, and a final extension step for 10 min at 72°C. Sequence analysis of the PCR amplicons was performed employing the PRISM dye Deoxy Terminator cycle sequencing kit (Perkin Elmer, Foster City, CA, USA).

Digoxigenin-labelling of cRNA and in-situ hybridization

For cRNA synthesis, 5 µg of the pGEM-T plasmid clone containing the insert for human RLF were digested with the restriction enzymes Not I (antisense cRNA) and Sac II (sense cRNA; both New England Biolabs, Schwalbach/Taunus, Germany), extracted in phenol and precipitated. Employing a cRNA synthesis kit (AMS Biotechnology, Wiesbaden, Germany) and a digoxigenin-RNA 10× labelling mix (Boehringer, Mannheim, Germany), cRNA synthesis was performed with 1 µg of digested and extracted plasmid as described previously (Klonisch et al., 1999a). For non-radioactive in-situ hybridization, dewaxed 6 µm thick serial sections of human endometrial and placental tissues were digested with proteinase K at 20 µg/ml and 30 µg/ml (Boehringer), respectively, postfixed in 4% parafomaldehyde and processed according to a published method (Lewis and Wells, 1992). Briefly, fixed sections were washed 2×5 min in phosphate-buffered saline (PBS) containing 5 mmol/l MgCl2 and endogenous alkaline phosphatase was inactivated for 30 s in cold 20% acetic acid. After blocking non-specific binding sites for 1 h with 20% glycerol, the hybridization mixing containing the sense or antisense cRNA plus 100 µg each of salmon sperm DNA and yeast tRNA (both Sigma), 50% deionized formamide and dextran sulphate, 2×SSC [1×SSC is 150 mmol/l NaCl, 15 mmol/l Na citrate, (pH 7.0)] and 1×BFP [0.2% each of polyvinylpyrrolidone, bovine serum albumin (BSA), Ficoll 400] were added to the sections. After incubation at 42°C overnight, sections were washed 4×15 min in 4×SSC at room temperature, RNase-digested for 30 min at 37°C and subsequently washed in 4×SSC (5×7 min, 37°C), 2×SSC (20 min, 60°C), 0.2×SSC (20 min, 42°C), 0.1×SSC and 2×SSC (5 min each at room temperature) and finally 1×TNMT (100 mmol/l Tris-HCl, pH 7.5, 100 mmol/l NaCl, 4 mmol/l MgCl2, 0.05% Triton X-100) for 10 min at room temperature. After blocking non-specific binding sites with 3% BSA, hybridization signals were detected by employing a 1:1000 dilution of an anti-digoxigenin alkaline phosphatase conjugated Fab-antibody in 1% BSA (Boehringer) and chromogenic detection. Some sections were counterstained with haematoxylin, mounted in glycerol gel and examined under bright-field microscopy.

Immunohistochemistry

Human endometrial and placental tissue serial sections (6 µm thick) were dewaxed and rehydrated in PBS containing 0.1% Tween-20 (PBS-T). For the alkaline phosphatase detection procedure, endogenous alkaline phosphatase was inactivated in 20% acidic acid for 30 s at 4°C. For horseradish peroxidase (HRP) detection techniques, endogenous peroxidase was inactivated with 3% H2O2 in methanol. Non-specific binding sites were blocked with 10% non-immune goat serum. Tissue sections were incubated overnight at 4°C with a rabbit polyclonal RLF antiserum diluted 1:200 in PBS-T (alkaline phosphatase detection) or 1:750 in PBS-Tween (HRP detection) containing 3% BSA. The RLF antiserum had been generated against a synthetic peptide epitope of human RLF and was previously shown to specifically detect human RLF (Hombach-Klonisch et al., 2000b). After three washing steps, the sections were incubated for 1 h at room temperature with an alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (Dianova, Hamburg, Germany) at 1:250 or with an HRP-conjugated goat anti-rabbit secondary antibody (Southern Biotechnology Associates, Birmingham, AL, USA) at 1:300. Specific binding was visualized with the alkaline phosphatase substrate HistoMark Red (KPL, Maryland, USA) or with the peroxidase-substrate DAB (Pierce, Rockford, IL, USA). Sections were counterstained with haematoxylin and mounted in glycerol gel. Mouse monoclonal antibodies were used for the detection of cytokeratin.
Proteins were separated on a 15% sodium dodecyl sulphate with a protein assay kit (Biorad, Munich, Germany). The extracted used for Western analysis. The amount of protein was determined Berlin, Germany). After an overnight blocking step at 4°C containing a sequence of the B-domain of human RLF (BioGenes, had been generated by immunizing rabbits with a synthetic peptide (Amersham, Braunschweig, Germany). For immunodetection of (mouse APAAP; all reagents from Dako). For immunodetection of detected with a peroxidase-conjugated goat anti-rabbit Ig (Dianova) powder. After thorough washing with PBS-T, speci- diluted antibodies. Specific binding was visualized using the mouse APAAP technique as described above.

**Western analysis**

Protein was isolated from snap-frozen samples of human term placental tissue using lysis buffer containing 63 mmol/l Tris, 2% SDS and 10% saccharose. After boiling for 5 min at 95°C and subsequent centrifugation, the supernatant was stored at –20°C until used for Western analysis. The amount of protein was determined with a protein assay kit (Biorad, Munich, Germany). The extracted proteins were separated on a 15% sodium dodecyl sulphate–polyacrylamide gel and blotted onto a Hybond nitrocellulose membrane (Amersham, Braunschweig, Germany). For immunodetection of human RLF, a previously characterized RLF antisera was used that had been generated by immunizing rabbits with a synthetic peptide containing a sequence of the B-domain of human RLF (BioGenes, Berlin, Germany). After an overnight blocking step at 4°C in PBS-Tween containing 5% milk powder and 1% BSA, blots were incubated for 1 h at room temperature with the rabbit polyclonal serum, diluted 1:2000 in PBS plus 0.1% Tween-20 (PBS-T) containing 5% milk powder. After thorough washing with PBS-T, specific binding was detected with a peroxidase-conjugated goat anti-rabbit Ig (Dianova) diluted at 1:5000 in PBS-T and visualized with the ECL Western blotting detection reagent (Amersham). As a negative control, blots were incubated with a rabbit non-immune serum (Dako) diluted 1:2000 in PBS-T with 5% milk powder.

**Results**

Employing RT-PCR analysis with a primer pair specific for human RLF, an amplicon of the expected size of 317 bp was detected in a human placenta cDNA library and in cDNA preparations of term placental tissue from two patients and of human postpubertal testicular tissue used as a positive control. Sequence analysis confirmed the amplicons to encode for human RLF. Alternative splice variants were not detected. The three human choriocarcinoma cell lines JEG-3, JAr and BeWo were devoid of RLF transcripts (data not shown).

Non-radioactive in-situ hybridization and immunostaining with a rabbit polyclonal RLF antisera were performed for the detection of RLF mRNA and RLF protein, respectively, in the normal human cyclic endometrium and in placental tissues of normal and pathological pregnancies. A summary of the results is presented in Table II. The endometrial tissues investigated had previously been characterized histologically and shown to display expression patterns for both the oestrogen receptor and the progesterone receptor typical during the regular menstrual cycle (Küchenhoff et al., 1999). In the endometrial tissues of all patients, luminal epithelial cells, endometrial gland cells and stromal cells expressed RLF mRNA (Figure 1A) and immunoreactive RLF (Figure 1C,E). Expression of RLF transcripts and protein within the endometrium varied with the stage of the cycle and was consistently stronger during the proliferative phase (Figure 1C) and less intense during the secretory phase (Figure 1E). Independent of the stage of the cycle, endometrial gland cells located adjacent to the myometrium displayed more intense staining for immunoreactive RLF and displayed stronger RLF hybridization signals than apical glandular tissue located close to the uterine lumen (data not shown).

Extravillous trophoblasts located within the cell columns of anchoring villi (Figure 1F) and in maternal blood vessels (Figure 1I) of first trimester placentae, as well as the extravillous trophoblasts of fetal membranes and basal plate of third trimester placentae, expressed RLF transcripts and stained immunopositive for RLF protein (Figure 1K). In the first trimester, both the proliferating and the invading extravillous trophoblast of cell columns, as revealed by immunostaining for ErbB2 and HLA-G (Mühlhausen et al., 1993; McMaster et al., 1995), expressed RLF mRNA (Figure 1F) and RLF protein (data not shown). In the placental bed of first trimester specimens, immunostaining for cytokeratin was used to identify extravillous trophoblast (Figure 1G,J). In serial sections, immunohistochemistry was performed for cytokeratin, RLF and vimentin. Decidual cells [positive for vimentin, negative for cytokeratin (Figure 1J)] weakly expressed RLF transcripts (Figure 1I) and RLF protein (data not shown), while extravillous trophoblast cells were strongly RLF immunopositive (Figure 1K). The amniotic epithelial cell layer was also a source of RLF (Figure 1K). Within the villous tissue of human placenta throughout gestation, strong expression of RLF mRNA (Figure 1M) and RLF protein were detected in villous cyto- trophoblast, whereas in the syncytiotrophoblast and in stromal cells only weak expression of RLF mRNA was observed (Figure 1M). All tissue sections were examined for the expression of both RLF transcripts and immunoreactive protein and the consistent results were obtained in all samples studied (Table II). The extravillous trophoblasts at ectopic tubal implantation sites also strongly expressed RLF protein (Figure 1L). Patients with clinically symptomatic pre-eclampsia dis-

| Table II. In-situ hybridization and immunohistochemical results for relaxin-like factor |
|---------------------------------|-----------------|-----------------|
| Tissues                        | Hybridization   | Immunohistochemistry |
| Endometrium                    |                 |                  |
| Proliferative phase luminal/    | + +             | + +              |
| glandular epithelium           |                 |                  |
| Secretory phase luminal/glandular epithelium | + | + |
| Stroma                         | (+)             | (+)              |
| Placenta                       |                 |                  |
| Villous cytotrophoblast        | + +             | + +              |
| Villous syncytiotrophoblast    | +               | +                |
| Extravillous cytotrophoblast   | + +             | + +              |
played placental RLF expression patterns similar to those obtained in normal pregnancies (data not shown). All endometrial and placental sections treated with a sense cRNA probe were devoid of hybridization signals (Figure 1B,H). When endometrial and placental sections were treated with a rabbit non-immune serum instead of the rabbit RLF antiserum, the sections were devoid of immunoreactivity (Figure 1D).

Western analysis on human term placental tissue with the previously characterized RLF antiserum (Hombach-Klonisch et al., 2000b; Klonisch et al., 2001) revealed specific immunoreactive bands at approximately 14 and 6 kDa consistent with human precursor and processed RLF respectively (Figure 2A). Rabbit non-immune serum served as a negative control and did not reveal specific bands (Figure 2B).
expression of RLF is increased during cellular proliferation and would be consistent with the observations in proliferating villous cytotrophoblast of the dog placenta (Klonisch et al., 2001) and, as demonstrated here, in the human placental villous tree.

The structurally closely related hormone, relaxin, has also been localized to the glandular and luminal epithelium of the human endometrium during both phases of the cycle. In contrast to RLF, expression of the relaxin protein appears to be restricted to the late secretory phase in the endometrial stromal cell compartment (Bryant-Greenwood et al., 1993). This suggests a role for relaxin during progesterone-dependent decidualization of endometrial stromal cells, as also suggested in the non-pregnant uterus of the marmoset. Here, increasing levels of relaxin protein are found in the endometrial epithelium during the late proliferative and secretory phase (Einspanier et al., 1997). Human endometrial cells contain high-affinity binding sites for relaxin (Osheroff and King, 1995). In the non-pregnant rat uterus, oestrogens appear to mediate the effects of relaxin which, at least in part, is achieved by the 17β-oestradiol-dependent up-regulation of relaxin binding sites (Pillai et al., 1999; Tan et al., 1999). In cell cultures derived from human endometrium, relaxin alone, or in combination with progestins, has been shown to modulate the expression of vascular endothelial growth factor (Unemori et al., 1999), placental protein 14 (Tseng et al., 1999; Taylor et al., 2000), aromatase (Tseng et al., 1987), HOXA-10 (Gui et al., 1999), prolactin (Huang et al., 1987; Zhu et al., 1990), prorenin (Poisner et al., 1990) and insulin-like growth factor binding protein-1 (Tseng et al., 1992; Liu et al., 1997). These diverse effects appear to be mediated by protein kinase C- and cAMP-dependent signalling pathways (Chen et al., 1988; Fei et al., 1990; Kalbag et al., 1991).

From studies of relaxin-negative mice, relaxin has been shown to be the paracrine stromal factor that induces proliferation and differentiation of uterine and vaginal epithelial cells upon oestrogen stimulation (Zhao et al., 2000). The presence of specific RLF receptors in the mouse uterus (Büllersbach and Schwabe, 1995) and the ability, as shown in the mouse, of RLF to cross-react with relaxin binding sites which are probably similar to those in human uterine cells (Osheroff and King, 1995; Palejwala et al., 1998) suggests a functional interplay between relaxin and RLF within the endometrium and decidua. The likely presence of specific RLF receptors in the human uterus as well as the temporal differences in the expression patterns of both relaxin and RLF within human endometrial stromal cells indicate that RLF possesses specific paracrine/autocrine functions during endometrial cell proliferation and differentiation of the normal menstrual cycle in women.

Expression of RLF in the human placenta has been reported by Northern and RT-PCR analysis (Tashima et al., 1995) but the cellular localization of RLF was not shown. We have previously described the differential expression of RLF transcripts during differentiation of migratory binucleate trophoblast cells within the syncytiotrophoblastic placenta of the fallow deer (Hombach-Klonisch et al., 2000a). The ruminant placenta displays only limited trophoblast invasion due to the cellular

Discussion

The interrelationship between the cyclic transformations of the uterine endometrial epithelial and stromal cell compartments and the ovarian steroid hormones oestrogen and progesterone have been described in detail (Spona et al., 1979; Tabibzadeh, 1996). Here we demonstrate RLF mRNA and protein expression in luminal and glandular epithelial cells and in the stromal cell compartment of human endometrial tissues throughout the cycle. The same endometrial tissues had previously been found to show an expression pattern of receptors for oestrogen and progesterone that is typical for the normal cycling endometrium (Küchenhoff et al., 1999). We did not observe a correlation between the age of the patients and the intensity of RLF staining. However, the increased RLF signals, both RNA and protein, during the proliferative as compared to the secretory phase of the cycle suggested that oestrogens have a stimulatory effect on endometrial RLF gene activity. We have previously demonstrated RLF mRNA expression in the uterine epithelium of the non-pregnant and pregnant uterus of the fallow deer (Hombach-Klonisch et al., 2000a) and the dog (Klonisch et al., 2001) indicating that RLF plays an as yet undefined role in normal uterine epithelial cell physiology in various species. As in the endometrium of the canine non-pregnant uterus (Klonisch et al., 2001), basal glands in the human endometrium displayed stronger expression of RLF as compared to apical glands or luminal epithelium. This might suggest that the

Figure 2. Employing a previously characterized rabbit antiserum specific for human relaxin-like factor (RLF) (Hombach-Klonisch et al., 2000b), Western analysis revealed immunoreactive bands at ~14 and 6 kDa consistent with the presence of unprocessed and cleaved RLF in term placental tissue extracts (A). When rabbit non-immune serum was used instead of the rabbit polyclonal RLF antiserum, the blot was devoid of specific immunostaining (B).
fusion of terminally differentiated binucleate trophoblast cells with uterine luminal epithelial cells (Wango et al., 1990; Wooding, 1992; Wooding et al., 1997). In contrast, placenta in the human is a highly invasive process that involves specialized epithelial placental cells, the extravillous trophoblast. Placental cytotrophoblast differentiation leads to two different populations of cells, extravillous and villous trophoblast (Benirschke and Kaufmann, 1995). Trophoblast cells remaining in the fetal compartment fuse to create a multinucleate syncytiotrophoblast covering the floating chorionic villi, the syncytiotrophoblast. Extravillous trophoblasts proceed along the invasive pathway from the cell columns of anchoring villi. These cells invade the uterine wall and reach the maternal blood vessels as far as the first third of the myometrium (Zhou et al., 1997). Extensive extravillous trophoblast invasion is observed in the first two trimesters of pregnancy resulting in the attachment of the placenta to the uterus and the establishment of sufficient maternal blood flow into the intervillous space (Kaufmann and Castellucci, 1997).

Villous and extravillous trophoblast were the major sources of RLF in the human placenta at all gestational stages studied, with the strongest expression in the first trimester placenta. Differentiation of villous cytotrophoblasts toward the syncytiotrophoblast resulted in down-regulation of the RLF expression. Thus, weak expression of RLF in placental tissues in the third trimester may be due to the rarification of villous cytotrophoblast and the resulting presence of villous trees exclusively covered by syncytiotrophoblast.

The detection of 14 and 6 kDa immunoreactive bands by Western analysis revealed the presence of a RLF precursor and a processed form of RLF within third trimester placental tissues. Human prorelaxin has previously been demonstrated to engage in a high-affinity interaction with the relaxin receptor (Tan et al., 1998) suggesting that both unprocessed and enzymatically cleaved RLF may function as active placental hormones during pregnancy (Büllesbach et al., 1999a).

The human placenta is also a source of the two isoforms of human relaxin, H1 and H2 relaxin (Sakbun et al., 1990; Hansell et al., 1991; MacLennan et al., 1991). The presence of relaxin binding sites within the human fetal membranes (Koay et al., 1986; Qin et al., 1997a) indicates that the human placenta is a relaxin target tissue. The expression of RLF in villous and extravillous trophoblasts as well as fetal amniotic cells (Sakbun et al., 1990; Bogic et al., 1995) indicates that RLF may co-operate with human relaxins, for example in facilitating the rupture of fetal membranes at term. H2 relaxin induces the release of tissue plasminogen activator, collagenases and matrix-metalloproteinases, MMP-1, MMP-3 and MMP-9, as has previously been demonstrated in cultured human fetal membranes (Qin et al., 1997a,b). The presence of specific receptors for both relaxin (Osheroff et al., 1995) and RLF (Büllesbach and Schwabe, 1999a,b) in the mouse uterus and the fact that placental insufficiency is not an apparent feature in relaxin-negative mice (Zhao et al., 1999) nor RLF-negative mice (Nef and Parada, 1999; Zimmermann et al., 1999) may provide indirect evidence for the complimentary functions of both hormones within the mouse placenta. Relaxin-negative/RLF-negative double-knockout mice should prove helpful to test this hypothesis.

RLF expression in ectopic tubal implantation sites and placental tissues of patients with symptomatic pre-eclampsia, involving placental insufficiency and inadequate trophoblast invasion, revealed that the constitutive expression of RLF in trophoblast is not dependent on (i) the site of implantation, (ii) maternal decidualization which is lacking at the tubal implantation site, (iii) placental sufficienty, or (iv) adequate trophoblast invasion. The loss of RLF transcription in the human choriocarcinoma cell lines JEG-3, JAr and BeWo may reflect a process of de-differentiation in these trophoblastic tumour cells similar to that previously demonstrated in human testicular Leydig cell adenoma (Klonisch et al., 1999b), which also show loss of RLF gene activity. However, in different trophoblast populations of the human placenta, RLF appears to be constitutively expressed.

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References


Relaxin-like factor in endometrium and placenta


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