Enhanced Expression of Uterine Stathmin during the Process of Implantation and Decidualization in Rats

KAZUHIRO TAMURA, TAKAHIKO HARA, MIKIHIRO YOSHIE, SHINYA IRIE, ANDRE SOBEL, AND HIROSHI KOGO

Department of Pharmacology (K.T., M.Y., S.I., H.K.), Tokyo University of Pharmacy and Life Science, Horinouchi, Hachioji, Tokyo 192-0392, Japan; Tokyo Metropolitan Institute of Medical Science (T.H.), Honkomagome, Bunkyo-ku, Tokyo 113-0032, Japan; and Institut National de la Santé et de la Recherche Médicale (A.S.), Unite-440, Institut du Fer a Moulin, Paris 75005, France

We used the library subtraction technique to identify genes specifically expressed in the rat uterus during early pregnancy. One such gene was that for stathmin, a factor that is associated with tubulin binding and the destabilization of microtubules. Stathmin was expressed at higher levels in implantation sites than in interimplantation sites on d 6 and 7 of pregnancy; the levels on d 6 and 7 were higher in implantation sites than in the entire uterus on d 3–5 of pregnancy or in nonpregnant uteri. Intense expression of stathmin mRNA was primarily limited to the subluminal stromal cells at the implantation site. Expression was also detected in the decidual zones and was accentuated during the period of decidualization (d 7–12). In the delayed implantation pregnant rat model, uterine stathmin expression was low, but increased after implantation induced by administration of 17β-estradiol to the progesterone-primed animal. Further, decidualization in the pseudopregnant rat, induced by intrauterine infusion of oil, enhanced stathmin expression. Stathmin expression clearly increases in the uterus when stimulated by embryo implantation and decidualization and may play a role in the early stages of pregnancy. (Endocrinology 144: 1464–1473, 2003)

COORDINATION of a developing conceptus and a receptive uterine epithelium is necessary for successful implantation (1–5). The uterus undergoes dramatic morphological and molecular changes during the preimplantation period, which are controlled by the ovarian steroid hormones estradiol (E2) and progesterone (P4) (1–7). In rats, a nidatory surge of estrogen on the afternoon of d 5 of pregnancy is essential for initiating implantation later that evening or in the early morning of d 6 (1, 5). Once this estrogen surge occurs, blastocysts are activated, and the receptive uterus allows them to attach to its epithelial layers (3, 7). Upon stimulation first by E2 and then by P4, uterine epithelial cells differentiate and undergo apoptosis, whereas stromal cells underneath the implantation sites proliferate and transform into decidual cells (6, 7). Increased vascular permeability in the uterine epithelium is necessary for and is the first obvious indication of the implantation process (1, 6). Thus, extravasation of an intravascularly administered macromolecular dye on the morning of d 6 indicates the location of the implantation sites (1, 6).

Because of the complex tissue reorganizations required for implantation, it is assumed that a unique set of genes involved in preparing the uterus for accepting invading blastocysts is induced during the early stages of pregnancy (Ref. 5 and references therein). Gene targeting has proven useful for understanding the genes essential for the implantation process in the mouse (reviewed in Ref. 5). For example, female mice deficient in leukemia inhibitory factor (LIF) are infertile, but administration of exogenous LIF restores fertility and can even replace the need for the nidatory estrogen surge (8). The genes encoding IGF-I and Hoxa-10 are expressed extensively at the implantation site, and mice deficient in these genes have implantation and decidualization failures (reviewed in Ref. 5). Recently, Yoshioka et al. (9) and Reese et al. (10), using gene chip arrays, reported on global gene expression profiles during implantation in mice. Although normal blastocyst implantation and decidualization are still poorly understood, the analysis of gene products that are expressed selectively and abundantly in the implantation site is one way to find the factors that participate in the process.

Stathmin is a member of a highly conserved cytosolic protein family that has been studied in relation to cell growth and differentiation (11). This 19-kDa protein has recently been proposed to function solely by sequestering tubulin and not by changing the structures of microtubules (12). Phosphorylation of stathmin is triggered by a large variety of regulatory agents, including hormones and growth factors whose signaling involves kinases (13). Stathmin has been suggested to participate in both oocyte maturation and preimplantation embryonic development (14, 15) because it is expressed in inner cell mass mass cells of expanded and hatched blastocysts. In the present study we systematically isolated genes that are expressed in the rat uterus on the day of implantation (d 6) and further characterized their expression patterns to determine whether they are associated with implantation and decidualization. We show in the present study increased expression of stathmin in the uterus at the time of implantation and decidualization and therefore con...
clude that stathmin should be considered an implantation-associated gene in the rat.

Materials and Methods

Animals and protocols

The animal care and surgery protocols used in these studies were reviewed and approved by the institutional animal care committees of Tokyo University of Pharmacy and Life Science in compliance with institutional guidelines for experimental animal care. Eight-week-old female rats of the Wistar-Imamichi strain (Imamichi Institute for Animal Reproduction, Ibaraki, Japan) were mated at proestrus with 10-wk-old males of the same strain. Day 1 of pregnancy was determined by the presence of a vaginal plug or sperm. To construct two uterine cDNA libraries from d 5 and 6 of pregnancy, uteri were excised on the morning (0900–0930 h) of each day and flushed with sterilized PBS to remove blastocysts. Injection via the tail vein of 0.2 ml of a 1% solution of Chicago Sky Blue dye in saline 5 min before killing was used to identify implantation sites, which appear as blue bands around the uterus. The tissue between the blue bands was defined as interimplantation sites.

Paraffin-embedded sections (6 μm) of the uterus were used for immunohistochemical analyses. The sections were exposed for 2 h to 10% normal goat serum. The sections were incubated for 1 h with goat antirabbit IgG conjugated to horseradish peroxidase in Tris-HCl buffer (pH 7.45, room temperature) containing 4% paraformaldehyde. Paraffin-embedded sections (6 μm) were prepared for in situ hybridization and immunocytochemistry. DIG-labeled antisense and sense riboprobes were prepared using the stathmin cDNA fragment (nucleotide 252–653) in the pBluescript vector and an RNA transcription kit (Takara, Siga, Japan) according to the manufacturer’s instructions. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) or β-actin was used as an internal control. The bands on the Kodak scientific imaging film (X-OMAT XB-1, Eastman Kodak Co., Rochester, NY) were analyzed using NIH Image, and each value was normalized against that of the G3PDH band in the corresponding lane.

In situ hybridization

Uterine tissues were homogenized in 10 vol 4 mM guanidine isothiocyanate using a Polytron (Brinkmann Instruments, Inc., West Orange, NY). Extraction of polyadenylated [poly(A)^+] RNA from total RNA was performed using the Oligotex-dT30-<super>-mRNA<sub>purification kit (TaKaRa, Siga, Japan) according to the manufacturer’s instructions. Ten to 20 μg total RNA or 100 ng poly(A)^+ RNA were separated on 1.5% agarose gels containing 2-4% formaldehyde and transferred to positively charged nylon membranes (Roche). For preparation of cDNA probes, a plasmid vector carrying rat stathmin cDNA was digested with EcoRI, NotI, and PsiI, and the digested cDNA (~300 bp) was subcloned into the plasmid vector pBluescript. After fixation of the RNA on the membranes, prehybridization was carried out for 4 h in high sodium dodecyl sulfate buffer containing the DIG-labeled cDNA. The blot was reacted with alkaline phosphatase-labeled anti-DIG antibody (Roche) and a chemiluminescent substrate (CDP-star) according to the manufacturer’s instructions. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) or β-actin was used as an internal control. The bands on the Kodak scientific imaging film (X-OMAT XB-1, Eastman Kodak Co., Rochester, NY) were analyzed using NIH Image, and each value was normalized against that of the G3PDH band in the corresponding lane.

Immunoblot and immunohistochemical analyses

Uterine tissues were homogenized with a Polytron in ice-cold lysis buffer [50 mM Tris-HCl buffer (pH 7.5) containing 0.15 mM NaCl, 10 mM EDTA, 0.1% Tween-20, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 0.1 mM phenylmethylsulfonylfluoride, and 0.1% (vol/vol) β-mercaptoethanol]. The homogenate was centrifuged twice at 42 C for 18 h, and bound probes were visualized using an alkaline phosphatase-conjugated anti-DIG antibody with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega Corp., Madison, WI) as substrates.

Verification of implantation-selective expression by Southern blotting and RT-PCR

Each cDNA library (1 μg) from d 5 uterus or d 6 implantation sites was digested with EcoRI and NotI, loaded on a 1.2% agarose gel, and transferred to a nylon membrane. The cDNA fragments obtained by library subtraction were labeled with digoxigenin (DIG) using a DIG-NDNA labeling kit (Roche, Mannheim, Germany). For Southern hybridization with cloned individual cDNA fragments, hybridization was performed for 17 h at 47 C with high sodium dodecyl sulfate buffer containing the DIG-labeled cDNA probe (10 ng/ml) as previously described (19).

In Northern blot analysis

Uterine tissues were homogenized in 10 vol 4 mM guanidine isothiocyanate using a Polytron (Brinkmann Instruments, Inc., West Orange, NY). Extraction of polyadenylated [poly(A)^+] RNA from total RNA was performed using the Oligotex-dT30-<super>-mRNA<sub>purification kit (TaKaRa, Siga, Japan) according to the manufacturer’s instructions. Ten to 20 μg total RNA or 100 ng poly(A)^+ RNA were separated on 1.5% agarose gels containing 2-4% formaldehyde and transferred to positively charged nylon membranes (Roche). For preparation of cDNA probes, a plasmid vector carrying rat stathmin cDNA was digested with EcoRI, NotI, and PsiI, and the digested cDNA (~300 bp) was subcloned into the plasmid vector pBluescript. After fixation of the RNA on the membranes, prehybridization was carried out for 4 h in high sodium dodecyl sulfate buffer containing the DIG-labeled cDNA. The blot was reacted with alkaline phosphatase-labeled anti-DIG antibody (Roche) and a chemiluminescent substrate (CDP-star) according to the manufacturer’s instructions. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) or β-actin was used as an internal control. The bands on the Kodak scientific imaging film (X-OMAT XB-1, Eastman Kodak Co., Rochester, NY) were analyzed using NIH Image, and each value was normalized against that of the G3PDH band in the corresponding lane.

Library subtraction and sequencing of cDNAs

cDNA library construction and library subtraction were performed as previously described by Hara et al. (18). mRNA from d 5 or 6 uteri was used to generate the driver cDNA and the tracer cDNA, respectively. Each cDNA library consisted of size-selected (>1 kb) cDNA fragments in pME18S vectors. One hundred micrograms of purified plasmid DNA from the d 5 library were digested with EcoRI, NotI, and ScaI before biotinylation using Photoprobe biotin (Vector Laboratories, Inc., Burlingame, CA). Five micrograms of plasmid DNA from the d 6 library (implantation sites) were digested with EcoRI and NotI before hybridization with the biotinylated tracer DNA in hybridization buffer [0.75 M NaCl, 5 mM EDTA, 25 mM HEPES buffer (pH 7.5), and 0.1% sodium dodecyl sulfate] containing 0.25 mg/ml Escherichia coli RNA at 68 C for 20 h. Biotinylated DNA was removed by incubation with streptavidin, followed by four extractions with phenol/chloroform/isoamyl-alcohol (25:24:1). The subtracted DNA was further hybridized for 2 h with the same biotinylated driver DNA. After removing biotinylated double-stranded DNAs, residual DNA was ligated into a pCRII vector (Invitrogen, Groningen, The Netherlands), digested with EcoRI and NotI, and used to transform electrocompetent E. coli (ElectroMax DH10B, Life Technologies, Inc., Grand Island, NY) for generation of the subtracted library. Randomly isolated clones were further screened by direct sequencing and Southern hybridization using both cDNA libraries and/or Northern hybridization using rat uterine RNA preparations from d 5 and 6 of pregnancy. Each clone was sequenced and analyzed by using the BLAST program to search public DNA databases.
Collagen α1 type I, γ-enteric smooth muscle actin isoform, collagen α1 type III, and lipoprotein lipase were found in the subtracted library with a high frequency. cDNAs encoding some constituents of the extracellular matrix, such as collagen α1 type 3, pro-α2(I) collagen, and dermatan sulfate proteoglycan as well as collagen α1 type 1, were isolated as implantation-associated genes. To analyze the expression of known genes whose physiological functions in the uterus have not been reported, the individual cDNA inserts in the subtracted library were used as probes for Southern blot analyses of the cDNA libraries (d 5 and 6). We observed increased expression of mRNAs for cathepsin S, cyclophilin, ferritin-H subunit, non-histone chromosomal high mobility group 1 protein, and stathmin. Of these, stathmin exhibited the biggest change, showing a distinct signal in the cDNA from the d 6 uterine library, in contrast to its faint band on d 5 (Fig. 1A).

Statistical analysis

The results of densitometric analyses were obtained from two or three independent experiments. Data are expressed as the mean ± SEM, and statistical significance (P < 0.05) was determined using t test and ANOVA. The results of Northern and Western blots that are shown represent single experiments.

Results

Identification of stathmin as a gene highly expressed during implantation and verification of its implantation-selective expression

In the present study subtraction cloning was carried out using implantation sites from d 6 pregnant rats and the entire uterus from d 5 pregnant rats to identify genes that are highly expressed at the time of implantation. In the subtracted uterine cDNA library, 226 independent clones were sequenced. Among them, 9 genes (7.0%) were unknown whereas 119 clones were known genes, including several cDNAs that have previously been reported to be expressed in the uterus during the periimplantation period (22). For example, cathepsin, whose expression is known to be associated with embryo development and uterine decidualization (23), was one of the clones identified in the subtracted library (Table 1). Among the clones sequenced, mRNAs for collagen I type I, γ-enteric smooth muscle actin isoform, collagen α1 type III, and lipoprotein lipase were found in the subtracted library with a high frequency. cDNAs encoding some constituents of the extracellular matrix, such as collagen α1 type 3, pro-α2(I) collagen, and dermatan sulfate proteoglycan as well as collagen α1 type 1, were isolated as implantation-associated genes. To analyze the expression of known genes whose physiological functions in the uterus have not been reported, the individual cDNA inserts in the subtracted library were used as probes for Southern blot analyses of the cDNA libraries (d 5 and 6). We observed increased expression of mRNAs for cathepsin S, cyclophilin, ferritin-H subunit, non-histone chromosomal high mobility group 1 protein, and stathmin. Of these, stathmin exhibited the biggest change, showing a distinct signal in the cDNA from the d 6 uterine library, in contrast to its faint band on d 5 (Fig. 1A).

Statmin mRNA is abundantly expressed at implantation sites

To evaluate the pattern of expression during the periimplantation period, the levels of statmin mRNA were analyzed by Northern hybridization on gestational d 3–7. Expression of the 1.1-kb statmin transcript was detected on all 5 d. However, the expression level was low in uteri until d 5 and markedly increased at implantation sites on d 6 and 7 (6-6 and 7-6 in Fig. 1B). Furthermore, on d 6 there was a distinct difference in the intensity of the statmin mRNA signals in preparations from implantation sites compared with interimplantation sites (6-6 vs. 6* in Fig. 1C). Densitometric analyses are shown in Fig. 1D. Uterine RNA from nonpregnant rats at various stages of the estrous cycle was compared with that from pregnant animals. Only faint signals were found in uteri from nonpregnant rats and were similar to those found on d 3 of pregnancy.

In situ hybridization of statmin mRNA in the periimplantation rat uterus

Figure 2 shows the temporal and cell-specific localization of statmin expression using in situ hybridization. On d 3 and 4, signals were detected in glandular epithelial cells and endometrial cells close to the luminal epithelium (Fig. 2, A and B). On d 5 just before implantation, an intense signal was detected in uterine stromal cells underlying epithelial cells (Fig. 2, C and D). mRNA was also detected in blastocysts that were unattached in the uterine lumen. At the site of implantation on d 6 (Fig. 2, E and F), statmin mRNA was observed primarily in subluminal stromal and smooth muscle cells. When longitudinal sections of uterus were examined, expression of statmin mRNA was never limited to a specific region of endometrial cells beneath the luminal epithelium, but higher levels were always found in the area surrounding the implanting blastocyst. Signals were found in both mesometrial and antimesometrial areas as well as in the decidua zone on d 7 (Fig. 2G). The specificity of hybridization was confirmed by the lack of signals in tissue sections hybridized with a statmin sense probe (Fig. 2H).

Expression of statmin protein in the rat uterus

We next analyzed the localization of statmin protein during the periimplantation period. On d 3 and 4, staining of the protein was detected in the glandular epithelial and stromal cells underlying the luminal epithelium (Fig. 3, A and B). On d 5 the area of staining had expanded in the endometrium compared with that on d 4 (Fig. 3D). At implantation sites on d 6, intense staining was detected in uterine stromal cells, especially those underlying the luminal epithelium (Fig. 3F). The intensity of staining of the stromal cells lying just below the luminal epithelium on d 6 was stronger than that found on d 4 or 5 (Fig. 3F vs. Fig. 3, B or D). Stathmin protein was also found in decidua cells (Fig. 3G). The lack of staining in control slides confirmed the specificity of the staining pattern (Fig. 3H).
To further examine the changing expression of stathmin protein during the perimplantation period, protein extracts were analyzed by Western blotting. The stathmin protein was identified as a 19-kDa single band in uterine lysates (Fig. 4A). The levels at the implantation sites on d 6 and 7 were prominently higher than those on d 3–5. Densitometric analysis (Fig. 4C) indicated a significant increase in stathmin levels on d 6 (1.32 ± 0.09) and d 7 (1.60 ± 0.11) compared with that on d 5 (1.00 ± 0.07). Further, levels were higher in implantation sites recovered on d 6 or 7 than in interimplantation sites on the same days (data not shown). To determine the fate of this protein as pregnancy progressed, levels of stathmin were analyzed in uteri between d 8 and 20 (Fig. 4, B and C). The expression increased markedly on d 6 and remained elevated through d 12 (P < 0.05 vs. d 5), but decreased on d 14–16. However, the levels gradually increased again between d 16 and 20.

**Effects of ovarian hormones on stathmin expression in the delayed implantation and ovariectomized nonpregnant rats**

To examine whether stathmin expression is associated with the induction of implantation and is regulated by ovarian hormones, especially the nidiary surge in estrogen, Northern blot analyses were carried out using the P₄-treated delayed implantation model (Fig. 5). Low levels of the stathmin transcript were found in the uteri of rats treated only with P₄, similar to those found in hypophysectomized rats (data not shown). After the termination of delayed implantation by an injection of E₂, only faint expression of stathmin mRNA was seen within 6 h, but the expression was pronounced after 24 h. Similar patterns in the levels of stathmin protein were observed in the delayed implantation model using Western blot (data not shown). However, a significant influence of ovarian steroids on stathmin expression was not observed in ovariectomized rats under the same doses as those used in delayed implantation model (Fig. 6).

**Effect of artificial decidual stimulation on stathmin expression in pseudopregnant uterus**

The levels of stathmin expression in uteri of pseudopregnant rats, which lack both implantation sites and decidualization, were analyzed on d 5 and 6 (Fig. 7A). The same levels of stathmin protein were detected in uteri from d 5 pregnant or pseudopregnant animals. On d 6, however, stathmin did not increase in pseudopregnant uteri and were significantly below the level found in pregnant animals on d 6. In fact, on d 6 the stathmin levels in pseudopregnant animals had significantly decreased from d 5 (d 6, 0.53 ± 0.002; d 5, 0.93 ± 0.070; P < 0.05; Fig. 7C, left panel). Decidualization is initiated immediately in the endometrial cells adjacent to the implantation sites during blastocyst attachment and invasion (7). To examine the relationship between decidualization and stathmin expression, an artificial stimulus was given to the pseudopregnant rats (Fig. 7B). After injection of sesame oil into the uterine lumen, stathmin levels started to increase within 24 h (P < 0.01 vs. 0 h, Fig. 7C, right panel), peaked at 48 h, and continued to be expressed for at least 120 h. Thus, there were increases in stathmin expression during the development of deciduoma under conditions that
FIG. 2. Localization of uterine stathmin mRNA during the early stage of pregnancy in rats (A, d 3; B, d 4; C and D, d 5; E and F, H, d 6; G, d 7). Original magnification, ×63 (except C, ×315; and E, ×3.2). The section in H was hybridized with a sense cRNA probe. ge, Glandular epithelium; le, luminal epithelium; s, stroma; bl, blastocyst; am, antimesometrial; m, mesometrial; em, embryo; pdz, primary decidual zone; sdz, secondary decidual zone; IS, implantation sites.
FIG. 3. Immunostaining of uterine stathmin during early pregnancy in rats (A, d 3; B, d 4; C and D, d 5; E, F, and H, d 6; G, d 7). Original magnification, ×63. H, Negative control (d 6), only secondary antibody was added in the procedure. ge, Glandular epithelium; le, luminal epithelium; s, stroma; am, antimesometrial; m, mesometrial; em, embryo; pdz, primary decidual zone; sdz, secondary decidual zone; IS, implantation sites.
lacked blastocysts, in contrast to the time-dependent decreases in the levels in nontreated intact uteri.

Discussion

The present study was undertaken in an attempt to identify genes in the rat that are associated with implantation and, therefore, may be related to the establishment of pregnancy. Based on the results obtained by Southern blot analyses, attention was focused on the profile of stathmin expression in the uterus during the periimplantation period. The uterus undergoes dramatic developmental changes during early pregnancy (1–7). With the increase in progestin that follows the formation of the ovarian corpora lutea, the endometrial luminal epithelium ceases to proliferate and undergoes differentiation in preparation for embryo implantation. After the cell to cell reactions associated with the attachment of blastocysts to epithelial cells, the underlying stromal cells undergo proliferation and differentiation into decidual cells (decidualization; Refs. 1–7). Recent studies using mice lacking estrogen receptor α (ERα) (24) have emphasized that implantation, but not decidualization, requires the action of estrogen. In contrast, the action of the P4 receptor is essential for both implantation (1–4) and decidualization (25). The latter study established that increased expression of the P4 receptor in the uterus could be accomplished by the action of either ERα or ERβ as well as by a mechanical stimulus in the presence of P4.

The results of the present study reveal that stathmin expression, as evidenced by increases in its mRNA and protein, is a characteristic of embryo implantation in the rat. Uterine stathmin expression does not increase in the delayed implantation model, which has intraluminal blastocysts and is exposed to P4 but not E2. However, the increases in expression found in pregnant animals were mimicked in the delayed model after providing an estrogen stimulus to induce implantation. During normal pregnancy, increased expression of stathmin was seen between d 5 and 6, particularly at implantation sites. In the delayed implantation model, increased expression was found 24 h after exposure to E2, which would be equivalent to d 6 of pregnancy. Therefore, the hormonal environment associated with implantation, i.e., estrogen acting on a P4-primed uterus (1–6), is necessary to

FIG. 4. Western blot analysis of uterine stathmin expression during pregnancy in rats. A and B, Numbers at the top of the figures indicate the day of pregnancy. 6-IS and 7-IS, Implantation sites on d 6 and 7; 6*, interim-implantation site on d 6. A, Each lane shows the data from an individual animal (n = 2). C, Densitometric analysis of stathmin levels detected by two independent experiments (n = 4), including the data in B. Quantitation of stathmin protein contents was conducted using NIH Image. The levels are expressed relative to the density of the band on d 5. *, P < 0.05; **, P < 0.01.
cause increased expression of stathmin. However, the hormones are not sufficient, because the interimplantation sites, which are exposed to the same hormonal milieu as the implantation sites, express significantly less stathmin. Furthermore, the uteri of pseudopregnant rats, exposed only to the action of P4, actually showed decreased, rather than increased, stathmin expression between d 5 and 6. However, intraluminal instillation of sesame oil, an artificial stimulus to induce decidualization, increased expression of the stathmin gene in the pseudopregnant rat. Therefore, the important physiological changes in the endometrium that regulate the expression of stathmin appear to be those that signal decidualization. Several gene products could be involved in this signaling, including IGF-I, cyclooxygenase-2, LIF, HOX-10, HOX-11, or the IL-11 receptor (reviewed in Ref. 5), all of which are essential for implantation in the mouse. Whether increased stathmin is a cause or an effect of implantation/decidualization, and whether it has any specific effects on these processes remain to be determined. The function of stathmin in cell division and differentiation is unresolved, but is generally considered to involve microtubule stabilization (12, 13). Recent studies have been interpreted to show that most, if not all, of the effects of stathmin can be accounted for by its sequestering of tubulin, which controls the microtubule dynamics of the cell cycle (12). Phosphorylation of 4 serines (serines 16, 25, 38, and 63) in stathmin inactivates its microtubule-destabilizing activity and is necessary for progression through the cell cycle (12). Phosphorylation of 4 serines (serines 16, 25, 38, and 63) in stathmin inactivates its microtubule-destabilizing activity and is necessary for progression through the cell cycle (12). Phosphorylation of 4 serines (serines 16, 25, 38, and 63) in stathmin inactivates its microtubule-destabilizing activity and is necessary for progression through the cell cycle (12). Phosphorylation of 4 serines (serines 16, 25, 38, and 63) in stathmin inactivates its microtubule-destabilizing activity and is necessary for progression through the cell cycle (12). Phosphorylation of 4 serines (serines 16, 25, 38, and 63) in stathmin inactivates its microtubule-destabilizing activity and is necessary for progression through the cell cycle (12).
cyclins (D type) are associated with cdk4 or cdk6 and are involved with the entry of cells into the S (DNA synthesis) phase of the cycle. The retinoblastoma proteins negatively regulate the G1 cyclins and are inactivated by phosphorylation through the action of the cyclin/cdk complex. Increased expression of cyclin D3 has been associated with decidualization at implantation sites in mice and interpreted as indicating increased cell proliferation of the decidualizing stromal cells (30, 31). This increase in proliferation and particularly the inhibition of cyclin B/cdk1 for the production of polyploid decidual cells might involve the action of stathmin. However, there is currently no evidence to support this view. The localization of cyclin D3 in the uterus resembles that of stathmin in rats, but there are some differences. Cyclin D3 is primarily expressed in decidualizing stroma, but cyclin D3 signals have also been found in epithelial cells, whereas we did not detect distinct signals for stathmin in epithelial cells during the perimplantation period.

Stathmin appears to be unnecessary for the establishment or maintenance of pregnancy, because female mice deficient in the stathmin gene are not infertile nor do they have any major disorders in the nervous system, which normally expresses stathmin (32, 33). However, no detailed examination of reproductive function in stathmin-deficient animals has been made. Stathmin is a member of a phosphoprotein family that includes the developmentally regulated neuronal protein SCLIP; Ref. (34); thus, other members of the gene family might substitute for it in stathmin-deficient animals.

In conclusion, our data suggest that stathmin is an implantation- and decidualization-associated gene that may be involved in the proliferation and differentiation of endometrial stromal cells during early pregnancy. Further studies will be required to determine whether the stathmin expression induced by implantation of embryos is controlled by the same signaling system as that occurring after artificially induced decidualization.

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Address all correspondence and requests for reprints to: Dr. Kazuhiro Tamura, Department of Pharmacology, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo, Japan. E-mail: hiro@ps.toyaku.ac.jp.

References
14. Carson DD, Bagchi I, Dey SK, Enders AC, Fazlebas AT, Lessey BA, Yoshi-
15. Paria BC, Song H, Dey SK 2001 Implantation: molecular basis of embryo-
16. Paria BC, Reese J, Das SK, Dey SK 2002 Deciphering the cross-talk of im-
plantation: advances and challenges. Science 296:2185–2188
17. Abrahamsohn PA, Zorn TM 1995 Implantation and decidualization in ro-
Abrahamsohn PA, Zorn TM 1991 The effects of ovarian steroids in controlling
20. Reese J, Das SK, Paria BC, Lim H, Song H, Matsumoto H, Knudtson KL,
DuBois RN, Dey SK 2001 Global gene expression analysis to identify mo-
lecular markers of uterine receptivity and embryo implantation. J Biol Chem
276:44137–44145
21. Sobel A 1991 Stathmin: a relay phosphoprotein for multiple signal transduc-
22. Amazyed P, Pantalone D, Carlier M-F 2002 The effect of stathmin phosphor-
ylation on microtubule assembly depends on tubulin critical concentration.
J Biol Chem 277:22718–22724
92:3100–3104
pression of the phosphoprotein p19/SCG10 gene family in mouse preimplan-
tation embryos, uterus, and placenta. Reprod Fertil Dev 4:205–211
Cellular and subcellular localization of stathmin during oocyte and preim-
rat uterine prostaglandin F2α during the peri-implantation period. Prostaglan-
din Leukotriene Essent Fatty Acids 43:29–43
27. Paria BC, Huet-Hudson YM, Dey SK 1993 Blastocyst’s state of activity de-
termines the “window” of implantation in the mouse receptive uterus. Proc
Natl Acad Sci USA 90:10159–10162
28. Harad T, Harada N, Mitsuji H, Miura T, Ishizaka T, Miyajima A 1994 Char-
acterization of cell phenotype by a novel cDNA library subtraction system: expression of CD8α in a mast cell-derived interleukin-4-dependent cell line. Blood 84:189–199
29. Tamura K, Kawaguchi T, Harad T, Sakamoto T, Tohei A, Miyajima A, Tsuch-
iya S, Kogo H 2000 Interleukin-6 decreases estrogen production and messenger ribonucleic acid expression encoding aromatase during in vitro cytodif-
Ovarian immune cells express granulocyte-macrophage colony-stimulating
factor (GM-CSF) during follicular growth and luteinization in gonadotropin-
opmental tissue expression and phylogenetic conservation of stathmin, a phos-
32. Brown JRG, Papaoanoun VE 1995 Extracellular matrix remodeling at im-
on Molecular and Cellular Aspects of Perimplantation Processes. New York: Springer Verlag; 125–152
33. Afonso S, Romagnano L, Babiarz B 1997 The expression and function of
ovarian cystatin C and cathepsin B during mouse implantation and placentation. Development 124:3415–3425
34. Hewitt SC, Goulding EH, Eddy EM, Korach KS 2002 Studies using the
estrogen receptor a knockout uterus demonstrate that implantation but not
decidualization-associated signaling is estrogen dependent. Biol Reprod 67:
1268–1277
35. Kurita T, Lee Kj, Saunders PTK, Cooke PS, Taylor JA, Lubahn DR, Zhao C,
Makela S, Gusstafsson JA, Dahlia R, Cunha GR 2001 Regulation of pro-
gesterone receptors and decidualization in uterine stroma of estrogen receptor
a knockout mouse. Biol Reprod 64:227–283
36. Holmfield P, Larson N, Segerman B, Howell B, Morabito, J Cassimeris L,
Gullberg M 2001 The catastrophe-promoting activity of ectopic Op18/stath-
m is required for disruption of mitotic spindles but not interphase micro-
1992 Induction of stathmin expression during liver regeneration. FEBs Lett
331:65–70
38. Andersen SSL 2000 Spindle assembly and the art of regulating microtubule
39. Cassimeris L 1999 Accessory protein regulation of microtubule dynamics
throughout the cell cycle. Curr Opin Cell Biol 11:134–141
40. Tan J, Raja S, Davis MK, Tawfik O, Dey SK, Das SK 2002 Evidence for coordi-
nated interaction of cyclin D3 with p21 and cdk6 in directing the de-
velopment of uterine stromal cell decidualization and polyploidy during im-
plantation. Mech Dev 111:99–113
41. Das SK, Lim H, Paria BC, Dey SK 1999 Cyclin D3 in the mouse uterus is
associated with the decidualization process during early pregnancy. J Mol
Endocrinol 22:91–104
Normal development of mice lacking metabolin (F19), a phosphoprotein
43. Ozon S, Mauccuer A, Sobel A 1997 The stathmin family-molecular and bio-
logical characterization of novel mammalian proteins expressed in the nervous
44. Koppel J, Boutilier MC, Doye Y, Peyro-Saint-Paul H, Sobel A 1999 Devel-
opmental tissue expression and phylogenetic conservation of stathmin, a phos-