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# Dramatic Elevation of Plasma Metastin Concentrations in Human Pregnancy: Metastin as a Novel Placenta-Derived Hormone in Humans

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**Metastin is a novel peptide that was recently isolated from human placenta as the endogenous ligand of an orphan heptahelical receptor, hOT7T175. Metastin has been shown to suppress the motility of hOT7T175-transfected melanoma cells; however, studies of the physiological function of metastin have begun only recently. To investigate the possibility that metastin is an endocrine peptide, we determined the immunoreactive (ir-) metastin concentration in human plasma using our newly developed, sensitive, and specific two-site enzyme immunoassay. The plasma concentrations of ir-metastin in males and females were 1.30 ± 0.14 (n = 12) and 1.31 ± 0.37 fmol/ml (n = 10), respectively. As metastin is known to be abundant in human placenta, the ir-metastin concentration in the maternal plasma was then determined. The ir-metastin concentrations were 1230 ± 346 fmol/ml (n = 11) in the first trimester, 4590 ± 555 (n = 16) in the second trimester, and 9590 ± 1640 (n = 12) in the third trimester. On d 5 after delivery, the ir-metastin concentration returned to nearly the nonpregnant level (7.63 ± 1.33 fmol/ml; n = 10), suggesting that ir-metastin increases in pregnancy and is derived mainly from the placenta. The plasma from both nonpregnant and pregnant women showed a single ir-metastin peak at the same retention time as authentic metastin on reverse phase HPLC analysis, indicating that the major portion of the circulating metastin, as determined by our two-site enzyme immunoassay, represents endogenous metastin. Histochemical studies of human placenta localized metastin mRNA and immunoreactivity to the syncytiotrophoblasts. The present study provides evidence for metastin as a novel placenta-derived hormone in humans. (*J Clin Endocrinol Metab* 88: 914–919, 2003)**

WE RECENTLY ISOLATED a novel regulatory peptide, metastin, as the cognate ligand of an orphan seven-transmembrane domain receptor, hOT7T175 (1). Metastin was purified from human placenta after observations of its activity in inducing an increase in the intracellular calcium ion concentration in hOT7T175-transfected Chinese hamster ovary (CHO) cells (1). Metastin is encoded by a putative metastasis suppressor gene named *KiSS-1* and consists of 54-amino acid residues and an amidated C-terminus. Two other groups have shown independently that the same peptide from the KiSS-1 gene product is the natural ligand of hOT7T175 [named AXOR12 (2) and GPR54 (3), respectively].

In a previous study we demonstrated that metastin inhibited chemotaxis, invasion, motility, and growth of hOT7T175-transfected CHO cells *in vitro* and attenuated pulmonary metastasis of hOT7T175-transfected B16-BL6 melanomas *in vivo* (1, 4). Recently Ringel *et al.* (5) reported that the metastin receptor was overexpressed in papillary thyroid cancer, and that metastin activated both ERK and Akt in ARO thyroid cancer cells. These data suggest that metastin possesses antimetastasis activity in some tumors.

Kotani *et al.* (3) reported that the metastin receptor gene was strongly expressed in the placenta, pituitary, pancreas, and spinal cord and that iv administration of metastin stimulated oxytocin release in rats, suggesting that metastin plays a role in endocrine function. However, the presence of metastin in plasma has not been confirmed. In addition, both KiSS-1 and its receptor gene were highly expressed in placenta (1, 2, 6), and metastin was isolated from human placental extracts (1, 3), suggesting that metastin has a physiological role in human pregnancy. It is inferred from these results that metastin may have various biological activities, and there remains the possibility that metastin might have physiologically important functions in addition to antimetastatic activities and stimulation of oxytocin release. To obtain evidence about the physiological significance of metastin, it is very important to establish a sensitive and specific method for determining metastin concentrations in human plasma.

In the present study we developed a specific and sensitive two-site enzyme immunoassay (EIA) for human metastin and investigated the metastin concentration in human plasma. Using the EIA system, we found that immunoreactive (ir-) metastin was present in human blood and then measured plasma metastin levels in human pregnancy. We also characterized the immunoreactivities of metastin in plasma from pregnant and nonpregnant women using chromatography. Further, to determine the localization of metastin-positive cells in human placenta and to investigate the possibility of a role for metastin in pregnancy, we performed *in situ* hybridization and immunohistochemical staining of metastin in human placenta.

Abbreviations: CHO, Chinese hamster ovary; E2, estradiol; EIA, enzyme immunoassay; hCG, human chorionic gonadotropin; hPL, human placental lactogen; HRP, horseradish peroxidase; ir-, immunoreactive; RF, arginine-phenylalanine; RP-HPLC, reverse phase HPLC; SSC, standard saline citrate; TFA, trifluoroacetic acid.

## Materials and Methods

### *Synthetic peptide*

Metastin and metastin-related peptides were synthesized with an automated peptide synthesizer (model 430A, PE Applied Biosystems, Foster City, CA).

### *Human subjects*

The present study included 39 pregnant women: 11 in the first trimester (mean ± SEM, age, 25.5 ± 1.7 yr), 16 in the second trimester (27.8 ± 1.6 yr), 12 in the third trimester (29.1 ± 1.3 yr), and 10 who had delivered 5 d previously (25.5 ± 1.5 yr). Plasma samples from 10 nonpregnant women (32.4 ± 2.6 yr) and 12 men (36.8 ± 1.7 yr) were used to provide baseline controls. All of the nonpregnant patients were enrolled at random, and the respective samples were collected without bias for stage of the menstrual cycle. Umbilical cord arterial and venous plasma samples were obtained within 1 min after delivery from four neonates. The maternal samples were not collected from the same mothers used for the umbilical samples. The plasma samples from men were obtained from healthy volunteers, and the plasma samples from women were purchased from Direct Clinical Access, Inc. (Marlborough, MA). The present study was approved by the institutional review boards of Takeda Chemical Industries Ltd. (Osaka, Japan) and University of Tsukuba (Tsukuba, Japan), and all adults participating in this study gave informed consent.

### *Preparation of plasma samples*

All plasma samples were collected in chilled tubes containing 1 mg/ml EDTA-2Na between 0900 and 1200 h, centrifuged at 1000 × *g* for 25 min at 4 C, and then stored at −80 C until assay. The plasma was diluted with the buffer C [0.02 M sodium phosphate buffer (pH 7.0) containing 1% BSA, 0.4 M NaCl, and 2 mM EDTA-2Na] and subjected to the two-site EIA.

### *Preparation of antibodies*

For immunogens, [Cys$^{13}$]metastin-(1–13) and [Cys$^{38}$]metastin-(38–54) (1.7 μmol) were conjugated with 30 nmol bovine thyroglobulin previously maleimidated with *N*-(γ-maleimidobutyryloxy)succinimide. These immunogens (40 μg/mouse) together with complete or incomplete Freund's adjuvant were injected sc into 8-wk-old, female BALB/c mice at 3-wk intervals. Four days after each mouse had been injected iv with 200 μg immunogen, spleen cells from each immunized mouse were fused with a mouse myeloma cell line, P3-X63Ag8-U1, as described previously (7). Monoclonal antibodies, KIS-1Na (IgG2b κ) and KIS-1Ca (IgG1 κ), were selected and purified from ascites fluid using a protein A-immobilized column (IPA-300, Repligen, Cambridge, MA). KIS-1Na was directed against the N-terminal region of metastin, and KIS-1Ca was directed against the C-terminal region of metastin. The antibody reactivity was investigated using horseradish peroxidase (HRP)-labeled [Cys$^{13}$]metastin-(1–13) or [Cys$^{38}$]metastin-(38–54) in competitive EIAs, as described previously (8). All experiments were performed in accordance with institutional guidelines for animal care at Takeda Chemical Industries Ltd.

### *Two-site EIA for metastin*

KIS-1Ca was conjugated with HRP according to the methods of Suzuki *et al.* (7). The two-site EIA for metastin was performed as described previously (8). Briefly, 100 μl standard metastin or the samples to be tested were placed in separate wells of a KIS-1Na-coated microtest 96-well plate (Nunc, Naperville, IL) and incubated at 4 C for 24 h. After washing with PBS, each well was reacted with 100 μl HRP-labeled KIS-1Ca at 4 C for 24 h. After the wells had been washed with PBS, the bound enzyme reactivity was measured using a TMB microwell peroxidase system (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

### *Measurement of placenta-related steroid or peptide hormones*

Human chorionic gonadotropin (hCG) levels in plasma were determined using an HCG-CTP test WAKO (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Human placental lactogen (hPL) levels in plasma were measured using an Eiken hPL kit (Eiken Kagaku, Tokyo, Japan). The plasma concentrations of estradiol (E2) and progesterone were measured using specific RIA kits (Diagnostic Products, Los Angeles, CA). The intra- and interassay coefficients of variation using these kits did not exceed 10%. The cross-reactivities with other peptides and steroid hormones in these kits did not exceed 4%. The detection limits of the hCG, hPL, E2, and progesterone assay kits are 0.2 mIU/ml, 60 ng/ml, 8 pg/ml, and 20 pg/ml, respectively.

### *Reverse phase HPLC (RP-HPLC) analyses*

The plasma was added to an equal volume of $CH_3CN$ and centrifuged at 15,000 rpm for 5 min at 4 C. The supernatant was lyophilized and reconstituted with 5% $CH_3CN$ containing 0.05% trifluoroacetic acid (TFA). An aliquot of plasma extract was injected onto a RP-HPLC column on a TSK ODS 80 TM (4.6 × 250 mm; Tosoh, Tokyo, Japan) column. The solvents used were: A, 5% $CH_3CN$ containing 0.05% TFA; and B, 60% $CH_3CN$ containing 0.05% TFA. During the elution, the concentration of B was increased linearly from 0% to 45.5% over 5 min, from 45.5% to 63.6% over 30 min, and from 63.6% to 100% over 5 min at a flow rate of 1.0 ml/min. Each fraction was lyophilized, dissolved in buffer C, and assayed for ir-metastin using the two-site EIA.

### *In situ hybridization*

Plasmid DNA containing 481-bp human KiSS-1 cDNA was linearized by digestion with *Bam*HI or *Xho*I. Digoxigenin-labeled probes were generated by labeling with digoxigenin-11-UTP (Roche Molecular Biochemicals, Indianapolis, IN) using T7 or Sp6 RNA polymerase (Nippon Gene Co., Ltd., Tokyo, Japan) in a 20-μl transcription mixture containing 2.0 μg of the linearized plasmid; 4 μl ribonuclease polymerase buffer; 1 μl 0.2 mM dithiothreitol; 1 μl of 10 mM stocks of ATP, GTP, and CTP; 6.5 μl 1 mM UTP; 3.5 μl of 10-mM stocks of digoxigenin-11-UTP; and 1 μl ribonuclease Inhibitor (Nippon Gene Co., Ltd.). The transcription mixtures were incubated for 1 h at 37 C (for T7) or 40 C (for Sp6). The labeled probes were hydrolyzed to approximately 150-base fragments. *In situ* hybridization was performed according to the manufacturer's instructions (ISHR Kit, Nippon Gene Co., Ltd.). In brief, the human term placenta slides (DAKO Corp., Kyoto, Japan) were rehydrated in xylene and graded concentrations of ethanol. The slides were incubated with proteinase K (5 μg/ml) for 10 min at room temperature before treatment with glycine (2 mg/ml). The slides were then treated with 0.1 M triethanolamine for 5 min, followed by 0.25% acetic anhydride for 15 min. The sections were hybridized in an oven at 42 C for 16 h in diluted digoxigenin-labeled probes (1 μg/ml)/hybridization buffer containing 50% formamide, 2× standard saline citrate (2× SSC), 1 μg/μl tRNA, 1 μg/μl salmon sperm DNA, 1 μg/μl BSA, and 10% dextran sulfate. The slides were washed three times with 50% formamide/2× SSC for 20 min at 42 C. After treatment with ribonuclease A (20 μg/ml) for 30 min at 37 C, the sections were rinsed three times in 0.1× SSC for 20 min at 42 C. After three washes in PBS, the sections were preincubated in 10% normal horse serum for 30 min and incubated with alkaline phosphatase-conjugated antidigoxigenin Fab for 8 h (1:2000; Roche Molecular Biochemicals). The sections were washed in PBS and visualized with Vector Blue (Vector Laboratories, Inc., Burlingame, CA) for 24 h.

### *Immunohistochemistry*

Normal human term placenta tissue slides (DAKO Corp.) were deparaffinized and incubated in 1 mM EDTA buffer (pH 8.0) at 95 C for 30 min. After treatment with 20% normal horse serum for 30 min, these sections were incubated with 60 μg/ml KIS-1Ca at 4 C overnight. These sections were incubated in biotin-conjugated horse antimouse IgG (Vector Laboratories, Inc.) for 30 min at room temperature and then incubated in avidin-biotinylated HRP complex (Vector Laboratories, Inc.) for 30 min at room temperature. The immunolabeling was visualized with a mixture of diaminobenzidine and $H_2O_2$ in 0.05 M Tris-buffered saline solution (pH 7.2). Counterstaining was performed with hematoxylin. In preabsorption studies, KIS-1Ca antibody was incubated with 1 μM human metastin for 1 h at room temperature.

### *Statistical analysis*

Data are expressed as the mean ± SEM. Statistical analysis was performed by *t* test with Holm's correction and by Dunnett's test to determine the significance of the differences, with a level of $P < 0.05$ accepted as statistically significant. Correlations were estimated using linear regression analysis.

## Results

### *Reactivity of antibodies*

We obtained two monoclonal antibodies, KIS-1Na recognizing metastin-(1–13) and KIS-1Ca recognizing metastin-(38–54) amide. These antibodies were shown to have high affinity for metastin by competitive EIAs using HRP-labeled [$Cys^{13}$]metastin-(1–13) or [$Cys^{38}$]metastin-(38–54) ($IC_{50}$ values, 0.1 nM for KIS-1Na and 1 nM for KIS-1Ca; Fig. 1A). The concentrations of KIS-1Na and KIS-1Ca using the competitive EIAs were both 50 ng/ml. KIS-1Ca did not react with the unamidated form of metastin (Fig. 1A), suggesting that the antibody recognizes the C-terminal arginine-phenylalanine (RF) amide structure. KIS-1Ca inhibited the metastin-induced increase in intracellular calcium ion concentration of hOT7T175-transfected CHO cells at equimolar concentrations (data not shown). This result is consistent with our previous finding that the amidated C-terminal structure of metastin is indispensable for its receptor interaction and agonistic activity (1).

### *Two-site EIA*

The two-site EIA was further established using KIS-1Na and KIS-1Ca for sensitive and specific detection of metastin.

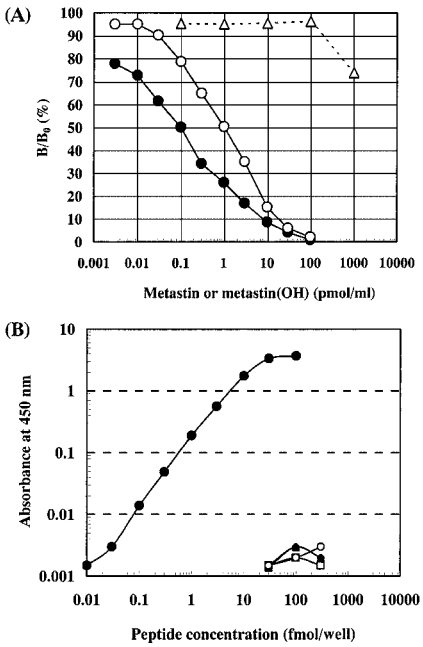


FIG. 1. A, Reactivity of monoclonal antimetastin antibodies (KIS-1Na and KIS-1Ca) with metastin. The reactivities of KIS-1Na (●) or KIS-1Ca (○) with metastin and of KIS-1Ca with metastin-(1–54)OH (△) were examined by competitive EIA using HRP-labeled [$Cys^{13}$]metastin-(1–13) or [$Cys^{38}$]metastin-(38–54). B, Standard curve of metastin in the two-site EIA. Metastin (●) and RF-$NH_2$ peptides including calcitonin gene-related peptide (□), cholecystokinin-4 (▲), neuropeptide FF (♦), and PRL-releasing peptide 31 (○) are indicated.

The detection range was 0.03–30 fmol metastin/0.1 ml/well (Fig. 1B). No cross-reactivity was found for other peptides possessing an RF-$NH_2$ structure (Arg-Phe-$NH_2$) in the C-terminus, such as calcitonin gene-related peptide, cholecystokinin-4, neuropeptide FF, and PRL-releasing peptide-31, even at 300 fmol/well (Fig. 1B). The intra- and interassay coefficients of variation (n = 10) were 12% and 4.9%, respectively. The recovery in this method was 112 ± 12.3% (n = 5) when 50 fmol metastin were added to human plasma.

### *Plasma metastin concentrations in men and nonpregnant women*

The two-site EIA method allowed direct measurement of ir-metastin in human plasma due to its high sensitivity. The mean plasma concentrations of ir-metastin in normal human plasma were 1.30 ± 0.14 fmol/ml (n = 12) for men and 1.31 ± 0.37 fmol/ml (n = 10) for nonpregnant women (Table 1). There was no difference in plasma metastin concentration between the sexes.

### *Plasma metastin concentrations during pregnancy and the postpartum period*

The mean concentrations of ir-metastin in maternal plasma were 1230 ± 346 fmol/ml (n = 11) in the first trimester, 4590 ± 555 (n = 16) in the second trimester, 9590 ± 1,640 (n = 12) in the third trimester, and 7.63 ± 1.33 (n = 10) in 5 d (120–125 h) postdelivery (Table 1 and Fig. 2).

We also measured plasma hCG, hPL, E2, and progesterone levels using the same samples taken from maternal plasma. As plasma ir-metastin levels increased, corresponding increases in plasma hPL, E2, and progesterone concentrations were observed. Significant positive correlations were found between the concentration of ir-metastin and those of E2 (r = 0.41; $P < 0.005$), progesterone (r = 0.46; $P < 0.005$), and hPL (r = 0.59; $P < 0.0005$); however, there was no significant correlation between ir-metastin and hCG concentrations (Table 2). The metastin concentrations in the plasma of umbilical cord arterial and venous blood were 474 ± 71.0 (n = 4) and 617 ± 88.7 (n = 4) fmol/ml. There was no significant difference in plasma metastin concentration between these two sources of blood.

**TABLE 1.** Mean concentrations of ir-metastin and hCG in plasma sampled during pregnancy and the postpartum period, and from nonpregnant women

| | ir-metastin (fmol/ml) | hCG (mIU/ml) |
|---|---|---|
| Men (n = 12) | 1.30 ± 0.141 | |
| Nonpregnant women (n = 10) | 1.31 ± 0.373 | 0.525 ± 0.229 |
| 1st trimester (n = 11) | 1,230 ± 346[b] | 65,200 ± 16,100[b] |
| 2nd trimester (n = 16) | 4,590 ± 555[b] | 17,100 ± 2,500[b] |
| 3rd trimester (n = 12) | 9,590 ± 1,640[b] | 20,500 ± 3,890[b] |
| Postpartum (n = 10) | 7.63 ± 1.33[a] | 1,090 ± 496[b] |

Values are mean ± SEM.
[a] $P < 0.005$ and [b] $P < 0.001$ *vs.* the values in nonpregnant women by Dunnett's test.

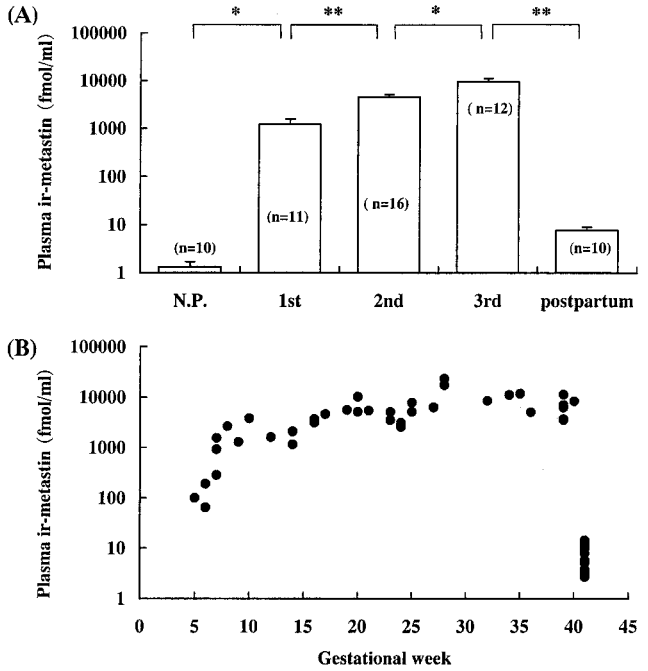


FIG. 2. A, Mean concentrations of ir-metastin in nonpregnant women (N.P.); those in the first trimester (1st), second trimester (2nd), and third trimester (3rd) of pregnancy; and postpartum (mean ± SEM). *, $P < 0.05$; **, $P < 0.01$ (by $t$ test with Holm's correction). B, Plasma ir-metastin levels in 39 pregnant women during wk 5–40 of pregnancy and in 10 women postpartum (shown at 41 wk).

**TABLE 2.** Correlations by linear regression analysis between ir-metastin and placental hormone concentrations in maternal plasma during pregnancy

| Hormone | No. of samples | Correlation coefficient *vs.* ir-metastin | Statistical significance |
|---|---|---|---|
| E2 | 48 | 0.41 | $P < 0.005$ |
| Progesterone | 48 | 0.46 | $P < 0.005$ |
| hPL | 48 | 0.59 | $P < 0.0005$ |
| hCG | 48 | 0.20 | NS |

NS, Not significant.

### *Characterization of ir-metastin in plasma from nonpregnant and pregnant women*

The ir-metastin in plasma from nonpregnant and pregnant (7 and 28 wk gestation) women was analyzed by RP-HPLC. Each of the plasma samples provided almost a single peak of immunoreactivity that was eluted in the same fractions as authentic metastin (Fig. 3). The ir-metastin concentration in the peak fraction was 2.7 fmol for plasma from nonpregnant women and 160 and 2600 fmol for plasma from 7- and 28-wk pregnant women. This indicates that the major part of ir-metastin found in human plasma represents endogenous metastin.

### *In situ hybridization and immunohistochemical staining of metastin in human placenta*

To elucidate the localization of metastin-positive cells in human term placenta, we performed *in situ* hybridization and immunohistochemical analysis using KIS-1Ca, which recognized the extreme C-terminal RF amide sequence of

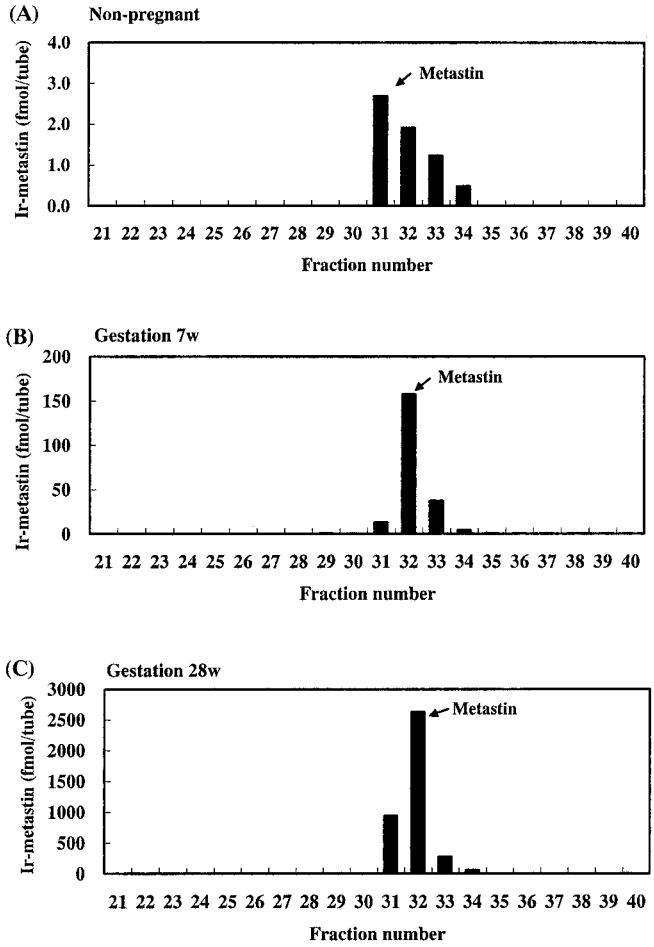


FIG. 3. RP-HPLC elution profiles of ir-metastin extracted from plasma in nonpregnant and pregnant women (7 and 28 wk gestation). The *vertical arrows* indicate the position of authentic metastin. A, Nonpregnant plasma (5 ml); B, plasma at 7 wk gestation (1 ml); C, plasma at 28 wk gestation (0.5 ml).

metastin, the site that is essential for the bioactivity of metastin. KiSS-1 mRNA-expressing cells were detected in the syncytiotrophoblasts (Fig. 4, A and B). The negative control with a digoxigenin-labeled KiSS-1 sense riboprobe showed an absence of staining (Fig. 4C). Immunohistochemical staining revealed that metastin was positive in syncytiotrophoblasts of human term placenta (Fig. 4, D and E). The specificity of immunostaining was confirmed by preabsorption studies (Fig. 4F). *In situ* hybridization and immunohistochemical studies of human placenta at term located it in the outer syncytiotrophoblasts, which are ideally positioned to secrete the peptide into maternal blood.

## Discussion

In the present study we report for the first time the establishment of a sensitive and specific two-site EIA for metastin and dramatic changes in plasma metastin concentrations during human pregnancy. The plasma metastin concentration increased to 1230 fmol/ml [900-fold that in nonpregnant plasma (1.3 fmol/ml)] in the first trimester, reached a maximum level of 9590 fmol/ml (7000-fold that in nonpregnant plasma) in the third trimester, and then returned to 7.6

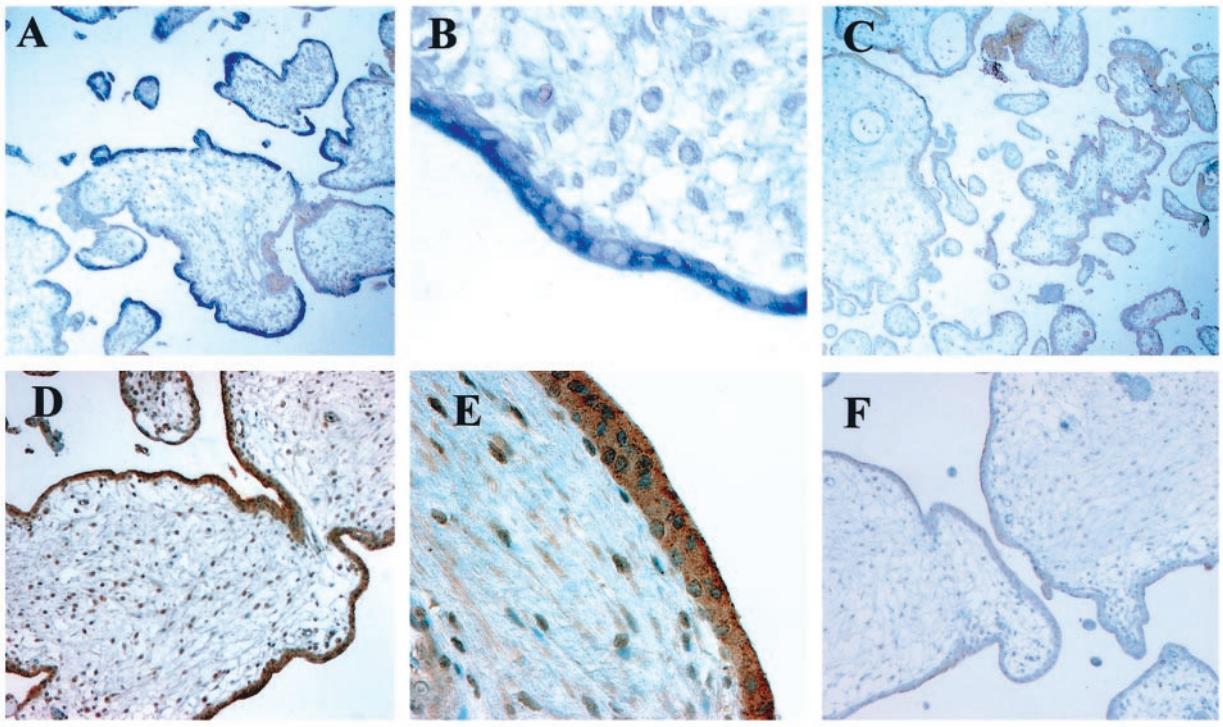


FIG. 4. Photomicrographs showing KiSS-1 mRNA-expressing cells (A and B) and metastin-immunoreactive cells (D and E) in human term placenta. A, Human term placenta with human antisense probe. B, High magnification of A. C, Human term placenta with human sense probe. D, Immunohistochemical staining of metastin in human term placenta using KIS-1Ca. E, Enlargement of the section shown in D. F, Preabsorption with 1 $\mu$M human metastin blocked the staining reaction. Both KiSS-1 mRNA-expressing cells (*blue*) and metastin-immunoreactive cells (*brown*) are found in the syncytiotrophoblasts. Magnification, ×100 (A, C, D, and F) and ×400 (B and E).

fmol/ml by postpartum d 5. The increase in plasma ir-metastin levels was notably in accord with the progression of pregnancy. A significant correlation was found between the concentrations of ir-metastin and those of sex steroids or hPL, suggesting that the release of ir-metastin during pregnancy is related to the size of the placenta. The expression of KiSS-1 (metastin) mRNA is notably predominant in the human placenta (1–3). Furthermore, we have shown that metastin is concentrated in the syncytiotrophoblasts, which are well known placental endocrine cells that produce several hormones, including hCG (9, 10) and hPL (11). Taken together, these findings strongly suggest that metastin is produced in human placenta and circulates in maternal blood throughout pregnancy. The plasma metastin levels postpartum were, however, still about 6 times higher than the nonpregnant levels. This may be due to delayed clearance of metastin released from placenta before delivery and/or a possible contribution from nonplacental metastin. As the placenta is an endocrine organ that produces a wide variety of hormones that play important roles in pregnancy, the findings of the present study have added metastin to the list of placental hormones.

In addition, a low level of metastin exists in nonpregnant plasma. This indicates that metastin is also released from nonplacental tissues and may have an endocrine-like function in nonpregnancy. It has been reported that KiSS-1 mRNA is expressed in pancreas, pituitary, and peripheral blood leukocytes, although the level of mRNA expression in those tissues is lower than that in placenta (1–3). Endocrine release of metastin from these tissues as well as possible changes in metastin levels under various conditions, including diseases, menstruation, and aging, will require further investigation.

It has been reported that many regulatory peptides, such as CRH (12, 13), neuropeptide Y (14), leptin (15), and neurokinin B (16), are produced in the placenta and released into the maternal plasma. The plasma levels of these peptides in pregnant women are 4- to 50-fold higher than those in nonpregnant women. Although a physiological role of these peptides has yet to be ascertained, it is postulated that CRH and NPY contribute to the stress-related responses of parturition, because plasma concentrations of these peptides are known to increase in pregnant women during labor or parturition (12–14). In this study we did not measure changes in the time course of plasma metastin concentrations on the day of delivery. To clarify differences in the physiological role of metastin from those in CRH and neuropeptide Y, it will be necessary to measure its concentration in plasma during gestation, labor, and delivery.

There are striking similarities between the behavior of invasive placental cells and that of invasive cancer cells (17–19); like tumor cells, cytotrophoblastic cells migrate through and invade the uterine wall at the time of implantation. Unlike tumor invasion, however, this unique interaction between genetically dissimilar trophoblasts and uterine cells is closely regulated and is limited both temporally and spatially by mechanisms that are largely unknown. In a previous study we demonstrated that metastin suppressed the motil-

ity, invasion, and growth of hOT7T175-transfected CHO cells *in vitro* (4). We also showed that the inhibitory activities of invasion were based on the regulation of cell motility related to the formation of stress fibers, not to the inhibition of cell adhesion to the extracellular matrix (1, 4). Considering the localization of metastin in syncytiotrophoblasts and the dramatic elevation of plasma ir-metastin in the first trimester, it is possible that metastin may be involved in the regulation of trophoblast invasion. To clarify the role of metastin during implantation, future studies using human placenta in the first trimester should be undertaken.

Some peptides and proteins derived from placenta, such as hCG, Schwangerschafts protein-1 and $\alpha$-fetoprotein, are used as tumor markers. hCG and Schwangerschafts protein-1 are used as tumor markers not only of chorionic tumors, but also uterine, ovarian, lung, and bladder tumors (20). $\alpha$-Fetoprotein has also been used as a marker of hepatic tumors (21). Metastin shows antimetastatic effects *in vitro* and *in vivo* (1, 4), and a recent *in vivo* investigation of human melanomas showed significant loss of KiSS-1 expression in deeply invasive primary tumors and metastases (22). These results led us to speculate that metastin could be a new type of tumor marker that can detect the metastatic potential of some tumors. To clarify the possibility of metastin as a tumor marker, the correlation between a tumor and its plasma metastin concentration should be investigated in future studies.

In the present study, using two region-specific monoclonal antibodies, a specific and sensitive EIA for metastin was established. This study provides the first evidence that metastin is secreted in high concentrations throughout gestation from the placenta, and that its production starts very early in pregnancy. This evidence suggests that metastin is a novel placenta-derived hormone and may have an important physiological role in human reproduction, from implantation to delivery. In addition, measurements of the plasma and tissue concentrations of metastin under various conditions using this EIA will help to elucidate the physiological role of metastin.

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