J. Tokyo Med. Univ., **64**(3): 285-293, 2006

Expression and function of midkine in human endometrium

Li-Hsing HO

Department of Medicine, Chung Shan Medical University

Abstract

Midkine (MK) is a heparin binding-growth factor, which shows strong expression during the mid-gestation period of mice and has a deep relation with tissue reconstruction. It would be of interest to know whether MK plays any part in the human endometrium, which repeats exfoliation and regeneration. In this study, we investigated the production and dynamics of MK in the endometrium.

Surgical specimens obtained from patients with gynecological benign tumor were used for the study after obtaining informed consent. MK mRNA expression in tissues was examined by RT-PCR and that of cells by in situ RT-PCR. MK localization was studied by the enzyme antibody method and MK in culture supernatant was measured by enzyme immunoassay. Endometrium was separated into glandular and stromal cells and cultured respectively. MK production in culture media was measured and the influences of steroid hormones and growth factors were also examined.

MK mRNA expression was seen in 78.1% (25/32) of the endometrium and was over-expressed in that of the early and mid secretory phase. MK mRNA expression in cells was recognized in both glandular and stromal cells. However, marked MK localization was seen only in the cytoplasm of glandular cells, whereas it was absent or only faintly stained in stromal cells. MK concentration in the culture supernatant of glandular cells increased with the cultivation progress. However, it was not influenced by the addition of the steroid hormones and the growth factors. On the other hand, marked increase of MK concentration in stromal cells was recognized with the addition of progesterone, although it did not increase with the cultivation progress. As for the cell culture on Matrigel, the MK concentration of supernatant in both glandular and stromal cells showed a higher level on the third day of the cultivation.

MK expression increases gradually from the menstrual phase toward the secretory phase, and it is suggested that MK expressed at the time of cell differentiation plays an important role in the period of implantation and differentiation of fertilized eggs.

Introduction

Midkine (MK) is a heparin-binding growth factor discovered in 1988 as a gene product. The expression increases during the mid-gestation period of mouse embryogenesis, in the early stage of differentiation of embryonal carcinoma stem cells treated with retinoic acid[1]. MK is structurally distinguished from other heparin binding growth factors such as fibroblast growth factor (FGF). However, MK is part of a new family of growth factors with pleiotrophin (PTN)[2][3], which has a 50% sequence homology with MK[4].

Recently, though various biological activities of MK have become clarified, its functions can be summarized as linked to nerve survival and differentiation, carcinogenesis and tissue reconstruction. There are various reports that MK promotes the expansion of neurites in the process of cultivation of mouse embryo neurons[5]

Received January 5, 2006, Accepted February 8, 2006
Key words: Midkine, Endometrium, Glandular cell, Stromal cell
Corresponding author: Li-Hsing Ho No. 110, Sec. 1, Chien-Kuo N. Road, Taichung, Taiwan 402

and acts to promote nervous survival[6]. MK is over-expressed in many types of human carcinoma tissues compared with normal tissues. High expression of MK is recognized in 80–90% of digestive tract cancers such as colorectal carcinoma, liver cancer, pancreatic cancer, esophageal cancer and stomach cancer[7] as well as lung cancer[8] and breast cancer[9], and it has been reported that over-expression of MK in neuroblastoma[10], glioma[11] and bladder cancer[12] is associated with poor prognosis. Serum MK levels are also increased in patients with various types of carcinomas[13]. On the other hand, there are many reports about inflammation and the tissue reconstruction, emphasizing the activities of MK in migration of blood neutrophils[14]. MK increases the activation of plasminogen-activating enzyme of vascular endothelial cells[15], It has also been shown to be present in the synovial liquid of rheumatism patients[15]. Moreover, when MK is preadministered subretinally in albino rats, degeneration resulting from continuous optical irradiation can be prevented[16], while it is markedly deposited on the senile plaque of Alzheimer's disease[17].

Nevertheless, there are few reports to date on MK in the reproductive and gynecological fields[18–20]. It would be of interest to know whether MK, which is closely related to tissue reconstruction plays any part in the human endometrium, and its process of repeated exfoliation and regeneration. In this study, we therefore investigated the production and dynamics of MK in the endometrium.

Materials and Methods

Surgical specimens obtained from patients with gynecological benign tumors were used for the study after obtaining informed consent. The benign disease group was composed of 24 patients who underwent open or laparoscopic surgery for uterine myoma or ovarian tumor, and who had regular menstrual cycles. The phase of the menstrual cycle at which the functioning layer of the endometrium was collected was the menstrual phase in 6 cases, the early and mid-proliferative phase in 6 cases, the late proliferative phase in 5 cases, the early and mid-secretory phase in 7 cases, and the late secretory phase in 8 cases. The accuracy of the phase of the menstrual cycle was confirmed by measurement of the serum E2 and P levels and by pathological endometrial dating[21].

1) RT-PCR

A portion of the aseptically collected tissue specimens was immediately cut with a scalpel into pieces, rapidly frozen with liquid nitrogen, and stored at −70°C until the RNA extraction. Total RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan). Five μg of total RNA was reverse-transcribed into cDNA using an RT-PCR kit (Stratagene, La Jolla, CA) according to the manufacturer's recommendations. The prepared cDNA was stored at −20°C until use. The MK was amplified using the following primer pairs designed by Tsutsui et al.[22]:

5′-ATGCAGCACCGAGGCTTCCT-3′ (MK-F)
5′-ATCCAGGCTTGGCGTCTAGT-3′ (MK-R).

One-microliter aliquots of the cDNA and 10 pmol of each primer were subjected to PCR using Ready-to-Go PCR bead kit (Amersham Pharmacia Biotech, Uppsala, Sweden). MK was amplified with 30 cycles of 94°C for 30s, 50°C for 45s and 72°C for 90s. The amplified products were fractionated on 1.5% agarose gel. The gel was stained with ethidium bromide. The efficiency of cDNA synthesis from each sample was estimated by PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers.

2) In situ RT-PCR

Formalin-fixed, paraffin-embedded sections were deparaffinized in xylene and rehydrated. Then they were soaked in 0.01 M citrate buffer and treated by microwave at 500 W for 10 minutes twice to obtain an enhancement of the RT-PCR signal[23]. RNase-free DNase (final concentration of 100 units/ml) (Nippon Gene) in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ at 37°C for 7 h was used to degrade the DNA. DNase was inactivated by heating at 75°C for 10 min. RNA PCR Kit (AMV) Ver. 2.1 (TaKaRa, Shiga, Japan) and MK oligonucleotide antisense primer were used in the reverse transcriptase reaction. Reverse transcription was carried out in a total volume of 20 ml on each tissue slide. The final concentrations for the reaction mixture were as follows ; 1X RNA PCR buffer, 5 mM $MgCl_2$, 1 mM dNTP mixture, 0.5 U RNase inhibitor, 1 U AMV reverse transcriptase, 10 mM antisense primer of MK and DPEC H_2O. The slides on which the reaction mixture was mounted were covered with EasiSeal for in situ PCR (Hybaid, London, UK) and reacted for 10 minutes at 30°C, 30 minutes at 42°C and 90°C at 5 minutes. The slides as a negative control did not undergo this reverse transcriptase reaction. The PCR amplification was carried out in 50 ml mixture of 0.2 mM of dNTP mixture (TaKaRa), 0.02 mM digoxigenin-11-dUTP (dig-11-dUTP) (Boehringer Mannheim, Tokyo, Japan), 0.17 U EX Taq and EX Taq buffer (TaKaRa), 0.8 mM each of the sense and antisense primers, 25 mM $MgCl_2$ and H_2O. The slides were heated to 82°C for seven minutes on the Omni slide in situ thermal cycler (Hybaid). After cooling to 55°C, PCR mixed solution was added to the specimens and tissue sections soaked in the solution were covered with EasiSeal. The PCR amplification was carried out five times with denaturing at 94°C for 60s, annealing at 55°C for 30s, and primer extension at 68°C for 30s. The

slides were incubated for 30 min with 0.3% Triton X-100 and 0.5% non-fat dry milk at room temperature. Immunodetection was performed using anti-digoxigenin (Fab) antibody conjugated to alkaline phosphatase (Boehringer Mannheim) and the mixture of nitro blue tetrazolium (NBT) and 5-brom-4-chloro-3-indolyl phosphate (BCIP) (Promega, Madison, USA). Binding was demonstrated microscopically by the presence of blue staining.

3) Immunohistochmistry

Immunochistochemical staining was carried out using Histofine SAB-PO (R) kit (Nichirei, Tokyo, Japan). Paraffin sections were deparaffinized, rehydrated and their endogenous peroxidase activity and non-specific binding were blocked with methanol containing 3% H_2O_2 and 10% goat normal serum, respectively. Sections were incubated with a rabbit anti-MK antibody (kindly donated by Dr. Sakuma of Meiji Cell Technology Center) diluted 100 times with 0.01 M phosphate-buffered saline for 1 h at room temperature. Sections were then treated with a biotinylated goat anti-rabbit antibody, and horseradish peroxidase (HRP) labeled streptoavidin. Development was done by treating the sections with a Liquid DAB-Plus Substrate kit (Zymed, San Francisco, CA, USA). Normal rabbit serum instead of anti-MK was used as negative control. After counterstaining with hematoxylin, immunostaining of MK on the tissue sections was detected by a light microscope.

4) Preparation of endometrial glandular and stromal cells

Endometrial glandular and stromal cells were isolated from normal endometrial tissues by modified procedures of Satyaswaroop et al.[24]. In brief, normal human endometrial tissues obtained at operation were washed with calcium and magnesium-free Hanks' solution (CMF-Hanks') several times, minced into small fragments, and incubated with CMF-Hanks' containing 0.25% collagenase (type IA, Sigma, St. Louis, MO, USA) in a flask at 37°C in an atmosphere of 5% CO_2 in air for 3 hrs. After the flask was skaken gently for 3 minutes, the suspension of glands and cells were strained through a 250 μm stainless steel sieve (SANPO, Tokyo, Japan) to remove any undigested tissues and mucous material. The filtrate was then strained through a 38 μm sieve (SANPO). The sieves were washed thoroughly with CMF-Hanks'. Then the sieve was turned upside down and washed with CMF-Hanks' several times. The washing solution containing endometrial glands was centrifuged and the precipitate was resuspended with Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12 ; Life Technologies, Tokyo, Japan) without phenol red containing 50 U/ml penicillin, 50 mg/ml streptomycin (Life Technologies), 0.25 mg/ml fungizone (Life Technologies), 10% fetal calf serum. After 60 min incubation, the floating endometrial epithelial cells were collected and used for experiments. While the filtrate passed through a 250 μm which was containing much volume of stromal cells was centrifuged and washed with CMF-Hanks'. They were further purified by centrifugation on a Ficoll-Paque (Amersham Pharmacia Biotech). The purified human endometrial stromal cells were washed and resuspended with DMEM/F12 medium and used for the experiments. Immunohistochemical analysis for keratin or vimentin staining indicated that the purity of these cell preparations was greater than 99%.

5) Cell culture

The separated cells were resuspended in basal medium composed of 1 : 1 Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 without phenol red containing 50 U/ml penicillin, 50 mg/ml streptomycin (Life Technologies), 0.25 mg/ml fungizone (Life Technologies), 10% charcoal stripped bovine serum. Glandular and stromal cells were plated out in 24-well plastic or Matrigel multiplate (Collaborative Research) at a concentration of 1×10^6 cells per 250 μl well, and incubated at 37°C in 95% air and 5% CO^2. On the 3rd day of cultivation on plates, glandular colonies consisted of tightly packed polygonal or tadpole shaped cells that grew in swirls, and stromal colonies consisted of elongated spindle cells (Fig. 1). While on Matrigel, glandular colonies formed three-dimensional structures like tube formation, and stromal colonies formed cell islands accompanied with small three-dimensional structures (Fig. 2).

Culture media were exchanged and we added estradiol (10^{-8} M), progesterone (10^{-7} M), EGF (100 ng/ml) and TGFβ (1 ng/ml) in triplicate to the culture wells respectively, then the culture media were exchanged every 4 days, and culture supernatants were kept at −20°C until measurement.

6) Enzyme-linked immunoassay for midkine

Measurement of MK concentration was performed by the method of Ikematsu et al.[13]. In brief, 96 well multititer plates absorbed with rabbit anti-human MK antibodies were made and kept at 4°C until assay. The first reaction was done by the reaction of antigen and labelled antibody. Aliquots of 50 μl of MK standard solutions or sample solutions with peroxidase-labelled chicken anti-human MK antibodies were added into the wells. After incubation for 30 minutes at room temperature, the wells were washed five times with washing buffer and emptied by tapping. In second reaction, aliquots of 100 μl of a substrate solution were added into the wells and the plates were incubated for 20 minutes at room temperature. The reaction was stopped by adding 100 μl of 2N sulphuric acid and

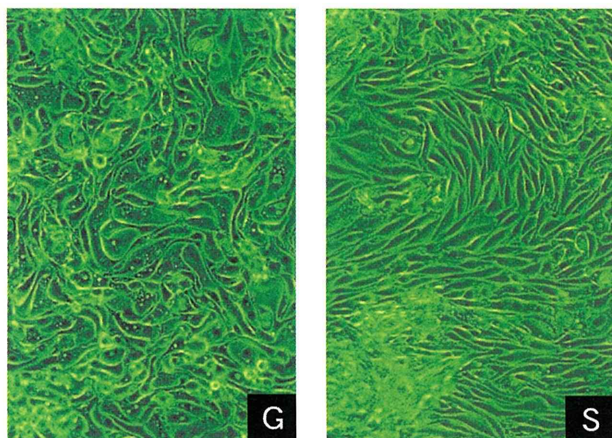


Fig. 1 Growth features of endometrial glandular cells (G) and stromal cells (S) on plain plates

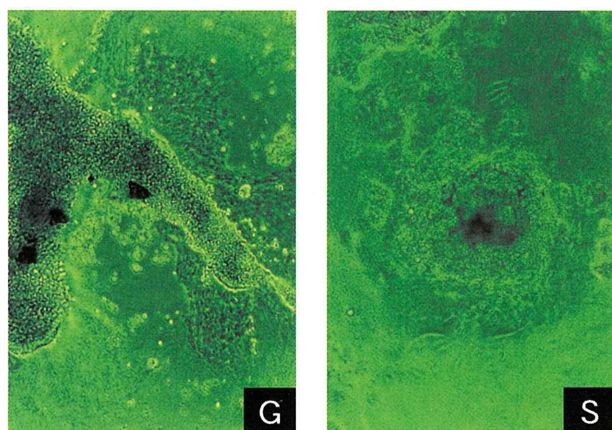


Fig. 2 Growth features of endometrial glandular cells (G) and stromal cells (S) on Matrigel

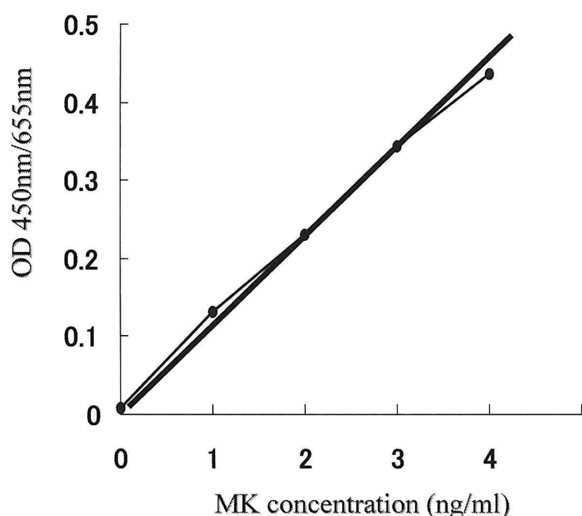
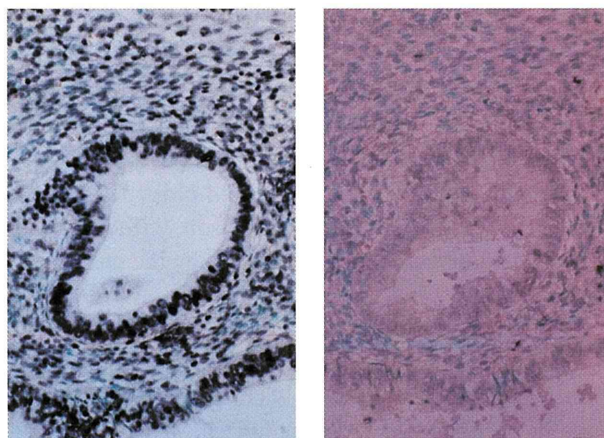


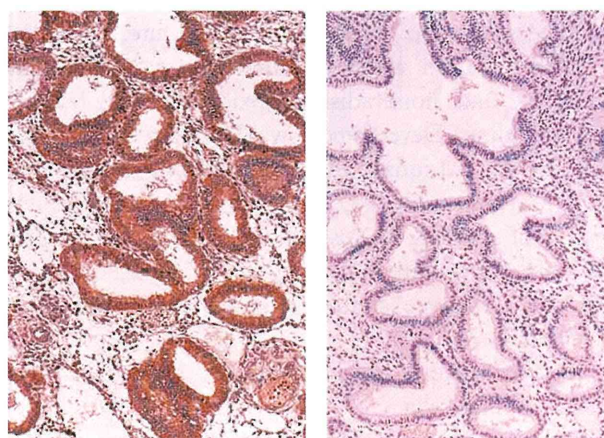
Fig. 3 MK ELISA standard curve

detection was performed at OD450 was detected using a multiplate reader. The MK standard curve showed an almost linear line between 0.5 and 4 μg/ml of MK standard (Fig. 3). Both coefficient of variations of intra- and inter-assay in this assay were within 10%.



6A　6B

Fig. 6 The expression of MK mRNA in the endometrium at the cellular level using in situ RT-PCR
6A : MK mRNA localization in an endometrium section, 6B : control in a serial section, 200 magnification



6A　6B

Fig. 7 Immunohistochemical staining of MK in endometrium
6A : MK localization in an endometrium section, 6B : control in a serial section, 100 magnification

7) Statistical analysis

Student's t-test was used for analysis of differences between groups. The statistical package used was SPSS version 10.07 (SPSS Inc. Chicago, IL, USA). A p-value of lower than 0.05 was considered to indicate a statistically significant difference.

Results

1) RT-PCR analysis of MK in endometrium

MK mRNA expression was seen in 78.1% (25/32) of specimens of the functional layer of the endometrium. The intensity of its expression became stronger from the menstrual toward the secretory phases (Fig. 4). When its detection rate was analyzed in the endometrium during the menstrual cycle, it increased gradually from the menstrual phase to early and mid secretory phase, and decreased to the late secretory phase and the

menstrual phase, as shown in Fig. 5.

2) Study using in situ RT-PCR

The expression of MK mRNA in the endometrium, as observed at the cellular level, was visualized as high signals in the nuclei of the endometrial glandular cells. Less strong signals were noted in the uterine stromal cells (Fig. 6A). In the control group, for which a sense probe was used, no cellular signals were detected (Fig. 6B).

3) Immunohistochemical study

We examined MK expression in protein level by immunohistochemical analysis using anti-MK antibodies. Marked MK localization was seen in the cytoplasm of endometrial glandular cells whereas it was absent or faintly stained in stromal cells (Fig. 7).

4) MK production of separated endometrial cells

MK concentration in the culture supernatant of glandular cells increased with the cultivation progress, showing high levels of an average of 89.5 ng/ml on the 11th

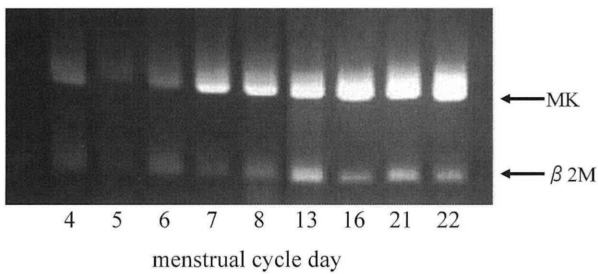


Fig. 4 MK mRNA expression in the endometrium during the menstrual cycle

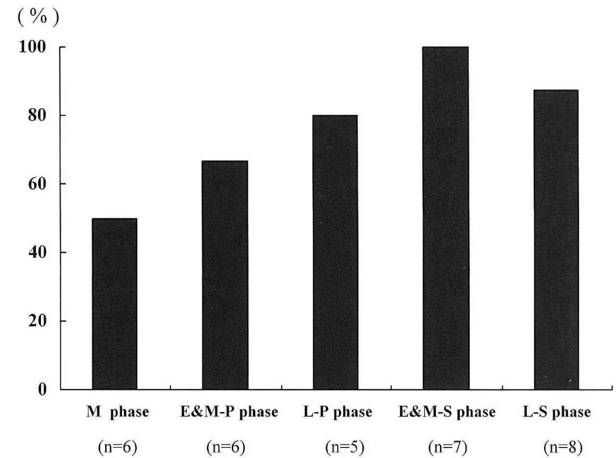


Fig. 5 The detection rate of MK mRNA expression in different menstrual phases
E&M: early and mid, L: late, M: menstrual, P: proliferative, S: secretory

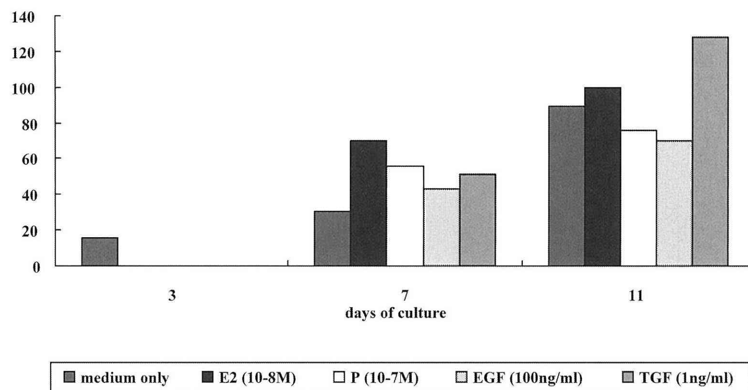


Fig. 8 MK concentrations in culture media of endometrial glandular cells
Influence by steroid hormones and growth factors

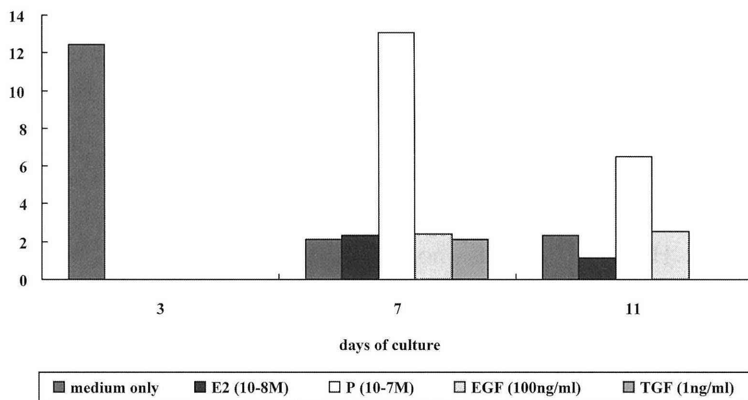


Fig. 9 MK concentrations in culture media of endometrial stromal cells
Influence of steroid hormones and growth factors

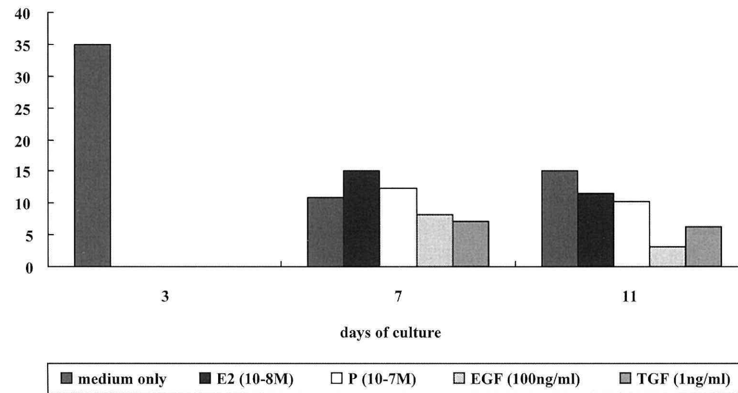


Fig. 10 MK concentrations in Matrigel culture media of endometrial glandular cells
Influence of steroid hormones and growth factors

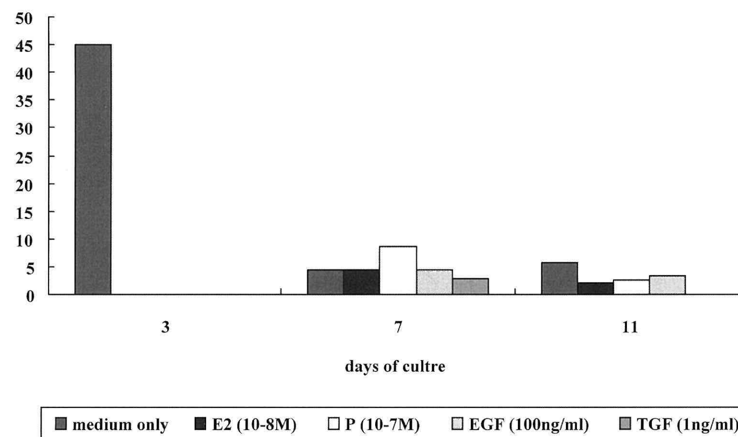


Fig. 11 MK concentrations in Matrigel culture media of endometrial stromal cells
Influence of steroid hormones and growth factors

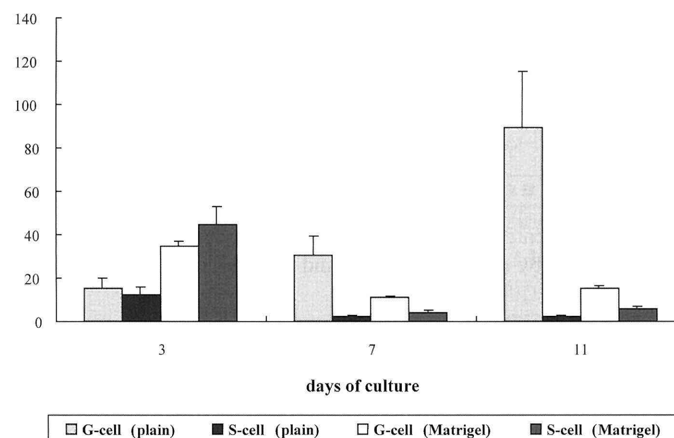


Fig. 12 MK concentrations in culture media of endometrial glandular and stromal cells
Comparison of culture on Matrigel with culture on plain plates

day of cultivation (Fig. 8). However, it was not influenced by the addition of the steroid hormones and the growth factors (Fig. 8). On the other hand, marked increase of MK concentration in stromal cells was recognized by the addition of progesterone, although it did not increase with the cultivation progress, and showed lower levels (average 2.3 ng/ml) compared with glandular cells (Fig. 9). As for the cell culture on Matrigel, the MK concentration of supernatant in both glandular and stromal cells was not influenced by the addition of the steroid hormones and growth factors (Fig. 10, 11), but those on the third day of the cultivation showed a higher level compared with those in plastic multiplates (Fig. 12).

Discussion

There is no report on the dynamics of MK in the endometrium durng repeated endometrial exfoliation and regeneration. We showed that MK was produced from the endometrium by proving its expression and localization. However, it is reported that serum MK levels were low in women who have normal menstrual cycles and is not influenced by the period of the menstrual cycle[20]. This shows that the MK produced by the endometrium is different from that by carcinoma patients and works in the autocrine or paracrine system. On the other hand, it is reported that the measurement of the serum MK level in patients with carcinomas is diagnostically useful by detection of higher levels in comparison with the low levels in normal healthy persons and cases of benign tumors[13,18]. Although the truncated MK is expressed in carcinoma tissues[25,26], it was not recognized in the endometrial tissues examined in this study. This also suggests that the function of endometrial MK is different from that in carcinoma tissue.

As regards the dynamics of the MK expression in the endometrium of the menstrual cycle, we found that its expression increased gradually from the menstrual toward the early and mid-secretory phase. This suggests that MK production from the endometrium is influenced by estrogen and progesterone. Therefore, we separated endometrial glandular cells and stromal cells from the endometrium of normal healthy women, and established a culture system of endometrial cells containing three-dimensional structure. It was reported that human endothelial cells formed tube-like structures on the gel composed of basement membrane proteins within 24 hours[27], and endometrial cells used in this study formed tube-like structures on Matrigel within 48 hours (data not shown).

The MK level in the culture supernatant was high in the early stage of culture and thereafter decreased when the cells were cultured on Matrigel, suggesting that MK influences the formation of the three-dimensional structure. In the hormone addition experiment, the MK level in culture supernatant showed significantly high values when progesterone was added to the stromal cells. MK protein of stromal cells was not recognized immunohistochemically, but its mRNA was found to be expressed by in situ RT-PCR, which might indicate that its production is controlled by certain conditions. It can be said that the deciduation of stromal cells is one of those reasons.

Since MK expression increases gradually from the menstrual phase toward the secretory phase, this suggests that MK expressed at the time of cell differentiation plays an important role in the period of implantation and differentiation of fertilized eggs.

References

1) Kadomatsu K, Tomomura M, Muramatsu T: cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis. Biochem Biophys Res Commun **151**: 1312–1318, 1988
2) Li YS, Milner PG, Chauhan AK, Watson MA, Hofman RM, Kodner CM, Milbrant J, Deuel TF: Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. Science **250**: 1690–1694, 1990
3) Merenmies J, Rauvala H: Molecular cloning of the 18 kDa growth-associated protein of developing brain. J Biol Chem **265**: 16721–16724, 1990
4) Muramatsu T: Midkine (MK), the product of a retinoic acid responsive gene, and pleiotrophin constitute a new protein family regulating growth and differentiation. Int J Dev **37**: 183–188, 1993
5) Muramatsu H, Sirakawa H, Yonezawa S, Murata H and Muramatsu T: Midkine, a retinoic acid-inducible growth/differentiation factor: immunochemical evidence for the function and distribution. Dev Biol **159**: 392–402, 1993
6) Michikawa M, Kikuchi S, Muramatsu H, Muramatsu T: Retinoic acid responsive gene product, midkine (MK), has neurotrophic functions for mouse spinal cord and dorsal root ganglion neurons in culture. J Neurosci Res **35**: 530–539, 1993
7) Aridome K, Tsutsui J, Takao S, Kadomatsu K, Ozawa M, Aikou T, Muramatsu T: Increased midkine gene expression in human gastrointestinal cancers. Jpn J Cancer Res **86**: 655–661, 1995
8) Garver RI, Radford DM, Donis-keller H, Wick MR, Milner PG: Midkine and pleiotrophin expression in normal and malignant breast tissue. Cancer **74**: 1584–1590, 1994
9) Garver RI, Chan CS, M, Milner PG: Reciprocal expression of pleiotrophin and midkine in normal versus malignant lung tissue. Am J Respir Cell Mol Biol **9**: 463–466, 1993
10) Nakagawa A, Milbrand J, Muramastu T, Deuel TF, Zhao H, Cnaan A, Muramastu T: Differential expression of pleiotrophin and midkine in advanced neuroblastomas. Cancer Res **55**: 1792–1797, 1995
11) Mishima K, Asai A, Kadomatsu K, Ino Y, Nomura K, Narita Y, Muramatsu T, Kurino T: Increased expression of midkine during the progression of human astrocytomas. Neurosci Lett **233**: 29–32, 1997
12) O'Brien T, Cranston D, Fuggle S, Bicknell R, Harris AL: The angiogenic factor midkine is expressed in bladder cancer, and over expression correlates with invasive cancers. Cancer Res **56**: 2515–2518, 1996
13) Ikematsu S, Yano A, Aridome K, Kikuchi M,

Kumai H, Nagano H, Sakuma S, Muramatsu H, Kadomatsu K, Muramatsu T : Serum midkine levels are increased in patients with various types of carcinomas. Br J Cancer **83** : 701-706, 2000

14) Takada t, Torimatsu K, Muramatsu H, Song XJ, Torii S, Muramatsu T : Midkine, a retinoic acid-inducible heparin-binding cytokine in inflammatory responses : chemotactic activity to neutrophils and association with inflammatorysynovitis. J Biochem **122** : 453-458, 1997
15) Kojima S, Muramatsu H, Amamura H, Muramatsu T : Midkine enhances fibrinolytic activity of bovine endothelial cells. J Biol Chem **270** : 9590-9596, 1995
16) Unoki K, Ohba N, Arimura H, Muramatsu H, Muramatsu T : Rescue of photoreceptors from the damaging effect of constant light by midkine, a retinoic acid responsive gene product. Invest Ophthalmol Vis Sci **35** : 4063-4068, 1994
17) Yasuhara O, Muramastu H, Kim S U, Muramatsu T, Murata H : Midkine, a novel neurotrophic factor, is present in senile plaques of Alzheimer disease. Biochem Biophys Res Commun **192** : 246-251, 1993
18) Nakanishi T, Kadomatsu K, Okamoto T, Tomoda Y, Muramatsu T : Expression of midkine and pleiotrophin in ovarian tumors. Obstet Gynecol **90** : 285-290, 1997
19) Chung HW, Wen Y, Choi EA, Li h, Moon HS, Yu H-K, Polan ML : Pleiotrophin (PTN) and midkine (MK) mRNA expression in eutopic and ectopic endometrium in advanced stage endometriosis. Mol Hum Reprod **8** : 350-355, 2002
20) Sato C, Nishi H, Isaka K : Expression of midkine in gynecological tumors and its clinical spplication. J Tokyo Med Univ **62** : 381-388, 2004
21) Noyes R, Hertig A, Rock, J : Dating the endometrial biopsy. Fertil Steril **1** : 3-25, 1950
22) Tsutsui J, Kadomatsu K, Matsubara S, Nakagawara A, Hamanoue M, Takao S, Shimizu H, Ohi Y, Muramatsu T : A new family of heparin-binding growth/differentiation factors : increased midkine expression in Wilms' tumor and other human carcinomas. Cancer Res 53 : 1281-1285, 1993
23) Sibony M, Commo F, Callard P, Gasc JM : Enhancement of mRNA in situ hybridization signal by microwave heating. Lab Invest **73** : 586-91, 1995
24) Satyaswaroop PG, Bressler RS, De La Pena MM, Gurpide E : Isolation and culture of human endometrial glands. J Clin Endocrinol Metab **48** : 639-41, 1979
25) Kaname T, Kadomatsu K, Aridome K, Yamashita S, Sakamoto K, Ogawa M, Muramatsu T and Yamamura K. The expression of truncated MK in human tumors. Biochem Biophys Res Commun **219** : 256-260, 1996
26) Aridome K, Takao S, Kamane T, Kadomatsu K, Natsugoe S, Kijima F, Aikou T, Muramatsu T : Truncated midkine as a marker of diagnosis and detection of nodal metastases in gastrointestinal carcinomas. Br J Cancer **78** : 472-477, 1998
27) Kubota Y, Kleinman HK, Martin GR, Lawley TJ : Role of laminin and basement membrane in the morphological differentiation of human endothekial cells capillary-like structures. J Cell Biol **107** : 1589-1598, 1988

子宮内膜におけるMKの発現について

何　　黎星

中山醫學大學医学部

【要旨】 本研究では、組織再構築と深い関わりがあるMidkine (MK) が剝離再生を繰り返すヒト子宮内膜において如何なる役割を果たすか検討した。

患者のinformed consentを得た後、良性疾患手術検体から新鮮な子宮内膜を採取した。MK mRNAの発現はRT-PCR法およびin situ RT-PCR法により検索した。MKの組織局在は酵素抗体法、濃度測定は、MK-ELISA法を用いた。次に子宮内膜細胞の培養系を確立し、添加実験ならびに形態学的変化に伴うMKの機能解析を行なった。

MK-mRNAは、子宮内膜に発現しており、黄体期にその発現が増強された。腺細胞培養上清中のMK濃度は、培養経過とともに増加したが、ステロイドホルモンなどの添加による有意の差は認めなかった。間質細胞培養では、培養経過よる増加は認めず、progesterone添加により著明な増加が認められた。腺および間質細胞は、Matrigel上で培養初期にMK高値を示した。

三次元管腔構造を構築するMatrigel上で内膜細胞は高濃度のMKを産生することから、MKの子宮内膜再構築への関与が示唆された。子宮内膜からのMK産生は、分泌期に向かい漸増することから、MKは受精卵の分化や着床の時期に重要な役割を演じていることが示唆された。

〈キーワード〉 Midkine、子宮内膜、子宮内膜腺細胞、子宮内膜間質細胞