Serum levels of midkine, a heparin-binding growth factor, increase in both malignant and benign gynecological tumors

Ragaa M. H. SALAMA\textsuperscript{a,e}, Hisako MURAMATSU\textsuperscript{a,c}, Honami KOBAYASHI\textsuperscript{b}, Seiji NOMURA\textsuperscript{b}, Shigehiko MIZUTANI\textsuperscript{b} and Takashi MURAMATSU\textsuperscript{a,d}**

\textsuperscript{a}Department of Biochemistry, \textsuperscript{b}Department of Gynecology and \textsuperscript{c}Division of Disease Models, Center for Neurological Disease and Cancer, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku Nagoya 466-8550, Japan
\textsuperscript{d}Department of Health Science, Faculty of Psychological and Physical Sciences, Aichi Gakuin University, 12 Araike, Iwasaki-cho, Nisshin, Aichi 470-0195, Japan
\textsuperscript{e}Present address: Department of Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence: Takashi MURAMATSU E-mail: tmurama@dpc.aichi-gakuin.ac.jp

Abstract
Midkine (MK) is a heparin-binding growth factor, the expression of which is strong in the midgestation period, is highly restricted in the adult, and is increased in many human carcinomas. In the present investigation, we determined serum levels of MK by an enzyme immunoassay, and found that in many patients with gynecological tumors, the values increased above the cut off value of 500 pg/ml. All control subjects (n=49) including pregnant women exhibited values not more than 500 pg/ml. The percentage of positive cases was as follows: ovarian carcinoma (n=15), 87%; uterine cervical carcinoma (n=18), 67%; uterine endometrial carcinoma (n=10), 50%; ovarian cyst (n=25), 72%; leiomyoma (n=29), 72%. CA-125 and MK levels were not correlated in ovarian carcinoma and ovarian cyst; a combination of the two assays may be helpful in increasing specificity or sensitivity. Furthermore, increased MK levels were found even in patients with early stages of uterine cervical and endometrial carcinomas. Serum MK levels might become helpful in the initial screening of gynecological tumors.

Key words: Enzyme-linked immunosorbent assay, growth factor, midkine, tumor marker

Introduction
Midkine (MK) is a heparin-binding growth factor with a molecular weight of 13 kDa [1-3] and has about 50% sequence identity to pleiotrophin /HB-GAM [4-6]. MK promotes the growth, survival and migration of various cells [1, 7-10]. It is strongly expressed during midgestation embryogenesis, while its expression in adult tissues is restricted [11, 12]. MK becomes overexpressed in many human carcinomas including gynecological tumors [1, 13-23]; in cervical carcinoma, MK mRNA increases more than 100-fold compared to levels in normal cervical tissue [23]. Among ovarian tumors, malignant epithelial tumors expresses significantly more MK mRNA than benign tumors [22]. MK is considered to promote tumor invasion through its activities. Indeed, an antisense oligodeoxyribono-
nucleotide to MK inhibits growth of colorectal carcinoma cells in nude mice [24].

We also found that serum MK levels increase in patients with various carcinomas [25-30]. Since MK levels in normal subjects are low, and frequency of elevated MK levels in carcinomas are high, MK has become a promising tumor marker [25-30]. However, no data are available on serum MK levels in patients with gynecological tumors. Here, we report MK levels in patients with malignant gynecological tumors, in patients with benign gynecological tumors and in control subjects including pregnant women. We wished to observe possible correlation between MK expression and biological properties of the tumor, and to evaluate potential value of serum MK levels as a tumor marker in gynecological field. Since CA-125, an antigen on a mucin-type glycoprotein, is a useful marker in the diagnosis of ovarian carcinomas [31-34], we also compared the values of MK and CA-125 in sera from patients with malignant and benign ovarian tumors.

**Materials and Methods**

*Specimens*

Serum samples were collected from 97 female patients and 49 female controls at Nagoya University Hospital. The controls included healthy volunteers (n=13), sterile subjects (n=12), pregnant woman (n=19), and patients with non-tumor diseases (n=5, uterian prolapse, anemia, infection, endometritis, and ovarian dysfunction). The ages of the patients with the following tumors were as follows: ovarian carcinoma (n=15, 58.5 ± 14.9, range 36-81), uterine cervical carcinoma (n=18, 51.7 ± 18.7, range 25-85), uterine endometrial carcinoma (n=10, 63.1 ± 11.7, range 37-80), ovarian cyst (n=25, 38.6 ± 11.0, range 25-65), leiomyoma (n=29, 44.6 ± 7.5, range 24-54). The age of the control subjects was 33.4 ± 7.9 (range 22-55). Sera were collected by centrifugation after standing plasma samples at room temperature for 15-18 hours, and were stored at -80°C before analysis. All patients and volunteers completed and signed an informed consent document that was approved by the Nagoya University Review Board.

**Assay**

The enzyme-immunoassay of MK was performed as described previously [25], using chemically synthesized human MK (Peptide Institute, Osaka, Japan) as a standard. Briefly, affinity-purified rabbit antibody to MK was coated to a well of a microtiter plate. Serum sample was incubated in the well, and the bound MK was reacted with biotinylated anti-MK antibody. Then, streptavidin-β-galactosidase conjugate was allowed to react. The bound enzyme was assayed using 4-methylumbelliferyl-β-D-galactoside as a substrate.

CA-125 was determined by an immuno-radiometric assay employing M11 anti-CA-125 monoclonal antibody. The assay kit, CA125II TFB, was produced by Fujirebio Diagnostic Inc (Halvern, PA, USA) and was obtained from TFB, Inc (Tokyo, Japan).

**Results**

**MK values in control subjects**

Serum MK values were determined in control subjects. Since the value can change according to the procedure to obtain serum, we thought it important to compare MK values using samples obtained at the same hospital by the same method.

All of the 49 control specimens gave MK values of less than 500 pg/ml (Figure 1). In a previous

![Figure 1. Average MK values in sera of control subjects. N, normal volunteer; St, sterile; Pr, pregnant; NT, non-tumor disease; A, all of the control subjects (N + St + Pr + NT).](image-url)
Figure 2. MK values in sera from gynecological tumors. Open squares in uterine cervical carcinoma and uterine endometrial carcinoma indicate cases of early stage tumors (stage 0 and 1). Marks above 5,000 indicate values above 5,000. Dashed line indicates the cut off level of 500 pg/ml.

In this study, a survey of 135 normal subjects also gave MK values of less than 500 pg/ml [27]. Although another study gave a value greater than 500 pg/ml [25], this level was encountered only rarely in normal subjects [25]. In the present study, we adopted a cut off value of 500 pg/ml.

The 49 control subjects consisted of 13 normal volunteers, 19 pregnant women, 12 sterile subjects, and 5 patients with non-tumoral diseases such as anemia. MK values did not differ significantly between the groups (Figure 1). Although the average age of the control subject was different from that of the patients with tumor, we previously observed that age did not affect the MK value in the controls [27].

Ovarian carcinoma

Among 15 specimens from patients with ovarian carcinomas, 13 exhibited MK values of more than 500 pg/ml (Figure 2).

Among 12 patients with records in both MK and CA-125 values, 11 were positive for MK and 10 were positive for CA-125 (more than 35 IU/ml) (Table 1). All patients were positive in at least one of the assays. Thus, MK values appear to be a valuable aid for screening in ovarian carcinoma.

Uterine carcinomas

Of the patients with cervical carcinomas, 12 among 18 exhibited serum MK values greater than the cut off value. Furthermore, 5 of 10 patients with carcinomas in an early stage (stage 0-1) showed positive values (Figure 2). Among the uterine endometrial carcinomas in the uterine body, 5 of 10 were positive. Among stage 1 tumor, 3 out of 6 cases were positive (Figure 2). Therefore serum MK values are helpful in the detection of uterine carcinomas, especially those in the early stages.

Leiomyoma and Ovarian cysts

Among 29 leiomyomas, 21 exhibited serum MK values higher than the cut off level (Figure 2). Among 25 ovarian cysts, 18 showed elevated MK values (Figure 2). Therefore, a high level of MK is not specific for malignancy in the gynecological field.

A CA-125 value was available in 17 cases of ovarian cyst. Among these specimens, 6 were positive for CA-125, and 13 were positive for MK. Since the MK and CA-125 values behaved independently, cases positive for both CA-125 and MK numbered 4 among 17 (Table 1). On the other hand in 11 cases of ovarian carcinomas, still 9
Table 1. Status of MK and CA-125 in patients with ovarian carcinoma or ovarian cyst

<table>
<thead>
<tr>
<th>Number of Cases</th>
<th>Ovarian carcinoma</th>
<th>Ovarian cyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>CA-125</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Twelve patients with ovarian carcinoma and 17 patients with ovarian cyst were classified into 4 groups according to the mode of expression of MK and CA-125: MK +, MK values > 500 pg/ml; MK -, MK values ≤ 500 pg/ml; CA-125 +, CA-125 values > 35 IU/ml; CA-125 -, CA-125 values ≤ 35 IU/ml.

patients were positive for both MK and CA-125 (Table 1).

Finally, as summarized in Fig. 3, the average serum MK values were always higher in patients with gynecological tumors than in control subjects (Figure 3).

Discussion

In control subjects, serum MK values were less than 500 pg/ml. It is interesting to note that the value was also at the low level in pregnant women, even though fetuses should have secreted much MK into amniotic fluid [35]. The placenta appears to function rigorously as a barrier. On the other hand, in many patients with malignant or benign tumors, serum MK levels were elevated. The high value of MK in leiomyoma might be related to the invasive character of the tumor. Recently, MK has been proposed to be involved in etiology of endometritis [36]. It remains to be investigated whether serum MK levels increase in patients of endometritis.

The present investigation provided information regarding suitability of MK as a tumor marker in gynecological field. CA-125 is a useful marker in the diagnosis of ovarian carcinomas, but markers to detect carcinomas at early stages are still eagerly sought [31-34, 37]. The present investigation confirmed the utility and limitation of CA-125: positive results were obtained in most but not all of patients with ovarian carcinoma, and patients with ovarian cyst occasionally gave positive results. In ovarian carcinoma, MK was not superior to CA-125; again it did not detect 100% of ovarian carcinomas, and gave positive results in many cases of ovarian cysts. However, CA-125 value and MK value changed independently. Therefore, if we pick up patients positive in either MK or CA-125, more cases of ovarian carcinoma might be detected. On the other hand, if we pick up patients positive in both MK and CA-125, specificity to detect ovarian carcinoma might be increased. Examination of larger number of patients will be required for definitive conclusion.

Another important point is that serum MK levels increased at an early stage in the development of uterine cervical carcinoma and uterine body carcinoma, enabling early detection. An increase of serum MK levels at the early stages of tumorigenesis has been noted in esophageal carcinoma [29].

The expression of MK aids in the progression of cancer due to activities to promote growth, survival, migration and angiogenesis. Indeed, in neuroblastoma [20], bladder carcinoma [19] and glioblastoma [21], patients with tumors of strongly expressing MK showed a worse prognosis than those with tumors having weak MK expression. Furthermore, serum MK levels correlated with a
worse prognosis in patients with esophageal carcinoma [30]. Indicators of a poor prognosis also correlated with high MK values in sera of neuroblastoma patients [28]. Whether there is a correlation between MK levels and prognosis in patients with gynecological tumors remains to be clarified.

In conclusion, serum MK levels exhibited interesting characteristics as a tumor marker in gynecological fields, and further extensive studies on serum MK levels in various gynecological diseases are much required.

Acknowledgements
We thank Ms. T. Adachi and H. Inoue for secretarial assistance.

References


Biol. 9: 463-466.