〔Chiba Med J 68, 333～342, 1992〕

〔原著〕

A CLINICAL AND FUNDAMENTAL STUDY ON THE CORRELATION BETWEEN SERUM CONCENTRATIONS AND TISSUE LOCALIZATION OF CANCER ANTIGEN 125 (CA125) AND TISSUE POLYPEPTIDE ANTIGEN (TPA) IN OVARIAN CARCINOMA

Haruo YOSHINO, Noriyuki INABA, Ichio FUKASAWA, Yuko OKAJIMA, Yoriko OTA, Nobuo SUZUKI* and Hiroyoshi TAKAMIZAWA

(Received October 6 1992, Accepted October 20, 1992)

SUMMARY

There is a strong correlation between tissue localization and the serum concentrations of tumor markers such as human chorionic gonadotropin (hCG) and Schwangerschaftsprotein 1 (SP_1) in choriocarcinoma. However, in the case of ovarian cancer there is usually a partial dissociation between tissue localization and serum levels, with the exception of α-fetoprotein (AFP). The present study investigated the correlation between the blood levels and tissue localization of cancer antigen 125 (CA 125) and tissue polypeptide antigen (TPA), tumor-produced markers which are commonly used in clinical practice. The following results were obtained:

1. The maximum pretreatment blood concentrations of both CA 125 and TPA (positivity rates: 68.6 % and 54.3 %, respectively) were found in serous cystoadenocarcinoma (CACa). Since the results for specificity were reversed (62.7 % and 87.3 %), the correlation between cancer and blood levels (diagnostic value) was 43.0 % and 47.4 %, respectively.

2. Positivity for the 2 tumor markers increased with advancing clinical stage, suggesting the relationship between the blood level and the volume of cancer tissue or the extent of invasion/destruction of the surrounding tissues.

3. In serous CACa patients with high pretreatment blood levels, they decreased in 96 % (CA 125) and 93 % (TPA) of the patients undergoing complete resection and they increased in 100 % (CA 125 and TPA) of the patients with recurrence, indicating a strong correlation between the presence of cancer and blood levels.

4. CA 125 and TPA were respectively localized in the tumors of 86 % and 71 % of

Department of Obstetrics and Gynecology and *Second Department of Biochemistry, School of Medicine, Chiba University, Chiba 260.
芳野春生，稲葉憲之，深沢一雄，岡嶋祐子，太田順子，鈴木信夫*，高見澤裕吉：卵巣悪性腫瘍における腫瘍マーカー CA 125 及び TPA の組織内局在と血中値相関に関する臨床的，基礎的研究
千葉大学医学部産科婦人科学講座，*生化学第二講座
〒260 千葉市中央区亥鼻 1-8-1
(平成4年10月6日受付，平成4年10月20日受理)

21 patients serous CACa, and in 0 % and 75 % of 20 patients with mucinous ovarian CACa.

5. Tissue localization was dissociated from blood levels in 19 % (CA 125) and 24 % (TPA) of serous CACa patients and in 30 % (CA 125) and 0 % (TPA) of mucinous CACa patients. Nonspecificity of tumor markers is a major reason for such dissociation. Even in patients with confirmed tumor marker localization in the tumor cells, blood levels were not necessarily correlated with the proportion of marker-positive cells or the immunohistochemical positivity rate.

6. In basic experiments using cultured cell lines, intracellular localization agreed with the intracellular concentration in all cases, but the intracellular localization was not always in proportion to the concentration in the culture supernatant.

7. Recovery tests were performed for the 2 tumor markers in various mixtures of serum, culture supernatant, and amniotic fluid of known concentrations. Recovery rates decreased significantly to 51.3 % for CA 125 and 57.0 % for TPA when amniotic fluid was mixed with human serum, suggesting that tumor markers may be modified or masked during the process from production to release into the blood.

Key words: Ovarian cancer, CA 125, TPA, Serum concentration, Tissue localization

I. Introduction

The tissue localization of tumor markers often differs from their serum levels under the present circumstances where no strictly cancer-specific markers are available. Various pathways and many bottlenecks need to be negotiated before tumor-produced substances enter the blood. Apart from technical limitations including the inadequate sensitivity of immunohistochemical tests and blood level assays, various problems exist concerning the relationship of blood levels to the tissue localization of tumor markers.

Even when the localization of tumor markers in cancer cells is confirmed, the degree of release into the blood is variable. Cytoarchitectural proteins and tissue enzymes, including placental tissue protein 5 (PP_5), PP_{11}[1], and P 450 HFLa[2], show little extracellular release, while some tumor markers like cancer antigen 125 (CA 125)[3] and tissue polypeptide antigen (TPA)[4] are readily transferred extracellularly.

CA 125 and TPA have been extensively used as tumor markers for ovarian cancer and are released into the blood in high concentrations. In this study, we investigated the following points:

(1) The percentage of patients with high pretreatment blood levels (positivity rate, sensitivity, and diagnostic value).

(2) The percentage of patients with high pretreatment blood levels in relation to clinical stage.

(3) Changes in tumor markers in patients with complete resection, incomplete resection, and tumor recurrence.

(4) The localization of the markers in cancer tissues.

(5) The correlation between blood levels and tissue localization.

In addition, a fundamental study was carried out on masking phenomena occurring during the release of tumor markers into the blood which involved the following experiments:

(1) Determination of the intracellular localization and concentrations of these tumor markers as well as their release into culture medium using 3 cultured ovarian cancer cell lines.

(2) Recovery tests for CA 125 and TPA that involved comparing the measured and theoretical tumor marker levels in various mixtures of serum, amniotic fluid, and culture medium of known initial concentrations.

II. Materials and Methods

Serum and tissue samples

Serum samples were pretherapeutically obtained from 35 patients with serous ovarian cystadenocarcinoma (CACa), 31 patients with mucinous ovarian CACa, and 102 patients with other gynecologic diseases including endometriosis. Serum samples were also collected before treatment from 135 patients with ovarian malignancies; 64 in stage I, 23 in stage II, 33 in stage III, and 15 in stage IV. In addition, serum was obtained at one month after operation or at the time of that recurrence was suspected in 34 patients with serous ovarian CACa, in whom the CA 125 and TPA levels were elevated preoperatively. Serum samples were stored at −80 °C until use.

Tissue specimens were obtained at operation from 21 patients with serous ovarian CACa, 20 with mucinous ovarian CACa, and 11 with benign gynecologic diseases. These specimens were immediately cut into small cubes and embedded in paraffin after fixation in a mixture of 90 % ethanol and acetic acid[5].

Measurement of CA 125 and TPA levels

The CA 125 level was measured by a radioimmunoassay (RIA) employing the sandwich solid-phase method. The antibody applied was a mouse anti-human CA 125 monoclonal antibody (Centocor, Pennsylvania, USA). TPA levels were measured using a double-antibody RIA; the first antibody was horse anti-TPA (Sangtec Medical, Bromma, Sweden) and the second was rabbit anti-horse IgG (Sangtec Medical). The normal upper levels were set at 35 u/ml (CA 125) and 110 U/L (TPA).

Immunohistochemistry of CA 125 and TPA

CA 125 and TPA were demonstrated in the 3 to 4 μm sections by the avidin-biotin immunoperoxidase (ABC) technique[6] with a VECTASTAIN™ ABC kit (PK-4001, 40002, Vector Laboratory, California, USA). To unmask tissue antigens, the sections were briefly exposed to 0.1 % trypsin after deparaffinization[7]. The sufficient blocking of endogenous peroxidase activities was achieved by pretreatment of the sections with 1.0 % hydrogen peroxide in phosphate-buffered saline (PBS, pH 7.2) for 60 min at room temperature[8]. Murine monoclonal antibodies against CA 125 and TPA were kindly donated by Dr RC Bast (Duke University, North Carolina, USA) and Dr B Wiklund (Sangtec Medical), respectively, and were used as the primary antibody. The reagents contained in the ABC kit were diluted as indicated. The substrate solution contained 0.015 % 3, 3'-diaminobenzidine tetrahydrochloride (Riedel de Haen AG, Hannover, Germany) in PBS. The solution was prepared fresh each time and 5 μl of 30 % hydrogen peroxide was added to 5 ml of the solution immediately before use. Specimens were incubated with the substrate for 5–10 min at room temperature.

Control sections were incubated with PBS or normal nonimmune mouse IgG (NMI, Miles Laboratories, Illinois, USA) in place of the first specific murine monoclonal antibodies.

Synthesis of CA 125 and TPA by cultured tumor cell lines

Three cultured ovarian carcinoma cell lines (HAC 2: mesonephroid Ca, SHIN 3: serous CACa, HOC 21: serous CACa) were used to evaluate the production of these two tumor markers by cultured cells and their release into culture medium. These cell lines were cultured in Eagle's minimal essential medium (MEM) supplemented with 10 % fetal calf serum (Gibco, New York, USA) and were incubated in Falcon tissue culture dishes (60 mm) at 37 °C in 5 % CO_2 in air. The concentrations of CA 125 and TPA in the culture medium were below the cutoff levels (8 μ/ml and 5 U/L) of the used RIA kits used. When the cultured cells reached 10×10^6 in number, the cells attached to the dishes were treated with 0.25 % trypsin, and the resulting cell suspensions were centrifuged at 1,000 rpm for 5 min. These cells were then subjected to the immunohistochemical investigation and to sonication. The culture medium supernatant and 1 ml of PBS containing 10^7 cells sonicated for 30 sec were prepared for tumor marker assays using the above-mentioned RIA kits. The cell

Table 1. Pretherapeutic positvie rates of CA 125 and TPA in ovarian neoplasms

	n	CA 125	TPA	CA 125/TPA
Carcinoma				
Serous CACa	35	24 (68.6)	19 (54.3)	28 (80.0)*1
Mucinous CACa	31	12 (38.7)	10 (32.3)	14 (45.2)
Total	66	36 (54.5)	29 (43.9)	42 (63.6)
Benign disease*2	102	38 (37.3)	13 (12.7)	38 (37.3)

*1 : positive cases (%) (upper limits, CA 125 ; 35u/ml, TPA ; 110U/L)
*2 : include ovarian adenomyosis
CACa : cystadenocarcinoma

Table 2. Diagnostic values of CA 125 and TPA in ovarian malignacies

	n	CA 125	TPA	CA 125/TPA
Serous CACa	35	69/63/43	54/87/47	80/63/50*
Mucinous CACa	31	49/63/25	32/87/28	45/63/28
Total	66	55/63/35	44/87/38	64/63/40

* : sensitivity/specificity/diagnostic value

suspension was centrifuged at 500 rpm for 5 min and the sediment was fixed in the mixture of 90 % ethanol and acetic acid[5]. Using the rapid cell block method of Fukushima[9], cultured cells were embedded in paraffin, cut into 3–4 μm sections, and prepared for immunocytochemistry by the ABC method.

Recovery of CA 125 and TPA from various mixtures

Three different sera (CA 125/TPA ; 19 u/ml/25 U/L, 32/25, 63/35), amniotic fluid (2 300/9 200) and culture supernatant (OMC 3 ; mucinous CACa, 11/5 100) were mixed with each other at dilutions of 1 : 1, 1 : 3, 1 : 7 and 1 : 15. The concentrations of CA 125 and TPA were then measured in each mixture, and were compared with the values calculated based on the original concentrations and dilutions of the mixtures.

III. Results

Table I shows the pretreatment CA 125 and TPA levels in 66 patients with CACa (35 serous and 31 mucinous), who accounted for the majority of the patients with malignant ovarian tumors, and 102 patients with benign ovarian disease including endometriosis. Positivity for e ther of the markers is indicated as CA 125/TPA. Although positivity for CA 125 was only 38.7 % in patients with mucinous CACa, it was 68.6 % in serous CACa, with an overall positivity rate of 54.5 %. The pretreatment positivity rate for TPA was 32.3 % in patients with mucinous CACa, while it was 54.3 % in serous CACa. With simultaneous measurement of the 2 tumor markers, positivity for TPA increased from 32.3 % so 45.2 % (a rise of 12.9 %) in mucinous CACa and that from CA 125 increased from 68.6 % to 80.0 % (a rise of 11.4 %) in serous CACa, with the overall increase being 9.1 %. However, the pretreatment posivity rate for CA 125 was 37.3 % in benign disease, and the rate was higher in endometriosis. On the other hand, 12.7 % of patients with benign ovarian disease were low positive rate for TPA and the false positivity rate for the marker did not increase with simultaneous measurement, resulting in a pretreatment positivity rate of 37.3 % for CA 125.

Table 2 shows the sensitivity determined from the percentage of patients with high pretreatment blood levels (positivity rate) and specificity determined from the false positive rate in patients with benign disease. The specificity of CA 125, TPA, and CA 125/TPA was respectively 63 %, 87 %, and 63 % as determined from the false positive rates in benign disease, resulting in diagnostic va-

Table 3. Positive rates of serum CA 125 and TPA levels in relation to clinical stagings

Staging	CA 125				TPA			
	n	Range*1	Mean*1	PR (%)	n	Range*2	Mean*2	PR (%)
I	64	8- 180	46	16/64 (13)	64	25- 90	51	19/64 (30)*3
II	23	15- 170	38	9/23 (39)	23	47-310	95	14/23 (61)
III	33	18-16000	4986	26/33 (79)	33	55-770	328	20/33 (61)
IV	15	34- 2200	1580	11/15 (73)	15	25-928	188	12/15 (80)

*1 : u/ml *2 : U/L, *3 : No-positive/No-examined (%)
PR : positive rate (CA 125> 35u/ml, TPA> 110U/L)

Table 4. Postoperative fluctuation of serum CA 125 and TPA in the clinical courses

	CA 125				TPA			
	n	<35u/ml	≧35u/ml	Increased	n	<35u/ml	≧35u/ml	Increased
Post CO*1	23	22 (96)	1	—	15	14 (93)*3	1	—
Post ICO*1	7		6 (86)	1	4		4 (100)	—
Recurrence*2	4	—	4	—	4	—	4	—

*1 : evaluated at one month after operation *2 : evaluated at the point of suspicious diagnosis of the recurrence based on clinical symptoms, medical electronics or serum tumor markers, *3 : cases/No-examined (%), CO : complete operation, ICO : incomplete operation

lues for all ovarian cancer of 35 %, 38 %, and 40 %, respectively. A diagnostic value of 50 % was only achieved for CA 125/TPA in serous CACa.

Table 3 shows the relationship between the clinical stage (I-IV) and the pretreatment positivity rates of the 2 tumor markers. Positivity rates for both markers increased as the clinical stage advanced, and reached 79 % for CA 125 in stage III and 80 % for TPA in stage IV. Changes in the blood tumor marker levels with surgery or recurrence were investigated in 34 patients with serous CACa who showed high pretreatment blood levels (Table 4). Postoperative blood levels decreased in 96 % (CA 125) and 93 % (TPA) of the patients with complete resection, while they increased in 100 % of the patients with recurrence, indicating a strong correlation between the presence of cancer and the blood level of each marker.

Immunohistochemical detection of CA 125 and TPA was performed in 41 cancer patients (21 with serous CACa and 20 with mucinous CACa) as well as 11 patients with benign ovarian tumors. The findings shown in Figs. 1 and 2 were defined as positive for tumor marker localization. CA 125

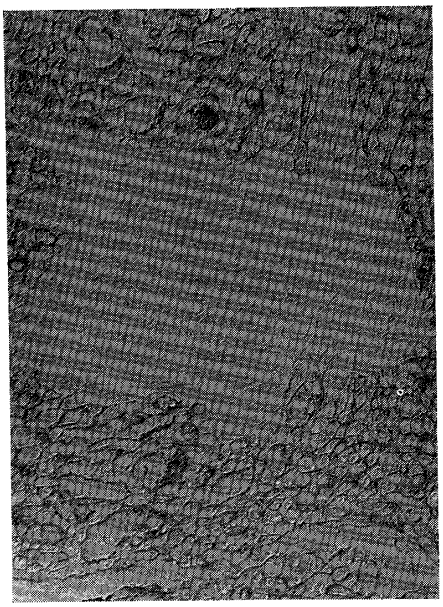

Fig. 1. Localization of CA 125 in serous cystadenocarcinoma (10×25, under interference phase contrast)

and TPA were respectively detected in 86 % and 71 % of serous CACa and in 0 % and 75 % of mucinous CACa (Table 5). Positivity was also noted in 1 patient each with serous cystadenoma and luteal cyst.

Table 6 shows the relationship between tissue

localization of tumor markers and blood levels in these patients. Patients with an increased blood level despite the absence of tissue localization were defined as showing dissociation. All other cases, including those of confirmed tumor marker localization with increased or normal blood levels and those of no localization with normal blood levels were defined as showing accordance. A high percentage (81 %) of accordance was observed for CA 125 in serous CACa, while the accordance for this marker was 70 % in mucinous CACa. Although accordance for TPA was 76 % in serous CACa, it was 100 % in mucinous CACa, with a high overall rate of 88 %.

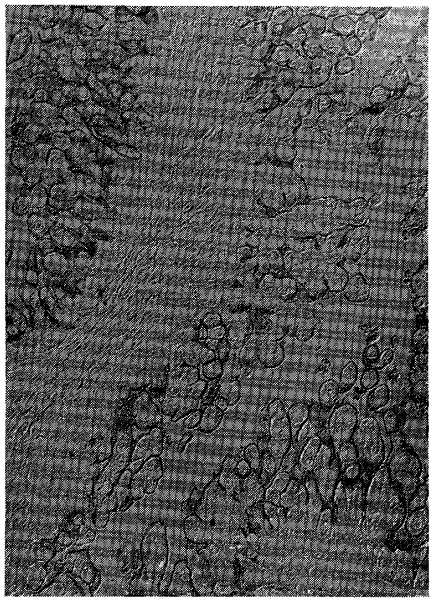

Fig. 2. Localization of TPA in serous cystadenocarcinoma (10×25, under interference phase contrast)

Table 7 shows the relationship of the intracellular localization and concentration of the tumor markers in 3 cultured ovarian cancer cells lines as well as the concentrations in culture supernatant. The production of CA 125 was observed in SHIN 3 and HOC 21 cells and that of TPA was confirmed in all 3 cell lines. The intracellular concentration of CA 125 was relatively high (190 u/ml) in SHIN 3 cells, while it was low (21 u/ml) in HOC 21 cells. The intracellular concentration of TPA was very high in all 3 cell lines, ranging from 320,000 to 940,000 U/L. Regarding culture supernatant, CA 125 was only detected in SHIN 3 supernatant and TPA was measurable in all 3 supernatants at levels from 19 to 14,600 U/L.

Mixtures of human serum (CA 125/TPA ; 19 u/

Table 5. Immunohistochemical detection rates of CA 125 and TPA in ovarian neoplasms

	CA 125	TPA	CA 125/TPA
Carcinoma			
Serous CACa	18/21 (86)	15/21 (71)	19/21 (90)*1
Mucinous CACa	0/20	15/20 (75)	15/20 (75)
Total	18/41 (44)	30/41 (73)	34/41 (83)
Benign neoplasm	1*2/11 (18)	1*3/11 (18)	2/11 (18)

*1 : No-positive/No-examined (%), *2 : serous cystadenoma *3 : lutein cyst

Table 6. Correlation between tissue localization and serum levels of CA 125 and TRA in ovarian carcinoma

	CA 125		TPA	
	Concordance	Separation	Concordance*1	Separation*2
Carcinoma				
Serous CACa	17/21 (81)	4/21 (19)	16/21 (76)	5/21 (24)*3
Mucinous CACa	14/20 (70)	6/20 (30)	20/20 (100)	0/20
Total	31/41 (76)	10/41 (24)	36/41 (88)	5/41 (12)

*1 : localization (+)/serum levels (normal or ⇧), lacalization (−)/serum levels (normal),
*2 : lacalization (−)/serum leves⇧, *3 : cases /No-examined (%)

Table 7. CA 125 and TPA-intracellular localization and concentrations, and release into the medium

	HAC 2	SHIN 3	HOC 21
CA 125	−/ — / —	+/ 190/ 30	±/ 21/ —
TPA	++/940 000/14 600	+/320 000/2 200	+/600 000/19

*: intracellular localization/intracellular concentration (per 10^7 cells in 1 ml of PBS)/ levels in the medium with 10^7 cells, −, ±, +, ++: negative and graded positive laocalization, units: u/ml(CA125), U/L(TPA)

Table 8. CA 125 and TPA-recovery tests in various mixtures

	Culture medium added human serum	Amniotic fluid added human serum	Culture medium added amniotic fluid
CA 125	99.0	51.3	98.7*
TPA	97.1	57.0	83.0

*: measured concentrations/calculated concentrations (mean %)

ml 25 U/L, 32/25, 63/35), amniotic fluid (2,300/9,200), and culture supernatant (OMC 3; 11/5,100) were analyzed and the measured values were compared with the theoretical values. The tests were performed at 4 different mixture ratios and repeated a mumber of times. Results were expressed as the mean recovery rate (%) (Table 8). The recovery rate was 83 % when culture supernatant was diluted with human serum or amniotic fluid, but the rate fell to about 50 % for both tumor markers when amniotic fluid was diluted with human serum.

IV. Discussion

In this study, the diagnostic values of CA 125, TPA, and CA 125/TPA were respectively 35 %, 38 %, and 40 % for all CACa, and a diagnostic value of 50 % was achieved only for CA 125/TPA in serous CACa (Table 2). The correlation of these tumor markers with ovarian cancer thus appears comparable with those of ferritin, carbohydrate antigen 19-9 (CA 19-9), SP_3, and carcinoembryonic antigen (CEA)[12,13]. In contrast, alpha-fetoprotein in yolk sac tumor[10] and human chorionic gonadotropin (hCG) and Schwangerschaftsprotein 1 (SP_1) in choriocarcinoma[11] show higher correlations. Positivity rates for both CA 125 and TPA increased with advancing clinical tumor stage and reached 79 % in stage III for CA 125 and 80 % in stage mIV for TPA (Table 3). This finding was confirmed by the changes in blood levels after surgery and recurrence in patients with high pretreatment tumor marker levels. Blood levels decreased in 96 % (CA 125) and 93 % (TPA) of patients undergoing complete resection and increased in 100 % (both markers) of patients with recurrence (Table 4), indicating a strong correlation between the presence of cancer and the blood level of markers. This finding supported the report[12] that tumor size correlated well with the tissue localization of tumor markers and the blood levels in mice with ovarian cancer. These results confirmed that tumor markers can play an important role in the monitoring of treatment or the early detection of recurrence[13], and they suggest the existence of a strong correlation between tumor volume and blood levels in patients with increased pretreatment blood levels of tumor markers[14].

In our study of the correlations between tissue localization and blood levels, it was impossible to examine all tissues removed. However, specimens subjected to the study were examined for the detection of tumor marker localization as extensively as possible so as not to miss any positive tissues. A high rate of concordance (81 %) was found for CA 125 in serous CACa and a rate of 100 % was noted for TPA in mucinous CACa, with the overall concordance rate being 88 % (Table 6). The dissociation rate in benign tumors was 64 % (CA 125) and

45 % (TPA), suggesting that the production of these markers also occurs in normal tissues. These results indicate that, under the current circumstances where there are no true cancers-pecific markers, tumor marker blood levels do not always agree with tissue localization, even if the markers are produced by tumors. We should bear this in mind when clinically applying immunodetection[15] and immunochemotherapy[16] utilizing tumor markers.

Our study of the intracellular localization and concentration of CA 125 and TPA as well as the concentration of each marker in culture supernatant utilized 3 ovarian cancer cell lines. No dissociation was noted between the intracellular localization and the concentration of each marker, and the intracellular concentration generally agreed with the concentration in culture supernatant (Table 7). However, there were significant differences between the 2 markers and among the cultured cell lines in relation to extracellular release. The intracellular concentration of CA 125 in SHIN 3 cells was 6.3 times higher than the concentration in the culture supernatant. That of TPA was 64.4 times in HAC 2 cells, and was 145.5 and 31,578.9 times in SHIN 3 and HOC 21 cells, respectively. Thus, there were considerable differences of tumor marker release between the various cell lines.

The mechanism of the release of tumor markers into the blood is much more complex in cancer tissue due to (1) the variable distribution of tumor markers in cancer cells (e. g., polarity), (2) the variations of tumor vascularity, and (3) differences in the extent of invasion/destruction of surrounding tissues[17]. Thus, all these factors need consideration in addition to the intrinsic properties of the cancer cells and the tumor markers themselves. Furthermore, (4) the masking of tumor markers in blood and other body fluids can create further problems. A basic study was conducted to assess the effect of masking. Amiotic fluid contains a large amount of CA 125 and TPA, and the maternal blood levels of these substances increase slightly immediately after delivery while fetal blood levels stay within normal limits[18]. Since amniotic CA 125 and TPA would be expected to cause the slight increase in maternal blood levels immediately after delivery[19], it appears strange that there is no influence on fetal blood levels. We also had the impression that the change in maternal blood levels was too small considering the very high amniotic concentrations of these markers. Accordingly, we performed experiments using mixtures of human serum, amniotic fluid, and culture superantant containing known concentrations of CA 125 and TPA, and compared the measured and theoretical values (Table 8). There was no difference between the measured and theoretical values when culture supernatant was diluted with human serum or amniotic fluid. However, when amniotic fluid was diluted with human serum, the measured value decreased to about 50 % of the theoretical value. This masking phenomenon may occur under a variety of conditions and would further complicate the relationship between the intracellular concentrations and blood levels of tumor markers.

Acknowledgement

We are grateful for the heartiful collaboration given by all staffs at our department. We also thank Dr Souei Sekiya at our department for his kind donation of culture ovarian cancer cell lines. We deeply appreciate the excellent technical assistance given by Mrs Naomi Sato and Mrs Hatsumi Sudo at our department. This study was supported by grant from the Ministry of Education, Science and Culture (Inaba N: 62570702).

要　旨

絨毛癌における human chorinonic gonadotropin (hCG) や Schwangerschaftsprtein 1 (SP_1) のように組織局在と血清値が極めて明瞭に相関する場合もあるが，卵巣癌においては α-fetoprotein (AFP) を除いて一部解離するのが通常であろう．本研究では日常臨床で頻繁に用いられている腫瘍産生性の腫瘍マーカー，cancer antigen 125 (CA 125) 及び tissue polypeptide antigen (TPA) を取り上げ，血中値と組織局在の相関を検討し，以下の結論を得た。

1) CA 125, TPA 共に漿液性嚢胞腺癌で最高の治療前血中高値率（陽性率，各々68.6%及び54.3%）が得られた。しかしながら特異性が62.7%および87.3%と逆

転したため，癌の血中値の相関（診断効率）は43.0%ならびに47.4%に留まった。

2) 両腫瘍マーカー共臨床進行期が進むに連れて陽性率が上昇し，血中値と癌容積，あるいは周囲組織の侵襲破壊度との関連が示唆された。

3) 治療前血中高値を示した漿液性嚢胞腺癌では治療による血中値の推移は完全手術群で96%（CA 125）および93%（TPA）が減少し，また再発群では両者とも100%上昇し，癌の存在と血中値間で高い相関が認められた。

4) 漿液性嚢胞腺癌21例にて CA 125は86%，TPAは71%，また粘液性嚢胞腺癌20例にてそれぞれ0%，75%の局在率が得られた。

4) 組織局在と血中値の解離は漿液性嚢胞腺癌ではそれぞれ19%（CA 125）および24%（TPA），また，粘液性嚢胞腺癌では30%（CA 125）及び0%（TPA）認められた。解離の要因として腫瘍マーカーの非特異性があげられるが，癌細胞内に局在が認められた症例でも血中濃度は必ずしも局在陽性細胞の占める割合，あるいは免疫組織学的陽性度と比例しない症例も見られた。

5) 培養細胞系における基礎的検討で細胞内局在と細胞内濃度は全例一致したが，細胞内濃度と培養上清中濃度は必ずしも正比例では無かった。

6) 両腫瘍マーカーの濃度が既にわかっている血清，培養液，および羊水を種々組み合わせて両マーカーの回収試験を行ったが，羊水にヒト血清を加えると，回収率は CA 125が51.33%，TPA は57.0%まで有意に低下する事が判明した。すなわち，産生組織から血中へ移行する際に腫瘍マーカーが修飾を受け，マスキングされ得る可能性が実験的に示唆された。

References

1) Nito A, Inaba N, Fukazawa I, Ishige H, Takamizawa H, Lüben G and Bohn H: Immunoradiometrical and histochemical investigation of placenta-"specific" tissue proteins 5, 10 and 11 ($PP_{5,10}$ and$_{11}$) in normal pregnancy and ovarian cystadenocarcinoma. Chiba Med J **62**: 143-152, 1986.

2) Okajima Y, Inaba N, Sato N, Takamizawa H, Itahashi K, Kitada M and Kamataki T: Cytochrome P-450 of human fetal livers (P-450 HFLa)-Immunohistochemical investigation and its possible clinical application. In Placental and Endometrial Proteins: Basic and Clinical Aspects (eds: Tomoda Y, Mizutani S, Narita O and Klopper A) VSP (Utrecht, The Netherlands, Tokyo) pp 263-266, 1988.

3) Bast RC Jr, Feeneny M, Lazarus H, Nadler LM, Colvin RB and Knapp RC: Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest **68**: 1331-1337, 1981.

4) Björklund B and Björklund V: Antigenicity of pooled human malignant and normal tissues by cytoimmunological technique: presence of an insoluble, heat-labil tumor antigen. Int Arch Allergy Appl Immunol **10**: 153-184, 1957.

5) Wurster K, Kuhlman WD and Rapp W: Immunohistochemical studies on human gastric mucosa. Procedures for routine demonstaration of gastric proteins by immunoenzyme techniques. Virchows Arch Pathol Anat 328: 213-228, 1978.

6) Hsu S-M, Raine L: Protein A, avidin and biotin immunohistochemistry. J Histochem Cytochem **29**: 1349-1353, 1981.

7) Curran RC and Gregory J: The unmasking of antigens in paraffin sections of tissue by trypsin. Experientia **33**: 1400-1401, 1977.

8) Weir EE, Pretlow TG, Pitts A and William EE: Destruction of endogenous peroxidase activity in order to locate cellular antigens by peroxidase-labelled antibodies. Histchem Cytochem **22**: 51-54, 1974.

9) Fukushima N: Cell block technique. In: Color Atlas of Cancer Cytology, Takahashi M (ed). p 47, Thieme, Stuttgart

10) Abelev GI: Alpha-fetoprotein as a marker of embryo-specific differentiations in normal and tumor tissues. Transplant Rev **20**: 3-37, 1974.

11) Inaba N, Lüben G and Bohn H: Schwangerschaftsprotein 1 (SP_1). Medical Technology **11**: 254-260, 1983.

12) Fukazawa I, Inaba N, Ota Y, Sato N, Shirotake S, Iwasawa H, Sekiya S, Takamizawa H, Suzuki N and Tokita H: Experiments with tissue cultures from a human ovarian serous cystadenocarcinoma producing cancer antigen 125 (CA 125), tissue polypeptide antigen (TPA) and carcinoembryonic antigen (CEA). Arch Gynecol Obstet **243**: 69-81, 1988.

13) Fukazawa I, Inaba N, Ota Y, Sato N, Shiroke S, Iwasawa H, Sato T, Takamizawa H and Wiklund B: Serum levels of six tumor markers in patients with benign and malignant gynecological diseases. Arch Gynecol **243**: 61-68, 1988.

14) Inaba N, Fukazawa I and Takamizawa H: Reliability of tumor markers in ovarian car-

cinomas. Obstet Gynecol **55** : 853-858, 1988.
15) Inaba N, Fukazawa I, Ijichi M, Sato N, Iwasawa H, Ishige H, Sekiya S, Takamizawa H, Suzuki N, Uematsu S, Akezuma H, Takahashi S, Tokita T, Fujiwara T and Mizuno K : A fundamental investigation on radioimmudetection with use of PLC/PRF/5 hepatocellular carcinoma cell line producing heaptitis B surface antigen in nude mice. Oncol Chemother **2** : 174-182, 1986.
16) Ota, Y, Oehr P, Schultes B, Liu Q, Wagner U, Sundstroem B, Biersack HJ, Krebs D, Inaba N and Takamizawa H : The application of immunotargeting into cancer chemotherapy with Carboplatin : in vivo studies. Asia-Oceania J Obstet Gynecol, (submitted).
17) Kiyozuka Y, Noda T, Noda T, Adachi S, Adachi S, Akada S and Ichijo M : A study on the release of CA 125 into blood circulation. Acta Obstet Gynecol Jpn **41** : 1951-1958, 1989.
18) Itahashi K, Inaba N, Fukazawa I and Takamizawa H : Immunoradiometrical measurement of tissue polypeptide antigen (TPA) and cancer antigen 125 (CA 125) in pregnancy and at delivery. Arch Gynecol Obstet **243** : 191-197, 1988.
19) Inaba N, Fukazawa I, Ota Y, Nitto A, Ijichi M, Sato T and Takamizawa H : Tissue polypeptide antigen and cancer antigen 125 in pregnancy-These two cancer-related antigens are oncoplacental? Acta Obstet Gynecol Jpn **37** : 2139-2140, 1986.