Table 3. *Result of hierarchical regression analysis on patients' willingness to continue living at home (n = 73)*

	Model 1		Model 2		Model 3		Model 4	
	OR[a]	(95% CI[b])	OR[a]	(95% CI[b])	OR[a]	(95% CI[b])	OR[a]	(95% CI[b])
Patient characteristics								
Age (years)		—		—		—		—
Sex (1. Male/0. Female)		—		—		—		—
Performance Status (0–4)		—		—		—		—
No. of medical treatments (0–4)	0.49*	(0.23–0.97)	0.44*	(0.19–0.90)	0.39*	(0.13–0.94)	0.20*	(0.05–0.72)
Desire for home care (1. Present/0. Absent)	3.32	(0.74–17.34)	3.26	(0.64–20.71)		—		—
Patient discharge-related information								
Consistency with care envisioned by patient			2.70*	(1.34–6.41)	2.39	(0.95–7.19)	2.77*	(1.08–8.62)
Patient QOL								
Physical well-being					0.86	(0.67–1.01)	0.83	(0.61–1.02)
Social well-being						—		—
Emotional well-being						—		—
Functional well-being					1.36*	(1.06–1.94)	1.45*	(1.08–2.17)
PFC status								
Age (years)								—
Gender (1. Male/0. Female)								—
Additional support								—
Satisfied with life (satisfied with current QOL)							2.37*	(1.15–5.77)
R^{2c}	0.09		0.16		0.		0.26	
MR^{2d}	0.17		0.30		0.39		0.50	

*$p < .05$
— denotes item was not selected by backward elimination ($p > .2$)
[a]Adjusted odds ratio
[b]95% confidence interval
[c]Coefficient of determination
[d]Max-rescaled coefficient of determination

was found to be associated with the desire to maintain the current home care.

The support of the caregiver is an essential part of home care, and it seems that patients are sensitive to the situation of the caregivers close to them and worry about their caregivers' well-being since it relates to their giving care at home. The caregivers' satisfaction with life appears to bolster the willingness of patients to continue home care.

In the present study, the model contribution ratios were increased by adding the variables of caregiver status to those of patient characteristics, indicating that the attitudes and well-being of caregivers are important factors in the willingness of patients to continue home care and should be taken into account.

Assistance during the Early Phase of Home Care of Terminally Ill Cancer Patients to Promote Its Continuance

Taking account of the caregiver's status is essential if appropriate assistance is to be given during the early phase of home care. Our results indicate that efforts to promote consistency between the care envisioned by the patient and its reality are important, as are measures to reduce patients' fears of difficulties resulting from medical treatments. Thus, there is a substantial need to improve discharge assistance and continuing care, for example, via outpatient counseling for both patients and caregivers (Naylor et al., 1999, 2000; Naylor, 2000).

The importance of the role of caregivers, who are in closest contact with patients, was confirmed by the finding that the level of satisfaction with life of caregivers is associated with the willingness of patients to continue home care. Therefore assessment over time and finding a place to discuss such matters as the feelings about their current lives, not only of patients but also of caregivers, is desirable.

Our results suggest that the following aspects of care should be considered in the development of high quality transitional care from CCCs to the patient's own home in Japan: tailoring a support system for cancer pain relief and other physical suffering, coordinating care with other medical fa-

cilities, explaining the medical condition to the patient, coordinating the patient/family relationship with regard to telling the patient the diagnosis, and coordinating the provision of welfare services such as nursing, equipment rental, and provision of home helpers.

We recognize that this study has several limitations. The percentage of valid responses was low. However, virtually no studies exist in which an equal number of responses have been obtained from terminally ill cancer patients and their caregivers (Rinck et al., 1997). In addition, this study was cross-sectional. Future work should measure changes in the willingness of patients to continue home care over time and to further elucidate factors affecting this outcome, such as changes occurring in the living environment during home care.

This is the first quantitative study of the transition period from CCC to home care experienced by both terminally ill cancer patients and their caregivers in Japan. An understanding of the factors that determine the willingness of patients to continue living at home is necessary for planning the support required for a smooth transition to care at home and for providing solutions to problems encountered by health care professionals who provide home care.

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Paradoxical Discrepancy Between the Serum Level and the Placental Intensity of PP5/TFPI-2 in Preeclampsia and/or Intrauterine Growth Restriction: Possible Interaction and Correlation with Glypican-3 Hold the Key

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Abstract

There have been controversies whether maternal serum placental protein 5 (PP5)/tissue factor pathway inhibitor (TFPI)-2 is increased in the patients with preeclampsia and/or intrauterine growth restriction (IUGR). Here, we have estimated the serum PP5/TFPI-2 in these patients by a sandwich enzyme-linked immunosorbent assay with a newly developed monoclonal antibody, coupled with placental immunohistochemical studies of their placentae with semiquantitative scoring.

Serum PP5/TFPI-2 level was significantly elevated only in the patients with preeclampsia alone ($p = 0.033$), while PP5/TFPI-2 was detected significantly less intensely in the placentae of the same patients ($p = 0.035$) in immunohistochemistry, as compared to Controls. A proteoglycan present on the placental villous surface, glypican-3, showed the same pattern of staining as PP5/TFPI-2, and there was a positive correlation (C.I. $= 0.506$, $p = 0.004$) between the immunohistochemical scores for these. Further experiments using HepG2 cells transfected with *PP5/TFPI-2* suggested that glypican-3 could anchor PP5/TFPI-2 on the placental villi.

A possibility that a decrease in glypican-3 in the placenta increases the outflow of PP5/TFPI-2, which in turn increases its serum level, was proposed. Preeclampsia and IUGR, often regarded to have the same pathological basis in common, showed distinct distributions of PP5/TFPI-2, which could be a clue to elucidate the pathogenesis of preeclampsia and IUGR.

Keywords: Placental protein 5/tissue factor pathway inhibitor-2; Glypican-3; Preeclampsia; Intrauterine; Growth restriction; Syndecan-1

1. Introduction

Preeclampsia and intrauterine growth restriction (IUGR) are difficult to predict clinically, and are some of the severe complications of pregnancy. Although part of the mechanisms underlying these disorders has been elucidated, the ultimate causes of preeclampsia and IUGR remain unknown [1–3].

Placental protein 5 (PP5) is a soluble protein produced in the human placenta and is detected in the serum of the pregnant woman [4]. We previously have found from amino acid sequence comparisons that PP5 is identical to a 29-kDa Kunitz type proteinase inhibitor [5]. The same protein, named tissue factor pathway inhibitor (TFPI)-2, was cloned independently

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as a homologue of TFPI from a human placental cDNA library by others [6].

PP5/TFPI-2 is a potent inhibitor of trypsin, plasmin, plasma kallikrein, factor XIa and factor VIIa/TF complex, and also weakly inhibits amidolytic activity of factor Xa [7]. The expression of PP5/TFPI-2 has been demonstrated in various human tissues other than the placenta [5,8–10], and its contribution to angiogenesis [9–11] and carcinogenesis [12–15] has been the focus of several studies. Recently, the function of PP5/TFPI-2 as a mitogen for vascular smooth muscle cells [16] and retinal pigment epithelial cells [17] has been demonstrated.

Despite its abundant presence in the placenta, the function of PP5/TFPI-2 during pregnancy is not fully understood. We have demonstrated that PP5/TFPI-2 is localized on the surface of microvilli and the endoplasmic reticulum membrane of syncytiotrophoblasts by immunoelectron-microscopy, and that incubation with heparin releases PP5/TFPI-2 from the villous surface of the placenta [18,19].

TFPI is known to bind to glypican-3, a member of the transmembrane heparan sulphate proteoglycans (HSPGs), on the cell surface of hepatocellular carcinoma cell line, HepG2 cells. When HepG2 cells are incubated with heparin, TFPI is released from the cell surface into the culture medium [20]. TFPI possesses a highly positively charged region in its carboxyl terminus, for which heparin competes with glypican-3 to release TFPI [21].

As PP5/TFPI-2 has a similar structural domain to TFPI, we hypothesized that PP5/TFPI-2 might be retained on the surface of the placental villi by proteoglycans such as members of the glypican and syndecan families, and that PP5/TFPI-2 might play a role to maintain intervillous blood flow [19]. Glypican-3 is known to be expressed abundantly in the placenta [22], along with syndecan-1, a member of the HSPGs syndecan family [23].

It has been reported that the maternal serum level of PP5/TFPI-2 is elevated in the patients with severe preeclampsia [24–26]. Some investigators have reported the elevated maternal serum level of the same protein also in the patients with IUGR [25,26], while others have failed to demonstrate the elevation [27,28], using the same rabbit polyclonal antibody raised against a fraction of purified PP5/TFPI-2 as for the radioimmunoassay [29,30]. Another evaluation with new specific monoclonal antibody and with a more specific technique (sandwich ELISA) than radioimmunoassay may serve to clarify the association between the maternal serum PP5/TFPI-2 levels and preeclampsia and/or IUGR. In addition, the mechanism underlying the increase in PP5/TFPI-2 in the maternal serum remains to be elucidated. To date, there have been no reports on the in situ expression of PP5/TFPI-2 in the placentae of the patients with preeclampsia and/or IUGR as compared with their serum PP5/TFPI-2 levels.

Here we have attempted to clarify the maternal serum levels of PP5/TFPI-2, along with the in situ expression of the same protein in the placentae of the patients with preeclampsia and/or IUGR. We have also sought for the association of PP5/TFPI-2 with some proteoglycans in the placentae.

2. Materials and methods

2.1. Placental tissue and serum samples

The experimental protocol was peer-reviewed and approved by the Ethical Committee of Yokohama City University Graduate School of Medicine. Placentae, maternal and umbilical venous sera were collected from the patients who were scheduled to undergo caesarean section. After receiving a detailed explanation, each of the patients who agreed to be enrolled in this study gave written informed consent.

Preeclampsia was diagnosed according to the definition established by the National High Blood Pressure Education Program [31]. IUGR was diagnosed if the estimated weight of the fetus was less than the 10th percentile for its gestational age according to the Japanese standard fetal growth curve [32], and the presence of growth arrest and non-reassuring fetal status were inferred from the fetal monitoring. For each of the patient, the gestational age had been confirmed in the first trimester by ultrasound.

The maternal serum was sampled 10–60 min before the mothers moved to the operation room, before the administration of anesthesia. The maternal serum was also sampled 4 days after delivery. The umbilical venous serum was collected carefully from the cord to avoid contamination with maternal blood. All serum samples were stored at −80 °C until the assay.

Placental tissues were sectioned into samples of approximately 3 cm × 3 cm × whole thickness taken from five different portions, fixed in 10% neutral-buffered formalin and embedded in paraffin for histopathological studies.

2.2. Preparation of mouse monoclonal anti-PP5/TFPI-2 antibody

Monoclonal antibody was raised against a synthetic peptide antigen consisting of 14 amino acid residues, NH_2-DAAQEPTGNNAEIC-COOH, corresponding to the N-terminus of the mature PP5/TFPI-2 protein after cleavage of the putative signal peptide. Specificity of the antigenic peptide to PP5/TFPI-2 was verified by searching the peptide sequence against other proteins with the BLAST program at the National Center for Biotechnology Information, National Institute of Health, Bethesda, MD (http://www.ncbi.nlm.nih.gov/BLAST/). The cysteine residue at the carboxy terminus was conjugated to keyhole limpet hemocyanin.

To use as the standard PP5/TFPI-2 protein for screening of the hybridoma cell clones of new antibodies and for the enzyme-linked immunosorbent assay (ELISA), recombinant PP5/TFPI-2 was prepared as follows. Histidine-tagged *PP5/TFPI-2* cDNA was transfected into the yeast (*Pichia Pastoris*) by using an EasySelect *Pichia* Expression Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The expressed recombinant PP5/TFPI-2 was affinity purified against the histidine-tag by using a Ni-NTA Spin Kit (QIAGEN, Valencia, CA).

Five-week-old BALB/c mice, gained from Oriental Yeast Co., Ltd., Tokyo, Japan, were immunized with the antigenetic peptide every 2 weeks. Three days after the last injection of 250 μg of the immunogen, the mouse spleen cells were sampled and fused with a mouse myeloma cell line P3U1 using polyethyleneglycol. From the antibody produced by the hybridomas, a clone 28Aa was selected for use in the study by Western blotting against the recombinant PP5/TFPI-2 expressed in the yeast described above. The antibody of the selected clone was purified from the ascites of the BALB/c mice that had been injected intraperitoneally with the hybridoma cells by column chromatography using protein A (Amersham Biosciences Co., Piscataway, NJ).

2.3. Sandwich ELISA

Serum levels of PP5/TFPI-2 were assayed by Sandwich ELISA using the clone 28Aa mouse monoclonal antibody against human PP5/TFPI-2 as described above, and a previously described rabbit polyclonal antibody against human PP5/TFPI-2 [18].

PP5/TFPI-2 antibody clone 28Aa diluted to 10 μg/ml was applied to a 96-well plate (Greiner Bio-one, Longwood, FL). After incubation at 4 °C overnight, the plate was blocked with 1% bovine serum albumin (Sigma) in

phosphate-buffered saline (PBS) at room temperature for 1 h. Serum samples diluted five times or the recombinant PP5/TFPI-2 protein diluted to different concentrations was added to each well. The plate was then incubated at 37 °C for 1 h. After washing, the rabbit polyclonal antibody against human PP5/TFPI-2 diluted to 10 μg/ml was added to the wells, and the plate was incubated at 37 °C for an additional hour. For detection, a horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin (Ig) G H+L (Molecular Probes, Invitrogen, Carlsbad, CA), diluted to 1:16000 was added to each well. After incubation at 37 °C for 1 h, *O*-phenylenediamine (Sigma) was added for color development. Absorbance at 490 nm was read by a Benchmark Plus spectrophotometer (Bio Rad, Hercules, CA) and the results were analysed by Microplate Manager Ver. 5.2 (Bio Rad).

2.4. Immunohistochemical analyses

Paraffin sections of the placentae were routinely stained with Hematoxylin and Eosin. The samples were also subjected to immunohistochemical staining for PP5/TFPI-2 and glypican-3.

Deparaffinized and rehydrated slides were immersed in 0.01 M citrate buffer, pH 6.0 (Sigma), and heated in a microwave oven for antigen retrieval. The slides were then cooled, washed in PBS, and immersed in 3% H_2O_2 diluted in methanol at room temperature.

The clone 28Aa mouse monoclonal antibody against human PP5/TFPI-2 or a mouse monoclonal antibody against human glypican-3 (clone 1G12, Biomosaics, Burlington, VT), diluted to 5 μg/ml or 40 μg/ml, respectively, was used as the primary antibody. Histofine SAB-PO multikits (Nichirei, Co., Tokyo, Japan) were used to detect the labeled antigens. Histochemically labeled antigens were visualized by reaction with 3,3′-diaminobenzidine (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Immunohistochemical staining for syndecan-1 (CD138) was also performed with a mouse monoclonal antibody against human CD138 (clone B-B4, Serotec, Oxford, UK) (diluted 1:200), as described above except for the antigen retrieval. Adjacent sections were used for immunohistochemical stainings for PP5/TFPI-2, glypican-3, and syndecan-1.

The results of the immunohistochemistry were analysed by using a modified German immunoreactive score [33–35]. The immunostaining intensity was rated as follows: 0, none; 1, weak; 2, moderate; and 3, intense. The quantity of immunohistochemically positive trophoblasts was also graded as follows: 0, none; 1, 1–10%; 2, 11–50%; 3, 51–80%; and 4, 81–100%. A score per slide was calculated as the summation of the areas of intensity multiplied by the quantity of each area. Each slide was evaluated of its score three times by two independent examiners who were blinded to its origin. The average of the scores from all of the slides of the placenta was determined as the representative data for that sample.

2.5. Transfection, immunoprecipitation and Western blotting

HepG2 cells were obtained from the Cell Bank, RIKEN BioResource Center (Tsukuba, Japan), and cultured in RPMI1640 (Kohjin Bio, Co., Itado, Japan) containing 10% fetal bovine serum (Moregate Biotech, Balimba, Australia) under an atmosphere of humidified 5% CO_2. A mammalian expression vector pcDNA3 (Invitrogen) or the vector containing the whole coding region of human *PP5/TFPI-2* cDNA was transfected to HepG2 cells with Lipofectamine 2000 transfection reagent (Invitrogen) under the manufacturer's instruction.

Forty-eight hours after transfection, the cells were lysed at room temperature for 10 min in 1 ml of a lysis buffer (25 mM Tris–Cl, pH 7.5; 100 mM NaCl; 2 mM EDTA (Sigma); 1% Triton X-100 (Sigma)) containing protease inhibitors (Complete Mini, Roch Diagnostics, Indianapolis, IN). After cell debris was removed by centrifugation, each lysate was further pre-cleared with Protein G Sepharose 4 fast flow (Amersham Biosciences).

Immunoprecipitation was carried out with 2.5 μg of the 28Aa mouse monoclonal antibody against human PP5/TFPI-2 and 50 μl of the Protein G Sepharose at 4 °C, and then the Sepharose phase was washed four times with the lysis buffer. Each immunoprecipitate was recovered by adding 50 μl of 2× SDS containing sample buffer and incubating at 70 °C for 10 min. Equal amount of immunoprecipitate (10 μl each) was subjected to SDS PAGE, followed by Western blotting for PP5/TFPI-2 (with the clone 28Aa antibody) or glypican-3 (the clone 1G12 mouse monoclonal antibody against human glypican-3, Biomosaics), respectively. An HRP conjugated sheep anti-mouse IgG (Amersham Biosciences) was used as the secondary antibody, and the signals were detected with the Supersignal West Pico chemiluminescent substrate (Pierce).

2.6. Statistics

Data are expressed as the mean ± standard error (SE). Statistical comparison was performed by either Student's *t* test, Welch's *t* test, Mann–Whitney's *U* test, or analysis of covariance (ANCOVA). The correlation index was calculated by using either Pearson's test or Spearman's test. SPSS software (Basic 11.0, SPSS Inc., Chicago, IN) was used for calculation. Significance was set at $p < 0.05$.

3. Results

3.1. Patients

Fifty-five patients who had been scheduled to undergo caesarean section at 24–39 weeks of pregnancy agreed to the collection of samples for research usage. Four patients were excluded from the study because they had previously taken medication for other pre-existing diseases. Hence, the 51 patients who had not been diagnosed of any pre-existing disease such as hypertension, renal disease, diabetes mellitus, or other chronic disease before pregnancy were enrolled in the study. There were no neonates with congenital or chromosomal abnormalities.

3.2. Maternal serum PP5/TFPI-2 levels in preeclampsia and/or IUGR

Maternal serum samples at delivery were available from the 51 patients, whose obstetrical complications are summarized in Table 1. Nineteen patients had preeclampsia, 10 of whom had preeclampsia only (Group P), and the other nine of whom had been also diagnosed as IUGR (Group P + IUGR). Seven had been diagnosed with IUGR alone (Group IUGR). The other 25 patients did not have the above-mentioned obstetric complications (the Control).

We confirmed from the clinical records that none of the patients in Group P, Group P + IUGR, and Group IUGR had been hypertensive or had proteinuria early in pregnancy, nor had they persisted hypertension or proteinuria at the time of their follow-up visits 1 month after delivery. All of the patients in Group P and Group P + IUGR had received antihypertensive medications for as long as 1–14 days.

The patients with preterm premature rupture of the membrane and premature labor in the Control had mild, if any, pathological changes in the placentae (Blanc stage [36] one, i.e., intervillositis at most), and had no clinical sign of severe chorioamnionitis or maternal systemic infection such as uterine tenderness, foul smelling amniotic fluid, maternal fever more than 38 °C, maternal tachycardia (≥120 beats/min), or maternal leukocytosis (≥20000/μl).

The clinical features of the study groups are summarized in Table 2. The maternal mean arterial pressure (MAP) and

Table 1
Distribution of the patients

Complications/indications for C/S	Number of the patients	
Preeclampsia (Group P)		10
Non-reassuring fetal status	3	
Incontrollable maternal hypertension/renal insufficiency	6	
Both of the above	1	
Preeclampsia with IUGR (Group P + IUGR)		9
Non-reassuring fetal status	7	
Incontrollable maternal hypertension/renal insufficiency	2	
IUGR (Group IUGR)		7
Non-reassuring fetal status	7	
No above complications (the Control)		25
History of C/S	9	
Breech presentation	5	
Placenta previa	3	
Preterm PROM	4	
Preterm labor	2	
History of uterine surgery	1	
Operated atresia ani	1	
Total		51

IUGR, intrauterine growth restriction; C/S, caesarean section; PROM, premature rupture of the membrane.

urinary protein (UP) were measured at the time of blood sampling. Although none of the patients in Group IUGR had been diagnosed as hypertensive, the maternal MAP was significantly higher not only in Group P and Group P + IUGR ($p < 0.001$ for both Groups), but also in Group IUGR ($p = 0.032$), as compared with the Control. However, the maternal MAP was significantly lower in Group IUGR than in Group P ($p = 0.001$). There were no differences in the maternal MAP and UP between Group P and Group P + IUGR.

The mean gestational age at delivery was significantly younger in Group P ($p = 0.039$), in Group P + IUGR ($p = 0.005$), and in Group IUGR ($p = 0.037$) than in the Control. Even controlling for the gestational age at delivery, the neonatal birth weight was still significantly lower in Group P + IUGR ($p < 0.0001$) and in Group IUGR ($p < 0.0001$) than in the Control.

PP5/TFPI-2 has been reported to be detectable early in pregnancy, and rise to a maximum at gestational weeks 36–37 [29,30]. To adjust for the effect of gestation, we compared the serum PP5/TFPI-2 levels in the maternal samples obtained at delivery by analysis of covariance (ANCOVA) (Fig. 1), after controlling for gestational age at delivery and neonatal birth weight. The detection limit of the sandwich ELISA for PP5/TFPI-2 was 1 ng/ml, and the intra- and inter-assay coefficients of variances were 5.0% and 10.0%, respectively. The analytical recovery was 80%.

The maternal serum PP5/TFPI-2 levels were 530.8 ± 111.3 ng/ml in Group P, 362.1 ± 146.0 ng/ml in Group P + IUGR, 223.9 ± 149.8 ng/ml in Group IUGR, and 233.2 ± 83.8 ng/ml in the Control. The maternal serum PP5/TFPI-2 level was significantly higher in Group P than in the Control ($p = 0.033$), but there were no significant differences in this value between Group IUGR and the Control, and between Group P + IUGR and the Control.

The PP5/TFPI-2 levels in the umbilical serum samples and in the maternal serum samples obtained 4 days after delivery were too low to be measured (data not shown).

3.3. Immunohistochemistry for PP5/TFPI-2, glypican-3, and syndecan-1

Placental samples were available from eight patients in Group P, seven patients in Group P + IUGR, six patients in Group IUGR, and from 24 patients in the Control. We selected 12 placental samples from the patients in the Control who were matched in gestational age at delivery with the patients in the other three study groups randomly. There was no significant difference in the maternal age, body mass index, and umbilical arterial pH among the patients in the three study groups and the Control whose placental samples were subjected to immunohistochemical analysis.

Table 2
Comparison of the characteristics of the study groups

	P ($n = 10$)	P + IUGR ($n = 9$)	IUGR ($n = 7$)	Control ($n = 25$)
Maternal age (years)	31.8 ± 1.9	29.8 ± 2.1	31.0 ± 2.4	31.8 ± 1.0
Maternal BMI	24.6 ± 1.7	24.0 ± 1.7	22.7 ± 1.4	21.8 ± 0.7
Maternal MAP at delivery (mmHg)	118.2 ± 4.4*[a]	110.1 ± 6.3*[b]	88.8 ± 5.5*[c]	78.1 ± 2.0
Maternal UP (mg/dl)	296.3 ± 75.1*[d]	491.8 ± 179.5*[e]	21.4 ± 10.1	13.7 ± 7.5
% of primiparas	50.0	44.4	42.9	52.0
Gestational age at delivery (weeks)	32.5 ± 1.6*[f]	31.1 ± 1.6*[g]	31.1 ± 1.9*[h]	36.0 ± 0.8
% of male babies	30.0	44.4	57.1	48.0
UApH	7.270 ± 0.017	7.232 ± 0.019*[i]	7.295 ± 0.028	7.273 ± 0.018
Neonatal birth weight (g)	1922 ± 311	1124 ± 185*[j]	1344 ± 230*[k]	2550 ± 130

BMI, body mass index; MAP, mean arterial pressure; UP, urinary protein; and UApH, umbilical arterial pH.
Student's t test, otherwise noted.
*[a] $p < 0.001$ (compared to the Control), $p = 0.001$ (compared to Group IUGR), *[b] $p < 0.001$ (compared to the Control), *[c] $p = 0.032$ (compared to the Control), *[d] $p < 0.001$ (compared to the Control), $p = 0.009$ (compared to Group IUGR), *[e] $p < 0.001$ (compared to the Control), $p = 0.01$ (compared to Group IUGR), *[f] $p = 0.039$, *[g] $p = 0.005$, *[h] $p = 0.037$ (compared to the Control), *[i] $p = 0.037$ (compared to the Control, Mann–Whitney's U test), *[j] $p < 0.0001$ and *[k] $p < 0.0001$ (compared to the Control, ANOVA, where gestational age at delivery was set as a covariate.)

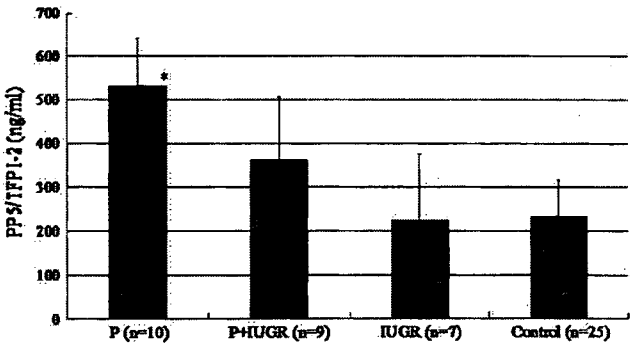


Fig. 1. Comparison of the PP5/TFPI-2 levels in maternal serum samples in the different groups obtained at delivery. Data are expressed as the mean ± SE and are adjusted for gestational age at delivery and the neonatal birth weight. $*p = 0.033$ (ANCOVA, where gestational age at delivery and the birth weight of the neonate are set as covariates).

PP5/TFPI-2 was detected in the cytoplasm of syncytiotrophoblasts, but not in any other type of cell such as cytotrophoblasts, decidual cells, stromal cells, or chorionic vascular endothelial cells (Fig. 2), as we have previously described [18,19]. Glypican-3 showed the same pattern as PP5/TFPI-2, that is, it was present only in the cytoplasm of syncytiotrophoblasts. Syndecan-1 was limited to the surface of the syncytiotrophoblasts.

3.4. Immunohistochemical evaluation

The results of the immunohistochemical analyses are summarized in Fig. 3. The coefficients of variation (CVs) of the scores from each of the two independent examiners were 19.7% and 13.8%, respectively. The CV between the scores from the two examiners was 17.9%. In contrast to the increase in maternal serum levels, PP5/TFPI-2 was detected scarcely in the placenta of Group P (Fig. 2), and so was glypican-3. The scores for PP5/TFPI-2, and also for glypican-3, in the placenta were significantly lower in Group P than in the Control ($p = 0.035$ for PP5/TFPI-2, 0.047 for glypican-3).

The scores for syndecan-1 in the placenta were significantly higher in Group P and Group IUGR ($p = 0.023$ and $p = 0.003$, respectively) than in the Control.

There was a positive correlation between the score for glypican-3 and that for PP5/TFPI-2 among the 33 placental samples examined (C.I. = 0.506, $p = 0.004$) (Fig. 4). The score for syndecan-1 correlated with neither that for PP5/TFPI-2 nor that for glypican-3 (data not shown).

3.5. Interaction of PP5/TFPI-2 and glypican-3

HepG2 cells abundantly produced both the core protein (approximately 60 kDa) and the glycated form (observed as a broad band around 97 kDa) of glypican-3, and no detectable amount of PP5/TFPI-2 was observed (Fig. 5, lanes 1 of (A) & (B)). With the antibody against PP5/TFPI-2, only the glycated form of glypican-3 and PP5/TFPI-2 were co-immunoprecipitate from the lysates of the PP5/TFPI-2 expression vector transfectants, and the core protein of glypican-3 was not detectable (Fig. 5, lanes 4 of (A) & (B)). From the lysates of the empty vector transfectants, which were prepared as a negative control, no detectable bands of PP5/TFPI-2 or glypican-3 were observed (Fig. 5, lanes 2 of (A) & (B)).

4. Discussion

First, we found that PP5/TFPI-2 interacts with glypican-3. In immunohistochemistry, glypican-3 was detected in a pattern identical to that of PP5/TFPI-2, with a positive correlation between the immunohistochemical scores for the two. The biochemical interaction of PP5/TFPI-2 with glycated glypican-3 was demonstrated in the HepG2 cells transfected with *PP5/TFPI-2*. These findings strongly support our previous proposal that glypican-3 serves as the anchor for PP5/TFPI-2 on the placental villi [19]. It is known that some proteins anchored to HSPGs can be shed together to the extracellular space [37]. Glypican-3 may not only anchor PP5/TFPI-2 but also play more roles in the secretory pathway of PP5/TFPI-2. Future studies should identify the precise localization of glypican-3 in the syncytiotrophoblasts and whether PP5/TFPI-2 and glypican-3 interact in the maternal serum.

Second, we highlighted the discrepancy that the maternal serum PP5/TFPI-2 level was increased, whereas placental PP5/TFPI-2 was detected significantly less intensely, in Group P as compared to the Control. This is the first study to investigate in parallel the maternal serum level and the placental immunohistochemistry of PP5/TFPI-2. Most glycoproteins that are produced by the placenta and detected in the maternal serum are known to be increased in the maternal serum of the patients with preeclampsia as compared to Controls [38–44]. It is thought that in preeclampsia, increased apoptosis of trophoblasts occurs in early pregnancy and that newly differentiated trophoblasts later in pregnancy overfunction as a compensation [40,42], based either on the assays of the extracts from the placenta at term [42] or on immunohistochemical studies of the placenta [41,43]. It is obvious from our data that the increase in PP5/TFPI-2 in maternal serum in preeclampsia must result from a mechanism different from that regulating other glycoproteins, which are detected strongly in the placenta in preeclampsia as compared to Controls [41,43].

Glypican-3, which was also detected significantly less intensely in the placenta of Group P as compared to the Control, may provide a clue to clarify the discrepancy in PP5/TFPI-2 levels. It is not clear whether the decreased amount of glypican-3 in the placenta in preeclampsia is caused by reduced expression of the protein through unknown mechanisms, and/or by increased cleavage of it. In either case, the amount of PP5/TFPI-2 anchored on villous surface might be decreased due to the smaller amount of glypican-3 on the villi. One could speculate that more PP5/TFPI-2 would flow out from the placenta to the maternal blood, as compared to normal pregnancy, which in turn would increase the level of PP5/TFPI-2 in maternal serum in preeclampsia. However, other mechanisms should be taken into account for the increase in PP5/TFPI-2 in

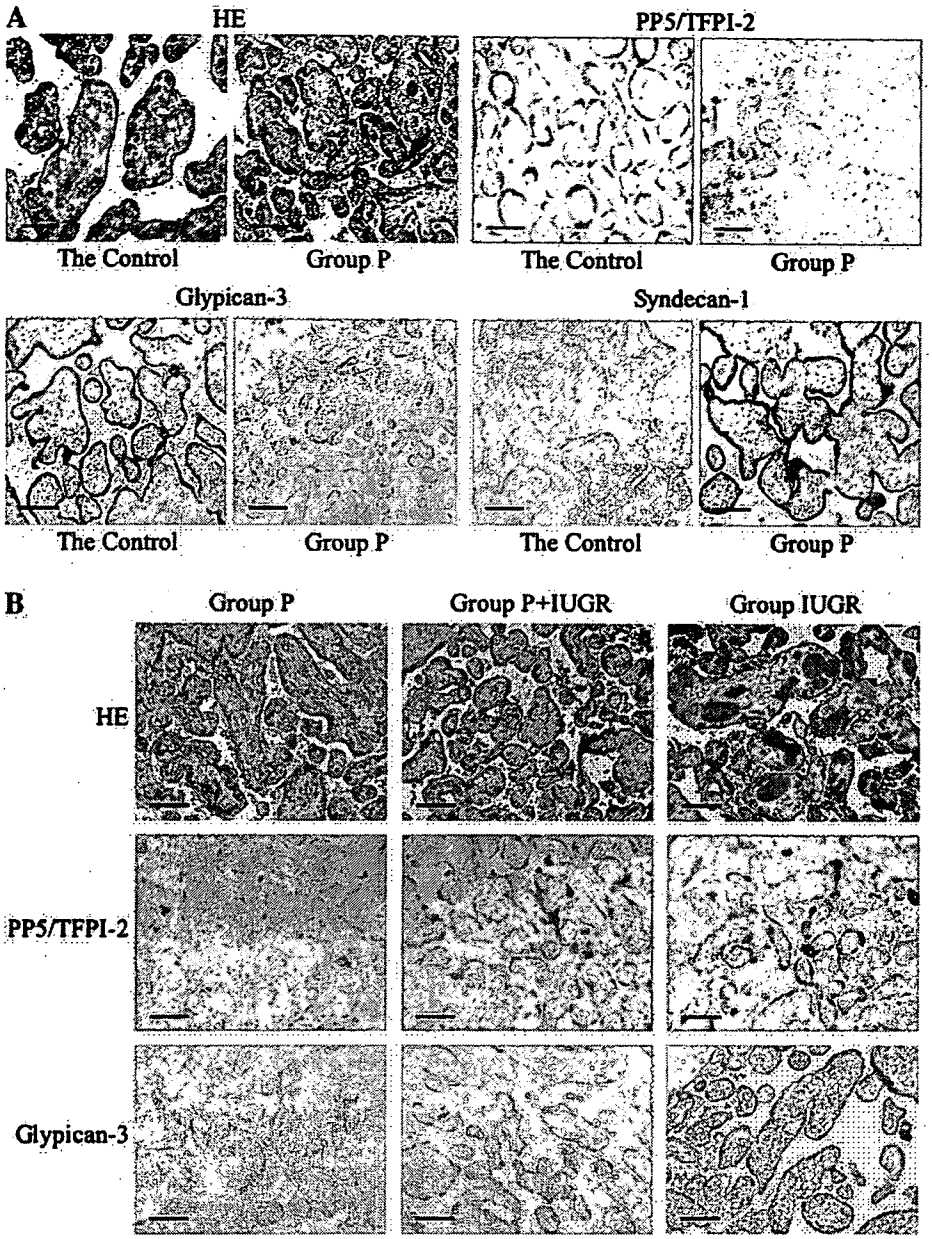
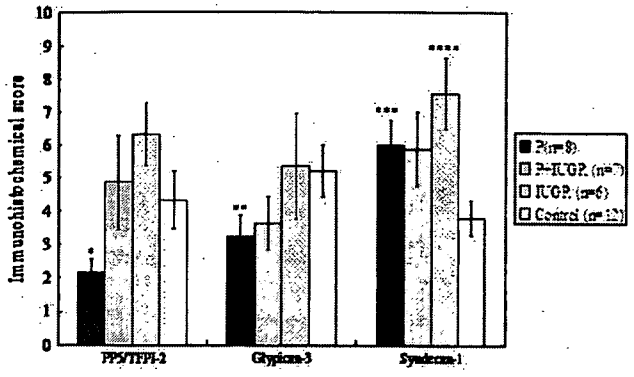


Fig. 2. Examples from the results of immunohistochemical studies. All images original magnification, ×100; scale bar, 100 μm. (A) HE staining, and immunohistochemical staining for PP5/TFPI-2, glypican-3, and syndecan-1 in the placental samples of the Control and Group P. (B) HE staining (the upper lane), and immunohistochemical staining for PP5/TFPI-2 (the middle lane) and glypican-3 (the lower lane) in the placental samples of Group P (the light column), Group P + IUGR (the middle column), and Group IUGR (the right column).

maternal serum, such as the metabolic pathway of the protein. Influences of impaired renal clearance of the glycoproteins, and of antihypertensive drugs in the patients with preeclampsia might not be ignored. As for the influence of renal function, even the clearance of human chorionic gonadotropin, a glycoprotein mainly excreted in urine, is shown to be not different between the patients with preeclampsia and normal Controls [45], which implies minimal influence of renal function. Further studies on the metabolic pathway of PP5/TFPI-2, as well as precise evaluation of the kinetics of PP5/TFPI-2 in preeclampsia, are required.

Third, syndecan-1 was immunohistochemically detected at significantly higher intensities in the placenta in Group P and Group IUGR than in the Control, contrary to another report [46]. This contradiction might be caused mainly by the different methods used for evaluation; for example, we used a semi-quantitative scoring system that focused on both the intensity and the quantity of the stained areas, whereas others had scored only for the intensity.

Fourth, we found that preeclampsia and IUGR, often considered to share the same pathological basis in common, presented distinct distributions of PP5/TFPI-2. In Group IUGR



	P(n=8)	P+IUGR (n=7)	IUGR (n=6)	Control (n=12)
PP5/TFPI-2	2.16±0.39*	4.86±1.43	6.31±0.95	4.33±0.85
Glypican-3	3.25±0.62**	3.64±0.79	5.96±1.62	5.22±0.77
Syndecan-1	6±0.74***	5.89±1.12	7.57±1.07****	3.8±0.53

Fig. 3. Comparison of the immunohistochemical scores for PP5/TFPI-2, glypican-3, and syndecan-1 in the placental samples. Data are expressed as the mean ± SE. Student's t test was used for all comparisons. *$p = 0.035$, compared to the Control, and $p = 0.001$, compared to Group IUGR, **$p = 0.045$, compared to the Control, ***$p = 0.023$, compared to the Control, and ****$p = 0.003$, compared to the Control.

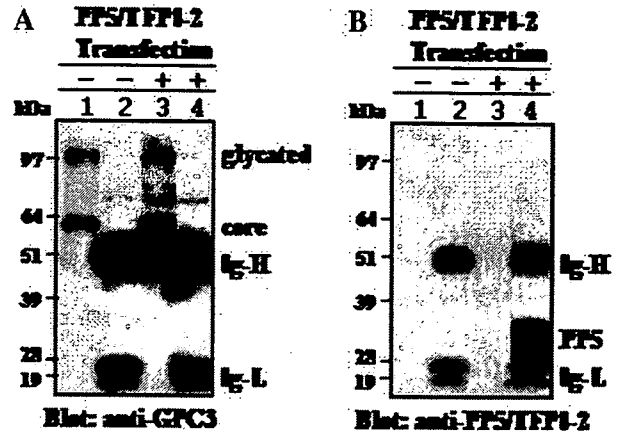


Fig. 5. Interaction of PP5/TFPI-2 and glypican-3 by immunoprecipitation experiments. HepG2 cell lysates from the PP5/TFPI-2 expression vector transfectant or the empty vector one were prepared as described in the text. Ten microliters of each sample before immunoprecipitation was loaded as input. Ten microliters from each 50 μl immunoprecipitant was also loaded. (A) Immunoblotted with anti-glypican-3 antibody and (B) with anti-PP5/TFPI-2. Molecular size from the marker bands was presented on the left side of each panel. Lanes 1 and 3, inputs; Lanes 2 and 4, immunoprecipitants. Ig-H, immunoglobulin heavy chain; Ig-L, immunoglobulin light chain; glycated, the glycated form of glypican-3; and core, the core protein of glypican-3.

and Group P + IUGR, the maternal serum PP5/TFPI-2 levels and placental immunohistochemical intensities of PP5/TFPI-2 were comparable to the Control. Although the patients in Group P and Group P + IUGR had preeclampsia to the same severity, they were not the same in the status of PP5/TFPI-2. The reason of the different status of PP5/TFPI-2 between preeclampsia and IUGR, as well as its relation to the clinical symptoms, is not known. Further studies would provide some available information on the pathogenesis of preeclampsia and IUGR.

Finally, we found that the umbilical serum levels of PP5/TFPI-2 were too low to be measured. The PP5/TFPI-2 levels were decreased in the maternal serum samples obtained 4 days after delivery, in agreement with another report [30].

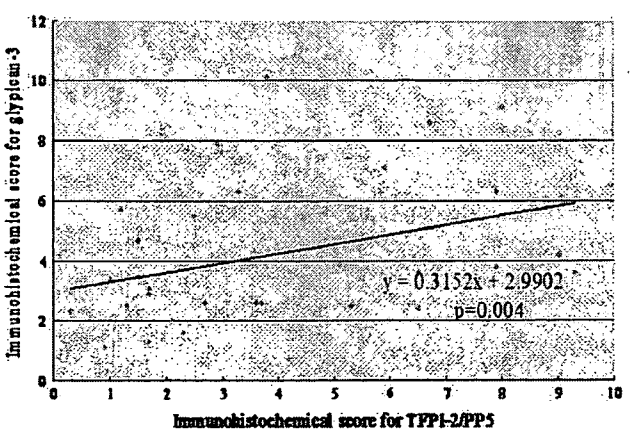


Fig. 4. Correlation between the immunohistochemical scores for PP5/TFPI-2 and those for glypican-3. C.I. = 0.506, $p = 0.004$ (Spearman's correlation test).

The role of PP5/TFPI-2 in pregnancy is not yet fully understood, but it is certain that PP5/TFPI-2 functions within the maternal serum and/or in the placenta, rather than in the fetal side. Our hypothesis has been that PP5/TFPI-2 works as an anticoagulant on the villous surface, which is not verified yet. Another group [47] has shown that the cognate tissue factor initiated coagulation inhibitor TFPI (or TFPI-1) is responsible for inhibiting coagulation in the placenta. Our finding in the present study, demonstrating the loss of PP5/TFPI-2 in the syncytium of the patients with preeclampsia, might imply its anticoagulant feature, because preeclampsia often encounters with elevated coagulation activity. Measuring the parameters of maternal coagulation activation in parallel with the examinations of placental events in situ should be considered as a further step to answer these questions.

In summary, the interaction of PP5/TFPI-2 with glypican-3 has been demonstrated from our studies. In patients with preeclampsia, there was a discrepancy in the PP5/TFPI-2 level in maternal serum, and the immunohistochemical intensity of the protein in the placenta. A decrease in the amount of glypican-3 in the placenta seems to hold the key for the discrepancy, but further studies are necessary to clarify the facts. Preeclampsia and IUGR, often regarded to share the same pathological basis, appeared to be totally distinct in terms of PP5/TFPI-2 distribution.

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がん対策と経済学①

米国における保険者のがん検診サービスの枠組みに関する調査

経営的視点に焦点を当てて

大重 賢治[1)] 岡本 直幸[2)] 水嶋 春朔[3)]

わが国においては，早期発見・早期治療を行う目的で，公的な保健事業として各種のがん検診が実施されてきた．公的な事業として行われる以上，その支出に見合うだけの効果が得られているかを評価することは重要なことである．

保健事業の経済的評価の手法としては，費用効果分析，費用便益分析などがあり[1~4]，多くの研究にて活用されている．がん検診の場合，「効果」の指標は，がん検診を行うことによって獲得された余命年数(life-year saved)や質調整生存年数(quality-adjusted life years)であり[5~7]，「便益」の指標は，がん検診に対して住民が支払っても良いと考える(willingness-to-pay)金額の総和となる[8~10]．すなわち，がん検診を経済学的に評価するためには，がん検診の「効果」や「便益」を数値で表すことが基本条件となる．しかしながら，これらを定量的に示すことが難しいこともあって，わが国においては，がん検診の経済的評価はまだ十分になされていないのが現状である．

「効果」や「便益」が，がん検診に投じた費用に見合っているかは，経済的に非常に重要な視点であるが，その他，もう1つ重要な視点(もしかしたら，政府や保険者にとっては最も重要な視点？)として，がん検診事業を行うことによって，将来の医療費が抑制されるか否か，がある．

経済的評価の手法としては費用分析の範疇に入り[2)]，検診事業を行う場合の費用と行わない場合の費用をいわば金銭的損得の観点から検討するものである．保険者が営利企業の場合，検診事業を行わない場合の費用が行う場合の費用を上回ると考えられる場合，保険者に検診事業を行う経済的インセンティブが発生する．逆に言うと，補助金などの制度がない限り，赤字になるような事業には取り組みにくいというのが現実であろう．たとえ保険者が，非営利団体であったとしても，恒常的に赤字を生み出すような事業には積極的にはなれないと考えられる．

われわれは，平成17年度厚生労働科学研究費補助金特別研究「がん検診の経済的効果及び制度の在り方に関する研究(主任研究者：水嶋春朔)」の一環として，医療が市場経済の仕組みの中で動いている米国において，がん検診がどのように提供されているかを調査した．米国の医療制度では，主体が，保険者およびサービス供給者ともに民間であることから，がん検診に対する考え方の中に経営的視点が反映されているのではないかと考えたからである．最も確認したかったのは，米国におけるがん検診が，政府の指導のもとにしぶしぶ行われているのか，それとも事業として積極的に行われているのかという点である．調査結果については，厚生労働科学研究費補助金特別研究報告書にて報告[11)]を行っているが，本稿では，そ

1) おおしげ けんじ：横浜市立大学医学部社会予防医学教室準教授 連絡先：〒236-0004 横浜市金沢区福浦3-9
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表　マネージドケア型組織 HMO と PPO の比較(文献[13]より)

	HMO	PPO
名称	Health Maintenance Organization	Preferred Provider Organization
主目的	医療費のコントロール	医療費のコントロール
特徴	医療機関と財政機関の両方を組織内に併せ持つ．組織外で行われた医療サービスには保険は支払われない．	医療保険者が独立した医療機関と契約を結び医療ネットワークを形作る．契約を行った医療機関は通常よりも安い金額で医療サービスを提供する．患者がネットワーク以外の医療機関を受診した場合，給付水準が減額される．
経済的インセンティブ	費用効果的なサービスを行おうとするインセンティブが働く	費用効果的なサービスを行おうとするインセンティブが働く
ゲートキーパーの存在	医療サービスの提供をコントロールする(ゲートキーパーの役割を担う)医師が存在する．ゲートキーパーである医師を介しない医療サービスには保険が支払われない．	ゲートキーパーの役割は存在しない．
医師の関わり方	組織に直接雇用される形態と，雇用ではなく契約を結ぶ形態がある．	組織と医師・医療機関との間で契約が結ばれる
組織の例	Keiser Parmanente 等	Health Net Inc. 等

註）この表は典型的な組織形態の比較であり，実際にはバリエーションが存在する．

の概要について紹介したい．

米国の医療保険者

米国では，高齢者と障害者を対象とした医療制度(メディケア)と貧困者のための医療制度(メディケイド)を除いては，医療は私的なサービスとして提供されている．医療保険は，主に福利厚生の一環として企業によって購入されてきた．

米国の国民医療費の対 GDP 比は，先進国の中でもずば抜けて高く，医療費の上昇は，医療保険の購入者である企業にとっても大きな負担となっていた．そのため 1980 年代頃より，医療費の抑制(企業側からみれば負担する保険料の抑制)に対して効果の期待できるマネージドケア型の医療システムが発達し，現在では，米国における民間医療保険の大部分が，この型のヘルスプランを採用している[12~15]．

マネージドケア型の医療システムの特徴は，保険者が，供給する医療，利用方法，価格などを一定の管理状態に置くところにある．このシステムの具体的な形態として，健康維持組織(Health Maintenance Organization：HMO)がある．HMO の基本的な形は，保険者と医療提供者(病院/医師)が一体となっているものである．

マネージドケア型の医療システムの形態にはバリエーションがある．例えば PPO(Preferred Provider Organization)のように，保険者が特定の医療サービス機関と契約を交わし，保険加入者にそれらのネットワーク内の医療機関を利用するよう奨励するシステムや，Point of Service(POS) Plan のように，HMO と PPO を併せたようなシステムもある(表)[13]．

調査地

2006 年 3 月，米国カリフォルニア州においてヘルスプランを提供しているマネージドケア型の組織を訪れ，がん検診サービスのあり方に関して聞き取り調査を行った．同州は，マネージドケア型の医療システムが最も発達している州の 1 つである[16]．訪問した機関は，HMO 型の Keiser Parmanente[17](以下，Keiser と略)と，PPO ネットワーク型の Health Net Inc.[18](以下，Health Net と略)である．聞き取り調査の相手は，両組織共に医師であり，Keiser の担当者の職位は，Assistant Medical Director for Quality and Clinical Analysis，Health Net の担当者の職位は，Regional Medical Director であった．

調査結果

1. がん検診の実施状況

両組織とも，がん検診は，United States Preventive Task Force[19] と American Cancer Society[20]のガイドラインに沿って実施していた．実施対象のがんも共通しており，積極的な検診の対象としているのが乳がん，子宮頸がん，大腸がんである．前立腺がん検診に関しては，「50歳以上の男性，ハイリスクの場合には45歳以上の男性に対して，PSA(prostate specific antigen)テストを，益と害を理解してもらった上で，希望があれば提供している(Keiser)」，「50歳以上の男性に対して，直腸診検査を毎年受けることを勧めている．PSA検査に関しては，まだ具体的な方針は立っていない．擬陽性が多いため判断保留中である(Health Net)」との回答であった．肺がん検診と胃がん検診は，有効性に関するエビデンス不足ということで，両組織とも実施を勧めていないとのことであった．

1) 乳がん検診の状況

Keiserでは，50～69歳の女性に対して2年に1度のマンモグラフィーによる検診を推奨している(40～49歳に関しては専門家との相談の上で実施)．受診率は最新の結果で84%とのことである(2年に1度の受診で，“受診者”にカウントされるため，対象者の84%が1年間に受診しているというわけではない．以下同様)．

Health Netでは，20～40歳の女性には3年に1度，40歳以上には毎年，医師による診察を受けるよう推奨している．また，40歳以上の女性にはマンモグラフィーによる検査を，1年もしくは2年に1回受けるよう推奨している．超音波検査は，ルーティンの検査としては行われていない．2005年，カリフォルニアにおける検診受診率(2年間で1回でも受診したもの)は，74.9%であった．

2) 大腸がん検診の状況

Keiserでは，50歳以上に対して，年に1度の便潜血テスト，5年に1度のS状結腸内視鏡検査(Flexible Sigmoidoscopy)による検査，10年に1度の大腸内視鏡(Colonoscopy)による検査を推奨している．受診率は最新の結果で45%である．

Health Netにおける大腸がん検診の取り組みも，Keiserと同様である．既往歴，家族歴があるような人には，より頻回の大腸内視鏡検査を勧めているという．2005年，カリフォルニアにおける検診受診率は45.7%であった．

3) 子宮頸がん検診の状況

Keiserでは，30～64歳までの女性に対して，3年に1度のPAPテストとHPV(human papilloma virus)検査を行うことを推奨している．18～29歳にも3年に1度のPAPテストを実施し，陽性者に対してHPV検査を追加して行うことを勧めている．受診率は最新の結果で79%である．

Health Netでは，21～65歳までの女性に対して，PAPテストを少なくとも3年に1回は行うように勧めている．2005年，カリフォルニアにおける検診受診率は，81.9%であった．

2. がん検診の経済的側面

検診受診料に関しては，「契約している医療保険の内容によって異なっており，無料から多少料金のかかる場合もある(Keiser)」，「どのような契約を行っているかによってバリエーションが多く，一概には言えないが，乳がん，大腸がん，子宮頸がん検診の受診者負担は大きくはない．無料の場合もある(Health Net)」との回答であった．

がん検診の実施に関して国の法律はあるか，という問いに対して，カリフォルニアの州法では「規定がある．また，パブリックリポート(保険契約の際の情報となる．毎年作成し加入者に配布)として出す必要がある(Keiser)」との回答を得た．がん検診の実施にあたっての政府の経済的援助は，「ない．ただし，メディケアの場合は，公的な枠組みの中で行われている(Health Net)」とのことである．がん検診に医療費抑制効果があると思うかという問いには，「ある．進行したがんになった場合，抗がん剤がものすごく高い．乳がんの化学療法の費用は，だいたい25万ドルぐらいかかる．がん検診は，とても費用効果的である

(Keiser)」,「ある．進行がんの場合，抗がん剤治療や集中治療など，医療費は莫大なものとなる．検診のコストのほうがはるかに安い(Health Net)」と，明確な回答が返ってきた．

3. 受診率向上の取り組み

がん検診の受診率を上げるためにはどうしたらよいかという問いに対して，「第一に，検診の重要性を会員ならびに医師に認識してもらうことである．特に現場の医師が検診の有効性に確信を持っていることが重要である．医師の認識を高めるための経済的インセンティブも必要である．第二に，がん検診受診勧奨の宣伝をメディアを利用して積極的に行うことが大切である．特に，有名人のがん罹患や死亡の発表に併せたキャンペーンは効果的である．第三に，がん検診の有効性に関するエビデンスを構築する必要がある．そのためには評価研究が欠かせない(Keiser)」,「教育が最も大事である．新聞，雑誌，TVなどを使って，がん検診の大切さについて教育を行っている．医師への教育も重要である．また，医師に対しては，検診受診率を高めるため，経済的なインセンティブが考えられている(Health Net)」との回答を得た．

患者(加入者)に対する経済的なインセンティブは，「グループ購入の場合など(企業による保険購入などを指す)，そのグループの受診率によって，保険料が変更されることもある．これも契約の内容による(Health Net)」とのことであった．検診を受けないことに対する患者側へのペナルティおよび医師側へのペナルティは「ない(Health Net)」ということである．

考察

今回の調査では，非営利組織と営利組織の両方の情報を得ることができた．若干の相違はあるものの，がん検診の取り組みはほぼ同様であった．有効性が明らかであるがん検診(乳がん検診，子宮頸がん検診，大腸がん検診)は強力に推進するが，有効性が十分に明らかにされていない検診の実施に関しては消極的であることも共通していた．

営利・非営利の違いがあるとはいえ，両組織とも民間の組織であり，米国の自由市場的な医療制度の中で，魅力的な保険料(保険購入者にとっては安いほうが魅力的)と魅力的なサービス提供で競争を行っている．がん検診は，医療費を抑え保険料を安くするという意味でも，消費者の満足度を高めるという意味でも，経営戦略的に重要な事業のようである．

まとめ

米国のマネージドケア型の組織を訪問し，がん検診サービスのあり方について聞き取り調査を行った．がん検診のサービスは，US Preventive Task Force等から出されているガイドラインに基づいて提供されており，乳がん検診，子宮頸がん検診に関しては，高い受診率が達成されていた．訪問した2つの組織の担当者とも，乳がん検診，子宮頸がん検診，大腸がん検診には，医療費抑制効果があるとの認識であった．がん検診の実施は，医師-患者関係の中で決定されており，検診の受診率を高めるための方策として，両組織の担当者とも，教育の重要性を強調していた．また，医師に対する経済的なインセンティブも重視していた．

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The model-based cost-effectiveness analysis of 1-year adjuvant trastuzumab treatment: based on 2-year follow-up HERA trial data

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Abstract *Background* Several randomized controlled trials have confirmed the usefulness of trastuzumab as an adjuvant therapy for HER2-overexpressed breast cancer patients; however, the costs for 1-year treatment are high. Therefore, we performed an economic analysis regarding the efficient distribution of medical resources. *Methods* To analyze the cost-effectiveness for a 1-year adjuvant trastuzumab treatment group compared with the observation group, we constructed a Markov model adopting a 3% per year discount rate for costs and outcomes. The time horizon was 50 years. The perspective was that of health-care payers, as only direct medical costs were calculated. The outcome was measured as life-year gained (LYG) from 2-year follow-up HERA trial data. *Results* The ICER of the standard setting (5 years efficacy and 50–60 kg patient weight) was JPY 2,600,000 (€17,000) per LYG. The calculation results of other weight class ICER were JPY 2,200,000 (€15,000) and JPY 3,300,000 (€22,000) per LYG for the patients, respectively, who weighed less than 50 kg, and 60–75 kg. In the sensitivity analysis, the period of trastuzumab efficacy was the most influential parameter for the result of cost-effectiveness. However, even if the trastuzumab efficacy were to continue for only 2 years, at least, which is a conservative setting judging from the joint analysis (NSABP B-31 and NCCTG N9831 trials), the ICER remains acceptable for any weight class. *Conclusion* These results suggest that the 1-year adjuvant trastuzumab treatment is cost-effective. Both clinical and economic benefits were superior for the 1-year adjuvant trastuzumab treatment group compared with the observation group.

Keywords Adjuvant treatment · Breast cancer · Cost-effectiveness · HERA trial · Trastuzumab

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Introduction

In Japan, the number of patient deaths due to breast cancer is increasing, while breast cancer mortality in Europe and the USA has generally improved since the 1990s [1]. The death toll from breast cancer is estimated as 10,000 persons per year, and reducing deaths due to breast cancer is one of the most important issues for women's public health.

Trastuzumab (Herceptin®) is a humanized monoclonal antibody that selectively targets the human epidermal growth factor type-2 (HER2) receptor. Amplification of the HER2 gene and overexpression of the HER2 protein, considered to be poor-prognosis factors, are observed among 20–30% of breast cancer patients [2]. Trastuzumab administered as combination therapy with chemotherapy has been proved to significantly improve disease-free survival, overall survival, and health-related quality of life (QoL) for metastatic breast cancer patients [3–5]. After 2005, several randomized control trials (RCTs) have confirmed the usefulness of trastuzumab as adjuvant therapy for HER2-positive patients, not only as metastatic therapy.

Ongoing large multicenter adjuvant trastuzumab RCTs: 1) the Herceptin Adjuvant (HERA) trial [6, 7], 2) the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial [8], 3) the North Central Center Treatment Group (NCCTG) N9831 trial [8], and 4) the Breast Cancer International Research Group (BCIRG) 006 trial [9], have shown good results, whereby the hazard ratio of the recurrence rate was about 0.5 even for HER2-positive patients who had a poor prognosis.

Though the cost of adjuvant trastuzumab treatment is high, the National Institute for Health and Clinical Excellence (NICE) in the UK has recommended adjuvant trastuzumab treatment for HER2-positive breast cancer patients based on the 1-year follow-up data of HERA trial [6, 10]; however, no such recommendation exists in Japan, and there have been no results of cost-effectiveness analysis based on the 2-year follow-up data of the HERA trial [7].

Therefore, this cost-effectiveness analysis (CEA) is designed to examine the economic efficiency of adjuvant trastuzumab treatment based on the 2-year follow-up data of HERA trial to support societal decision-making.

Patients and methods

Economic analysis

To analyze the cost-effectiveness of adjuvant trastuzumab treatment compared with observation alone, we used the Markov model by which the most common clinical transitions and health state transitions were simulated from multiple data sources. The model was build by TreeAge Pro 2006 (TreeAge Software, Inc, Williamstown, MA). One Markov cycle length corresponded to 1 month.

We adopted a 3% discount rate per year for costs and outcomes [11]. The discount-rate range for sensitivity analysis was 0–6%. The time horizon was 50 years (i.e., 600 Markov cycles), meaning that essentially all patients are considered as being dead.

The analysis perspective was that of health-care payers, and we calculated only the direct medical costs by the piece, because we were interested in the impact on the medical costs of adjuvant trastuzumab therapy. Neither indirect costs (work loss, etc.) nor direct non-medical costs (transportation cost, etc.) were considered. The primary result is indicated as the incremental cost per incremental life-year-gained (LYG). We used the exchange rate of €1 = JPY 150.

Hypothetic patients

Patients eligible for the HERA trial with HER2-positive breast cancer, who met the entry criteria, were considered as hypothetic patients of this economic analysis. Their median age was 49, and Japanese and node-negative patients were also included.

Based on the interim analysis of 2-year HERA follow up in 2007 [7], we only compared the economic efficiency for the 1-year of trastuzumab group (initial dose 8 mg/kg, maintenance dose 6 mg/kg, every 3 weeks for 1 year) and the observation group (adjuvant or neoadjuvant chemotherapy only). The hazard ratio for the risk of recurrence in the 1-year trastuzumab group, compared with the observation group, was 0.64 (95% confidence interval: 0.54–0.76; $P < 0.0001$), which was subject to probabilistic sensitivity analysis on the presumption of normal distribution on the log scale.

Major assumption

It is unknown how long the effect of trastuzumab continues, because HERA data cover only a 2-year median follow-up period. To take this uncertainty into account, the cost-effectiveness of trastuzumab was calculated for three hypothetic scenarios, with risk reduction continuing constantly for 2 years (conservative scenario), 5 years (standard scenario), and 10 years (optimistic scenario). After the end of the efficacy period of trastuzumab, the recurrence risk of the trastuzumab group is assumed to be equal to that of the observation group.

The next hypothesis is that trastuzumab is used for metastatic patients who have already been administered trastuzumab as adjuvant therapy. According to an inquiry survey of six Japanese leading hospitals participating in the HERA trial, most clinicians reported that they treated metastatic patients with trastuzumab after using it in an adjuvant setting and continued its combination therapy until a patient no longer responded to 3rd-line chemotherapy.

Patient weight may greatly influence the economic analysis result by determining the dose of trastuzumab. Japanese women, in their 50s, weigh an average of 54 kg. We assumed a patient weight of 50–60 kg (two 150 mg vials and one 60 mg vial) with a sensitivity analysis for patients weighing 50 kg (two 150 mg vials) and 60–75 kg (three 150 mg vials).

The assumed risk of recurrence during the first 5 years is higher than that during the next 5 years. The exact change of recurrence risk is not well defined, particularly not for HER2-positive patients. We presumed the recurrence risk after 5 years to be half that of the previous 5 years, continuing for the patients' lifetime [1]. This parameter was also subject to sensitivity analysis.

Furthermore, trastuzumab-caused cardiac events, which may affect QoL, are thought to be reversible [12, 13]; and thus may not affect life-year.

Markov model and therapeutic strategy

Figure 1(a) shows our constructed Markov model, modeling the therapeutic strategy for metastatic patients recommended by Hortobagyi [14], as both hormone therapy and chemotherapy until 3rd-line, followed by palliative care. This Markov model mainly consists of four parts, "without recurrence," "local recurrence," "metastatic recurrence," and "death," which are split into some parts corresponding to chemotherapy or hormone therapy stage.

Transition rate and model parameters were based on the HERA trial [7], and other published clinical trials [3, 15–19], (Table 1(a)). Transition rate was calculated from percentages of events or median time to progression and is assumed to follow a beta distribution in probabilistic sensitivity analysis. The percentage of cardiotoxicity is 0.6% (severe), 2% (symptomatic), and 3% (asymptomatic) [7], Although in Fig. 1(a) the arrows of each state to death were not drawn, this transition rate, which is the probability of death due to causes other than breast cancer, is considered to be equal to the natural death rate in Japan.

We also postulated a standard therapeutic strategy corresponding to each Markov state Fig. 1(b), by referring to the Japanese clinical practice guideline for breast cancer and multiple experts' opinions. In Japan, little cost-of-illness data exist; e.g., the treatment cost for

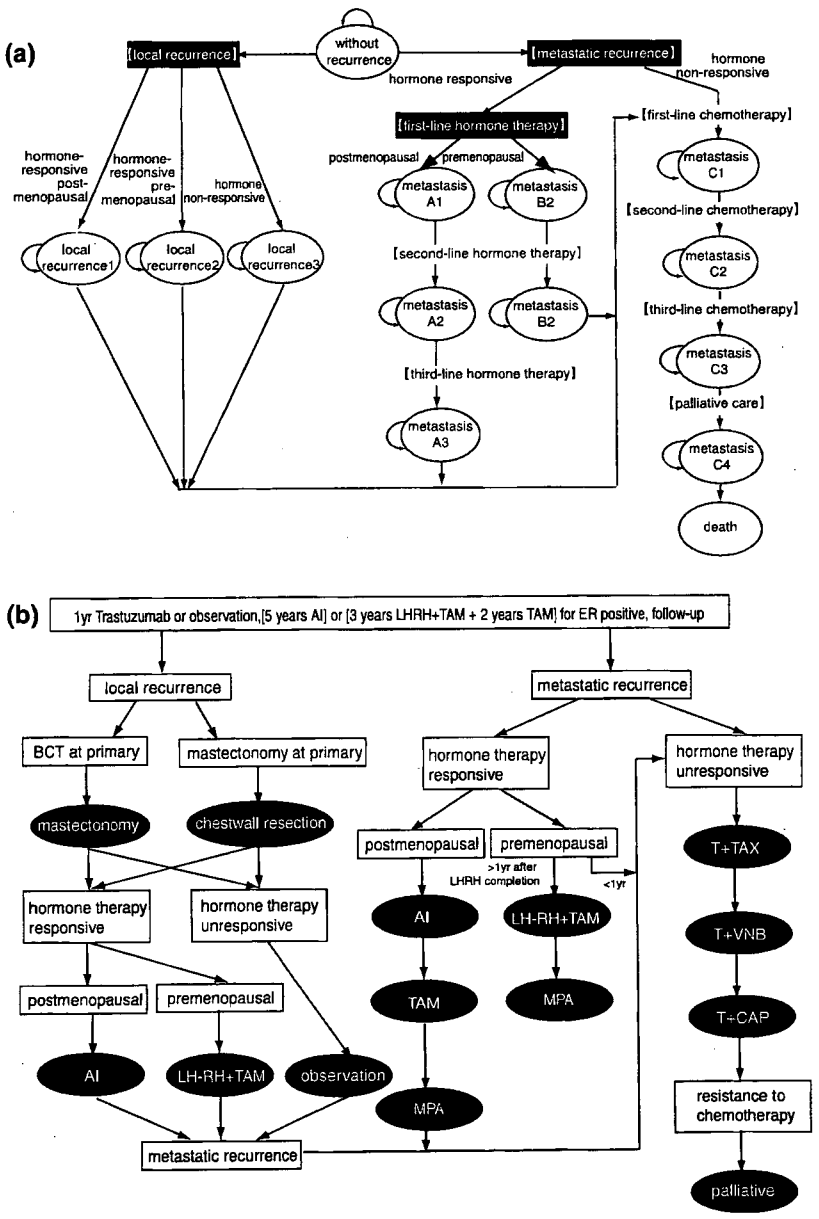

Fig. 1 (a) Markov model. All patients start in the "without recurrence." Patients move to an alternative health state with transition probability until they reach "death." Arrows indicate the passages from one state to another. (b) Assumed process of breast cancer treatment. The white letters on a dark eclipse background mean concrete treatment. AI: aromatase inhibitor, TAM: tamoxifen, LH-RH: LHRH agonist, MPA: medroxyprogesterone acetate T: trastuzumab, TAX: paclitacel, VNB: vinorelbine, CAP: capecitabine