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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