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Short communication

Circulating levels of maternal plasma cell-free pregnancy-associated placenta-specific microRNAs are associated with placental weight

K. Miura [a, *], S. Morisaki [a], S. Abe [a], A. Higashijima [a], Y. Hasegawa [a], S. Miura [a], S. Tateishi [a], H. Mishima [b], K. Yoshiura [b], H. Masuzaki [a]

[a] Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
[b] Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

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ABSTRACT

The aim of this study was to investigate the relationship between plasma concentration of cell-free pregnancy-associated placenta-specific microRNAs and clinical variables (placental weight, maternal body mass index, and neonatal birth weight). Circulating levels of cell-free pregnancy-associated placenta-specific microRNAs (miR-515-3p, miR-517a, miR-517c and miR-518b) in maternal plasma were measured by quantitative real-time RT-PCR in sixty-two pregnant women. The levels of cell-free pregnancy-associated placenta-specific microRNAs were significantly associated with placental weight, but not associated with body mass index or birth weight. Therefore, the measurement of cell-free pregnancy-associated placenta-specific miRNAs levels in maternal plasma may reflect the pregnancy status related to placenta volume.

1. Introduction

Recently, we have identified pregnancy-associated placenta-specific miRNAs (miR-515-3p, miR-517a, miR-517c, miR-518b and miR-526b), which locate on chromosome 19 miRNA cluster (C19MC) region, in the plasma of pregnant women [1,2]. To date, several placental miRNAs are involved in, or associated with, pregnancy-associated disorders, such as preeclampsia, fetal growth restriction or preterm delivery and, therefore, have a strong potential for use as sensitive and specific biomarkers [3–5]. A major source of cell-free placental miRNAs in maternal plasma is the villous trophoblast, which is able to release exosomes containing miRNAs into the maternal circulation [6,7], suggesting their potential for becoming novel biomarkers for prediction and detection of pathologies in pregnancy. Although the placenta is a source of supply for cell-free pregnancy-associated placenta-specific miRNAs (cfpp-miRNAs) [1,2], which factors affect the plasma concentration of cfpp-miRNA remain unknown.

Here, to increase knowledge of factors affecting the circulating levels of cfpp-miRNAs in maternal plasma, we investigated its association with placental weight (PW) as the placental factor, maternal body mass index (BMI) as the maternal factor and neonatal birth weight (BW) as the fetal factor. Also, as the pregnancy-associated but not placenta-specific miRNA [2], we measured the plasma concentration of cell-free miR-323-3p, and investigated its association with PW, BMI and BW.

2. Materials and methods

2.1. Sample collection

All samples were obtained after receiving written informed consent, and the Institutional Review Board of Nagasaki University approved the study protocol.

Women with smoking, multiple gestations, placenta previa, or invasive placentation, preterm labor, infection, fetal anomalies or aneuploidy, fetal growth restriction, or preeclampsia were excluded. Finally, we obtained maternal blood from 62 women with uncomplicated pregnancies of a singleton at 37–38 weeks to exclude the possibility of preterm labor. Gestational age was assessed using ultrasonography. All women had nothing to eat or drink for 8 h prior to blood collection. Maternal blood samples (7 mL) were collected within 3 h of elective cesarean section (CS) delivery. At the time of blood sampling, they had no signs of labor. Preparation and extraction of total RNA containing small RNA molecules were performed as described previously [2,8].

2.2. Real-time qRT-PCR analysis of miRNAs

All specific primers and TaqMan probes (miR-515-3p, miR-517a, miR-517c, miR-518b and miR-323-3p) were purchased from TaqMan MicroRNA Assays (Applied Biosystems, Warrington, UK). Absolute quantitative real-time RT-PCR (qRT-PCR) of miRNAs in plasma samples was performed as described previously [1,2,8,9]. For each miRNA assay, we prepared a calibration curve by 10-fold serial dilution of single-stranded cDNA oligonucleotides corresponding to each miRNA sequence from 1.0×10^2 to 1.0×10^8 copies/mL. Each sample and each calibration dilution was analyzed in triplicate. Each assay could detect down to 100 RNA copies/mL [2,8,9].

* Corresponding author. Tel.: +81 95 819 7363; fax: +81 95 819 7365.
E-mail address: kiyonori@nagasaki-u.ac.jp (K. Miura).

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Every batch of amplifications included three water blanks as negative controls for each of the reverse transcription and PCR steps. All data were collected and analyzed using a LightCycler® 480 Real-Time PCR System (Roche, Pleasanton, CA, USA).

2.3. Statistical analysis

Pearson product-moment correlation coefficients between plasma concentration of cfpp-miRNAs and clinical variables (PW, BMI or BW) were analyzed with SPSS version 19 (IBM Japan, Tokyo, Japan). Significances were defined as $P < 0.05$. To eliminate spurious correlation between circulating levels of cfpp-miRNAs and PW, BMI or BW, a partial correction coefficient analysis was performed.

3. Results and discussion

Median (minimum–maximum) levels of cfpp-miRNAs in maternal plasma were 5395.11 (1528.15–72734.98) copies/mL for miR-515-3p, 36338.12 (1789.61–909253.60) copies/mL for miR-517a, 29708.01 (1327.62–457445.24) copies/mL for miR-517c and 8492.21 (211.9–528026.51) copies/mL for miR-518b (Fig. 1a–d). As pregnancy-associated but not placenta-specific miRNA, median (minimum–maximum) levels of miR-323-3p in maternal plasma were 148131.34 (7669.99–2990112.52) copies/mL. Median (minimum–maximum) of PW, BMI, and BW were 573 (390–900) g, 20.2 (16.2–36.6), and 2995 (2274–3972) g, respectively. No relationship was detected between PW and BMI (r and *P*-value: 0.161 and 0.212). While, a significant association was seen between PW and BW, and between BMI and BW (*r* and *P*-values: 0.592 and <0.01, and 0.287 and <0.05, respectively).

The plasma concentrations of cfpp-miRNAs are associated with PW, but not associated with BMI or BW (Table 1, Fig. 1a–d). Previous reports support our data that PW affects the plasma concentrations of cfpp-miRNAs. Syncytiotrophoblast microparticles (STBM) and exosomes including fetal/placental DNAs/RNAs are released into maternal circulation of normal third trimester pregnancies, and their numbers are significantly higher in preeclampsia [10]. Their circulating levels may reflect the rate of syncytiotrophoblast apoptosis [11]. The placental factors, e.g. STBM and exosomes, in maternal blood damage the maternal endothelium, and then cause a syndrome of systemic endothelial dysfunction [12], indicating that increased concentration of STBM and exosomes is associated with the pathogenesis of preeclampsia [13–15]. Multiple pregnancy, which seems to have a larger volume of placenta in comparison to uncomplicated singleton pregnancy, is known to be one of the risk factors of preeclampsia [16,17]. Further, increased plasma concentration of cell-free placental miRNAs on C19MC region has been reported in preeclampsia [3]. Therefore, it seems to be reasonable that PW as a placental factor affects the plasma concentration of cfpp-miRNAs. Our previous study also

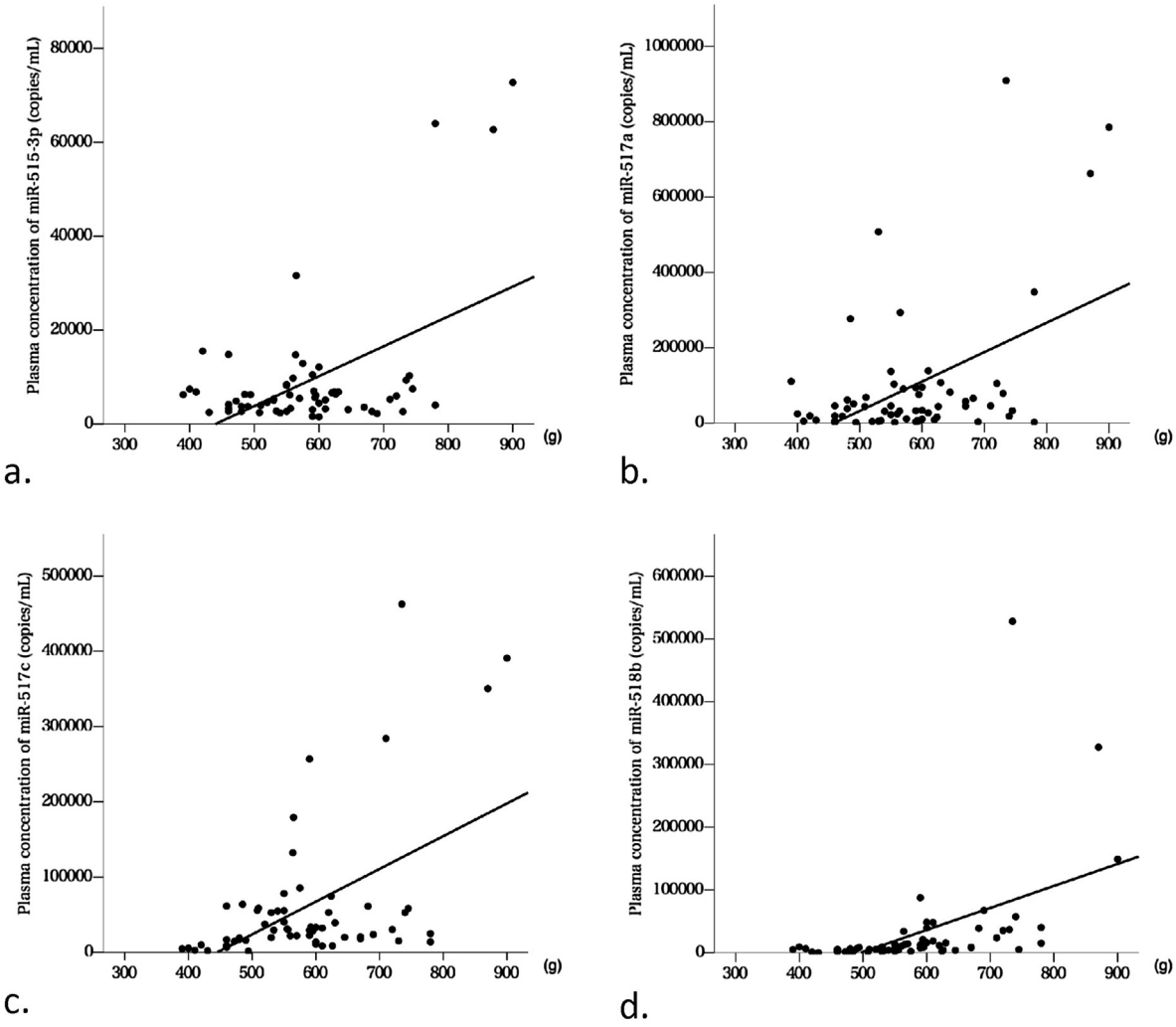

Fig. 1. Graph showing relationship between plasma concentration of cell-free pregnancy-associated placenta-specific miRNAs and placental weight. The correlation coefficient between placental weight and cell-free pregnancy-associated placenta-specific miRNAs levels for a. miR-515-3p is 0.506 (*P*-value: <0.01), for b. miR-517a is 0.475 (*P*-value: <0.01), for c. miR-517c is 0.512 (*P*-value: <0.01) and for d. miR-518b is 0.489 (*P*-value: <0.01).