Hydroxysteroid (17-β) Dehydrogenase 1 Is Dysregulated by Mir-210 and Mir-518c That Are Aberrantly Expressed in Preeclamptic Placentas
A Novel Marker for Predicting Preeclampsia


Abstract—In this study, to search for novel preeclampsia (PE) biomarkers, we focused on microRNA expression and function in the human placenta complicated with PE. By comprehensive analyses of microRNA expression, we identified 22 microRNAs significantly upregulated in preeclamptic placentas, 5 of which were predicted in silico to commonly target the mRNA encoding hydroxysteroid (17-β) dehydrogenase 1 (HSD17B1), a steroidogenetic enzyme expressed predominantly in the placenta. In vivo HSD17B1 expression, at both the mRNA and protein levels, was significantly decreased in preeclamptic placentas. Of these microRNAs, mir-210 and mir-518c were experimentally validated to target HSD17B1 by luciferase assay, real-time PCR, and ELISA. Furthermore, we found that plasma HSD17B1 protein levels in preeclamptic pregnant women reflected the decrease of its placental expression. Moreover, a prospective cohort study of plasma HSD17B1 revealed a significant reduction of plasma HSD17B1 levels in pregnant women at 20 to 23 and 27 to 30 weeks of gestation before PE onset compared with those with normal pregnancies. The sensitivities/specificities for predicting PE at 20 to 23 and 27 to 30 weeks of gestation were 0.75/0.67 (cutoff value = 21.9 ng/mL) and 0.88/0.51 (cutoff value = 30.5 ng/mL), and the odds ratios were 6.09 (95% CI: 2.35–15.77) and 7.83 (95% CI: 1.70–36.14), respectively. We conclude that HSD17B1 is dysregulated by mir-210 and mir-518c that are aberrantly expressed in preeclamptic placentas and that reducing plasma level of HSD17B1 precedes the onset of PE and is a potential prognostic factor for PE. (Hypertension. 2012;59:265-273.) • Online Data Supplement

Key Words: preeclampsia ■ microRNA ■ biomarker ■ placenta ■ prospective cohort study

The pathophysiology and etiology of preeclampsia (PE) remain largely unknown, and its final diagnosis can only be made when symptoms have regressed after delivery.4 Thus, it is of clinical significance to predict PE before its onset. Dysregulation of the serum levels of angiogenic/antiangiogenic factors has been demonstrated previously; examples include placental growth factor (PIGF), soluble fms-like tyrosine kinase 1 (sFlt-1), and soluble endoglin.2–4 However, these proteins may not sufficiently characterize the clinical features and pathophysiological mechanisms of PE onset.5 If there are any other parameters of which serum levels change in PE, they may illustrate the pathophysiology of PE in a manner different from the previous studies. MicroRNAs (miRNAs), small noncoding RNAs of ≈22 nucleotides in length, play a critical role in posttranscriptional gene regulation.6,7 Although many miRNAs are ubiquitously expressed in mammals, some miRNAs exhibit specific expression patterns in an organ- or cell-type–dependent manner.8 For instance, miRNAs derived from the miRNA cluster in human chromosome 19, a primate-specific miRNA cluster encompassing 46 miRNAs in the human genome,9 have been demonstrated to exhibit a placenta-specific expression pattern.10 Although a few studies have reported miRNAs that were dysregulated in preeclamptic placentas,11-13 these studies neither identify their targets nor elucidate their pathophysiological functions.

The purpose of this study was to elucidate novel mechanisms underlying the molecular pathology of PE and to identify novel prognostic factors for PE. In this study, we performed a comparative analysis of miRNA expression between normal and preeclamptic placentas by the combination of large-scale, high-throughput sequencing (HTS) and
quantitative PCR-based array analysis to identify miRNAs that were aberrantly expressed in preeclamptic placentas, that is, PE-related miRNAs. We demonstrated that the expression of hydroxysteroid (17-B) dehydrogenase 1 (HSD17B1), a steroidogenetic enzyme gene predominantly expressed in the placenta, was posttranscriptionally dysregulated by PE-related miRNAs. Furthermore, we found that plasma HSD17B1 protein levels in preeclamptic pregnant women reflected the decrease of its placental expression. Moreover, we performed a prospective cohort study of plasma HSD17B1 in pregnant women before PE onset to evaluate the prognostic value of plasma HSD17B1 in the prediction of PE, especially late-onset PE.

Methods
Further details are provided in the online Data Supplement. Please see http://hyper.ahajournals.org.

Specimens
Human placentas and blood plasma samples were obtained according to protocols approved by the Nippon Medical School Hospital Ethics Committee and the Jichi Medical University Ethics Committee.

Large-Scale Profiling of miRNA Expression by HTS
Large-scale profiling of miRNA expression was achieved by HTS on Genome Analyzer II (Illumina) and subsequent bioinformatic analyses.

Comprehensive Quantitative Analysis of miRNA Expression by Real-Time PCR-Based Array
miRNA expression in each placenta sample was determined comprehensively and quantitatively using TaqMan Human MicroRNA Arrays version 2.0 (A and B; Applied Biosystems, Foster City, CA).

Real-Time PCR
TaqMan MicroRNA Assays (Applied Biosystems) were used for the quantitative analysis of miRNA expression.

Luciferase Assays
Luciferase assays were performed using a reporter plasmid containing the 3′-untranslated region of the human HSD17B1 gene, pMIR-REPORT-HSD17B1.

Laser Microdissection Followed by Quantitative PCR
To examine the localization of miRNAs upregulated in preeclamptic placentas, laser microdissection (LMD) was performed using an LMD6000 system (Leica, Wetzlar, Germany).

Sandwich ELISA
HSD17B1 levels in plasma or placental tissue extracts were measured by sandwich ELISA.

Statistical Analysis
Statistical analyses were conducted using Student t test or the Mann-Whitney U test. P values <0.05 were deemed to be statistically significant.

Figure 1. Outline for the identification of preeclampsia (PE)-related gene.
Large-Scale Profiling of miRNA Expression in Normal and Preeclamptic Placentas by HTS

We conducted HTS-based miRNA profiling of human placentas during both normal and preeclamptic pregnancies (Figure 1). We obtained 26,618,424 and 26,695,898 sequence reads from the normal and preeclamptic placentas, respectively. These reads were filtered through multiple algorithms to identify noncoding RNA-derived sequences. The numbers of reads that passed through the final filtration step were 19,632,117 and 18,529,773 from the normal and preeclamptic placentas, respectively. The reads were then classified by referring to several RNA databases (Figure 1). Notably, 18,402,051 reads from normal placentas and 16,761,923 reads from preeclamptic placentas (93.7% and 90.5% of the noncoding RNA-derived reads, respectively) matched miRNA sequences (derived from 585 and 601 miRNA genes, respectively) registered in miRBase version 16.0.

Table S1 (available in the online Data Supplement) shows miRNAs corresponding with the entire reads from the normal and preeclamptic placentas. Corresponding with our previous findings,10 these miRNAs include several human placenta-specific miRNAs derived from human chromosome 19 (shown in bold letters in Table S1). We then performed a comparative analysis between normal and preeclamptic placentas based on the abundance of individual miRNAs. Provided that the level of the entire miRNA expression was equivalent between the normal and preeclamptic placentas, the proportions of individual miRNAs to the entire reads were considered to roughly reflect their expression levels. This assumption is supported by the result that the proportions of miR-191, which had been suggested to be suitable internal controls for the normalization of miRNA expression,14 are not much altered between normal and preeclamptic placentas. (Based on the data shown in Table S1; PE:normal ratio for miR-191 was 0.95.)

As a result of this analysis, we found that 62 miRNAs were upregulated in preeclamptic placentas compared with normal placentas (Table S2A) by selecting miRNAs, the proportion of which, to the entire reads, met the following criteria: the proportions to the entire reads from both normal and preeclamptic placentas were >0.001%, and the proportion to the entire reads increased by >1.5-fold in preeclamptic placentas compared with normal placentas. On the other hand, we identified 21 downregulated miRNAs, the proportions of which to the entire reads met the following criteria: the population in the entire sequence reads from both normal and preeclamptic placentas was >0.001%, and the proportion

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miRNA indicates microRNA; HTS, high-throughput sequencing analysis; PE, preeclampsia.
*Data show the percentage of individual miRNAs in the total miRNA read population in each placenta group.
†Data show the P values between the expression levels of individual miRNAs of normal (n=10) and preeclamptic (n=8) placentas.
‡Data show miRNAs that putatively target HSD17B1 by in silico analysis.
decreased by less than two thirds in preeclamptic placentas compared with normal placentas (Table S2B).

Quantitative PCR-Based Array Analysis of miRNA Expression in Normal and Preeclamptic Placentas

For the HTS-based miRNA profiling, we used pooled RNA samples from placentas (10 and 8 samples for normal and preeclamptic placentas, respectively); thus, only averaged profiles were available. Therefore, we performed real-time PCR-based array analysis to quantitatively and comprehensively examine the expression levels of 667 miRNAs in the individual placenta samples (Figures 1 and S1). This analysis revealed that 74 miRNAs were significantly (P<0.05) upregulated in preeclamptic placentas compared with normal placentas (Table S2A). By contrast, only 10 miRNAs were found to be significantly decreased in preeclamptic placentas compared with normal placentas (P<0.05; Table S2B).

HSD17B1 as a Putative Target of miRNAs Upregulated in Preeclamptic Placentas

By referring to the data from both HTS analysis and quantitative PCR array analysis, 22 miRNAs were identified as those commonly upregulated in preeclamptic placentas in both analyses (Table). In contrast, only 1 miRNA, miR-224, was found to be commonly downregulated in preeclamptic placentas (Table S2B). Herein, we designate these miRNAs “PE-related miRNAs.” In silico analysis predicted that 5 of the PE-related miRNAs (i.e., miR-20a, miR-210, miR-451, miR-518c, and miR-526b*) share HSD17B1 as their common target (Table). The putative recognition sites of these miRNAs in HSD17B1 3’-untranslated region and their sequences are shown in Figure S2A and S2B, respectively. All of these 5 miRNAs were upregulated in preeclamptic placentas. Therefore, we examined the expression level of HSD17B1 in the individual samples of the normal and preeclamptic placentas. Consistently, our quantitative PCR analysis revealed the significantly lower mRNA level of this gene in preeclamptic placentas than in normal placentas (P=0.0206; Figure 2A). Further, we also demonstrated by Western blotting that HSD17B1 expression was significantly decreased in preeclamptic placentas at the protein level as well (P=0.0096; Figures 2B and S3).

HSD17B1 Is a Target of miR-210 and miR-518c in Human Trophoblastic Cells

Next, we performed a luciferase assay to examine whether HSD17B1 is a direct target for these miRNAs. As shown in Figure 3A, miR-210 and miR-518c, but not miR-20a, miR-451, or miR-526b*, significantly decreased luciferase activity in BeWo cells cotransfected with pMIR-REPORT-HSD17B1. The suppressive effects of these miRNAs were significant compared with those in BeWo cells cotransfected with pMIR-REPORT as a control, although the luciferase activity in the control cells also appeared to be slightly influenced (Figure 3A). Thus, miR-210 and miR-518c suppressed luciferase expression by binding to the downstream HSD17B1 3’-untranslated region, indicating that HSD17B1 is indeed a target of these miRNAs.

We then examined the mRNA levels of HSD17B1 in BeWo cells overexpressing the above miRNAs. As shown in Figure 3B, miR-210 and miR-518c markedly downregulated the mRNA levels of HSD17B1, consistent with the result of the luciferase assay. We also demonstrated that these 2 miRNAs silenced HSD17B1 at a protein level by sandwich ELISA (Figure 3C). Furthermore, we performed antisense inhibition experiments and showed that locked nucleic acid–based functional inhibition of miR-210 and miR-518c upregulated the mRNA levels of HSD17B1 (Figure 3D), supporting the above results.

Localization of miR-210 and miR-518c in the Human Placenta

To examine which cell types express PE-related miRNAs, LMD followed by quantitative PCR was performed. LMD enabled us to isolate the syncytiotrophoblast (STB), fetal endothelium, and villous stroma separately from the paraffin-embedded sections of human placental villi. The application of LMD to miRNA localization in the placenta was validated by showing the predominant expression of miR-517a in the STB (Figure 3E), of which predominant localization in the STB had been shown previously by in situ hybridization.10 As shown in Figure 3E, both miR-210 and miR-518c were expressed predominantly in the STB in chorionic villi of the human placenta and much more weakly in villous stroma and fetal endothelium. Thus, these miRNAs are considered to play important roles in the STB by downmodulating the expression of their target genes.

Hypoxia Induced miR-210 and miR-518c Expression and Suppressed HSD17B1 Expression

Because several lines of study have indicated that hypoxia in the placenta should be involved in the onset and/or progres-
A

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** HSD17B1 silencing by miR-210 and miR-518c in human trophoblasts. A. Target validation of the preeclampsia (PE)-related microRNAs (miRNAs) by luciferase assay using BeWo cells (n=3). Normalized luciferase activities in pMIR-REPORT (mock)- and pMIR-REPORT-HSD17B1-transfected BeWo cells are indicated by gray and white columns, respectively. Luciferase activities were significantly downregulated by miR-210 and miR-518c via the 3′-untranslated region (UTR) of HSD17B1 compared with those in the cells transfected with an miRNA mimic for negative control (Cont). B. HSD17B1 expression is pronouncedly downregulated at a transcriptional level by miR-210 and miR-518c (40 nmol/L) compared with an miRNA mimic for negative control (Cont) 48 hours after the transfection of these miRNAs (n=3). The averaged value of the normalized HSD17B1 expression in the control cells was set at 1. C. HSD17B1 expression was also downregulated at a protein level by miR-210 and miR-518c. The relative levels of HSD17B1 protein in a fixed concentration of BeWo lysates were measured by sandwich ELISA (n=3). HSD17B1 protein levels 48 hours after the transfection of these miRNAs are indicated. The value of HSD17B1 protein concentration in the control cells was set at 1. D. Conversely, HSD17B1 mRNA expression is increased by the inhibitors for miR-210 (locked nucleic acid [LNA]-210) and miR-518c (LNA-518c) at 20 nmol/L compared with a negative control (Cont) 48 hours after transfection (n=3). E. Determination of miRNA localization by laser microdissection (LMD) followed by real-time RT-PCR (n=3). Regions corresponding to trophoblastic layer (TB), villous stroma (VS), and fetal endothelium (FE) were separately isolated by LMD and processed for total RNA isolation. The relative expression levels of miR-210, miR-518c, and a representative human chromosome 19–derived, placenta-specific miRNA, such as miR-517a, were assessed by real-time RT-PCR. The values of expression in trophoblastic layers were set at 1. Data are indicated as the mean±SEM. Statistically significant differences were determined by Student t test. ***P<0.001, **P<0.01, *P<0.05.

**Plasma HSD17B1 Protein Levels Are Decreased in Preeclamptic Pregnant Women**

Because a previous study reported that the enzymatic activity of HSD17B1 could be detected in sera from pregnant women, we measured the protein levels of HSD17B1 in maternal blood plasma collected from healthy pregnant women and preeclamptic pregnant women by ELISA. First, we determined the plasma HSD17B1 level with normal pregnancy collected at 20 to 23, 27 to 30, and 37 weeks of gestation (22.6, 30.2, and 32.7 ng/mL in median, respectively; Figure S4A). We then measured HSD17B1 protein levels in the plasma collected after the onset of PE and performed a comparative analysis of plasma HSD17B1 levels between normal and preeclamptic pregnancies. Analysis using whole plasma samples revealed that plasma HSD17B1 levels were significantly decreased in women with preeclamptic pregnancy (left graph in Figure 5). Similarly, there was a significant difference in HSD17B1 levels between the 2 groups when analysis was performed using plasma samples collected at nearly matched gestational ages, that is, the plasma at 26 to 34 weeks of gestation (average: 29.4 weeks) from women with early onset PE versus the normal plasma collected at 27 to 30 weeks of gestation (middle graph in Figure 5) and the plasma at 32 to 40 weeks of gestation (average: 36.4) from women with late-onset PE versus the normal plasma collected at 37 weeks of gestation (right graph in Figure 5).

**Prospective Cohort Study of Plasma HSD17B1 Levels in Pregnant Women Before PE Onset**

Furthermore, we performed a prospective cohort study of plasma HSD17B1 levels in pregnant women at 20 to 23 and...
27 to 30 weeks of gestation before PE onset. This study revealed that plasma HSD17B1 protein levels were significantly decreased in pregnant women before PE onset regardless of their gestational ages (Figure 6A). We also evaluated plasma HSD17B1 levels of pregnant women before the onset of late-onset PE. Plasma HSD17B1 levels were significantly decreased in pregnant women before the onset of late-onset PE compared with those with normal pregnancies, not only at 27 to 30 weeks of gestation (right graph in Figure 6B) but also at 20 to 23 weeks of gestation (left graph in Figure 6B). From a clinical point of view, it should be noted that there was a significant difference in plasma HSD17B1 protein levels between the 2 groups even at 20 to 23 weeks of gestation before the onset of PE, especially late-onset PE, because the results indicate that the dysregulation of plasma HSD17B1 levels occurs in advance of, not as a consequence of, the onset of PE.

Receiver Operating Characteristic Analysis to Evaluate the Prognostic Value of Plasma HSD17B1 Level

To test the prognostic value of plasma HSD17B1 level in the prediction of PE, we performed receiver operating characteristic curve analysis. We evaluated plasma HSD17B1 levels of pregnant women in the specific ranges of gestational weeks. At 20 to 23 and 27 to 30 weeks of gestation before the onset

Figure 4. Hypoxia-induced effects of the expressions of miR-210, miR-518c, and HSD17B1. The human trophoblastic cell lines BeWo and JEG-3 were exposed to hypoxia (1% O2) or normoxia (20% O2) for the indicated periods. A and B, The expressions of both miR-210 (A) and miR-518c (B) were upregulated by hypoxia (H) compared with normoxia (N) in BeWo and JEG-3 cells (n=3). C, Reciprocally, the expression of HSD17B1 was downregulated by hypoxia (H) compared with normoxia (N) in both BeWo and JEG-3 cells (n=3). Data are indicated as the mean±SEM. Statistically significant differences were determined by Student t test. **P<0.001, *P<0.01, *P<0.05.

Figure 5. Plasma HSD17B1 protein levels in preeclamptic pregnant women. Box plots of relative plasma HSD17B1 levels are shown. Comparative analysis using normal plasma samples and whole plasma samples collected after the onset of preeclampsia (PE) is indicated in the left graph. Comparison between the normal and preeclamptic groups when analysis was performed using plasma samples collected at nearly matched gestational ages, that is, the plasma at 26 to 34 weeks of gestation (average: 29.4 weeks) from women with early onset PE (PE-EO) vs the normal plasma collected at 27 to 30 weeks of gestation (middle graph) and the plasma at 32 to 40 weeks of gestation (average: 36.4 weeks) from women with late-onset PE (PE-LO) vs the normal plasma collected at 37 weeks of gestation (right graph) is indicated. The boxes mark the interval between the 25th and 75th percentiles. The lines inside the boxes denote medians. P values determined by Mann-Whitney U test are shown.
of PE, the area under the curve values of HSD17B1 were 0.713 and 0.729, respectively. At the cutoff values of 21.9 and 30.5 ng/mL, the sensitivities were 0.75 and 0.88, respectively (Figure 7A). Furthermore, we also evaluated plasma HSD17B1 levels of pregnant women at 20 to 23 and 27 to 30 weeks of gestation before the onset of late-onset PE. The area under the curve values of HSD17B1 at 20 to 23 and 27 to 30 weeks of gestation were 0.734 and 0.761, respectively. At the cutoff values of 21.9 and 22.0 ng/mL, the sensitivities were 0.82 and 0.80, respectively (Figure 7B). These data indicate that plasma HSD17B1 level is a potential prognostic factor for PE.

**Discussion**

In the present study, we identified PE-related miRNAs that were aberrantly expressed in preeclamptic placentas. We also demonstrated that HSD17B1, a placental steroidogenetic enzyme gene, is posttranscriptionally dysregulated by PE-related miRNAs. Moreover, a prospective cohort study of plasma HSD17B1 in pregnant women before PE onset revealed that a significant reduction of plasma HSD17B1 levels heralded the onset of PE. To date, a couple of studies have noted that miRNAs were dysregulated in preeclamptic placentas.\(^{11-13}\) Although these studies refer to the in silico targets of the identified miRNAs, they neither provide experimental evidence that they are the actual targets of the miRNAs nor elucidate their pathophysiological functions. To our knowledge, only 1 study published recently has identified a target of PE-related miRNAs, cysteine-rich, angiogenic inducer, 61 (CYR61) that is supported by experimental evidence.\(^{16}\) However, our findings were advantageous over the previous findings in that HSD17B1 was dominantly expressed in the placenta\(^{17}\) and was also detected in maternal plasma, which enables us to monitor the pathophysiological conditions of placentas without invasive clinical tests. In this study, we indeed demonstrated that the plasma, as well as the placental levels of HSD17B1, were dysregulated in PE.

In the present study, we used 2 alternative approaches to assess differential miRNA expression, the combination of which is expected to increase the accuracy of PE-related miRNA identification (Figure 1). As a result, we identified 22 PE-related miRNAs that were upregulated in preeclamptic placentas (Table), 5 of which putatively target HSD17B1 in silico. Furthermore, of these miRNAs, miR-210 and miR-518c were experimentally demonstrated to suppress HSD17B1 expression as a direct target.

Our LMD analysis revealed that both miR-210 and miR-518c were localized predominantly in the STB (Figure 3E), consistent with a previous report.\(^{19}\) Thus, it is likely that HSD17B1 expression is dysregulated in an miRNA-mediated manner in the STB of preeclamptic placentas. It should be noted that Winn et al\(^{19}\) also identified HSD17B1 previously as a pronouncedly downregulated gene in preeclamptic placentas by comprehensive analysis using microarray.

**MiR-210** is well known as a hypoxia-responsive miRNA.\(^{20-22}\) Hypoxia caused by insufficient blood supply into the intervillous space of the placenta is considered to affect trophoblast functions and to lead to preeclamptic features. Although this idea still remains controversial, our finding that miR-210, as well as miR-518c, in trophoblastic cells was transcriptionally upregulated in response to hypoxia (Figure 4) raises the possibility that hypoxia is involved, at least partly, in PE onset. Furthermore, HSD17B1 mRNA expression in these cells was reciprocally downregulated in response to hypoxia. These results indicate that dysregulation of the expression of miR-210, miR-518c, and...
HSD17B1 in preeclamptic placentas could be associated with events triggered by hypoxia. In addition, we showed in vitro that the overexpression of these miRNAs resulted in lowered HSD17B1 expression (Figure 3B and 3C), suggesting that the dysregulation of HSD17B1 expression is probably attributed to the altered expression of these miRNAs. Considering recent studies showing that miR-210 expression is induced downstream of hypoxia-inducible factor 1, HSD17B1 expression could, in turn, be under the control of hypoxia-inducible factor 1. On the other hand, mechanisms underlying the transcriptional regulation of miR-518c remain to be elucidated.

HSD17B1 is a steroidogenetic enzyme catalyzing the conversion of estrone to 17β-estradiol. The measurement of HSD17B1 levels in maternal plasma is advantageous because it is expected to be derived almost exclusively from the placenta. Although further technical improvement of HSD17B1 detection (eg, sensitivity) and larger-scale longitudinal studies are necessary in terms of clinical application, our prospective cohort study showing the lowered plasma HSD17B1 level before the onset of PE (Figure 6) suggests that plasma HSD17B1 serves as a biomarker to predict the onset of PE. Our receiver operating characteristic curve analysis revealed that plasma HSD17B1 has potential prognostic power for PE (Figure 7). It should be noted that plasma HSD17B1 levels were significantly decreased in pregnant women before the onset of late-onset PE at 20 to 23 weeks of gestation (left panel in Figure 6B). Some angiogenetic/antiangiogenic factors, including PIGF, sFlt-1, and soluble endoglin, have been suggested to serve as promising predictors for PE. However, in a prospective longitudinal analysis using a cohort of pregnant women with PE risk factor(s), Moore Simas et al found that the sFlt1:PIGF ratio, an index of antiangiogenetic activity, did not significantly increase at 22 to 26 weeks in women who developed PE after 34 weeks of gestation (ie, late-onset PE), although elevations in the angiogenetic ratio in the late second trimester were highly predictive of early onset (<34 weeks) PE. Similarly, a cross-sectional analysis using a nested case-control study of healthy nulliparous women within the Trial of Calcium for Preeclampsia Prevention by Levine et al showed that the serum levels of soluble endoglin and the sFlt1:PIGF ratio did not greatly increase at 21 to 32 weeks in women who developed PE at or after 37 weeks (term PE), whereas they began to rise at 17 to 20 weeks in women who developed PE before 37 weeks (preterm PE). These previous studies suggest that, in addition to angiogenetic/antiangiogenic factors, a novel, sensitive, and specific biomarker is still needed for the prediction of late-onset PE. After the cross-sectional analysis by Levine et al, we evaluated whether plasma HSD17B1 levels were altered in preterm and term PE, and we demonstrated that they significantly decreased at 20 to 23 weeks and 27 to 30 weeks of gestation in both types of PE compared with levels in normal pregnancy (Figure S4B). These findings, together with data from previous studies, suggest that decreases in plasma HSD17B1 levels are potentially predictive of term PE, as well as of preterm PE.
**Perspectives**

In this study, we showed PE-related miRNAs and subsequently identified one of their target genes, HSD17B1. However, we have not illustrated the whole picture of the pathophysiological functions of the PE-related miRNAs. To begin to elucidate the possible functions of the PE-related miRNAs, we performed a network-based analysis using ingenuity pathway analysis for the PE-related miRNAs; the PE-related miRNAs might play a role in the broad range of the human reproductive system (Table S3). Considering that a single miRNA targets multiple genes and also that multiple miRNAs target a single gene,28 further functional studies, including in vitro and in vivo studies using HSD17B1-deficient cells/animals, are warranted to comprehensively understand the role(s) of the PE-related miRNAs and HSD17B1 in the onset and/or progression of PE. We have reported previously that placenta-derived miRNAs are secreted via exosomes and enter into maternal circulation.10 Thus, the PE-related miRNAs, that placenta-derived miRNAs are secreted via exosomes and stand the role(s) of the PE-related miRNAs and

**Sources of Funding**

This work was supported in part by Grants-in-Aid and the “Research Core” Project for Private University: Matching Fund Subsidy from the Ministry of Education, Culture, Sports, Science and Technology, Japan; the Maruyama Memorial Research Fund from Nippon Medical School; a research grant from the Japan Society for the Study of Hypertension in Pregnancy; and a research grant from the National Natural Science Fund of China (81070216).

**Disclosures**

None.

**References**

Hydroxysteroid (17-β) Dehydrogenase 1 Is Dysregulated by Mir-210 and Mir-518c That Are Aberrantly Expressed in Preeclamptic Placentas: A Novel Marker for Predicting Preeclampsia


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Hydroxysteroid (17-Beta) Dehydrogenase 1 Is Dysregulated by Mir-210 and Mir-518c That Are Aberrantly Expressed in Preeclamptic Placentas: A Novel Marker for Predicting Preeclampsia


Short title: HSD17B1 as a prognostic factor for preeclampsia

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Materials and Methods

Definition of PE
We defined PE according to the definition and classification of pregnancy-induced hypertension (PIH) (2004) of the Japan Society for the Study of Hypertension in Pregnancy (JSSHP) (1). In brief, PE was defined as hypertension with proteinuria occurring after the 20th week of gestation but resolving by the 12th week postpartum. Hypertension was defined as systolic blood pressure (SBP) was ≥ 140 mmHg and/or diastolic blood pressure (DBP) was ≥ 90 mmHg on 2 occasions at least 4 hours apart. Proteinuria was defined as ≥300 mg/day from 24 h urine collection. If only test tape was available, semi-quantitative test results of 1+ on 2 occasions at least 1 day apart were considered to constitute a positive result. Early-onset (EO)-PE was defined as PE with onset at <32 weeks of gestation, and late-onset (LO)-PE was defined as PE with onset at ≥32 weeks of gestation. Severe PE was defined as SBP ≥ 160 mmHg or DBP ≥ 110 mmHg or proteinuria ≥2g/day or a semi-quantitative test 3+. Superimposed PE was defined as chronic hypertension diagnosed prior to pregnancy or prior to the 20th week of gestation, with proteinuria emerging after the 20th week of gestation, or chronic nephritis diagnosed prior to pregnancy or prior to the 20th week of gestation, with hypertension emerging after the 20th week of gestation. Superimposed PE was included in the category of PE in this study.

Subjects and procedures
The placental tissues used in this study were collected from patients with PE (n = 8) and women with normal pregnancies (n = 10). The clinical characteristics of the preeclamptic placentas are summarized in Supplementary Table S4. In all cases, tissues were processed as soon as possible following collection (within 20 min). Subsequent procedures for isolating total RNAs from the tissues were carried out as previously described (2).

Blood Pressure in Pregnancy and Puerperal Period
Blood pressure was measured with the validated Omron HEM-906® automated digital oscillometric sphygmanometer (OMRON Healthcare Japan), according to standard
procedures. The mean value of 2 blood pressure readings over a 60-s interval was documented for each participant.

**Large-scale Profiling of miRNA Expression by High-throughput Sequencing (HTS)**

Equal quantities of total RNA isolated from each placenta were pooled within either the normal or the PE group. The small RNA-derived cDNAs of normal and preeclamptic placentas were then generated using a Small RNA Sample Prep Kit provided by Illumina (San Diego, CA) according to the manufacturer’s instructions. Briefly, the pooled total RNAs were separated by denaturing polyacrylamide gel electrophoresis to isolate 18- to 30-nt small RNAs. The small RNAs were then ligated to 5’- and 3’-linkers at both termini. Complementary DNAs (cDNAs) of the small RNAs were synthesized by reverse transcription using a primer recognizing the sequence of the 3’-linker. This was followed by polymerase chain reaction (PCR) using primers recognizing the sequences of 5’- and 3’-linkers to synthesize double-stranded cDNAs. The cDNAs were subjected to quality examination using Bio-analyzer (Agilent) and HTS on Genome Analyzer II (Illumina).

HTS data were subjected to bioinformatic analyses as follows. First, trimming of adaptor sequences and elimination of invalid sequences were carried out using a Paracel Filtering Package (Paracel, Inc., Pasadena, CA). Second, reads that passed the above assessment were aligned using SOAP (short oligonucleotide alignment program) (3) to extract sequences matching the human genome. Finally, individual reads mapped to the human genome were annotated by referring to the databases as follows: miRBase v13.0 for miRNAs, Ensembl (release53) for non-coding RNAs except miRNAs, NCBI RefSeq for RefSeq transcripts, and UCSC hg18 for genomic sequences.

HTS-based analyses were supported by the Takara Bio Dragon Genomics Center (Yokkaichi, Japan).

**Comprehensive Quantitative Analysis of miRNA Expression by Quantitative PCR-based Array**

RT-PCR reactions were carried out according to the manufacturer’s instructions. Briefly, 1 μg of the total RNAs from each specimen was reverse transcribed using TaqMan® Universal Master Mix II (Applied Biosystems) and Megaplex RT Primers (Applied Biosystems) to synthesize cDNAs. Subsequent real-time PCR was performed using an Applied Biosystems 7900HT system and TaqMan® Universal PCR Master Mix. The relative quantity (RQ) of each miRNA was determined based on the ΔΔCt method using RQ Manager Software (Applied Biosystems). The data were normalized for the expression of RNU6.

**Quantitative PCR**

Monitoring of miRNA-derived PCR products was performed on the ABI 7300 Real-Time PCR System (Applied Biosystems). For the quantitative analysis of mRNA levels, SYBR® Premix Ex Taq™ II (Perfect Real Time) (Takara Bio, Ohtsu, Japan) was used. To normalize the expression levels of miRNAs and mRNAs, RNU6-2 (also known as U6) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as endogenous internal controls, respectively.

**Cell Culture**

Human trophoblastic cell lines derived from choriocarcinoma and BeWo and JEG-3 cell lines were purchased from the RIKEN Bioresource Center (Tsukuba, Japan) and DS Pharma Biomedical (Suita, Japan), respectively. BeWo cells were maintained in Ham’s F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 15% fetal bovine serum (FBS). JEG-3 cells were maintained in Eagle’s MEM (Invitrogen) supplemented with 10% FBS, 1%
nonessential amino acids, and 1 mM pyruvate. HeLa cells were obtained from the RIKEN Bioresource Center and maintained in DMEM containing 10% FBS. All cells were routinely cultured at 37°C in a humidified atmosphere containing 5% CO2. In experiments to examine the effect of hypoxia, BeWo and JEG-3 cells were exposed to 1% O2 for 12 to 48 hours.

**miRNA Overexpression and Inhibition**

Pre-miR precursor molecules (Applied Biosystems) and miRCURY LNA miRNA Power Inhibitors (Exiqon, Vedbaek Denmark) were used in order to achieve overexpression and inhibition of miRNAs, respectively. Transfection of these molecules was performed using Lipofectamine 2000 (Invitrogen).

Notes. Combination of antisense inhibitors of miR-210 and miR-518c was also analyzed. However, combination of these inhibitors did not enhance HSD17B1 mRNA inhibition.

**Luciferase Assays**

Molecular cloning of the 3’-UTR was carried out as follows. First, total RNAs isolated from BeWo, a trophoblastic cell line from a human gestational choriocarcinoma, were converted to cDNAs using PrimeScript reverse transcriptase (Takara Bio). The 3’-UTR of human HSD17B1 mRNA was then amplified from the cDNAs using the following primers: 5’-CCCACCTAGTAAAGGCTTCCTCAGCCG-3’ and 5’-TTTAAGCTTGTAGAGATGGGGGTCTCAGCT-3’. The PCR product was digested with HindIII and SpeI, and cloned into pMIR-REPORT (Applied Biosystems) via the restriction sites of these enzymes. The final construct was designated pMIR-REPORT-HSD17B1.

For luciferase assays, BeWo or HeLa cells were transfected with pMIR-REPORT-HSD17B1 or pMIR-REPORT (for the control) and pRL-tk (a constitutive Renilla luciferase expression plasmid) together with Pre-miR-20a, 210, 451, 518c, 526b*, or Pre-miR-negative control #1 at 20 nM (Applied Biosystems). At 48 hours after the transfection, the cells were processed using the Dual-Luciferase Reporter Assay System (Promega). Luminescence was detected on a TD-20/20 Luminometer (Promega).

**Laser Microdissection (LMD) Followed by Quantitative PCR**

Briefly, trophoblast layer, stroma, and fetal endothelium in intermediate villi of the normal placentas were separately isolated using this system, and total RNAs in these tissues/cells were extracted using ISOGEN-LS reagent (Wako, Osaka, Japan) according to the manufacturer’s protocol. The total RNAs were then subjected to reverse transcription followed by real-time PCR analysis, as previously described.

**miRNA Target Prediction in silico**

Putative targets of individual miRNAs were predicted using an algorithm available online as MicroCosm (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/).

**Sandwich ELISA**

Several 96-well FIA plates (#655077, Greiner, Frickenhasen, Germany) were coated with an anti-HSD17B1 mouse monoclonal antibody (M03, clone 2E5) (Abnova, Taipei City, Taiwan) diluted to 10 μg/mL in sodium carbonate buffer at 4°C overnight. The antibody-coated plates were washed with sodium phosphate buffer containing 0.05% Tween 20 (PBST) and treated with a blocking solution (PBST/5% skim milk) at room temperature for 2 hours. After removing the blocking solution, 50 μL of blood plasma or cell lysates were added to each well and incubated at 37°C for 90 minutes. The plates were then washed with PBST 4 times. An anti-HSD17B1 goat polyclonal antibody (50 μL) (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 30-fold in the blocking solution was added to each well and incubated at room
temperature for 1 hour. After washing the plates again with PBST, 50 μL of a horseradish peroxidase (HRP)-conjugated anti-goat IgG antibody diluted 5000-fold in the blocking solution was added to each well and incubated at room temperature for 1 hour. HRP activity in each well was detected using SuperSignal ELISA Pico (Thermo-Fisher) and GloMax-Multi Microplate Reader (Promega).

To use as a standard for the ELISA assay, we generated a recombinant HSD17B1 protein using a Baculovirus overexpression system. Briefly, the ORF of HSD17B1 was amplified by PCR from human placenta-derived cDNAs using primers to produce N-terminal His tag-linked HSD17B1 protein, and cloned into pPSC8 to generate the HSD17B1 expression plasmid, pPIC8-HSD17B1. Sf9 cells were cotransfected with pPIC8-HSD17B1 and AcNPV baculovirus DNA and cultivated at 28°C for 6 days. The supernatant of the culture was then added to ExpressSF+ cells to allow the infection of recombinant baculoviruses. After 72-h cultivation at 28°C, the infected cells were harvested and lysed. The His tag-linked recombinant HSD17B1 protein was purified using HisTrap FF (GE healthcare). A standard curve was made for each plate to minimize inter-assay variability. Measurement of all samples and standards were run in duplicate to determine intra-assay variability. The value of each sample was determined by interpolation from the standard curve using a five-parameter logistic model.

Statistical analysis

Statistical analyses were conducted using Student’s t-test or the Mann–Whitney U-test. P-values <0.05 were deemed to be statistically significant. These analyses were performed using Excel software embedding a macro software for statistical analysis (SAS Institute, Inc., Cary, NC, USA). The odds ratio and 95% confidence intervals (CIs) were calculated using the CONFIDENCE INTERVAL CALCULATOR (created by Rob Herbert in The University of Sydney) available at http://www.pedro.org.au/english/downloads/confidence-interval-calculator/.

References


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MiRNAs with bold letters indicate C19MC miRNAs. 
Chr, chromosome.
### Table S2 MiRNAs dysregulated in preeclamptic placentas identified by high-throughput sequencing analysis (HTS) and quantitative PCR-based array

(A) Upregulated miRNAs

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**Note:**
- †: Normal Hela (HTS) and PE (Quantitative PCR-based miRNA array) values for comparison.
- §: Fold change calculated as PE/Normal.
- **p**: Significance level of change (PE vs Normal).

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</tr>
</tbody>
</table>

C19MC miRNAs are shown in bold.

* More than 1.5-fold difference between preeclamptic and normal placentas in read frequency, and greater than 0.001% in both placentas.

† Percentage of individual miRNAs in the total miRNA read population in each placenta group.

‡ p values between the expression levels of individual miRNAs of normal (n=10) and preeclamptic (n=8) placentas.
(B) Downregulated miRNAs

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Normal (%)†</th>
<th>PE (%)†</th>
<th>Fold change</th>
<th>miRNA</th>
<th>p (PE vs Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-450b-5p</td>
<td>0.043943</td>
<td>0.029257</td>
<td>0.666</td>
<td>hsa-miR-145</td>
<td>0.0064</td>
</tr>
<tr>
<td>hsa-miR-1307</td>
<td>0.028649</td>
<td>0.018895</td>
<td>0.660</td>
<td>hsa-miR-143</td>
<td>0.0118</td>
</tr>
<tr>
<td>hsa-miR-3615</td>
<td>0.001581</td>
<td>0.001038</td>
<td>0.657</td>
<td>hsa-miR-188-5p</td>
<td>0.0118</td>
</tr>
<tr>
<td>hsa-miR-489</td>
<td>0.001951</td>
<td>0.001274</td>
<td>0.653</td>
<td>hsa-miR-107</td>
<td>0.0157</td>
</tr>
<tr>
<td>hsa-miR-200c</td>
<td>1.636152</td>
<td>1.043472</td>
<td>0.638</td>
<td>hsa-miR-133a</td>
<td>0.0268</td>
</tr>
<tr>
<td>hsa-miR-767-5p</td>
<td>0.003903</td>
<td>0.002473</td>
<td>0.634</td>
<td>hsa-miR-105</td>
<td>0.0343</td>
</tr>
<tr>
<td>hsa-let-7b</td>
<td>0.001011</td>
<td>0.000637</td>
<td>0.630</td>
<td>hsa-miR-224</td>
<td>0.0343</td>
</tr>
<tr>
<td>hsa-miR-1269</td>
<td>0.674988</td>
<td>0.424080</td>
<td>0.628</td>
<td>hsa-miR-551b</td>
<td>0.0343</td>
</tr>
<tr>
<td>hsa-miR-139-5p</td>
<td>0.011365</td>
<td>0.007036</td>
<td>0.619</td>
<td>hsa-miR-342-5p</td>
<td>0.0434</td>
</tr>
<tr>
<td>hsa-miR-584</td>
<td>0.002827</td>
<td>0.001680</td>
<td>0.594</td>
<td>hsa-miR-186</td>
<td>0.0435</td>
</tr>
<tr>
<td>hsa-miR-224</td>
<td>0.118292</td>
<td>0.070137</td>
<td>0.593</td>
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<tr>
<td>hsa-miR-653</td>
<td>0.076766</td>
<td>0.045240</td>
<td>0.589</td>
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</tr>
<tr>
<td>hsa-miR-744</td>
<td>0.002230</td>
<td>0.001279</td>
<td>0.573</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-223</td>
<td>0.043529</td>
<td>0.024807</td>
<td>0.570</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-100</td>
<td>0.001651</td>
<td>0.000934</td>
<td>0.566</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-let-7c</td>
<td>0.004430</td>
<td>0.002444</td>
<td>0.552</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-370</td>
<td>5.368577</td>
<td>2.944669</td>
<td>0.549</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-99a</td>
<td>0.128929</td>
<td>0.069779</td>
<td>0.541</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-125a-5p</td>
<td>0.025949</td>
<td>0.013949</td>
<td>0.538</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-99b</td>
<td>0.015290</td>
<td>0.008187</td>
<td>0.535</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-132</td>
<td>0.896251</td>
<td>0.473648</td>
<td>0.528</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Less than two third difference between preeclamptic and normal placentas in read frequency, and greater than 0.001% in both placentas.
† Percentage of individual miRNAs in the total miRNA read population in each placenta group.
‡ p values between the expression levels of individual miRNAs of normal (n=10) and preeclamptic (n=8) placentas.
Table S3 Ingenuity Pathway Analysis of PE-related miRNA predicting their involvement in reproductive system diseases.

<table>
<thead>
<tr>
<th>Disease/Disorder</th>
<th>p-value</th>
<th># Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive System Disease</td>
<td>1.72x10^-10 – 2.06x10^-2</td>
<td>11 miRNAs (miR-10, miR-17*, miR-19, miR-22, miR-126, miR-142, miR-193*, miR-210, miR-451, miR-515*, miR-590)</td>
</tr>
<tr>
<td>Cancer</td>
<td>8.94x10^-10 – 4.04x10^-2</td>
<td>11 miRNAs (miR-10, miR-17, miR-19, miR-22, miR-126, miR-142, miR-146, miR-193*, miR-210, miR-451, miR-515*)</td>
</tr>
<tr>
<td>Genetic Disorder</td>
<td>6.91x10^-9 – 4.04x10^-2</td>
<td>8 miRNAs (miR-17*, miR-19, miR-22, miR-126, miR-146, miR-193*, miR-210, miR-515*)</td>
</tr>
<tr>
<td>Skeletal and Muscular Disorders</td>
<td>6.91x10^-4 – 1.04x10^-2</td>
<td>4 miRNAs (miR-126, miR-146, miR-193*, miR-210)</td>
</tr>
<tr>
<td>Gastrointestinal Disease</td>
<td>8.40x10^-7 – 4.04x10^-2</td>
<td>5 miRNAs (miR-17*, miR-142, miR-146, miR-210, miR-515*)</td>
</tr>
</tbody>
</table>
Table S4 Clinical characteristics of preeclamptic pregnancies for the analysis of placental miRNAs

<table>
<thead>
<tr>
<th>No</th>
<th>Maternal age</th>
<th>Gestational age at delivery</th>
<th>Onset</th>
<th>Birth weight (g)</th>
<th>Gender</th>
<th>Type of PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>26w0d</td>
<td>Late</td>
<td>572</td>
<td>F</td>
<td>PH</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>28w6d</td>
<td>Early</td>
<td>856</td>
<td>M</td>
<td>PH</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>35w4d</td>
<td>Late</td>
<td>1280</td>
<td>M</td>
<td>Ph</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>35w4d</td>
<td>Late</td>
<td>1854</td>
<td>F</td>
<td>pH</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>39w2d</td>
<td>Late</td>
<td>3356</td>
<td>M</td>
<td>pH</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>34w2d</td>
<td>Early</td>
<td>1866</td>
<td>F</td>
<td>PH</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>37w0d</td>
<td>Late</td>
<td>2650</td>
<td>M</td>
<td>pH</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>37w0d</td>
<td>Late</td>
<td>2446</td>
<td>F</td>
<td>pH</td>
</tr>
</tbody>
</table>

* Early onset, the symptom occurred <32 weeks of gestation; Late onset, the symptom occurred at >=32 weeks of gestation.
† M, male; F, female.
‡ p, proteinuria <2g/day; P, proteinuria >=2g/day; h, SBP:140 to 159 mmHg and/or DBP: 90-109 mmHg; H, SBP>=160 mmHg and/or DBP>=110 mmHg
Table S5 Clinical characteristics of PE pregnancies for the analysis of plasma HSD17B1 levels

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>PE Referred group *</th>
<th>PE Prospective group †</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant woman (n)</td>
<td>35</td>
<td>32</td>
<td>141</td>
</tr>
<tr>
<td>Maternal age (yr)</td>
<td>31.3 ± 6.1</td>
<td>32.8 ± 5.6</td>
<td>32.0 ± 5.4</td>
</tr>
<tr>
<td>Collected plasma sample (n)</td>
<td>35</td>
<td>52 ‡</td>
<td>288 ‡</td>
</tr>
<tr>
<td>Gestational age at blood collection (wk)</td>
<td>25 – 40</td>
<td>19 – 37</td>
<td>19 – 38</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>33.9 ± 4.0</td>
<td>36.8 ± 4.1</td>
<td>39.5 ± 1.3</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>23.0 ± 4.1</td>
<td>25.8 ± 6.3</td>
<td>22.1 ± 4.1</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1814 ± 746</td>
<td>2480 ± 863</td>
<td>3001.3 ± 362.3</td>
</tr>
<tr>
<td>Small-for-gestational-age (%)</td>
<td>20.0</td>
<td>42.9</td>
<td>10.9</td>
</tr>
<tr>
<td>PE onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early onset (Plasma sample) (n)</td>
<td>13 (13)</td>
<td>10 (13)</td>
<td>–</td>
</tr>
<tr>
<td>Late onset (Plasma sample) (n)</td>
<td>22 (22)</td>
<td>22 (39)</td>
<td>–</td>
</tr>
<tr>
<td>Onset weeks (wk)</td>
<td>32.8 ± 4.2</td>
<td>34.3 ± 4.8</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
* Samples collected after PE onset.
† Samples collected for prospective cohort studies.
‡ For some pregnant women, blood was collected at 2 or 3 different gestational ages (20-23, 27-30, and/or 37 weeks of gestation) during pregnancy.
Figure S1. Quantitative analysis of the expression of 667 miRNAs by real-time PCR-based array. Hierarchical clustering of miRNAs based on expression patterns. For individual miRNAs, data are normalized for their expression levels in one of the normal placenta samples (Normal #1). A representative clustering brunch containing many C19MC-derived miRNAs (indicated by blue letters) is shown in red.
Figure S2. Putative recognition sites of miRNAs upregulated in preeclamptic placentas compared with normal placentas. (A) The nucleotide sequences of the 3’-UTR of human HSD17B1 mRNA. The stop codon of this mRNA is shown by boxed nucleotides (UAA). The underline shown as “a” indicates the sequence predicted to be recognized by miR-210. The underline shown as “b” indicates the sequence predicted to be recognized commonly by miR-20a, miR-451, miR-518c, and miR-526*.
Figure S3. Westernblots of normal (n=8) and preeclamptic (n=7) placentas for HSD17B1. Note that one specimen from the normal placentas and two specimens from preeclamptic placentas are missing because protein samples from these specimens were severely degraded. The band intensities of HSD17B1 were normalized for those of TUBA, and were compared between the normal and preeclamptic placentas (Figure 2).
Figure S4. (A) Plasma HSD17B1 levels in normal pregnant women after 20 wk. There was no significant difference in plasma HSD17B1 levels at 20-23 wk, 28-30 wk, and 37 wk. (B) Decreased plasma HSD17B1 levels with preeclamptic pregnancy. There were significant differences in plasma HSD17B1 levels between women with preterm PE (at <37 weeks) and term PE (>37 weeks) when analyses were performed using plasma samples collected at nearly matched gestational ages indicated in each graph. The boxes mark the interval between the 25th and 75th percentiles. The lines inside the boxes denote medians. Statistically significant differences were determined by Mann–Whitney U-test. *P*-values less than 0.05 were considered significant.