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Up-regulation of microRNA-202-3p in first trimester placenta of pregnancies destined to develop severe preeclampsia, a pilot study

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Abstract

MicroRNA expression has not been studied during placentation in pregnancies that develop preeclampsia, where it likely manifests. In this pilot study, miRNA expression in late first trimester placenta from four pregnancies that developed severe preeclampsia matched to controls using the Affymetrix GeneChip® miRNA 3.0 Array identified 9 miRNAs differentially expressed, with MiR-202-3p the most significantly overexpressed in severe preeclampsia. Real-time reverse transcription polymerase chain reaction (qRT-PCR) confirmed overexpression of MiR-202-3p in a validation cohort, with a 7-fold increase in pregnancies that developed severe preeclampsia ($p \leq 0.05$). Differential miRNA expression, specifically miR-202-3p, is seen in first trimester placenta in severe preeclampsia.

Keywords

Chorionic villus sampling (CVS); first trimester placenta; microRNA (miRNA); preeclampsia

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Introduction

Preeclampsia, affecting 2–8% of pregnancies, is associated with risk of maternal and fetal morbidity and mortality. (1–3) Pathogenesis is linked to aberrant trophoblast invasion leading to chronic placental malperfusion, maternal endothelial dysfunction and hypertension with adverse outcomes. (2) Gene expression changes in first trimester placenta occur (4) however, most studies are derived from placental tissue at delivery. (5) Since differences exist between the first and third trimester placenta, (6) detection of early changes are also critical for identification of potential mechanisms.

MiRNAs are small noncoding RNA that control target genes by blocking translation. Single miRNAs can impact hundreds of genes leading to cascading effects. Aberrant miRNA expression is linked to development of disease states (7, 8) which led to the use of miRNA profiling for prognostics and therapeutic targeting.(9–13)

Genome-wide studies of miRNAs in normal and pathological human placentas exist but none in first trimester. (5, 7, 14–17) Thus, the goal of this pilot study was to identify differential miRNA expression in first trimester placenta of pregnancies that developed severe preeclampsia to understand early pathogenesis.

Methods

Subjects were identified from the Cedars-Sinai Medical Center (CSMC) Prenatal Biorepository, through an IRB protocol. Chorionic villi from 11–13 week gestations were placed in RNAlater (Qiagen, Valencia CA) and stored as described. (18)

Severe preeclampsia was defined per American College of Obstetricians and Gynecologists Guidelines (19). Patients with medical complications (pregestational/gestational diabetes, chronic hypertension, tobacco dependence), or multiple gestation were excluded.

Four samples were identified from subjects that developed severe preeclampsia and matched with controls for gestational age at CVS (+/− 6 days), fetal sex, parity, which delivered at term without complications.

The validation cohort of 4 CVS samples from subjects that developed severe preeclampsia were matched with 4 controls for fetal sex and maternal parity.

Specimens were transferred to RNA-Bee reagent (Fisher Scientific) and RNA extracted using the RNA-Bee manufacturer's method. Quantity and quality was assessed with NanoDrop 8000 Spectrophotometer and Agilent 2100 Bioanalyzer.

Total RNA was labeled with FlashTag™ Biotin HSR RNA Labeling Kit. Hybridization was carried out on an Affymetrix GeneChip® miRNA 3.0 Array containing 5,639 human targets. Arrays were scanned using the Affymetrix® GeneChip® Scanner 3000. MiRNA expression was analyzed using Affymetrix Expression Console.

Comparative real-time reverse transcription polymerase chain reaction (qRT-PCR) was used in the validation cohort. RNA was reverse-transcribed using microRNA-specific primers

(Qiagen Inc). cDNA was amplified using Qiagen miScript PCR System on the Viia7 Real Time PCR machine (Applied BioSystems).

Descriptive statistics were performed utilizing StataIC 13. T-tests, Mann Whitney U and Chi-square was used as appropriate.

Exploratory Principal Component Analysis (PCA) was generated to evaluate the samples using Rgl package (Version 0.93) in R and Bioconductor. ANOVA was used for batch effects and compare data across groups. qRT-PCR was analyzed using ΔCT values quantitated from comparison to the reference miRNA, RNU6-2 using the Mann-Whitney U test.

Results

For the microarray study, maternal age, BMI, race, parity, and gestational age at CVS were similar. All fetuses were females. Gestational age at delivery (250 ± 32.9 vs 275 ± 9.1 days) and birth weight (2351 ± 1110.15 vs 3617 ± 564.2 g) were also similar. However, the median birth weight percentiles were lower in subjects that developed preeclampsia (10^{th} vs 71^{st}, p=0.0421).

For the validation cohort for qRT-PCR studies, maternal age, BMI, race, parity and gestational age at CVS were similar. Gestational age at delivery (233 ± 38 vs 279 ± 6 days, p=0.0548), birth weight (1670 ± 957 vs 3045 ± 143 g, p=0.0295) and birth weight percentiles (3^{rd} vs 19.5^{th}, p=0.0209) were lower in the pregnancies that developed preeclampsia.

Nine miRNAs were differentially expressed in severe preeclampsia compared to controls (Table 1). MiR-202-3p was the most differentially expressed miRNA and therefore qRT-PCR of miR-202-3p was performed in a validation cohort. It was increased 7-fold in preeclampsia compared to control pregnancies (Figure 1).

Discussion

Nine miRNAs were differentially expressed in severe preeclampsia compared to controls in late first trimester placenta. MiR-202-3p, the most significantly overexpressed miRNA in microarray studies was also overexpressed in a validation cohort using qRT-PCR.

Mi-202, encoded on chromosome 10, is the precursor of two different mature miRNA sequences from the same stem-loop, miR-202-3p and miR-202-5p, each having different targets. (http://www.ncbi.nlm.nih.gov/nuccore/NR_030170; http://mirbase.org/cgi-bin/mirna_summary.pl?fam=MIPF0000121) MiR202-3p was upregulated in first trimester placenta from pregnancies that developed severe preeclampsia. MiR-202-3p promotes apoptosis and inhibits proliferation in cancer cells by post-transcriptionally targeting expression of various proteins. (9, 20, 21) Among the other differentially expressed miRNA's, miR-451 and miR-548 also inhibit proliferation, miR-451 with tumor-suppressor properties in colon cancer cell lines and miR-548 with tumor-suppressor properties and pro-apoptotic effects in breast cancer cell lines. (22, 23) The overexpression of miR-202-3p, as well as miR-451 and miR-548, in the late first trimester placenta might have a similar role in

apoptosis and inadequate trophoblast proliferation and invasion, however, this remains to be determined.

Differential expression of miRNAs in the third trimester placenta that develop preeclampsia have been described.(14) However, miR202-3p was not described. Oxidative stress may alter the expression of miRNAs in placenta (24) and chronic hypoxia in preeclampsia may lead to changes in placental miRNA expression throughout gestation. Our studies utilized samples early in gestation, identifying potential events during placentation that can lead to severe preeclampsia, before clinical onset, which may permit assessment of cause rather than effects of disease.

Limitations include the small sample size. However, significant differences in miRNA expression by microarray in the initial cohort was confirmed in a validation cohort. The majority of samples were from Caucasian women of advanced reproductive age, thus results may not be applicable to all groups. Larger studies are necessary.

Our findings support existing evidence that fundamental biological processes leading to severe preeclampsia occur in the first trimester (2, 25) and miRNA expression is altered in first trimester placenta of women who develop severe preeclampsia, which differs from the third trimester. The biologic function of the identified novel candidate miRNA miR-202-3p in first trimester placenta warrants further investigation and may ultimately be used as a biomarker for development of preeclampsia.

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Highlights

MiR-202-3p is expressed early in gestations that develop severe preeclampsia.

MiR-202-3p is 7-fold higher in first trimester placenta developing preeclampsia.

Differences in miRNA expression exist among first and third trimester placenta.

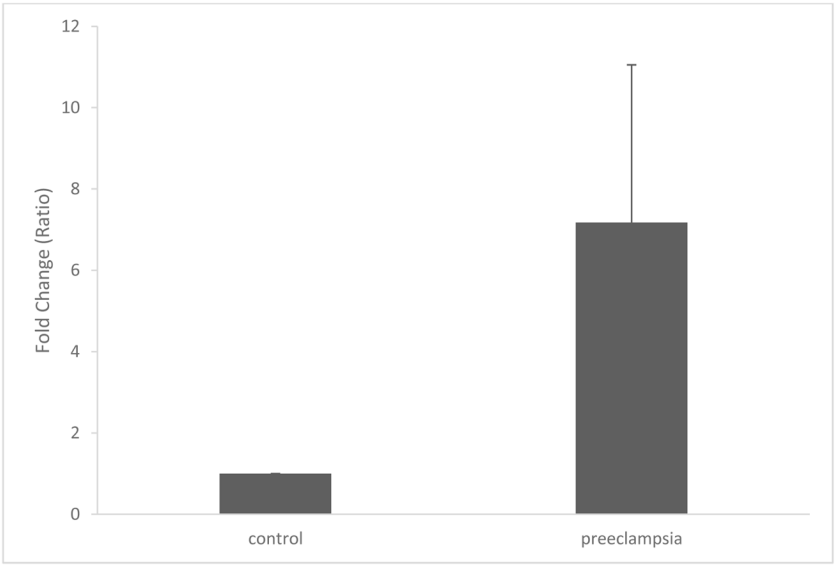

Figure 1. qRT-PCR with miR-202-3p

qRT-PCR of miR-202-3p is increased 7-fold in severe preeclampsia (fold change ± SD). P< 0.05.

Table 1

Differentially Expression MicroRNAs in Microarray Scan

MicroRNA	*P* value *	Fold change
has-miR-202-3p_st	0.000115112	2.21
hp_hsa-mir-320b-1_x_st	0.000215714	−1.5
has-miR-432-star_st	0.00749553	−1.7
ENSGOOOOO221611_st	0.0129797	1.53
hsa-miR-25-star_st	0.0261407	1.66
hsa-miR-4701-3p_st	0.0306885	−1.83
hsa-miR-451_st	0.0314716	2.15
hp_hsa-miR-933_st	0.0383562	−1.53
has-miR548aj_st	0.0384967	2.03

* ANOVA