BASIC SCIENCE: OBSTETRICS

Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies

Xiao-ming Zhu, MD; Tao Han, MD; Ian L. Sargent, PhD; Guo-wu Yin, MD; Yuan-qing Yao, MD, PhD

OBJECTIVE: The purpose of this study was to perform a comprehensive analysis of the microRNA expression profile in placentas from preeclamptic pregnancies vs normal placentas.

STUDY DESIGN: Placentas were obtained from patients with (1) mild preeclampsia ($n = 8$) and (2) severe preeclampsia ($n = 15$) and (3) in a normal control group ($n = 11$) with elective cesarean delivery. The microRNA expression profile was assessed by microRNA microarray and real-time reverse transcriptase-polymerase chain reaction analysis.

RESULTS: Thirty-four microRNAs were expressed differentially in preeclamptic placentas, compared with normal placentas. Of these, 11 microRNAs were overexpressed, and 23 microRNAs were underexpressed in preeclamptic pregnancies. Notably, several microRNA clusters on human chromosome 19q13.42, 13q31.3, Xq26.2, Xq26.3, and 14q32.31 (a human imprinted region) were expressed differentially in preeclamptic placentas. These results were confirmed with the use of real-time polymerase chain reaction for selected microRNAs (miR-210, -152, -411, and so on).

CONCLUSION: The results show that 34 microRNAs are deregulated in preeclamptic pregnancies, which suggests the involvement of these microRNAs in the pathogenesis of preeclampsia.

Key words: microarray, miRNA, placenta, preeclampsia

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Preeclampsia is a common pregnancy-specific syndrome that is characterized by hypertension and proteinuria. It is a disorder that affects at least 5% of all pregnancies worldwide[1] and is a leading cause of maternal and perinatal morbidity and death. Although the cause of preeclampsia is unknown, it is agreed generally that preeclampsia results from the presence of a placenta[2] because the only treatment for preeclampsia is the delivery of the placenta, after which the symptoms regress rapidly. Evidence shows that preeclampsia is associated with alterations in placental microRNA (miRNA) expression.[3]

miRNAs are noncoding RNAs of 21-24 nucleotides that function as negative regulators of gene expression by antisense complimentarily to specific messenger RNAs. The miRNAs are versatile and ubiquitous. Several hundred mammalian miRNAs have been identified, and speculations about the total number of human miRNAs exceed 1000.[4] Recent data indicate that miRNAs play a fundamental role in diverse biologic and pathologic processes, which include cell proliferation, differentiation, apoptosis, animal development, carcinogenesis, cardiovascular disease, and primary muscular disorders.[5,6] Studies of miRNA expression have revealed that miRNA expression is tissue specific and that miRNAs are expressed abundantly in human placenta.[7,8]

It has been reported previously that miR-210 and -182 are expressed differentially in the human placentas of patients with preeclampsia, compared with control subjects, with the use of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.[3] However, a comprehensive list of the human miRNAs has not been screened in preeclampsia placentas. In the present study, we performed a comprehensive analysis of the miRNA expression profile in placentas from preeclamptic pregnancies with the use of microarray analysis and real-time RT-PCR confirmation to elucidate the relationship between miRNA and preeclampsia.

MATERIALS AND METHODS

Sample collection

Placental tissues were obtained with informed consent from nulliparous

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From the Department of Obstetrics and Gynecology (Drs Zhu, Yin, and Yao), Tangdu Hospital, The Fourth Military Medical University, Xi'an, China; the Health Department, Automotive Management Institute of People's Liberation Army (Dr Han), Beng Bu, China; and the Nuffield Department of Obstetrics and Gynecology (Dr Sargent), University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.

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Reprints: Yuan-qing Yao, MD, PhD, Department of Obstetrics and Gynaecology, Tangdu Hospital, Xin Si St., Xi'an 710038, China. rao_yu_zxm@hotmail.com.

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TABLE 1

Clinical characteristics of normal and preeclamptic pregnancies

	Group			*P* value[a]		
Variable	**Control (n = 11)**	**MPE (n = 8)**	**SPE (n = 15)**	**Control vs mPE**	**Control vs sPE**	**MPE vs sPE**
Maternal age (y)	31.8 ± 3.7	29.5 ± 5.3	31.9 ± 3.8	.239	.977	.203
Gestational age (wk)	37.5 ± 2.2	37.6 ± 1.4	35.9 ± 2.7	.876	.109	.107
Birthweight (g)	3135 ± 377	3094 ± 322	2947 ± 532	.841	.293	.455
Prepregnancy body mass index (kg/m^2)	24.8 ± 2.2	25.9 ± 2.5	24.0 ± 2.4	.342	.381	.077
Proteinuria (g/24 h)	Not detected	0.9 ± 0.3	2.8 ± 1.6	.090	< .01	< .01
Systolic blood pressure (mm Hg)	111 ± 9.0	145 ± 7.1	170 ± 26.0	< .01	< .01	< .01
Diastolic blood pressure (mm Hg)	69 ± 5.8	96 ± 5.6	113 ± 18.1	< .01	< .01	< .01

All results are presented as mean ± SD.

[a] Obtained with the 1-way analysis of variance with SPSS software (version 11; SPSS Inc, Chicago, IL).

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TABLE 2

Primers used for real-time RT-PCR

Gene name	**Primer**	**Sequence (5′-3′)**
Has-miR-210	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtcagcc
	PCR	Forward: TGTGCGTGTGACAGCGGC
Has-miR-152	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcccaag
	PCR	Forward: GTCGTCAGTGCATGACAGAACTT
Has-miR-411	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcgtacg
	PCR	Forward: GCCGTAGTAGACCGTATAGCG
Has-miR-377	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacaaaa
	PCR	Forward: GCGATCACACAAAGGCAACT
Has-miR-518b	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacctct
	PCR	Forward: GCAAAGCGCTCCCCTTTAG
Has-miR-18a	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtatctg
	PCR	Forward: GTGCTAAGGTGCATCTAGTGCAG
Has-miR-363	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtacaga
	PCR	Forward: TCGAATTGCACGGTATCCATC
Has-miR-542-3p	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtttcag
	PCR	Forward: GCGGCTGTGACAGATTGATAAC
	Universal	Reverse: GTGCAGGGTCCGAGGT (for PCR)
U6	RT	AACGCTTCACGAATTTGCGT
	PCR	Forward: CTCGCTTCGGCAGCACA
		Reverse: AACGCTTCACGAATTTGCGT

Has, Homo sapiens.

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