Deregulated microRNA species in the plasma and placenta of patients with preeclampsia

SHENG YANG[1*], HAILING LI[2*], QINYU GE[3], LI GUO[1] and FENG CHEN[1]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu 211166;
[2]Department of Obstetrics and Gynecology, Zhongda Hospital, Southeast University, Nanjing, Jiangsu 210009;
[3]State Key Laboratory of Bioelectronics, Southeast University, Nanjing, Jiangsu 210096, P.R. China

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Abstract. Emerging evidence indicates that microRNAs (miRNAs), a class of small non-coding RNAs, are involved in a number of biological processes. The results of SOLiD™ sequencing were used to analyze differentially expressed miRNA profiles in the plasma and placenta of patients with preeclampsia (PE) and a subject who had had a pregnancy without complications. miRNAs were identified that were consistently expressed in the placenta, following normalization of the raw data. miRNAs that had increased and differential expression were selected, as defined by percentage >0.02% and a log2 fold change ≥|1.2|, respectively. This process was repeated in the plasma. Twenty such miRNAs were identified. These were: miR-126, miR-126*, miR-130a, miR-135b, miR-142-3p, miR-149, miR-188-5p, miR-18a, miR-18b, miR-203, miR-205, miR-224, miR-27a, miR-29a, miR-301a, miR-517c, miR-518-3p, miR-518e, miR-519d and miR-93. These miRNAs belonged to 13 clusters or families. However, only four clusters or families involved two or more of these miRNAs. These were the mir-16 cluster, the mir-17 family, the mir-130 family and the mir-517 family. These abnormally-expressed miRNAs and miRNA gene clusters or families are known to be involved in a number of biological processes. Gene enrichment analysis was used to investigate the pathways involved in the development of PE. In conclusion, the miRNAs identified in this study as being abnormally expressed in PE, may be useful as non-invasive diagnostic biomarkers. Co-regulated mRNAs and possible causal pathways involved in the pathogenesis of PE were also identified.

Correspondence to: Professor Feng Chen, Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, 818 East Tianyuan Road, Nanjing, Jiangsu 211166, P.R. China
E-mail: fengchen@njmu.edu.cn

*Contributed equally

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Introduction

Preeclampsia (PE) is a vascular disorder, which presents with hypertension and proteinuria during pregnancy. It is a consequence of a number of pathophysiological processes, including endothelial dysfunction and systemic inflammation. It is a key risk factor for maternal and fetal morbidity and mortality worldwide (1-3). Although the exact mechanisms underlying the development of PE remain unclear, disorders of maternal tissue, and maternal obesity and insulin resistance are likely to be involved. Pathological manifestations associated with this condition include poor placentation, shallow placental invasion and abnormal angiogenesis. (4-7).

MicroRNAs (miRNAs) are single-stranded non-coding RNAs (ncRNAs) composed of 18-24 nucleotides. They contribute to numerous biological regulatory processes, such as tumorigenesis, cell proliferation, cell differentiation and apoptosis, by inducing the silencing of target messenger RNAs (mRNAs) (8-11). During the formation of these molecules two types of intermediate miRNA are created: Primary miRNA (pri-miRNA) and precursor miRNA (pre-miRNA). pri-miRNA, a long transcript from the 3'-untranslated region, is cleaved by the Drosha enzyme to form pre-miRNA in the nucleus. The cytoplasmic enzyme, Dicer, then processes pre-miRNA into mature miRNA. The RNA-induced silencing complex is then generated by the miRNA and catalyzes cleavage of a single phosphodiester bond on the mRNA target (12-14). Differentially expressed miRNAs are involved in certain human diseases and may have a use as biomarkers of these conditions (6,15-18). For example, upregulated miR-155 is known to be a risk factor for PE, via its regulation of cysteine-rich angiogenic inducer 61 (19).

Recently, a number of studies have suggested that differentially expressed miRNAs may be involved in the development of PE, since the biological processes involving these miRNAs have been demonstrated to be similar to those involved in PE (20-22). miRNAs may be useful as biomarkers for the diagnosis of PE. However, the results from microarray studies are inconsistent, and thus further investigation is required in order to reliably use this method of analysis in clinical practice. For example, miR-182 exhibited different levels of expression levels in two different studies (21,22). Therefore in the present study, sequencing technology was used to detect

Table I. Clinical information from four patients with preeclampsia.

Patient	Severity	Age (years)	Gender of infants	Length of pregnancy (days)
m1	Mild	27	Male	280
m2	Mild	26	Male	283
s1	Severe	34	Female	244
s2	Severe	28	Female	244

m, mild preeclamsia; s, severe preeclampsia.

the differentially, highly and consistently expressed miRNAs that may be associated with the development of PE. miRNA expression was measured in the plasma and placenta of patients with mild and severe PE. Based on the results of these experiments, a genome-wide screen for the deregulated miRNAs was conducted. Simultaneously, the miRNA gene clusters or families to which these miRNAs belong were defined. Functional enrichment analyses were conducted in order to predict the pathways involved in PE and the interaction between the target mRNAs.

Materials and methods

Sample collection and small RNA sequencing. Samples were obtained from five subjects who had delivered by elective cesarean section. They comprised four patients with PE and one subject with a pregnancy without complications, who were recruited from Zhongda Hospital (Nanjing, China). The study protocol was approved by the Research Ethics Board of Zhongda Hospital. Written informed consent was obtained prior to blood sample collection. Maternal plasma and placenta samples were collected from a normal pregnant female and the patients with PE. Two patients were diagnosed with mild PE (mPE group) and two with severe PE (sPE group; Table I). TRIzol® (Invitrogen Life Technologies, Carlsbad, CA, USA) was used to extract total RNA. mirVana™ miRNA Isolation kit (Ambion Life Technologies, Austin, TX, USA) was used to isolate small miRNA from total RNA. An miRNA library was constructed according to the manufacturer's instructions for the use of SOLiD™ Small RNA Expression kit (Invitrogen Life Technologies). SOLiD sequencing platform (Applied Biosystems Life Technologies, Foster City, CA, USA) was used to sequence miRNAs. The sequencing process was completed at the State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, (Nanjing, China).

Raw sequencing datasets were obtained from other ncRNAs, including small nucleolar RNAs, transfer RNAs and ribosomal RNAs. Remaining reads were mapped to the known human pre-miRNAs in the miRBase database (Release 16.0, http://www.mirbase.org/) with Bowtie 0.12.7 (23). Regardless of the adaptor sequence, only one mismatch was permitted.

Identification of differentially expressed miRNAs and their targets. Raw data was normalized. The percentage of each miRNA from a single sample was taken as its expression level. miRNAs that were consistently expressed in all samples were selected, as these were assumed to be deregulated in patients with mild and severe preeclampsia. Differentially- and highly-expressed miRNAs were identified. Highly expressed miRNA were defined as those where the percentage of the expression levels in a single sample was >0.02. When the difference of the logarithmic value of the level of an miRNA between patients with PE and the subject with a normal pregnancy was >1.2 or <-1.2, it was defined as a deregulated miRNA. Differentially and highly expressed miRNAs in the plasma and placenta were thus selected for further analysis. Gene clusters or families of deregulated miRNAs were identified by miRBase (23). Three datasets, including miRanda, TargetScan and miRTarBase, were used to identify the targets of deregulated miRNAs (24-26). One target was found in at least two datasets. The use of more than one dataset was employed in order to reduce the rate of false positive results.

Functional enrichment analysis. The targets of common miRNAs were investigated by functional enrichment analysis. mRNAs in which the frequency of targeting by miRNAs was ≥2 were enriched by the Gene Ontology Biological Process (GOBP) database and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (27). P-values represented the probability of the involvement of the pathways enriched by the genes. q values represent the false discovery rate (28). $P<0.05$ was considered to indicate a statistically significant difference. The targets identified by this process may have a causal role in the development of PE.

Results

Deregulated miRNAs and their families. The total number of miRNAs detected was 905. The number of miRNAs consistently expressed in placenta and plasma were 734 and 269, respectively. Highly-expressed miRNAs were selected from these groups in the placenta and plasma (159 and 109, respectively). In the placenta, 71 differentially-expressed miRNAs (26 upregulated and 45 downregulated) were identified (Fig. 1A). In the plasma, 94 differentially-expressed miRNAs (81 upregulated and 13 downregulated) were identified (Fig. 1B). Notably, the abnormally-expressed miRNAs observed in the plasma and placenta were all upregulated, including miR-126, miR-126*, miR-130a, miR-135b, miR-142-3p, miR-149, miR-188-5p, miR-18a, miR-18b, miR-203, miR-205, miR-224, miR-27a, miR-29a, miR-301a, miR-517c, miR-518-3p, miR-518e, miR-519d and miR-93 (Table II). Although expression levels were abnormal, they differed between the placenta

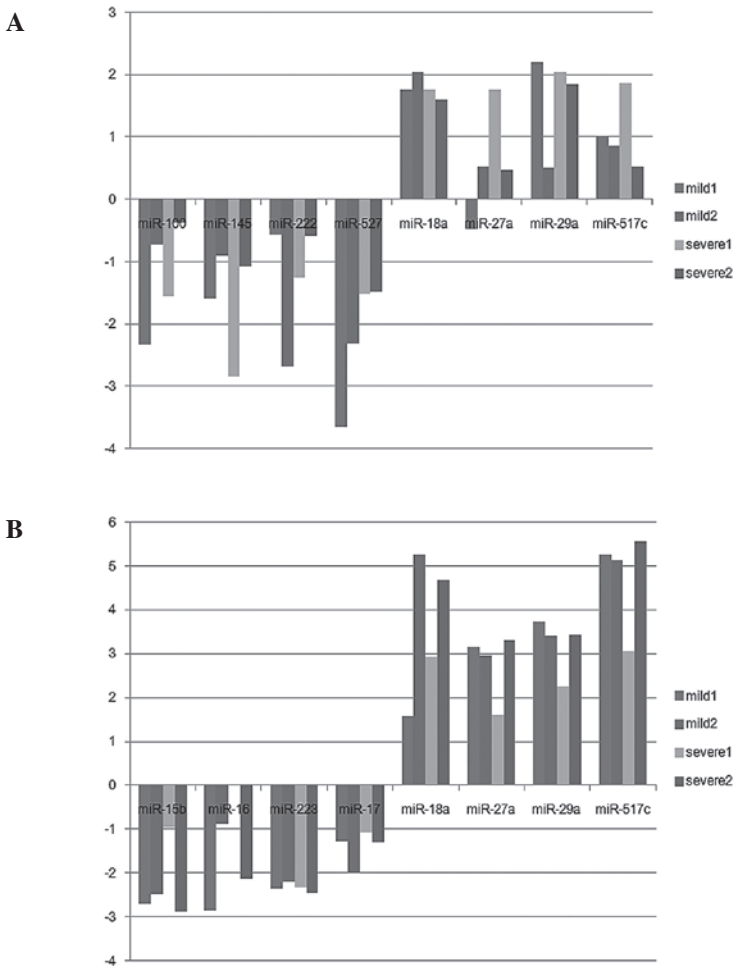

Figure 1. Dynamic expression levels of the differentially expressed miRNAs in the placenta and plasma. (A) Eight differentially expressed miRNAs in the placenta were identified. (B) Eight differentially expressed miRNAs in the plasma were identified. miRNA, microRNA.

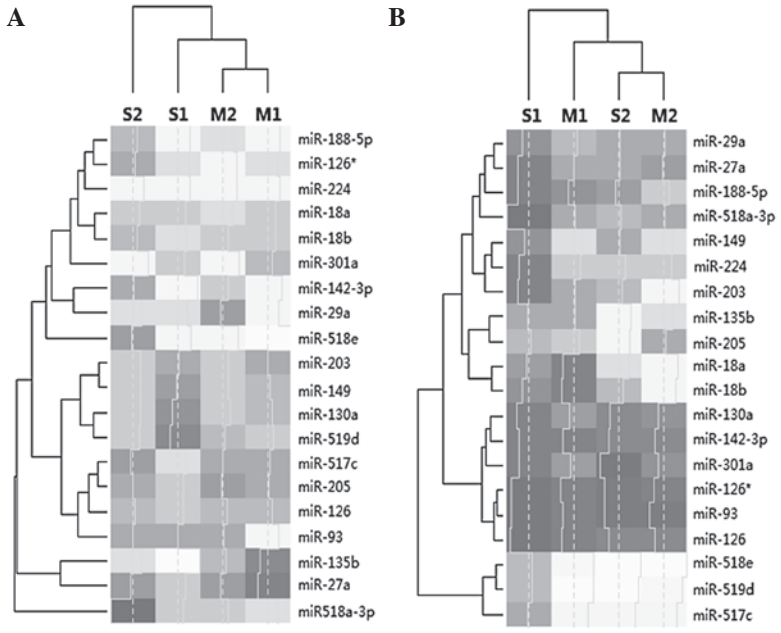

Figure 2. Heatmaps of common differentially expressed miRNAs in the placenta and plasma. Grey represents high levels of expression and white represents low levels of expression. (A) Expression level of differentially expressed miRNAs in the placenta. (B) Expression level of differentially expressed miRNAs in the plasma. miRNA, microRNA; s, severe preeclampsia; m, mild preeclampsia.

Table II. Association of differentially expressed miRNAs in placenta and plasma from patients with mild or severe preelampsia.

A, Placenta

miRNA	Mild		Severe	
	log2FC	%[a]	log2FC	%[a]
miR-126	1.36	0.89	1.29	0.85
miR-126*	2.35	0.99	1.68	0.62
miR-130a	1.57	2.16	1.15	1.61
miR-135b	0.71	0.02	2.96	0.11
miR-142-3p	2.10	0.07	2.49	0.10
miR-149	1.33	0.03	1.25	0.03
miR-188-5p	2.23	0.02	1.90	0.01
miR-18a	1.90	0.06	1.68	0.06
miR-18b	1.60	0.03	1.66	0.03
miR-203	1.23	0.02	1.40	0.02
miR-205	0.79	0.06	1.23	0.08
miR-224	2.45	0.17	2.23	0.15
miR-27a	0.10	0.49	1.25	1.09
miR-29a	1.58	1.80	1.94	2.31
miR-301a	2.23	0.07	2.23	0.07
miR-517c	0.94	2.53	1.34	3.35
miR-518a-3p	1.78	0.11	0.61	0.05
miR-518e	3.26	0.96	1.84	0.36
miR-519d	1.30	6.52	0.94	5.09
miR-93	1.65	0.05	0.81	0.03

B, Plasma

miRNA	Mild		Severe	
	log2FC	%[a]	log2FC	%[a]
miR-126	1.67	0.35	1.18	0.25
miR-126*	1.51	0.37	0.81	0.23
miR-130a	2.51	0.45	1.66	0.25
miR-135b	4.05	0.05	4.34	0.06
miR-142-3p	1.74	0.07	1.73	0.07
miR-149	4.52	0.06	3.03	0.02
miR-188-5p	3.35	0.03	2.20	0.01
miR-18a	4.36	0.34	4.05	0.27
miR-18b	4.05	0.18	3.26	0.11
miR-203	4.81	0.04	2.96	0.01
miR-205	3.75	0.13	4.35	0.20
miR-224	4.21	0.10	3.41	0.06
miR-27a	3.06	0.54	2.69	0.42
miR-29a	3.57	1.51	2.96	0.98
miR-301a	2.50	0.07	1.19	0.03
miR-517c	5.19	2.11	4.79	1.60
miR-518a-3p	3.35	0.04	2.62	0.03
miR-518e	6.35	0.45	5.65	0.28
miR-519d	6.06	5.24	5.37	3.24
miR-93	1.35	0.05	0.87	0.04

[a]Percentange of miRNA. miRNA, microRNA.

Table III. Summary of association of targets (frequency≥6) and their miRNAs.

Target	Frequency	miRNA
AAK1	7	miR-130a,miR-149, miR-188-5p, miR-203, miR-205,miR-27a, miR-93
ANKRD52	6	miR-149, miR-18a, miR-203, miR-224, miR-29a, miR-519d
FBXO45	6	miR-135b, miR-142-3p, miR-188-5p, miR-203, miR-27a, miR-29a
LCOR	6	miR-130a, miR-142-3p, miR-203, miR-205, miR-224, miR-27a
NFIB	6	miR-142-3p, miR-149, miR-203, miR-205, miR-224, miR-27a
NRP2	6	miR-130a, miR-149, miR-188-5p, miR-224, miR-27a, miR-93
PTEN	6	miR-188-5p, miR-18a, miR-205, miR-29a, miR-301a, miR-519d
RLIM	6	miR-130a, miR-203, miR-205, miR-27a, miR-29a, miR-518e

Frequency, frequency with which mRNA was targeted by dysregulated miRNAs. miRNA, microRNA; AAK1, adapto-associated protein kinase; ANKRD2, ankyrin repeat domain 2; FBXO45, F-Box protein 45; NFIB, nuclear factor I/B; NRP2, neuropilin-2; PTEN, phosphotase and tensin homoglog; RLIM, ring finger protein, LIM domain.

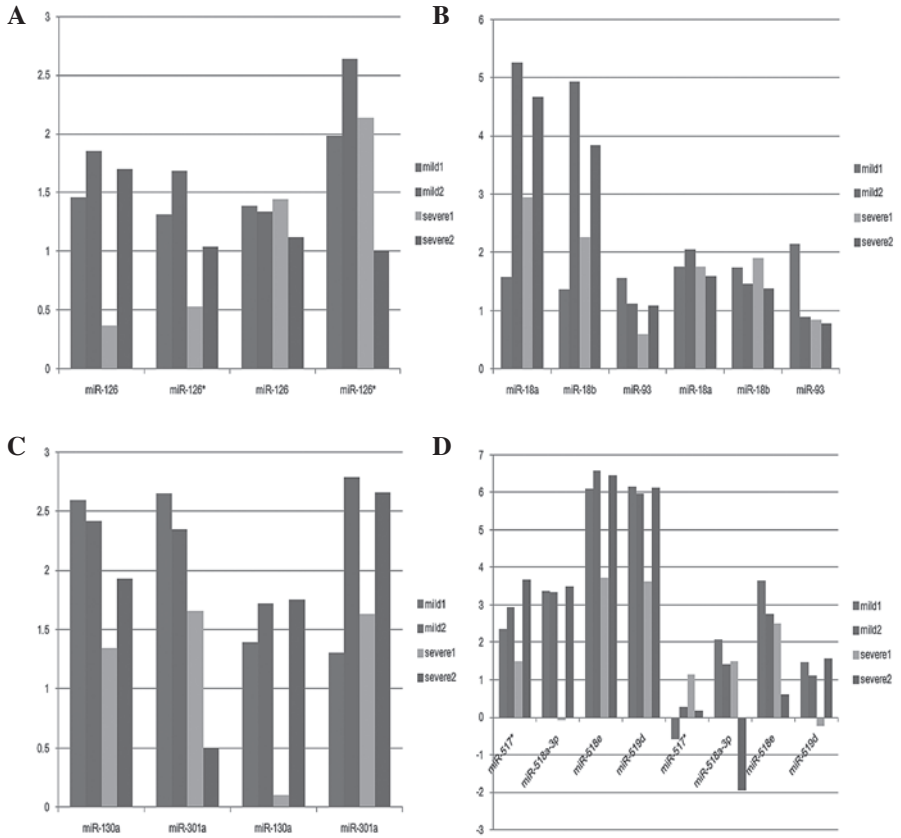

Figure 3. Dynamically expressed levels of miRNAs from the same family or cluster in patients with mild and severe preeclampsia. Solid lines, expression levels of miRNAs in plasma; dotted lines, expression levels of miRNAs in the placenta. (A) Expression levels of the miR-16 cluster, (B) expression levels of the miR-17 family, (C) expression levels of the mir-130 family and (D) expression levels of the mir-517 family. miRNA, microRNA.

and plasma (Fig. 2). The miRNAs identified, were members of 13 gene families or clusters, of which four families contained two or more of these aberrantly expressed miRNAs: miR 126, miR-126 and miR-126*; mir 17 family, miR-18a, miR-18b and miR-93; miR 130 family, miR-130a and miR-301a; and miR 515 family, miR-517*, miR-518a-3p, miR-518e and miR-519d. miRNAs involving the same family exhibited different expression levels (Fig. 3). These deregulated miRNAs were selected from 905 miRNAs following three experimental processes; therefore they may have been the causal miRNAs and their families may be the causal gene families.

Target mRNAs and gene enrichment analysis. The targets of deregulated miRNAs were predicted from three datasets, in order to increase the accuracy of this prediction. 3,818 mRNAs were identified as targets of the 20 deregulated miRNAs. 1,215 mRNAs were found to be regulated by ≥2 miRNAs. Adaptor-associated protein 1 (AAK1), ankyrin

Table IV. Top ten pathways of targets by GOBP.

GOBPID	Term	Count	P
GO:000635	Regulation of transcription, DNA-dependent	200	7.91 E-205
GO:0006350	Transcription	164	1.56 E-146
GO:0007275	Development	108	7.47 E-80
GO:0007165	Signal transduction	111	2.05 E-65
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	41	6.42 E-55
GO:0006468	Protein amino acid phosphorylation	54	1.91 E-53
GO:0003155	Cell adhesion	43	1.59 E-36
GO:0007399	Nervous system development	40	1.03 E-31
GO:0016568	Chromatin modification	26	1.49 E-31
GO:0019941	Modification-dependent protein catabolism	33	3.49 E-29

GOBP, Gene Ontology Biological Processes database; count, number of genes involved in the pathway.

Table V. Top ten pathways of targets by KEGG.

Pathway	Count	P	q
Focal adhesion	37	1.74 E-26	2.61 E-24
Colorectal cancer	23	2.32 E-21	4.97 E-20
MAPK signaling pathway	36	6.71 E-21	1.01 E-19
Wnt signaling pathway	28	1.27 E-20	1.74 E-19
ErbB signaling pathway	21	2.24 E-18	1.98 E-17
Axon guidance	23	8.84 E-17	5.76 E-16
Regulation of actin cytoskeleton	28	2.44 E-16	1.46 E-15
Insulin signaling pathway	21	4.39 E-14	2.12 E-13
Chronic myeloid leukemia	16	2.00 E-13	8.63 E-13
Prostate cancer	17	2.01 E-13	8.63 E-13

KEGG, Kyoto Encyclopedia of Genes and Genomes database; MAPK, mitogen-activated protein kinase; count, number of genes involved in the pathway.

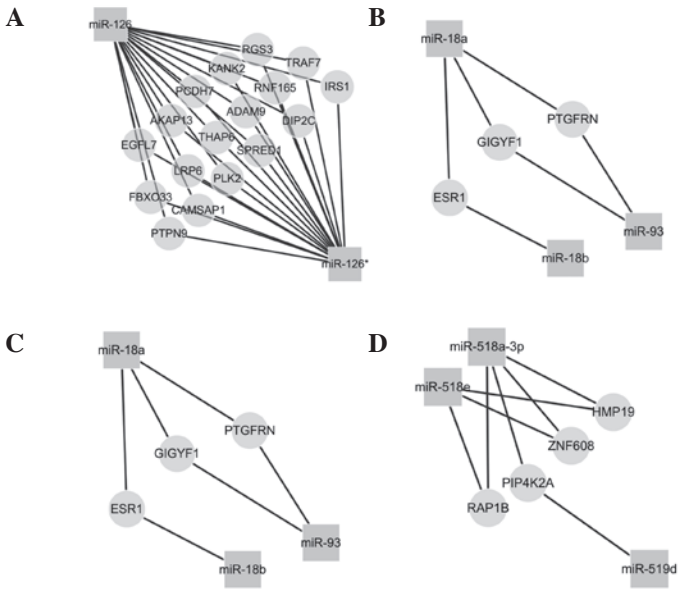

Figure 4. Associations between miRNAs from the same gene family and mRNAs. Triangles, miRNAs; circles, co-regulated mRNAs and squares, other targets of miRNAs. (A) mir-126 family, (B) mir-130 family, (C) mir-17 family and (D) mir-515 family. miRNA, microRNA; mRNA, messenger RNA.

repeat domain 2, F-Box protein 45, LOCR, nuclear factor I/B, neuropilin 2, phosphatase and tensin homolog (PTEN) and ring finger protein, LIM domain were found to be regulated by six or more deregulated miRNAs (Table III). It is hypothesized that these mRNAs were therefore associated with the development of PE. Within the same gene family, these miRNAs may regulate similar targets, and may thus be involved in similar biological processes (Fig. 4). Furthermore, these mRNAs were investigated using gene enrichment analysis by GOBP and KEGG (Tables IV and V). From GOBP, these targets were enriched into 1,337 pathways, amongst which 1,013 significant targets were identified ($P<0.05$). Targets (200) were enriched in the regulation of transcription (GOBPID: 0006355), which may be one of the causal pathways in the pathogenesis of PE. In addition, 75 significant pathways were identified from 119 pathways from the KEGG database. Focal adhesion, in which including 37 targets were enriched, may be another pathway involved in this disease process. Although the enrichment theories of GOBP and KEGG differed, the pathways enriched by each of them were essential.

Discussion

It was clear that the expression of miRNAs in the placenta was higher than that in the plasma. In addition, accounting for cluster analysis, the expression patterns in the placentas of the two patients with mild PE were more similar that those of the patients with severe PE. Therefore, the classification of the placenta should be performed prior to the analysis of plasma. However, miRNAs detected in plasma are more readily available as a non-invasive biomarker for screening in PE (29,30). It therefore appears logical to focus on those biomarkers that are deregulated in the plasma and placenta, and which are thus accessible and also discriminative.

Furthermore, a single miRNA may be expressed to a different degree between patients and between different tissues in the same patient. Common miRNAs may be utilized as non-invasive biomarkers, particularly in placental diseases and PE (30,31). miR-126 is generated from the EGFl 7 gene in mice and is known to indirectly increase the actions of proangiongenic factors, vascular endothelial growth factor (VEGF) and fibroblast growth factor by diminishing Spred-1 (32-34). Increased levels of proangiogenic factors may promote the development of PE (35). The deregulation of miR-126 in this disease may suggest a high level of expression of certain proangiogenic factors, and the consistency of expression further indicates that miR-126 is important in the pathogenesis of PE. Furthermore, certain deregulated miRNAs are related to the development of hypoxic trophoblasts which may be essential in the pathogenesis of PE (36,37). For example, when the trophoblast is exposed to hypoxia, miR-205 depresses mediator of RNA polymerase II transcription subunit 1 (MED1), improving placental development. This indicates that it is essential in trophoblast injury (32,38). In the present study, the consistently upregulated expression of this miRNA in patients with mild and severe preeclampsia provides further evidence for this hypothesis. Thus, the deregulated miRNAs may influence the development and generation of PE through regulation of their various targets, including VEGF and MED1.

The predicted targets and their downstream pathways are therefore also likely to be important factors in the development of PE. It is likely that the predicted targets are associated with PE as well as other diseases of pregnancy. For example, VEGFA, regulated by miR-126, miR-203, miR-205, miR-29a and miR-93, contributes to the development and maintenance of the glomerular filtration barrier (39). VEGF expression may reflect the degree of hypoxia in the placenta, which may also be the case for miR-126 (40). Therefore, miR-126 and VEGF may be a causal miRNA-mRNA module in the development of PE. This association requires verification in future studies. Although the enrichment aims of GOBP and KEGG differed, the pathways enriched by each were essential for the improvement in the diagnosis and treatment of PE. PTEN is regulated by six significant miRNAs, and was identified as being involved in the pathways of focal adhesion and prostate cancer by KEGG, and the pathway of protein amino acid phosphorylation by GOBP. Over-expression of PTEN induces soluble endoglin release from endothelial cells. This triggers endothelial dysfunction, a characteristic feature of PE (41). In an integrative view, gene enrichment analysis associates disease mechanisms with predicted targets. Notably, these enriched pathways are likely to be associated with the immune response, although one target is involved in different processes (21). The insulin signaling pathway may be associated with PE, as a second messenger of insulin is known to be involved in this disease (7).

In conclusion, the 20 deregulated miRNAs identified in the current study may be useful as non-invasive biomarkers. The pathways enriched from their targets and their gene clusters or families are likely to be involved in the development of PE.

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References

1. Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K and Williams MA: Placental microRNA expression in pregnancies complicated by preeclampsia. Am J Obstet Gynecol 204: e12-e21, 2011.
2. Redman CW and Sargent IL: Latest advances in understanding preeclampsia. Science 308: 1592-1594, 2005.
3. Grill S, Rusterholz C, Zanetti-Dällenbach R, *et al*: Potential markers of preeclampsia - a review. Reprod Biol Endocrinol 7: 70, 2009.
4. Roberts JM and Cooper DW: Pathogenesis and genetics of pre-eclampsia. Lancet 357: 53-56, 2001.
5. Broughton Pipkin F and Roberts JM: Hypertension in pregnancy. J Hum Hypertens 14: 705-724, 2000.
6. Huppertz B: Placental origins of preeclampsia: challenging the current hypothesis. Hypertension 51: 970-975, 2008.

7. Scioscia M, Gumaa K, Kunjara S, *et al*: Insulin resistance in human preeclamptic placenta is mediated by serine phosphorylation of insulin receptor substrate-1 and -2. J Clin Endocrinol Metab 91: 709-717, 2006.
8. Yi C, Wang Q, Wang L, *et al*: MiR-663, a microRNA targeting p21(WAF1/CIP1), promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. Oncogene 31: 4421-4433, 2012.
9. Yang Q, Lu J, Wang S, Li H, Ge Q and Lu Z: Application of next-generation sequencing technology to profile the circulating microRNAs in the serum of preeclampsia versus normal pregnant women. Clin Chim Acta 412: 2167-2173, 2011.
10. Salim H, Akbar NS, Zong D, *et al*: miRNA-214 modulates radiotherapy response of non-small cell lung cancer cells through regulation of p38MAPK, apoptosis and senescence. Br J Cancer 107: 1361-1373, 2012.
11. Guo L, Yang Q, Lu J, *et al*: A comprehensive survey of miRNA repertoire and 3' addition events in the placentas of patients with pre-eclampsia from high-throughput sequencing. PLoS One 6: e21072, 2011.
12. Yi R, Qin Y, Macara IG and Cullen BR: Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17: 3011-3016, 2003.
13. Lund E, Güttinger S, Calado A, Dahlberg JE and Kutay U: Nuclear export of microRNA precursors. Science 303: 95-98, 2004.
14. Schwarz DS, Tomari Y and Zamore PD: The RNA-induced silencing complex is a Mg^{2+}-dependent endonuclease. Curr Biol 14: 787-791, 2004.
15. Lu J, Getz G, Miska EA, *et al*: MicroRNA expression profiles classify human cancers. Nature 435: 834-838, 2005.
16. Mitchell PS, Parkin RK, Kroh EM, *et al*: Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 105: 10513-10518, 2008.
17. Kotlabova K, Doucha J and Hromadnikova I: Placental-specific microRNA in maternal circulation - identification of appropriate pregnancy-associated microRNAs with diagnostic potential. J Reprod Immunol 89: 185-191, 2011.
18. Guo L, Zhao Y, Yang S, Cai M, Wu Q and Chen F: Genome-wide screen for aberrantly expressed miRNAs reveals miRNA profile signature in breast cancer. Mol Biol Rep 40: 2175-2186, 2013.
19. Zhang Y, Diao Z, Su L, *et al*: MicroRNA-155 contributes to preeclampsia by down-regulating CYR61. Am J Obstet Gynecol 202: e1-e7, 2010.
20. Hu Y, Li P, Hao S, Liu L, Zhao J and Hou Y: Differential expression of microRNAs in the placentae of Chinese patients with severe pre-eclampsia. Clin Chem Lab Med 47: 923-929, 2009.
21. Pineles BL, Romero R, Montenegro D, *et al*: Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol 196: e1-e6, 2007.
22. Zhu XM, Han T, Sargent IL, Yin GW and Yao YQ: Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. Am J Obstet Gynecol 200: e1-e7, 2009.
23. Griffiths-Jones S, Saini HK, van Dongen S and Enright AJ: miRBase: tools for microRNA genomics. Nucleic Acids Res 36: D154-D158, 2008.
24. Lewis BP, Burge CB and Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15-20, 2005.
25. John B, Enright AJ, Aravin A, Tuschl T, Sander C and Marks DS: Human microRNA targets. PLoS Biol 2: e363, 2004.
26. Hsu SD, Lin FM, Wu WY, *et al*: miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 39: D163-D169, 2011.
27. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H and Kanehisa M: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 27: 29-34, 1999.
28. Ashburner M, Ball CA, Blake JA, *et al*: Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25: 25-29, 2000.
29. Kosaka N, Iguchi H and Ochiya T: Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 101: 2087-2092, 2010.
30. Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT and Sadovsky Y: The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. Placenta 31: 781-784, 2010.
31. Lázár L, Nagy B, Molvarec A, Szarka A and Rigó J Jr: Role of hsa-miR-325 in the etiopathology of preeclampsia. Mol Med Rep 6: 597-600, 2012.
32. Fish JE, Santoro MM, Morton SU, *et al*: miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 15: 272-284, 2008.
33. Wang S, Aurora AB, Johnson BA, *et al*: The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 15: 261-271, 2008.
34. Gellhaus A, Schmidt M, Dunk C, Lye SJ and Winterhager E: The circulating proangiogenic factors CYR61 (CCN1) and NOV (CCN3) are significantly decreased in placentae and sera of preeclamptic patients. Reprod Sci 14 (8 Suppl): 46-52, 2007.
35. Morales Prieto DM and Markert UR: MicroRNAs in pregnancy. J Reprod Immunol 88: 106-111, 2011.
36. Mouillet JF, Chu T, Nelson DM, Mishima T and Sadovsky Y: MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J 24: 2030-2039, 2010.
37. Maccani MA, Padbury JF and Marsit CJ: miR-16 and miR-21 expression in the placenta is associated with fetal growth. PLoS One 6: e21210, 2011.
38. Muralimanoharan S, Maloyan A, Mele J, Guo C, Myatt LG and Myatt L: MIR-210 modulates mitochondrial respiration in placenta with preeclampsia. Placenta 33: 816-823, 2012.
39. Eremina V and Quaggin SE: The role of VEGF-A in glomerular development and function. Curr Opin Nephrol Hypertens 13: 9-15, 2004.
40. Tsatsaris V, Goffin F, Munaut C, *et al*: Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin Endocrinol Metab 88: 5555-5563, 2003.
41. Cudmore MJ, Ahmad S, Sissaoui S, *et al*: Loss of Akt activity increases circulating soluble endoglin release in preeclampsia: identification of inter-dependency between Akt-1 and heme oxygenase-1. Eur Heart J 33: 1150-1158, 2012.