

MicroRNA expression profiles and networks in placentas complicated with selective intrauterine growth restriction

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Abstract. The microRNA (miRNA) profiles of placentas complicated with selective intrauterine growth restriction (sIUGR) are unknown. In the present study, the sIUGR-associated placental miRNA expression was investigated using microarray and confirmatory reverse transcriptase-quantitative polymerase chain reaction studies. Placenta samples around the individual insertion region for each umbilical cord were collected from monochorionic twins complicated with (n=17) or without sIUGR (control, n=16). miRNA profile analysis was performed on two sIUGR cases and one control using an Affymetrix microRNA 4.0 Array system. A total of 14 miRNAs were identified to be specifically differentially expressed (7 upregulated and 7 downregulated) among larger twins of sIUGR cases compared with smaller twins of sIUGR cases. The target genes of the identified miRNAs participate in organ size, cell differentiation, cell proliferation and migration. In addition, according to the miRNA-pathway network analysis, key miRNAs and pathways (transforming growth factor- β , mitogen-activated protein kinase and Wnt) were identified to be associated with the pathogenesis of sIUGR. To the best of our knowledge, the results of the current study have provided the most complete miRNA profiles and the most detailed miRNA regulatory networks of placental tissues complicated with sIUGR.

Introduction

MicroRNAs (miRNAs), 21-25 nucleotide long non-coding RNA molecules, are highly ubiquitous and conserved across many species (1). miRNA binds to the 3'-untranslated region

of target mRNA and silence gene expression by either translational repression or direct mRNA degradation (2). Human genome codes for more than 1,000 miRNAs, and each of them can potentially post-transcriptionally regulate a vast number of genes. By negatively regulating their mRNA targets, miRNA have been implicated in regulating a number of key cellular functions including cell migration, invasion, growth, differentiation and apoptosis (3,4). miRNA expression has been detected expressed in diverse tissues, including placenta (5). Altered expression of miRNAs has been showed in pregnancy-specific diseases, such as preeclampsia, ectopic pregnancy, fetal growth restriction and intrauterine growth retardation (6).

Selective intrauterine growth restriction (sIUGR) is used to define cases with an estimated fetal weight (EFW) of below the 10th percentile in one fetus (7,8). sIUGR occurs in 10 to 15% of monochorionic (MC) twins and is associated with an increased risk of intrauterine fetal demise (IUFD) and neurological adverse outcome for both twins (9). The presence of vascular anastomoses, the localization of umbilical cord and the unequal placental sharing are associated with the development of sIUGR in monozygotic twins, which have identical inherited backgrounds (10-12), while the molecular mechanisms underlying the pathogenesis of sIUGR are still unclear. Studies have showed that several angiogenic and antiangiogenic factors [vascular endothelial growth factor receptor-1 (VEGFR-1), endoglin and fms-Like Tyrosine Kinase-1 (Flt-1)] are involved in the pathogenesis of twin pregnancies complicated by sIUGR (13-15). Unbalanced placental expression of imprinted genes such as *PHLDA2* (16) and insulin-like growth factor 2 (*IGF2*) (17) may also contribute to the development of sIUGR. However, little is known about the dysregulated miRNAs in the placentas complicated sIUGR.

The aim of this study was to identify miRNA profiles in the placentas from pregnancies complicated by sIUGR. The placentas around the individual insertion region for each umbilical cord were collected and subjected for miRNA profile analysis using Affymetrix microRNA 4.0 Array System. We characterized 14 specific significant differentially expressed miRNAs (DEMs) in larger twin placenta compared to corresponding smaller twin placenta. The target genes of significantly changed miRNAs were predicted, and miRNA-Pathway network was established, which provided

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Key words: microRNA, placenta, selective intrauterine growth restriction, microarray

Table I. Primer sequence for qRT-PCR.

A, RT primer sequences.

miRNA	Primer sequence
has-miR-1	5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGATGGGC-3'
has-miR-370-3p	5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGATGGGC-3'
has-miR-5189-5p	5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGCCTGTC-3'
has-miR-373-3p	5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGACACCC-3'
has-miR-338-5p	5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGCACTCA-3'
has-miR-590-5p	5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGCTGCAC-3'

B, PCR primer sequences

miRNA	Primer sequence
has-miR-1	5'-ACACTCCAGCTGGGACATACTTCTTTATAT-3'
has-miR-370-3p	5'-ACACTCCAGCTGGGGCCTGCTGGGGTGGAA-3'
has-miR-5189-5p	5'-ACACTCCAGCTGGGTCTGGGCACAGGCGGATG-3'
has-miR-373-3p	5'-ACACTCCAGCTGGGGAAGTGCTTCGATTTTG-3'
has-miR-338-5p	5'-ACACTCCAGCTGGGAACAATATCCTGGTGC-3'
has-miR-590-5p	5'-ACACTCCAGCTGGGGAGCTTATTCATAAAA-3'
U6	5'-CTCGCTTCGGCAGCACA-3' and 5'-AACGCTTCACGAATTTGCGT-3'
Universal reverse	5'-TGGTGTCTCGTGGAGTCG-3'

Table II. Identified DEMs.

Category	Up-regulated	Down-regulated	Total
L1 vs. S1	84	46	130
L2 vs. S2	107	41	148
N1 vs. n2	50	83	133

Fold change >2, P<0.05.

comprehensive information on the molecular mechanisms of sIUGR.

Materials and methods

Collection of placenta samples. The study was performed with the approval of the Institutional Review Board of Zhejiang University. All participating women were given written, informed consent prior to the collection of samples. Thirty-three women were enrolled in this study, including 17 cases complicated with sIUGR and 16 cases with normal MC. The intertwin EFW discordance, calculated as [(larger twin-smaller twin)/larger twin], was above 20% and less than 5% for sIUGR and normal MC, respectively. Pregnancies complicated with twin-to-twin transfusion syndrome (TTTs), severe congenital anomalies and maternal complication were excluded from this study. The placentas around the individual insertion region for each umbilical cord were collected within 30 min after delivery. The tissue was excised from inside the placental lobules, avoiding both the maternal surface and

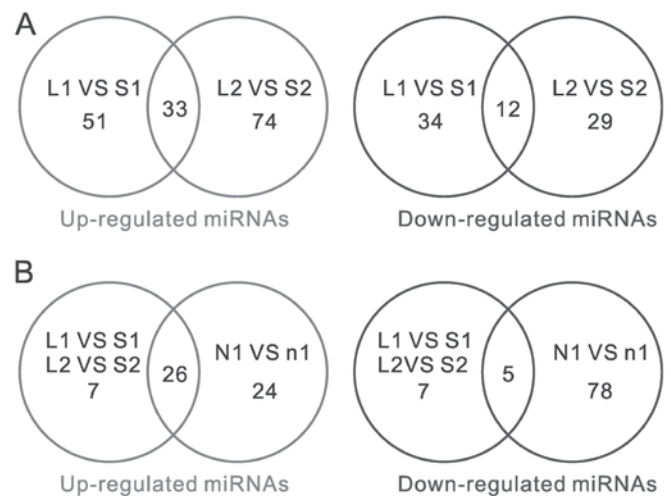


Figure 1. Venn-analysis of miRNAs. (A) Overlap of L1 vs. S1 and L2 vs. S2. (B) Overlap of (L1 vs. S1, L2 vs. S2) and N1 vs. n1. L1 and L2, placenta tissues supporting larger twins from case 1 and case 2 with sIUGR (intertwin EFW discordance was more than 25%), respectively; S1 and S2, placenta tissues supporting smaller twins from case 1 and case 2 with sIUGR, respectively. N1 and n1, placenta tissues supporting larger and smaller twin form normal MC (intertwin EFW discordance was less than 5%), respectively.

the amniotic membrane. The excised tissues were washed in sterilized ice-cold PBS to eliminate any blood and stored at -80°C until they were used to isolate RNA. Placenta samples from two cases with sIUGR [larger twin (L1 and L2), smaller twin (S1 and S2)] and one cases with normal MC [larger twin (N1) and smaller twin (n1)] were used for miRNA profiling; Placenta samples from other 15 cases with sIUGR and other

Table III. DEMs (L1 vs. S1).

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
Up-regulated	hsa-let-7c	2.574462	1.3642709	chr21	MIMAT0000064
	hsa-let-7g-5p	2.0787826	1.0557389	chr3	MIMAT0000414
	hsa-miR-1	6.5372915	2.708693	chr18	MIMAT0000416
	hsa-miR-101-3p	7.129267	2.8337538	chr1	MIMAT0000099
	hsa-miR-127-3p	2.284758	1.1920414	chr14	MIMAT0000446
	hsa-miR-1306-3p	2.2638326	1.1787672	chr22	MIMAT0005950
	hsa-miR-133b	38.066643	5.2504554	chr6	MIMAT0000770
	hsa-miR-144-5p	7.3764052	2.882918	chr17	MIMAT0004600
	hsa-miR-152	7.540236	2.9146097	chr17	MIMAT0000438
	hsa-miR-154-3p	6.04454	2.5956326	chr14	MIMAT0000453
	hsa-miR-154-5p	3.067223	1.6169331	chr14	MIMAT0000452
	hsa-miR-155-5p	7.5116105	2.9091222	chr21	MIMAT0000646
	hsa-miR-181c-5p	5.300414	2.406105	chr19	MIMAT0000258
	hsa-miR-193a-3p	31.360935	4.9708967	chr17	MIMAT0000459
	hsa-miR-194-5p	6.75868	2.7567415	chr1	MIMAT0000460
	hsa-miR-195-5p	2.3303063	1.2205195	chr17	MIMAT0000461
	hsa-miR-1973	7.752263	2.9546175	chr4	MIMAT0009448
	hsa-miR-199a-3p	2.136898	1.0955181	chr1	MIMAT0000232
	hsa-miR-199b-5p	2.0532408	1.0379028	chr9	MIMAT0000263
	hsa-miR-202-3p	37.374104	5.223967	chr10	MIMAT0002811
	hsa-miR-214-3p	2.0166378	1.0119519	chr1	MIMAT0000271
	hsa-miR-218-5p	6.466407	2.6929643	chr4	MIMAT0000275
	hsa-miR-221-3p	2.6082892	1.3831038	chrX	MIMAT0000278
	hsa-miR-222-3p	3.7729478	1.9156921	chrX	MIMAT0000279
	hsa-miR-28-5p	2.027596	1.0197701	chr3	MIMAT0000085
	hsa-miR-299-3p	28.977842	4.8568783	chr14	MIMAT0000687
	hsa-miR-299-5p	2.3908083	1.2574985	chr14	MIMAT0002890
	hsa-miR-30e-3p	5.7678924	2.5280442	chr1	MIMAT0000693
	hsa-miR-3125	7.3044753	2.8687806	chr2	MIMAT0014988
	hsa-miR-3127-5p	7.3825746	2.884124	chr2	MIMAT0014990
	hsa-miR-323a-3p	25.762617	4.687207	chr14	MIMAT0000755
	hsa-miR-33b-3p	6.059397	2.5991743	chr17	MIMAT0004811
	hsa-miR-342-3p	2.086935	1.0613856	chr14	MIMAT0000753
	hsa-miR-361-3p	7.1666164	2.8412921	chrX	MIMAT0004682
	hsa-miR-362-5p	3.0538094	1.61061	chrX	MIMAT0000705
	hsa-miR-3620-5p	88.913635	6.474333	chr1	MIMAT0022967
	hsa-miR-3622b-5p	6.9455557	2.7960901	chr8	MIMAT0018005
	hsa-miR-363-3p	5.4890895	2.4565668	chrX	MIMAT0000707
	hsa-miR-3682-3p	96.9888	6.599746	chr2	MIMAT0018110
	hsa-miR-370	6.1665797	2.6244705	chr14	MIMAT0000722
	hsa-miR-376c-3p	2.002838	1.0020456	chr14	MIMAT0000720
	hsa-miR-379-5p	107.84705	6.752843	chr14	MIMAT0000733
	hsa-miR-381-3p	2.1550357	1.1077118	chr14	MIMAT0000736
	hsa-miR-382-5p	2.920635	1.546282	chr14	MIMAT0000737
	hsa-miR-3917	6.9297132	2.7927957	chr1	MIMAT0018191
	hsa-miR-3923	178.99112	7.483744	chr3	MIMAT0018198
	hsa-miR-409-3p	4.9062624	2.2946243	chr14	MIMAT0001639
	hsa-miR-411-5p	7.6058702	2.9271133	chr14	MIMAT0003329
	hsa-miR-4476	41.244473	5.366129	chr9	MIMAT0019003
	hsa-miR-4535	80.93671	6.338722	chr22	MIMAT0019075
	hsa-miR-4539	91.96671	6.52304	chr14	MIMAT0019082

Table III. Continued.

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
	hsa-miR-4632-5p	2.642979	1.4021649	chr1	MIMAT0022977
	hsa-miR-4698	110.092384	6.782571	chr12	MIMAT0019793
	hsa-miR-4716-3p	2.1197543	1.0838971	chr15	MIMAT0019827
	hsa-miR-4740-5p	7.15094	2.8381329	chr17	MIMAT0019869
	hsa-miR-4743-5p	41.097565	5.360981	chr18	MIMAT0019874
	hsa-miR-4749-3p	7.212792	2.8505578	chr19	MIMAT0019886
	hsa-miR-4750-5p	7.316306	2.8711154	chr19	MIMAT0019887
	hsa-miR-4754	2.11079	1.0777831	chr19	MIMAT0019894
	hsa-miR-487a	32.68032	5.03035	chr14	MIMAT0002178
	hsa-miR-487b	2.2653325	1.1797228	chr14	MIMAT0003180
	hsa-miR-489	7.1415405	2.8362353	chr7	MIMAT0002805
	hsa-miR-493-5p	2.8272614	1.4994053	chr14	MIMAT0002813
	hsa-miR-495-3p	2.1915793	1.1319709	chr14	MIMAT0002817
	hsa-miR-5003-3p	74.188484	6.2131233	chr5	MIMAT0021026
	hsa-miR-500a-3p	5.8292727	2.543316	chrX	MIMAT0002871
	hsa-miR-502-3p	30.460754	4.9288797	chrX	MIMAT0004775
	hsa-miR-5096	5.691079	2.5087023	chr4	MIMAT0020603
	hsa-miR-513b	2.4948008	1.3189247	chrX	MIMAT0005788
	hsa-miR-5189	7.34395	2.8765562	chr16	MIMAT0021120
	hsa-miR-532-3p	30.798903	4.944807	chrX	MIMAT0004780
	hsa-miR-539-5p	7.7136526	2.9474142	chr14	MIMAT0003163
	hsa-miR-543	37.697845	5.23641	chr14	MIMAT0004954
	hsa-miR-5581-5p	2.7262392	1.4469122	chr1	MIMAT0022275
	hsa-miR-584-5p	39.059162	5.287589	chr5	MIMAT0003249
	hsa-miR-6075	7.67851	2.9408264	chr5	MIMAT0023700
	hsa-miR-6132	7.5204616	2.9108212	chr7	MIMAT0024616
	hsa-miR-6508-5p	6.424311	2.6835418	chr21	MIMAT0025472
	hsa-miR-6512-5p	29.820745	4.8982444	chr2	MIMAT0025480
	hsa-miR-652-3p	7.47874	2.9027953	chrX	MIMAT0003322
	hsa-miR-654-3p	2.6468177	1.4042588	chr14	MIMAT0004814
	hsa-miR-660-5p	2.1465678	1.1020317	chrX	MIMAT0003338
	hsa-miR-718	6.8719115	2.7807114	chrX	MIMAT0012735
	hsa-miR-887	33.127758	5.0499687	chr5	MIMAT0004951
Down-regulated	hsa-miR-1225-3p	-5.8417506	-2.5464008	chr16	MIMAT0005573
	hsa-miR-1238-3p	-8.677957	-3.1173553	chr19	MIMAT0005593
	hsa-miR-126-5p	-4.150803	-2.0533905	chr9	MIMAT0000444
	hsa-miR-1273f	-4.8539524	-2.27916	chr1	MIMAT0020601
	hsa-miR-141-3p	-2.0020258	-1.0014606	chr12	MIMAT0000432
	hsa-miR-142-3p	-5.4498663	-2.4462209	chr17	MIMAT0000434
	hsa-miR-1469	-2.2450392	-1.1667407	chr15	MIMAT0007347
	hsa-miR-193b-3p	-2.9037018	-1.5378933	chr16	MIMAT0002819
	hsa-miR-193b-5p	-12.566032	-3.6514573	chr16	MIMAT0004767
	hsa-miR-1972	-2.4747171	-1.3072636	chr16	MIMAT0009447
	hsa-miR-19a-3p	-6.396935	-2.6773808	chr13	MIMAT0000073
	hsa-miR-210	-11.999909	-3.5849516	chr11	MIMAT0000267
	hsa-miR-30b-3p	-4.5346327	-2.1809857	chr8	MIMAT0004589
	hsa-miR-3138	-4.882965	-2.2877574	chr4	MIMAT0015006
	hsa-miR-335-3p	-4.2380176	-2.0833895	chr7	MIMAT0004703
	hsa-miR-338-5p	-3.542421	-1.8247358	chr17	MIMAT0004701
	hsa-miR-3653	-3.0687642	-1.6176578	chr22	MIMAT0018073
	hsa-miR-3679-3p	-4.8174143	-2.268259	chr2	MIMAT0018105

Table III. Continued.

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
	hsa-miR-372	-3.2183118	-1.6863041	chr19	MIMAT0000724
	hsa-miR-373-3p	-4.5374827	-2.1818922	chr19	MIMAT0000726
	hsa-miR-3907	-4.354147	-2.12239	chr7	MIMAT0018179
	hsa-miR-4287	-12.793394	-3.6773272	chr8	MIMAT0016917
	hsa-miR-4324	-2.4016316	-1.2640148	chr19	MIMAT0016876
	hsa-miR-4429	-4.713071	-2.2366674	chr2	MIMAT0018944
	hsa-miR-4472	-2.5906193	-1.373297	chr12	MIMAT0018999
	hsa-miR-4484	-5.330914	-2.414383	chr10	MIMAT0019018
	hsa-miR-4486	-2.6245956	-1.3920952	chr11	MIMAT0019020
	hsa-miR-4649-3p	-35.55725	-5.152072	chr7	MIMAT0019712
	hsa-miR-4767	-2.7031322	-1.4346321	chrX	MIMAT0019919
	hsa-miR-4783-3p	-2.6541424	-1.4082458	chr2	MIMAT0019947
	hsa-miR-4800-5p	-2.005754	-1.0041447	chr4	MIMAT0019978
	hsa-miR-514b-5p	-2.4455242	-1.2901437	chrX	MIMAT0015087
	hsa-miR-516a-3p	-15.977234	-3.9979458	chr19	MIMAT0006778
	hsa-miR-518a-5p	-5.789181	-2.5333593	chr19	MIMAT0005457
	hsa-miR-518c-3p	-2.3767946	-1.2490172	chr19	MIMAT0002848
	hsa-miR-520b	-5.798103	-2.5355809	chr19	MIMAT0002843
	hsa-miR-523-3p	-2.0523486	-1.0372758	chr19	MIMAT0002840
	hsa-miR-5585-3p	-4.71074	-2.2359538	chr1	MIMAT0022286
	hsa-miR-590-5p	-3.056939	-1.6120877	chr7	MIMAT0003258
	hsa-miR-623	-2.361158	-1.2394946	chr13	MIMAT0003292
	hsa-miR-659-3p	-4.1545143	-2.0546799	chr22	MIMAT0003337
	hsa-miR-664b-3p	-2.9298499	-1.5508268	chrX	MIMAT0022272
	hsa-miR-765	-12.846773	-3.683334	chr1	MIMAT0003945
	hsa-miR-766-3p	-2.3770628	-1.24918	chrX	MIMAT0003888
	hsa-miR-770-5p	-2.219985	-1.1505499	chr14	MIMAT0003948
	hsa-miR-877-3p	-3.1021721	-1.6332787	chr6	MIMAT0004950

15 cases with normal MC were used for validation of microarray data.

RNA extraction. About 200 mg of homogenized placenta tissue was used for extraction of total RNA by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. After quantifying by using Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA), extracted RNA was aliquoted and stored at -80°C.

miRNAs expression analysis using miRNA array. miRNA profiling was performed using Affymetrix microRNA 4.0 Array (Santa Clara, CA, US), which covering 2,578 human microRNAs annotated in miRBase V2.0. Briefly, 1 µg of each sample was labeled with Biotin using the FlashTag™ Biotin HSR RNA Labeling Kit (Affymetrix) and then hybridized overnight with the array according to the manufacturer's protocols. After washing and staining, the hybridized slides were read by a GeneChip Scanner 3000 7G (Affymetrix). The raw data were exported by GeneChip Command Console Software Version 4.0 (Affymetrix). The microarray data have been deposited in NCBI's

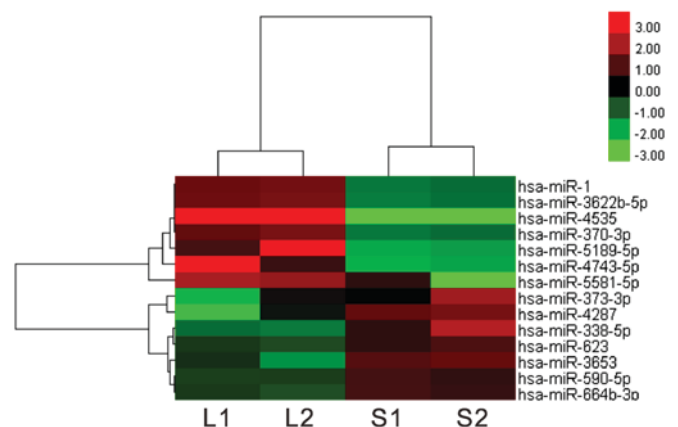


Figure 2. The 14 DEMs differentiated larger twins (L1 and L2) from smaller twins (S1 and S2). The hierarchical clustering analysis was shown.

Gene Expression Omnibus database (GEO, <http://www.ncbi.nlm.nih.gov/geo>) under accession number GSE98146. miRNAs exhibited Fold Change ≥ 2.0 and P-value < 0.05 were identified as significant differentially expressed miRNAs (DEMs). miRNA

Table IV. DEMs (L2 vs. S2).

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
Up-regulated	hsa-miR-1	6.2799373	2.6507502	chr18	MIMAT0000416
	hsa-miR-101-3p	6.4381766	2.6866522	chr1	MIMAT0000099
	hsa-miR-1236-5p	6.4806085	2.6961293	chr6	MIMAT0022945
	hsa-miR-1238-3p	6.655472	2.734541	chr19	MIMAT0005593
	hsa-miR-1290	152.34323	7.2511816	chr1	MIMAT0005880
	hsa-miR-133b	37.049896	5.2113976	chr6	MIMAT0000770
	hsa-miR-135b-5p	94.51581	6.562484	chr1	MIMAT0000758
	hsa-miR-136-3p	29.46167	4.8807673	chr14	MIMAT0004606
	hsa-miR-136-5p	6.712153	2.7467756	chr14	MIMAT0000448
	hsa-miR-139-3p	6.2614946	2.646507	chr11	MIMAT0004552
	hsa-miR-1469	26.883722	4.748661	chr15	MIMAT0007347
	hsa-miR-149-3p	5.559239	2.4748874	chr2	MIMAT0004609
	hsa-miR-154-3p	80.835304	6.3369136	chr14	MIMAT0000453
	hsa-miR-184	107.06464	6.742338	chr15	MIMAT0000454
	hsa-miR-191-3p	5.199818	2.3784611	chr3	MIMAT0001618
	hsa-miR-193a-3p	30.860113	4.9476714	chr17	MIMAT0000459
	hsa-miR-193b-5p	6.7797785	2.761238	chr16	MIMAT0004767
	hsa-miR-1972	31.809767	4.991398	chr16	MIMAT0009447
	hsa-miR-198	7.100607	2.8279424	chr3	MIMAT0000228
	hsa-miR-19a-3p	6.9222608	2.7912433	chr13	MIMAT0000073
	hsa-miR-204-5p	29.051617	4.8605466	chr9	MIMAT0000265
	hsa-miR-2114-5p	128.1817	7.0020466	chrX	MIMAT0011156
	hsa-miR-218-5p	6.376806	2.672834	chr4	MIMAT0000275
	hsa-miR-298	6.9036517	2.7873597	chr20	MIMAT0004901
	hsa-miR-299-3p	30.239016	4.9183393	chr14	MIMAT0000687
	hsa-miR-301a-3p	23.225285	4.5376244	chr17	MIMAT0000688
	hsa-miR-3127-5p	6.460783	2.691709	chr2	MIMAT0014990
	hsa-miR-3135b	123.808846	6.9519706	chr6	MIMAT0018985
	hsa-miR-3147	6.529907	2.7070625	chr7	MIMAT0015019
	hsa-miR-3173-3p	166.4288	7.3787613	chr14	MIMAT0015048
	hsa-miR-3180-3p	156.06705	7.286022	chr16	MIMAT0015058
	hsa-miR-3194-5p	106.03497	6.7283964	chr20	MIMAT0015078
	hsa-miR-33b-3p	5.4492188	2.4460495	chr17	MIMAT0004811
	hsa-miR-34b-5p	6.9539423	2.797831	chr11	MIMAT0000685
	hsa-miR-3610	7.0247335	2.8124435	chr8	MIMAT0017987
	hsa-miR-3620-5p	119.47107	6.9005175	chr1	MIMAT0022967
	hsa-miR-3622b-5p	6.797605	2.7650266	chr8	MIMAT0018005
	hsa-miR-3675-3p	5.4626126	2.4495912	chr1	MIMAT0018099
	hsa-miR-370	6.585063	2.7191973	chr14	MIMAT0000722
	hsa-miR-3911	136.38383	7.091529	chr9	MIMAT0018185
	hsa-miR-411-5p	6.842667	2.7745588	chr14	MIMAT0003329
	hsa-miR-4252	6.649911	2.733335	chr1	MIMAT0016886
	hsa-miR-4257	29.410418	4.8782554	chr1	MIMAT0016878
	hsa-miR-4274	6.556329	2.7128882	chr4	MIMAT0016906
	hsa-miR-4280	6.1641645	2.6239054	chr5	MIMAT0016911
	hsa-miR-4314	6.209586	2.6344972	chr17	MIMAT0016868
	hsa-miR-4317	29.65745	4.8903227	chr18	MIMAT0016872
	hsa-miR-4322	114.98171	6.8452606	chr19	MIMAT0016873
	hsa-miR-4327	168.50354	7.396635	chr21	MIMAT0016889
	hsa-miR-4428	6.98875	2.8050344	chr1	MIMAT0018943
	hsa-miR-4443	2.4926476	1.3176789	chr3	MIMAT0018961

Table IV. Continued.

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
	hsa-miR-4476	6.292596	2.6536553	chr9	MIMAT0019003
	hsa-miR-4482-3p	6.502412	2.700975	chr10	MIMAT0020958
	hsa-miR-4484	91.229645	6.5114307	chr10	MIMAT0019018
	hsa-miR-4486	37.18235	5.216546	chr11	MIMAT0019020
	hsa-miR-4496	5.89344	2.55911	chr12	MIMAT0019031
	hsa-miR-4513	131.67114	7.0407953	chr15	MIMAT0019050
	hsa-miR-4522	111.41926	6.7998548	chr17	MIMAT0019060
	hsa-miR-4535	84.8799	6.407351	chr22	MIMAT0019075
	hsa-miR-4539	156.92206	7.2939043	chr14	MIMAT0019082
	hsa-miR-4632-5p	6.771799	2.7595391	chr1	MIMAT0022977
	hsa-miR-4646-5p	80.15663	6.32475	chr6	MIMAT0019707
	hsa-miR-4656	112.753174	6.817024	chr7	MIMAT0019723
	hsa-miR-4690-5p	26.123838	4.707295	chr11	MIMAT0019779
	hsa-miR-4698	122.851776	6.940775	chr12	MIMAT0019793
	hsa-miR-4734	2.1158128	1.081212	chr17	MIMAT0019859
	hsa-miR-4740-5p	140.27261	7.1320896	chr17	MIMAT0019869
	hsa-miR-4743-5p	6.4767523	2.6952705	chr18	MIMAT0019874
	hsa-miR-4749-3p	6.195103	2.6311283	chr19	MIMAT0019886
	hsa-miR-4758-5p	72.96534	6.1891394	chr20	MIMAT0019903
	hsa-miR-4767	38.017128	5.2485776	chrX	MIMAT0019919
	hsa-miR-487a	34.672806	5.1157327	chr14	MIMAT0002178
	hsa-miR-5003-3p	99.04469	6.6300077	chr5	MIMAT0021026
	hsa-miR-502-3p	6.4271126	2.6841707	chrX	MIMAT0004775
	hsa-miR-5096	28.428453	4.8292637	chr4	MIMAT0020603
	hsa-miR-513a-5p	92.79643	6.5359974	chrX	MIMAT0002877
	hsa-miR-513b	5.130571	2.3591194	chrX	MIMAT0005788
	hsa-miR-513c-5p	5.255153	2.3937328	chrX	MIMAT0005789
	hsa-miR-514b-5p	86.46717	6.4340806	chrX	MIMAT0015087
	hsa-miR-5189	28.272223	4.8213134	chr16	MIMAT0021120
	hsa-miR-518a-5p	27.765553	4.795224	chr19	MIMAT0005457
	hsa-miR-5195-5p	6.754108	2.7557652	chr14	MIMAT0021126
	hsa-miR-520b	6.59573	2.7215323	chr19	MIMAT0002843
	hsa-miR-532-3p	6.8659596	2.7794614	chrX	MIMAT0004780
	hsa-miR-539-5p	28.060043	4.8104453	chr14	MIMAT0003163
	hsa-miR-543	36.802776	5.2017426	chr14	MIMAT0004954
	hsa-miR-557	87.43645	6.450163	chr1	MIMAT0003221
	hsa-miR-5581-5p	69.77128	6.1245613	chr1	MIMAT0022275
	hsa-miR-601	2.1609044	1.1116352	chr9	MIMAT0003269
	hsa-miR-602	5.791587	2.5339587	chr9	MIMAT0003270
	hsa-miR-605	12.47016	3.640408	chr10	MIMAT0003273
	hsa-miR-6075	35.023468	5.13025	chr5	MIMAT0023700
	hsa-miR-6081	6.4466505	2.6885498	chr9	MIMAT0023706
	hsa-miR-6086	107.75808	6.7516522	chrX	MIMAT0023711
	hsa-miR-6087	2.2509918	1.1705608	chrX	MIMAT0023712
	hsa-miR-610	150.35155	7.232196	chr11	MIMAT0003278
	hsa-miR-622	5.405943	2.4345462	chr13	MIMAT0003291
	hsa-miR-630	2.1389875	1.0969281	chr15	MIMAT0003299
	hsa-miR-6511b-5p	6.7421665	2.7532122	chr16	MIMAT0025847
	hsa-miR-659-3p	68.05403	6.0886087	chr22	MIMAT0003337
	hsa-miR-671-5p	116.28812	6.86156	chr7	MIMAT0003880
	hsa-miR-6722-3p	35.948414	5.167856	chr9	MIMAT0025854

Table IV. Continued.

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
Down-regulated	hsa-miR-758-3p	4.839683	2.2749126	chr14	MIMAT0003879
	hsa-miR-765	6.2472134	2.6432128	chr1	MIMAT0003945
	hsa-miR-769-3p	66.88174	6.0635405	chr19	MIMAT0003887
	hsa-miR-877-3p	34.31749	5.100872	chr6	MIMAT0004950
	hsa-miR-887	6.620651	2.726973	chr5	MIMAT0004951
	hsa-miR-10a-5p	-3.7419279	-1.9037818	chr17	MIMAT0000253
	hsa-miR-1281	-4.1730103	-2.0610886	chr22	MIMAT0005939
	hsa-miR-1306-3p	-6.1992292	-2.632089	chr22	MIMAT0005950
	hsa-miR-138-2-3p	-3.1503472	-1.6555109	chr16	MIMAT0004596
	hsa-miR-144-3p	-214.5132	-7.7449226	chr17	MIMAT0000436
	hsa-miR-148b-3p	-2.2464561	-1.1676509	chr12	MIMAT0000759
	hsa-miR-150-5p	-3.811245	-1.9302623	chr19	MIMAT0000451
	hsa-miR-151a-3p	-2.9817586	-1.5761634	chr8	MIMAT0000757
	hsa-miR-197-3p	-3.9836307	-1.9940839	chr1	MIMAT0000227
	hsa-miR-3064-5p	-2.292184	-1.196723	chr17	MIMAT0019864
	hsa-miR-3162-3p	-12.083851	-3.5950084	chr11	MIMAT0019213
	hsa-miR-335-3p	-13.996734	-3.8070183	chr7	MIMAT0004703
	hsa-miR-338-5p	-11.968582	-3.5811803	chr17	MIMAT0004701
	hsa-miR-363-3p	-2.986565	-1.5784872	chrX	MIMAT0000707
	hsa-miR-3651	-2.0020404	-1.001471	chr9	MIMAT0018071
	hsa-miR-3653	-7.8723674	-2.9767976	chr22	MIMAT0018073
	hsa-miR-3679-3p	-3.019187	-1.5941601	chr2	MIMAT0018105
	hsa-miR-373-3p	-3.2492068	-1.7000875	chr19	MIMAT0000726
	hsa-miR-378i	-6.4152656	-2.681509	chr22	MIMAT0019074
	hsa-miR-3923	-144.07567	-7.170683	chr3	MIMAT0018198
	hsa-miR-4287	-2.9859235	-1.5781772	chr8	MIMAT0016917
	hsa-miR-4324	-3.4396935	-1.78228	chr19	MIMAT0016876
	hsa-miR-4455	-2.6491299	-1.4055185	chr4	MIMAT0018977
	hsa-miR-4472	-3.141099	-1.6512694	chr12	MIMAT0018999
	hsa-miR-4481	-4.197097	-2.0693917	chr10	MIMAT0019015
	hsa-miR-4485	-3.3330746	-1.7368536	chr11	MIMAT0019019
	hsa-miR-455-3p	-2.3138413	-1.21029	chr9	MIMAT0004784
	hsa-miR-4707-5p	-2.0739546	-1.0523844	chr14	MIMAT0019807
	hsa-miR-4710	-22.40822	-4.485956	chr14	MIMAT0019815
	hsa-miR-4754	-3.394706	-1.7632866	chr19	MIMAT0019894
	hsa-miR-491-3p	-80.396675	-6.329064	chr9	MIMAT0004765
	hsa-miR-5190	-4.129208	-2.045865	chr18	MIMAT0021121
	hsa-miR-5196-5p	-2.629623	-1.394856	chr19	MIMAT0021128
	hsa-miR-574-5p	-2.571706	-1.3627257	chr4	MIMAT0004795
	hsa-miR-584-5p	-3.136044	-1.6489458	chr5	MIMAT0003249
	hsa-miR-590-5p	-2.542061	-1.3459988	chr7	MIMAT0003258
	hsa-miR-623	-3.541924	-1.8245332	chr13	MIMAT0003292
	hsa-miR-650	-12.4346285	-3.6362915	chr22	MIMAT0003320
	hsa-miR-652-5p	-2.726224	-1.4469041	chrX	MIMAT0022709
	hsa-miR-664b-3p	-3.0773356	-1.6216818	chrX	MIMAT0022272
	hsa-miR-766-3p	-7.31454	-2.870767	chrX	MIMAT0003888

target genes were predicted by miRanda (<http://www.microrna.org>) (18) and TargetScan (<http://www.targetscan.org/>) (19).

Pathway analysis. To find out the significant pathway of the differential genes, pathway analysis was performed according

Table V. DEMs (N1 vs. n1).

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
Up-regulated	hsa-let-7f-1-3p	4.762908	2.2518427	chr9	MIMAT0004486
	hsa-miR-1236-5p	4.8852377	2.2884288	chr6	MIMAT0022945
	hsa-miR-1290	3.285437	1.7160853	chr1	MIMAT0005880
	hsa-miR-138-2-3p	2.8810282	1.5265838	chr16	MIMAT0004596
	hsa-miR-142-3p	2.217947	1.1492249	chr17	MIMAT0000434
	hsa-miR-144-5p	98.02035	6.6150093	chr17	MIMAT0004600
	hsa-miR-149-3p	4.419525	2.1438913	chr2	MIMAT0004609
	hsa-miR-1914-3p	2.0559897	1.0398331	chr20	MIMAT0007890
	hsa-miR-197-5p	3.2845297	1.7156868	chr1	MIMAT0022691
	hsa-miR-19a-3p	2.7125516	1.4396505	chr13	MIMAT0000073
	hsa-miR-3138	5.336239	2.4158232	chr4	MIMAT0015006
	hsa-miR-3156-5p	2.5390592	1.3442941	chr10	MIMAT0015030
	hsa-miR-3180-3p	34.78387	5.1203465	chr16	MIMAT0015058
	hsa-miR-335-3p	4.254274	2.088913	chr7	MIMAT0004703
	hsa-miR-33b-3p	5.437156	2.4428523	chr17	MIMAT0004811
	hsa-miR-3675-3p	30.885275	4.9488473	chr1	MIMAT0018099
	hsa-miR-3679-3p	4.8394313	2.2748375	chr2	MIMAT0018105
	hsa-miR-378i	5.5282373	2.4668195	chr22	MIMAT0019074
	hsa-miR-382-5p	2.0238345	1.0170913	chr14	MIMAT0000737
	hsa-miR-4257	2.8030276	1.4869859	chr1	MIMAT0016878
	hsa-miR-4299	2.322742	1.2158289	chr11	MIMAT0016851
	hsa-miR-4324	2.5575805	1.3547796	chr19	MIMAT0016876
	hsa-miR-4442	2.2200553	1.1505957	chr3	MIMAT0018960
	hsa-miR-4472	2.564959	1.3589358	chr12	MIMAT0018999
	hsa-miR-4476	34.042854	5.08928	chr9	MIMAT0019003
	hsa-miR-4481	2.978939	1.5747986	chr10	MIMAT0019015
	hsa-miR-4486	2.60555	1.381588	chr11	MIMAT0019020
	hsa-miR-4497	2.0459917	1.0328002	chr12	MIMAT0019032
	hsa-miR-4505	2.1049914	1.0738144	chr14	MIMAT0019041
	hsa-miR-4513	31.151861	4.9612465	chr15	MIMAT0019050
	hsa-miR-4656	2.8565521	1.5142748	chr7	MIMAT0019723
	hsa-miR-4698	2.3614159	1.2396522	chr12	MIMAT0019793
	hsa-miR-4731-3p	3.035372	1.6018734	chr17	MIMAT0019854
	hsa-miR-4740-5p	2.4809349	1.3108839	chr17	MIMAT0019869
	hsa-miR-4746-3p	2.4210067	1.2756071	chr19	MIMAT0019881
	hsa-miR-4767	3.1487603	1.654784	chrX	MIMAT0019919
	hsa-miR-4788	2.6694448	1.4165397	chr3	MIMAT0019958
	hsa-miR-486-5p	2.3081188	1.2067175	chr8	MIMAT0002177
	hsa-miR-493-5p	2.2956579	1.1989076	chr14	MIMAT0002813
	hsa-miR-514b-5p	13.624513	3.7681327	chrX	MIMAT0015087
	hsa-miR-518a-5p	2.4678388	1.3032482	chr19	MIMAT0005457
	hsa-miR-520f	4.3717	2.1281943	chr19	MIMAT0002830
	hsa-miR-557	3.5224607	1.8165836	chr1	MIMAT0003221
	hsa-miR-6087	2.4719765	1.305665	chrX	MIMAT0023712
	hsa-miR-6127	2.003823	1.0027552	chr1	MIMAT0024610
	hsa-miR-650	3.9777071	1.991937	chr22	MIMAT0003320
	hsa-miR-652-5p	2.5595675	1.3559	chrX	MIMAT0022709
	hsa-miR-6722-3p	2.8041553	1.4875662	chr9	MIMAT0025854
	hsa-miR-769-3p	42.50052	5.4094086	chr19	MIMAT0003887
	hsa-miR-887	113.96282	6.8324194	chr5	MIMAT0004951

Table V. Continued.

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
Down-regulated	hsa-let-7c	-2.015736	-1.0113068	chr21	MIMAT0000064
	hsa-miR-101-3p	-30.543362	-4.932787	chr1	MIMAT0000099
	hsa-miR-1225-3p	-6.48305	-2.6966727	chr16	MIMAT0005573
	hsa-miR-126-5p	-7.1831927	-2.8446252	chr9	MIMAT0000444
	hsa-miR-1281	-2.6692638	-1.4164419	chr22	MIMAT0005939
	hsa-miR-133b	-6.7808595	-2.7614682	chr6	MIMAT0000770
	hsa-miR-136-3p	-32.852886	-5.037948	chr14	MIMAT0004606
	hsa-miR-136-5p	-3.713002	-1.8925861	chr14	MIMAT0000448
	hsa-miR-139-3p	-7.6147995	-2.928806	chr11	MIMAT0004552
	hsa-miR-1469	-7.4298234	-2.893328	chr15	MIMAT0007347
	hsa-miR-148a-3p	-2.1474736	-1.1026404	chr7	MIMAT0000243
	hsa-miR-152	-7.315202	-2.8708978	chr17	MIMAT0000438
	hsa-miR-154-3p	-40.05453	-5.3238935	chr14	MIMAT0000453
	hsa-miR-155-5p	-6.8148932	-2.768691	chr21	MIMAT0000646
	hsa-miR-181c-5p	-7.4166875	-2.890775	chr19	MIMAT0000258
	hsa-miR-183-5p	-7.3213196	-2.8721037	chr7	MIMAT0000261
	hsa-miR-184	-2.7409573	-1.4546798	chr15	MIMAT0000454
	hsa-miR-193a-3p	-3.5614202	-1.8324527	chr17	MIMAT0000459
	hsa-miR-193a-5p	-7.283015	-2.8645358	chr17	MIMAT0004614
	hsa-miR-1972	-33.596	-5.0702176	chr16	MIMAT0009447
	hsa-miR-198	-7.6496506	-2.9353938	chr3	MIMAT0000228
	hsa-miR-202-3p	-4.0569763	-2.0204048	chr10	MIMAT0002811
	hsa-miR-2114-5p	-102.90411	-6.685157	chrX	MIMAT0011156
	hsa-miR-218-5p	-6.8879266	-2.7840698	chr4	MIMAT0000275
	hsa-miR-222-3p	-2.7792513	-1.4746963	chrX	MIMAT0000279
	hsa-miR-299-3p	-7.6879406	-2.9425972	chr14	MIMAT0000687
	hsa-miR-301a-3p	-36.397095	-5.1857514	chr17	MIMAT0000688
	hsa-miR-3064-5p	-40.484715	-5.3393054	chr17	MIMAT0019864
	hsa-miR-30b-3p	-14.569369	-3.8648665	chr8	MIMAT0004589
	hsa-miR-3125	-2.843465	-1.5076501	chr2	MIMAT0014988
	hsa-miR-3127-5p	-2.7899294	-1.4802287	chr2	MIMAT0014990
	hsa-miR-3135b	-4.5898676	-2.1984525	chr6	MIMAT0018985
	hsa-miR-3147	-7.3836718	-2.8843384	chr7	MIMAT0015019
	hsa-miR-3173-3p	-3.5254762	-1.8178182	chr14	MIMAT0015048
	hsa-miR-3194-5p	-2.9631052	-1.5671098	chr20	MIMAT0015078
	hsa-miR-323a-3p	-7.243867	-2.85676	chr14	MIMAT0000755
	hsa-miR-345-3p	-3.170144	-1.6645484	chr14	MIMAT0022698
	hsa-miR-34b-5p	-89.56483	-6.4848604	chr11	MIMAT0000685
	hsa-miR-362-3p	-6.8308253	-2.77206	chrX	MIMAT0004683
	hsa-miR-3620-5p	-3.0599833	-1.6135237	chr1	MIMAT0022967
	hsa-miR-3660	-7.1529465	-2.8385377	chr5	MIMAT0018081
	hsa-miR-377-3p	-2.1646178	-1.1141124	chr14	MIMAT0000730
	hsa-miR-3907	-2.053038	-1.0377603	chr7	MIMAT0018179
	hsa-miR-3923	-86.46017	-6.433964	chr3	MIMAT0018198
	hsa-miR-411-5p	-7.0822854	-2.824215	chr14	MIMAT0003329
	hsa-miR-4252	-103.971664	-6.7000465	chr1	MIMAT0016886
	hsa-miR-4280	-6.009121	-2.587154	chr5	MIMAT0016911
	hsa-miR-4317	-34.604424	-5.1128845	chr18	MIMAT0016872
	hsa-miR-4322	-91.20365	-6.5110197	chr19	MIMAT0016873
	hsa-miR-4428	-6.9082146	-2.788313	chr1	MIMAT0018943
	hsa-miR-4522	-40.902264	-5.354109	chr17	MIMAT0019060

Table V. Continued.

Regulation	Systematic name	FC (L1 vs. S1)	Log FC (L1 vs. S1)	Chromosome	Mirbase accession no.
	hsa-miR-4539	-3.3245769	-1.7331707	chr14	MIMAT0019082
	hsa-miR-455-5p	-28.737402	-4.8448577	chr9	MIMAT0003150
	hsa-miR-4632-5p	-7.527243	-2.9121215	chr1	MIMAT0022977
	hsa-miR-4646-5p	-2.8159547	-1.4936241	chr6	MIMAT0019707
	hsa-miR-4649-3p	-7.12472	-2.8328333	chr7	MIMAT0019712
	hsa-miR-4690-5p	-7.212258	-2.850451	chr11	MIMAT0019779
	hsa-miR-4749-3p	-6.8722167	-2.7807755	chr19	MIMAT0019886
	hsa-miR-487a	-35.818806	-5.1626453	chr14	MIMAT0002178
	hsa-miR-489	-7.070114	-2.8217335	chr7	MIMAT0002805
	hsa-miR-491-3p	-5.64386	-2.4966822	chr9	MIMAT0004765
	hsa-miR-5003-3p	-7.107153	-2.8292718	chr5	MIMAT0021026
	hsa-miR-502-3p	-14.068433	-3.8143897	chrX	MIMAT0004775
	hsa-miR-5090	-5.9943867	-2.5836122	chr7	MIMAT0021082
	hsa-miR-5096	-7.0916066	-2.8261125	chr4	MIMAT0020603
	hsa-miR-513b	-3.0015473	-1.5857065	chrX	MIMAT0005788
	hsa-miR-516a-3p	-6.1154137	-2.6124501	chr19	MIMAT0006778
	hsa-miR-5190	-3.2782757	-1.7129372	chr18	MIMAT0021121
	hsa-miR-5195-5p	-31.958092	-4.9981093	chr14	MIMAT0021126
	hsa-miR-525-3p	-5.2564344	-2.3940845	chr19	MIMAT0002839
	hsa-miR-532-3p	-31.67539	-4.9852905	chrX	MIMAT0004780
	hsa-miR-539-5p	-7.4776726	-2.9025893	chr14	MIMAT0003163
	hsa-miR-543	-31.493706	-4.9769917	chr14	MIMAT0004954
	hsa-miR-574-3p	-2.0015302	-1.0011034	chr4	MIMAT0003239
	hsa-miR-602	-5.946781	-2.572109	chr9	MIMAT0003270
	hsa-miR-6075	-33.264282	-5.055902	chr5	MIMAT0023700
	hsa-miR-622	-6.371007	-2.6715214	chr13	MIMAT0003291
	hsa-miR-6511b-5p	-7.128962	-2.833692	chr16	MIMAT0025847
	hsa-miR-6512-5p	-14.816795	-3.8891616	chr2	MIMAT0025480
	hsa-miR-758-3p	-5.32387	-2.4124753	chr14	MIMAT0003879
	hsa-miR-765	-6.9221396	-2.791218	chr1	MIMAT0003945
	hsa-miR-766-3p	-2.33501	-1.2234287	chrX	MIMAT0003888
	hsa-miR-877-3p	-13.717739	-3.7779708	chr6	MIMAT0004950

to the KEGG database (20-22). The Fisher's exact test and chi-square test were used to select the significant pathway, and the threshold of significance was defined by P-value (<0.05).

miRNA-pathway network analysis. A miRNA-pathway network was built according to the relationship among miRNAs and pathways as previously described (23).

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). qRT-PCR was performed to measure the levels of miRNAs. A total of 0.5 µg of total RNA was reverse-transcribed using M-MLV reverse transcriptase (Thermo Fisher, Rockford, IL, USA) with a special stem-loop primer (Genepharma; Shanghai, China) for miRNAs. Real-time PCR was performed on ABI PRISM 7500 Real-time PCR system (Applied Biosystems; Foster City, CA, USA) using SYBR Green PCR kit (Thermo Fisher)

according to manufacturer's instruction. All samples were analyzed in triplicate. The primer sequences were listed in Table I. The relative expression level was determined by the $2^{-\Delta\Delta C_t}$ method and normalized to U6 expression. Statistical analysis was performed with ANOVA for multiple comparisons. P-value <0.05 were considered statistically significant.

Results

Identify differentially expressed miRNAs (DEMs). Placenta tissues around the individual insertion region for each umbilical cord were collected for RNA extraction and further analysis. Placenta tissues from two cases complicated with sIUGR [larger twin (L1 and L2), smaller twin (S1 and S2)] and one cases with normal MC [larger twin (N1) and smaller twin (n1)] were used for miRNA profile analysis by Affymetrix

Table VI. DEMs (L1 vs. S1 and L2 vs. S2).

Regulation	Systematic_name	FC (L1 vs. S1)	FC (L2 vs. S2)	Chromosome	Mirbase accession no.
Up-regulated	hsa-miR-1	6.5372915	6.2799373	chr18	MIMAT0000416
	hsa-miR-101-3p	7.129267	6.4381766	chr1	MIMAT0000099
	hsa-miR-133b	38.066643	37.049896	chr6	MIMAT0000770
	hsa-miR-154-3p	6.04454	80.835304	chr14	MIMAT0000453
	hsa-miR-193a-3p	31.360935	30.860113	chr17	MIMAT0000459
	hsa-miR-218-5p	6.466407	6.376806	chr4	MIMAT0000275
	hsa-miR-299-3p	28.977842	30.239016	chr14	MIMAT0000687
	hsa-miR-3127-5p	7.3825746	6.460783	chr2	MIMAT0014990
	hsa-miR-33b-3p	6.059397	5.4492188	chr17	MIMAT0004811
	hsa-miR-3620-5p	88.913635	119.47107	chr1	MIMAT0022967
	hsa-miR-3622b-5p	6.9455557	6.797605	chr8	MIMAT0018005
	hsa-miR-370	6.1665797	6.585063	chr14	MIMAT0000722
	hsa-miR-411-5p	7.6058702	6.842667	chr14	MIMAT0003329
	hsa-miR-4476	41.244473	6.292596	chr9	MIMAT0019003
	hsa-miR-4535	80.93671	84.8799	chr22	MIMAT0019075
	hsa-miR-4539	91.96671	156.92206	chr14	MIMAT0019082
	hsa-miR-4632-5p	2.642979	6.771799	chr1	MIMAT0022977
	hsa-miR-4698	110.09238	122.85178	chr12	MIMAT0019793
	hsa-miR-4740-5p	7.15094	140.27261	chr17	MIMAT0019869
	hsa-miR-4743-5p	41.097565	6.4767523	chr18	MIMAT0019874
	hsa-miR-4749-3p	7.212792	6.195103	chr19	MIMAT0019886
	hsa-miR-487a	32.68032	34.672806	chr14	MIMAT0002178
	hsa-miR-5003-3p	74.188484	99.04469	chr5	MIMAT0021026
	hsa-miR-502-3p	30.460754	6.4271126	chrX	MIMAT0004775
	hsa-miR-5096	5.691079	28.428453	chr4	MIMAT0020603
	hsa-miR-513b	2.4948008	5.130571	chrX	MIMAT0005788
	hsa-miR-5189	7.34395	28.272223	chr16	MIMAT0021120
	hsa-miR-532-3p	30.798903	6.8659596	chrX	MIMAT0004780
	hsa-miR-539-5p	7.7136526	28.060043	chr14	MIMAT0003163
	hsa-miR-543	37.697845	36.802776	chr14	MIMAT0004954
	hsa-miR-5581-5p	2.7262392	69.77128	chr1	MIMAT0022275
	hsa-miR-6075	7.67851	35.023468	chr5	MIMAT0023700
	hsa-miR-887	33.127758	6.620651	chr5	MIMAT0004951
Down-regulated	hsa-miR-335-3p	-4.238018	-13.99673	chr7	MIMAT0004703
	hsa-miR-338-5p	-3.542421	-11.96858	chr17	MIMAT0004701
	hsa-miR-3653	-3.068764	-7.872367	chr22	MIMAT0018073
	hsa-miR-3679-3p	-4.817414	-3.019187	chr2	MIMAT0018105
	hsa-miR-373-3p	-4.537483	-3.249207	chr19	MIMAT0000726
	hsa-miR-4287	-12.79339	-2.985924	chr8	MIMAT0016917
	hsa-miR-4324	-2.401632	-3.439694	chr19	MIMAT0016876
	hsa-miR-4472	-2.590619	-3.141099	chr12	MIMAT0018999
	hsa-miR-590-5p	-3.056939	-2.542061	chr7	MIMAT0003258
	hsa-miR-623	-2.361158	-3.541924	chr13	MIMAT0003292
	hsa-miR-664b-3p	-2.92985	-3.077336	chrX	MIMAT0022272
	hsa-miR-766-3p	-2.377063	-7.31454	chrX	MIMAT0003888

microRNA 4.0 Array system. The expression of 2,578 miRNAs were examined. miRNAs with Fold Change ≥ 2.0 , and P-value < 0.05 (g Student t test) were defines as DEMs.

Here, we identified a total of 130 (84 up-regulations and 46 down-regulations; Tables II and III) and 148 (107 up-regulations and 41 down-regulations; Tables II and

Table VII. Target genes of potential target genes of 14 DEMs.

Regulation	Systematic name	Target genes
Up-regulated	hsa-miR-1	ABCA1, ABHD2, ABI2, ABL2, ACER2, ADAM12, ADAR, AKAP11, AMOT, AMOTL2, ANKIB1, ANKRD29, ANKRD34B, ANO1, ANP32B, ANXA4, AP3D1, API5, ARF3, ARHGEF18, ARID2, ASH2L, ASPH, BCL11A, BDNF, BET1, BLCAP, BMPR1B, BOLL, BSCL2, BSN, BZRAP1, C1RL, CAGE1, CALN1, CAPRIN1, CASK, CDC42, CEBPZ, CHM, CLCN3, CLTC, CNN3, COIL, COL4A3, CPEB1, CREB5, CREM, DDX5, DHX15, DICER1, DLG4, DNAJC5, E2F5, EHMT2, EIF1AX, EIF4E, EML3, EPB41L4B, ETS1, FAM107B, FAM126A, FAM134A, FAM155A, FAM168B, FAM46C, FAM63B, FAM91A1, FBXL14, FBXL20, FBXO22, FNDC3A, FOXP1, FRS2, FZD4, G6PD, GABBR2, GAS2L1, GCH1, GDF6, GJA1, GLCC11, GLIS2, GMFB, GNPTAB, HACE1, HIAT1, HIGD1A, HMBOX1, HMGN1, HNRNPK, HNRNPU, HOOK1, HOXB4, HP1BP3, HS3ST3B1, HSP90B1, HSPD1, JARID2, KCNJ2, KCTD10, KDM5C, KDSR, KIAA1462, KTN1, LARP4, LASP1, LIN7C, LPPR4, LRCH1, LRRC8A, MAGI2, MAP3K1, MATR3, MEIS1, MEOX2, MET, MGAT4A, MIER1, MMD2, MMP8, MON2, MXD1, NAB1, NAMPT, NBEA, NCOA3, NDRG3, NET1, NFAT5, NR3C1, NR4A3, NRPI, NUP50, NXT2, OSBPL7, OSBPL8, OTX2, PABPC4L, PAX6, PAX7, PDE7A, PDGFA, PDIK1L, PFKFB2, PHAX, PHIP, PHLDA1, PKD2, PLEKHO2, POGK, PPIB, PREX1, PRIC285, PRKRIR, PTPLAD1, PTPN2, PTPRK, RAB43, RARB, RNF138, RNF141, RNF165, RNF213, RSNB1L, RUNX1, SEC22C, SEC23B, SEC63, SELT, SFRP1, SH3PXD2B, SH3TC2, SLC10A7, SLC16A6, SLC25A22, SLC25A30, SLC25A36, SLC29A3, SLC35B4, SLC35F1, SLC37A3, SLC39A9, SLC7A11, SLC8A2, SMAD4, SMAP1, SNED1, SNX13, SNX2, SOX9, SPRED1, SS18, STC2, STX12, SULF1, TAGLN2, TMED5, TMEM135, TMEM178, TMSB4X, TNKS2, TNPO1, TNRC6B, TNS3, TPPP, TRAPPC3, TRHDE, TRIM2, TTC3, TTC7B, UBE2H, UBE4A, UBR5, UTRN, VAMP2, WIPF2, WNK3, WSCD2, YPEL2, ZBTB4, ZBTB41, ZC3HAV1, ZFP91, ZNF148, ZNF236, ZNF652, ZZZ3
	hsa-miR-3622b-5p	ANKRD52, ATRNL1, BEND4, CADM4, CBX5, CCDC34, CCDC97, CNKSR2, COL5A3, CPNE5, DCX, DVL3, EDEM3, EXTL3, FAM126B, FAM20B, FBXL20, FKBP5, FOXP3, GRIK2, HUWE1, KCTD20, KIAA0317, KIAA1239, KLF12, LARP1, LCORL, LOXL4, LPPR2, MAP3K3, MBOAT2, MIB1, MUM1L1, MYO1D, NDRG3, NTRK2, NUCKS1, NUP98, PAX6, PDE7B, PHF20L1, PHF21A, PTP4A1, PVRL1, PXT1, QKI, RIMKLA, SH3TC2, SLC1A2, SNTB2, SP2, SRGAP3, SSH2, STAG2, TBC1D14, TCF20, TRIM46, TRIM66, TSGA10, TSPAN11, VPS53, ZBTB7B
	hsa-miR-4535	APBA1, CHD6, CLDN19, DNAJB12, EEF1A2, FKBP4, MAT2A, MYH7B, NDST1, PARVA, PTC1D1, RIBC1, SCN2B, SPIN3, SPOPL, TUB
	hsa-miR-370-3p	ABCG4, ABR, ACCN4, ACOX1, ACTR1A, ACVR2B, ADCY5, AFF1, ANGEL1, ANKH, ANKRD52, ARCN1, ARF3, ASB10, ATP11A, ATP1A2, ATXN7L3, BAG4, BMF, BSN, CIQTNF6, CCDC64, CCL21, CDC42EP4, CFL1, CFLAR, CHD2, CHRNA7, CIT, CNGB1, CRLF1, CYB5B, CYP2U1, DES, DGCR14, DHX35, DMRTB1, DNAJB1, DNAJC11, DND1, EML1, ENAH, ENOX2, FAM102A, FAM123B, FAM164C, FAM168B, FBLN5, FBXO46, FGF11, FGF7, FOSL2, FOXO1, GADD45B, GSG1L, HDAC4, HEMK1, HHIPL1, HIF1AN, HNRNPUL2, HPS5, HSPA12A, HTR4, IKZF4, INO80, IPPK, IVD, JMY, KCNJ11, KIAA2018, KIF1B, KLC2, KLF12, KLHL18, KRT80, LPHN2, MAP2K7, MOCS1, MRPS25, MTL5, NAPG, NCDN, NCOA5, NEK9, NF1, NFASC, NLGN2, NTRK2, ODF2, OPA3, ORA12, PACS1, PAPL, PCDH10, PCDH11X, PCDH19, PCLO, PDE7A, PHF19, PLEKHA6, PLEKHM1, POLR2F, POMT2, PPARGC1B, PRDM10, PRLR, PRRX1, PTC1D1, PXMP4, RAB11A, RAPIGDS1, RAPGEF1, RAPGEFL1, RBBP4, SAP30BP, SEMA6A, SH3BP2, SHE, SLC10A7, SLC46A1, SLC4A4, SMURF1, SOX12, SPRYD3, ST3GAL3, ST6GAL1, STK35, SYNGR1, SYNPO2, TGFBR2, TM9SF4, TMCO7, TMEM127, TMEM154, TMEM184A, TMEM40, TNRC6C, TP53I11, TRIM33, TRIOBP, TRIT1, UBE2R2, UBTF, USP37, USP47, USP5, VANGL1, VSTM2L, WDTC1, WNT10B, ZBTB39, ZBTB42, ZC3H7B, ZC3HAV1, ZCCHC17,

Table VII. Continued.

Regulation	Systematic name	Target genes
	hsa-miR-5189-5p	ZCCHC24, ZDHHC5, ZMYND11, ZNF148, ZNF185, ZNF37A, ZNF605, ZNF704 AAK1, ACVR1B, ACVR2A, ADAT2, ADCY2, ADRBK1, AGBL4, AHI1, AKT2, ANKS6, ARHGAP19, ARID4A, ARRDC3, ASTN1, ASXL1, ATG4B, ATOH8, ATP2B2, ATP6V0D1, ATP8A1, BAIAP2L2, BAK1, BMP8B, BMPR2, BRAP, BRD4, BTRC, CA5B, CACNA1I, CACNB3, CALM3, CBFA2T2, CCBE1, CCDC69, CCDC76, CD300LG, CDH24, CHL1, CNP, CPLX2, CSMD2, CSRNPI, CYP26B1, DACT3, DCBLD2, DCHS1, DENND1A, DIS3L2, DNAJC5G, DTX1, DUOX1, EEFSEC, ELFN2, FAM105B, FAM120C, FAM155B, FAM53C, FBXL19, FBXL20, FBXO33, FBXO41, FGD3, FGF14, FOXN3, FOXP1, GGT5, GLG1, GLUL, GNA12, GRIN1, GRIP2, GRK6, HEG1, HMI3, HOXA13, IGFN1, KATNB1, KCNK2, KCTD15, KIF21B, KPNA6, LCNL1, LHFPL4, LHX6, LHX8, LMAN2, LRRTM3, LYPLA2, LZTR1, M6PR, MAP3K3, MAPK1IP1L, MARCKSL1, MCTSI, MDGA1, MECP2, MLLT6, MMP19, MPP2, MYO1D, NAGS, NAT8L, NAV1, NDRG4, NFYA, NIPSNAP1, NOL3, NPTX1, NRPI, NUAKE1, NUP43, OBFC1, OFD1, OSBPL7, PBX1, PCDH11X, PHF15, PHF21A, PIGA, PLEKHM3, PODXL, POLDIP2, POLR2F, PPIL6, PPME1, PRMT2, PROSC, PTOV1, RAB11FIP3, RAB11FIP4, RAB11FIP5, RAB22A, RC3H1, REM1, RFX1, RHBDL3, RIMS3, RNF157, RNMTL1, RUNX3, SEC14L1, SEMA3G, SENP5, SETBP1, SH3PXD2B, SHANK2, SHANK3, SLC17A5, SLC23A2, SLC26A9, SLC30A6, SLC38A3, SLC6A4, SMARCD1, SNCA, SNX27, SRF, SRRM1, SS18L1, ST3GAL3, ST7L, STAC3, STK4, STX1B, SUV39H1, SV2A, SYNPO, SYT9, TBC1D13, TCF7L1, TFAP2A, THRB, TLN2, TMEM79, TRIM10, TRIM16, TRIM44, TRIM9, TSG101, TSPAN18, TSR2, TTBK1, USH1G, USP54, VAMP2, VCPIP1, VPS39, WBSR17, WDR37, WDTCL, WTAP, XYLT1, YEATS2, ZBTB7B, ZER1, ZHX3, ZNF76, ZNRFI
	hsa-miR-4743-5p hsa-miR-5581-5p	AKT1S1, ARL3, GRIN1, HIC1, NCDN, OLFML2A, SCRT2, ZDHHC8 APLNR, ATP6V1A, ATP8A1, BRD4, BTG4, CABP7, CADM1, CALN1, CAPN1, CCDC62, CCL22, CDON, CHRN2, CLDN2, CLIC4, CPEB2, CR2, CSNK1D, CTNND2, DCLK2, DRP2, FAF2, FAM13A, FGA, FNDC5, FOXP1, GGA1, GMEB1, GRIN1, HIF3A, HNRNPA3, IFFO2, IL4R, IPO7, ITPKB, KCNK3, KPNA6, LAMC1, LHX6, LIPH, MMP19, MTHFR, MYO5A, NACCI, NCOA3, NEDD4L, NTN1, NWD1, PARP16, PHF8, PHOSPHO1, PLCB3, PNKD, RALGPS1, RECQL5, RIMKLA, RMND5A, RNF169, SH3PXD2A, SHROOM4, SLC26A9, SYNGR3, SYT11, TBRG1, TGFB1, THSD7A, TP53I11, TPM3, TRIM47, TUB, UBAP2L, UBXN7, UNC119, VEZF1, ZKSCAN2, ZNF304, ZNF576, ZNF608, ZNF629, ZXDC
Down-regulated	hsa-miR-373-3p	A2LD1, AAK1, ABCA1, ABHD3, ABI2, ABL2, ACBD5, ACVR1C, ADAM9, ADAMTS18, AFF2, AGAP2, AHNK, AKAP5, AKTIP, ANKRD13C, ANKRD50, ANKRD52, ANO6, AP1M1, APBB2, ARHGAP30, ARHGEF10, ARHGEF18, ARHGEF3, ARHGEF7, ARID4A, ARID4B, ARL4A, ASAP1, ASB1, ASF1B, ASH1L, ATP2B2, ATXN1, BAHD1, BAMBI, BCAT1, BCL11A, BCL11B, BCL2L11, BCL6B, BMPR2, BNC2, BNIP3L, BRMS1L, BRWD1, BSCL2, BTBD7, BVES, CAMSAP1, CAMTA1, CASC4, CC2D1A, CCDC88A, CCND1, CCND2, CD44, CDC25A, CDC40, CDCA7, CFL2, CHD9, CLIP4, CNN1, CNOT6, CORIN, CREB1, CRK, CROT, CXCL12, CXCL14, CYB561D1, CYBB, CYBRD1, CYP26B1, DDHD1, DENND5B, DERL2, DGKE, DIRC2, DLGAP2, DMTF1, DNAJA2, DNAJC27, DPP3, DPP4, DPYSL5, DYNLT3, EDNRB, EGLN1, EIF4B, ELAVL2, ENDOD1, EPHA2, EPHA5, EPHA7, ERO1LB, EZH1, FAM102B, FAM117A, FAM18B2, FAM40B, FAM46C, FBXL4, FBXO10, FBXO41, FGD4, FGD5, FLT1, FMNL3, FOXK2, FOXO3, FRMD4A, FRMD4B, FYCO1, FZD6, GAB2, GALNT10, GALNT3, GATAD2B, GATC, GDA, GLIS3, GLS, GNB5, GNG12, GNPDA2, GOLGA1, GPR12, GPR137C, GPR180, GUCY1A3, HAUS8, HDAC4, HEG1, HIP1, HIPK3, HK1, HLF, HMGXB3, HN1, HNRNPUL2, HOOK3, HP1BP3, IGDCC3, IKZF2, IL28RA, IL8, INO80D, IPO7, IQSEC1, IRAK2, IRAK4, IRF2, IRF9, ISM1, ITGB8,

Table VII. Continued.

Systematic name	Target genes
	JUB, KDM2A, KIAA0226, KIAA0240, KIAA0513, KIAA1522, KIAA1549, KIAA1737, KIF3B, KLF12, KLF13, KLF3, KLHL28, KREMEN1, KSR2, LEF1, LEFTY1, LEFTY2, LHX6, LHX8, LIF, LMO3, LRIT1, LUC7L2, LYPD6, LYRM2, LYSMD3, LYST, MAML1, MAP1B, MAP3K1, MAP3K14, MAP3K2, MBD2, MBNL2, MBNL3, MCCD1, MCL1, MDM4, MECP2, MED13L, MFAP3L, MIB1, MICAL3, MKNK2, MKRN1, MLL, MLL3, MLLT6, MNT, MRPS25, MSL1, MTCH2, MTF1, MTMR3, MTUS1, MYO1D, NAPEPLD, NCOA3, NCOA7, NECAP1, NEK9, NFATC3, NFIB, NFYA, NHLRC2, NHLRC3, NNAT, NPAS3, NR2C1, NR2C2, OCRL, ODF2, OPCML, ORMDL3, OSBPL5, OSTM1, OTUD7B, PAFAH2, PAG1, PAK2, PAM, PAN3, PARP8, PBX3, PCDH7, PCGF5, PDCD4, PDLIM5, PFN2, PGBD5, PHACTR4, PHC3, PHF6, PHKA1, PHYHIP1, PIP4K2A, PKD2, PKN2, PLAG1, PLCL1, POFUT1, POLK, POU6F1, PPARA, PPARGC1B, PPP1R10, PPP1R9A, PPP6C, PRDM16, PRDM8, PRKACB, PRMT6, PRRT2, PRRX1, PSD3, PSEN1, PTGDR, PTPDC1, RAB11A, RAB11FIP1, RAB11FIP5, RAB22A, RABEP1, RAD18, RAD23B, RALGDS, RAPGEF2, RAPGEF5, RAPGEFL1, RASSF2, RBL1, RBMS2, RDX, RELA, RELL1, RGL1, RGMA, RHOC, RIMKLA, RNF180, RNF216, RNF38, RNF6, RORA, RPS6KA2, RRAGD, RSNB1, RSNB1L, RSF1, RSR2, RUNX2, RYR2, SAMD12, SAR1B, SASH1, SBF1, SCD5, SCN2A, SCN5A, SCRT2, SDC1, SETBP1, SETD7, SHCBP1, SIK1, SIPAIL3, SLC14A1, SLC16A12, SLC16A9, SLC35E1, SLC38A1, SLC39A6, SLC46A3, SLC6A9, SMARCC2, SNRK, SNTB2, SNX30, SNX5, SNX9, SOS1, SPRED1, SS18L1, SSX2IP, ST3GAL5, ST8SIA2, STX16, SUV420H2, SYAP1, SYDE1, SYNC, SYNPO2, TANC2, TAOK2, TAPT1, TARDBP, TBCEL, TCEB3, TET2, TET3, TGFB2, TIAM1, TMCC1, TMEM100, TMTC2, TMUB2, TNRC18, TNRC6B, TNRC6C, TNS1, TOX, TRAPPC2, TRHDE, TRIM2, TRIM44, TRIM66, TRPS1, TRPV6, TSEN34, TSHZ3, TTC9, TTPAL, TUSC2, UBASH3B, UBE2B, UBE2J1, UBE2Q2, UBE2R2, UBE2W, UBN1, UBN2, UHRF1, UHRF1BP1, ULK1, UNK, UNKL, UPF3A, USP24, USP42, USP46, USP53, VSX1, WDR26, WDR37, WDR45, WEE1, WIPF2, YTHDF3, ZBTB11, ZBTB41, ZBTB43, ZBTB44, ZBTB47, ZBTB7A, ZCCHC24, ZDHHC8, ZDHHC9, ZFP91, ZFYVE26, ZKSCAN1, ZMYND11, ZNF148, ZNF2, ZNF236, ZNF25, ZNF292, ZNF362, ZNF385A, ZNF436, ZNF473, ZNF512B, ZNF518A, ZNF566, ZNF597, ZNF697, ZNF862, ZNFX1"
hsa-miR-4287	AKT2, AP3M2, APLN, ASTN1, ATG9A, BAHDI1, BHLHE41, BSDC1, BTG2, CALB1, CAMK2A, CAMK2B, CCDC113, CECR6, COL17A1, CRTC2, DDX3X, DDX3Y, DNAJC21, EHF, EIF2S1, ENC1, EYA3, FAM117B, FAM76A, GCC1, GRAMD4, HELZ, HUNK, IGSF9B, KCNA6, KCNK10, KIAA1210, KLF12, KPNA6, KRT80, MDM1, MFAP3L, MID1, NARG2, NBN, NCAN, NFASC, OPCML, ORAI3, OSBP, PDE1B, PHF23, PI4K2A, PIK3C2B, PMEPA1, POLD3, RAB1B, RGL1, RIPK1, ROBO2, SGCZ, SGTB, SH3BP2, SH3RF2, TIGD3, TIMM17B, TOX2, UBN2, VBP1, ZNF48, ZNRF3, CREB1, CRK, CROT, CXCL12, CXCL14, CYB561D1, CYBB, CYBRD1, CYP26B1, DDHD1, DENND5B, DERL2, DGKE, DIRC2, DLGAP2, DMTF1, DNAJA2, DNAJC27, DPP3, DPP8, DPYSL5, DYNLT3, EDNRB, EGLN1, EIF4B, ELAVL2, ENDOD1, EPHA2, EPHA5, EPHA7, ERO1LB, EZH1, FAM102B, FAM117A, FAM18B2, FAM40B, FAM46C, FBXL4, FBXO10, FBXO41, FGD4, FGD5, FLT1, FMNL3, FOXK2, FOXO3, FRMD4A, FRMD4B, FYCO1, FZD6, GAB2, GALNT10, GALNT3, GATAD2B, GATC, GDA, GLIS3, GLS, GNB5, GNG12, GNPDA2, GOLGA1, GPR12, GPR137C, GPR180, GUCY1A3, HAUS8, HDAC4, HEG1, HIP1, HIPK3, HK1, HLF, HMGXB3, HN1, HNRNPUL2, HOOK3, HP1BP3, IGDCC3, IKZF2, IL28RA, IL8, INO80D, IPO7, IQSEC1, IRAK2, IRAK4, IRF2, IRF9, ISM1, ITGB8, JUB, KDM2A, KIAA0226, KIAA0240, KIAA0513, KIAA1522, KIAA1549, KIAA1737, KIF3B, KLF12, KLF13, KLF3, KLHL28, KREMEN1, KSR2, LEF1, LEFTY1, LEFTY2, LHX6, LHX8, LIF, LMO3, LRIT1, LUC7L2, LYPD6, LYRM2, LYSMD3, LYST, MAML1,

Table VII. Continued.

Regulation	Systematic name	Target genes
		MAP1B, MAP3K1, MAP3K14, MAP3K2, MBD2, MBNL2, MBNL3, MCCD1, MCL1, MDM4, MECP2, MED13L, MFAP3L, MIB1, MICAL3, MKNK2, MKRN1, MLL, MLL3, MLLT6, MNT, MRPS25, MSL1, MTCH2, MTF1, MTMR3, MTUS1, MYO1D, NAPEPLD, NCOA3, NCOA7, NECAP1, NEK9, NFATC3, NFIB, NFYA, NHLRC2, NHLRC3, NNAT, NPAS3, NR2C1, NR2C2, OCRL, ODF2, OPCML, ORMDL3, OSBPL5, OSTM1, OTUD7B, PAFAH2, PAG1, PAK2, PAM, PAN3, PARP8, PBX3, PCDH7, PCGF5, PDCD4, PDLIM5, PFN2, PGBD5, PHACTR4, PHC3, PHF6, PHKA1, PHYHIPL, PIP4K2A, PKD2, PKN2, PLAG1, PLCL1, POFUT1, POLK, POU6F1, PPARA, PPARGC1B, PPP1R10, PPP1R9A, PPP6C, PRDM16, PRDM8, PRKACB, PRMT6, PRRT2, PRRX1, PSD3, PSEN1, PTGDR, PTPDC1, RAB11A, RAB11FIP1, RAB11FIP5, RAB22A, RABEP1, RAD18, RAD23B, RALGDS, RAPGEF2, RAPGEF5, RAPGEFL1, RASSF2, RBL1, RBMS2, RDX, RELA, RELL1, RGL1, RGMA, RHOC, RIMKLA, RNF180, RNF216, RNF38, RNF6, RORA, RPS6KA2, RRAGD, RSBN1, RSBNIL, RSF1, RSR2, RUNX2, RYR2, SAMD12, SAR1B, SASH1, SBF1, SCD5, SCN2A, SCN5A, SCRT2, SDC1, SETBP1, SETD7, SHCBP1, SIK1, SIPA1L3, SLC14A1, SLC16A12, SLC16A9, SLC35E1, SLC38A1, SLC39A6, SLC46A3, SLC6A9, SMARCC2, SNRK, SNTB2, SNX30, SNX5, SNX9, SOS1, SPRED1, SS18L1, SSX2IP, ST3GAL5, ST8SIA2, STX16, SUV420H2, SYAP1, SYDE1, SYNC, SYNPO2, TANC2, TAOK2, TAPT1, TARDBP, TBCEL, TCEB3, TET2, TET3, TGFB2, TIAM1, TMCC1, TMEM100, TMTC2, TMUB2, TNRC18, TNRC6B, TNRC6C, TNSI, TOX, TRAPPC2, TRHDE, TRIM2, TRIM44, TRIM66, TRPS1, TRPV6, TSEN34, TSHZ3, TTC9, TTPAL, TUSC2, UBASH3B, UBE2B, UBE2J1, UBE2Q2, UBE2R2, UBE2W, UBN1, UBN2, UHRF1, UHRF1BP1, ULK1, UNK, UNKL, UPF3A, USP24, USP42, USP46, USP53, VSX1, WDR26, WDR37, WDR45, WEE1, WIPF2, YTHDF3, ZBTB11, ZBTB41, ZBTB43, ZBTB44, ZBTB47, ZBTB7A, ZCCHC24, ZDHHC8, ZDHHC9, ZFP91, ZFYVE26, ZKSCAN1, ZMYND11, ZNF148, ZNF2, ZNF236, ZNF25, ZNF292, ZNF362, ZNF385A, ZNF436, ZNF473, ZNF512B, ZNF518A, ZNF566, ZNF597, ZNF697, ZNF862, ZNFX1
	hsa-miR-338-5p	AAK1, ADAMTS17, ADARB2, AEBP2, AMMECR1, APPL1, ARFGAP3, ARID2, ARNT, ATAD1, ATF7, ATP2C1, ATRX, AUTS2, B4GALT6, BAZ1B, BCL11B, BCL2L11, BTG3, CADM2, CALM3, CAST, CCDC140, CCNT2, CD28, CD82, CD9, CDK5R1, CDYL2, CHST12, CLIC4, CLTC, CNR1, CNTN4, CPEB4, CPNE3, CREB3L1, CRIM1, CSNK1G1, CUL3, DGKG, DICER1, DLAT, DMXL2, DNAJC6, DNM3, DYRK4, EML1, EP300, EPAS1, EPHA7, ERRF1, EXOC5, FAM126A, FAM129B, FAM135B, FAM177A1, FAM18B2, FMNL2, FNDC3B, FOXJ3, FUT9, GATAD2B, GREM2, GRIA4, GRM7, GTF3C2, GUCY1A3, HCN1, HDAC9, HIF1A, HIPK2, HSPA12A, IKZF1, IMPACT, INO80D, IREB2, JMJD1C, KAL1, KDM5B, KIAA1024, KIAA1467, KLF11, KLHL14, KLHL6, KLRAQ1, KRAS, LMO4, LRP1, MACF1, MBNL1, MBNL2, MCTSI, MEF2C, MIPOL1, MKL2, MLL4, MLLT4, MN1, MON2, MPPED2, NCK2, NCOA3, NDFIP1, NPAS4, NRP1, NUDT4, NUFIP2, OCIAD1, ONECUT2, PARD6B, PCDH17, PCDH20, PCGF5, PCNX, PELI1, PHC3, PHIP, PKN2, PLAGL2, PLEKHA5, PPARGC1A, PPM1B, PPP2R5A, PRDM10, PRLR, PTCHD1, PTGS1, R3HDM2, RAB14, RAB1A, RAB22A, RAB6B, RAP2C, RAPGEF5, RAPH1, RCOR1, RICTOR, RND3, RNF138, RORA, SAMD12, SBNO1, SEC16B, SEMA6A, SERTAD2, SIRT1, SKP1, SLC4A7, SLIT1, SLMAP, SNTB1, SOX6, SPAST, SPOP, SSX2IP, STAG2, SUB1, SYNCRIP, SYPL1, TAF4, TANC2, TARDBP, TBX18, TBX2, TCERG1L, TEAD1, TET2, TLK1, TRA2B, TRAF3, TRPM7, TSHZ3, UBE2N, UBR2, USP25, WASF1, WDFY3, WWC3, ZBTB44, ZFAND5, ZNF292
	hsa-miR-623	AAK1, ACSM2A, ADARB2, AGPAT4, ALPL, AP3M2, APPL1, ATG9A, BAHD1, CACNA1C, CAMK2B, CCDC117, CCDC3, CELSR3, CLUAP1, CORO2A, CRTCL2, CXCL12, DCLK1, DCLK3, DSEL, ECE1, EGFLAM,

Table VII. Continued.

Regulation	Systematic name	Target genes
	hsa-miR-3653	EIF1, ELAVL2, EZR, FAM126A, FAM134C, FOXN2, GATAD2B, GLIS3, HAS3, HGSNAT, HLCS, HM13, HMGA2, HOXC10, HOXC9, IGF2R, ILDR2, KIAA1199, KPNA1, MAPK1, MECP2, MEIS1, MFSD11, MON2, MTMR7, NIPBL, NMT1, NR3C2, NTRK2, NTRK3, OBFC2A, ODZ4, PCMT1, PDE4A, PI4KB, PLCD4, POLD3, PRIMA1, RBM24, RHOBTB3, RIMKLA, RIN3, RNF144A, RNF169, RPRD2, SECISBP2L, SH3PXD2A, SH3TC2, SIGLEC1, SKI, SLC12A2, SLC44A5, SNX13, SUPT16H, TAOK2, TET3, TNFRSF8, TNRC6B, TPM3, TRIM31, TRPS1, ZMIZ1
	hsa-miR-590-5p	ACVR1C, ADCY2, AEBP2, AMIGO2, ATP1B4, ATRNL1, ATXN7, BMP3, BMPR2, BNC2, BRD3, BRPF3, BRWD3, BTG1, CCDC88A, CPEB4, DBT, DGCR2, DIXDC1, DUSP19, EFNB3, ESRRB, FAM107B, FASLG, GALNT2, GJC1, GPC2, GPC6, HCFC2, HIPK3, KIAA0947, KIAA2018, KLHL28, LPCAT2, LRRTM2, MED12L, MKLN1, MYSM1, NCOA1, NIP7, ODZ3, PCDH11X, PDE11A, PHLDA1, PI15, PRPF4B, R3HDM1, RBBP4, SEC62, SERBP1, SORT1, SPATA5, SV2B, TMEM215, TMEM50B, TRIM67, TRPM8, VPS33A, YAF2, ZADH2, ZDHHC21, ZFAND5, ZFY, ZNF280C, ZNF507, ZYG11B
	hsa-miR-664b-3p	ARHGAP24, ARHGEF12, ARMCX1, BAHD1, BMP3, BMPR2, CADM1, CCL22, CEP68, CNOT6, CREB5, DAG1, DSC2, EIF2C4, EIF4EBP2, ELF2, ENAH, EPHA4, FAM13A, FAM3C, FASLG, FBXO28, FGD4, FGF1, FRS2, GABRB2, GATAD2B, GLCCI1, GPR64, ITGB8, JHDM1D, JPH1, KCNT2, KLF12, KLHDC5, LCORL, LRRC57, MATN2, MBNL1, MICALL1, MTMR12, NELL2, NFAT5, NFIB, OSR1, PAG1, PAIP2B, PAN3, PBRM1, PCBP2, PDZD2, PER2, PGRMC2, PIK3R1, PLAG1, PLEKHA1, PPP1R3B, PTPN9, RAB22A, RASGRP1, RAVR2, RBPI, RECK, RFFL, RP2, RPRD1A, SATB1, SECISBP2L, SESTD1, SETD1B, SKI, SLC7A6, SNTB2, SNX29, SPRY2, ST3GAL6, STAG2, TAGAP, TBX2, TET1, TGFB2, TGFB2, TNRC6B, UBE2D3, UBN2, UBR3, YOD1, ZCCHC3, ZNF704
	hsa-miR-664b-3p	AASS, ABCE1, ABI2, ACVR2B, ADD3, AKNA, APAF1, APC, ARPM1, ASB13, ATG7, ATP2C1, BACH1, BACH2, BCAS1, BDNF, BHLHB9, BNC2, CA5B, CACHD1, CCNC, CENPL, COPA, CREG2, DCP2, DENND4C, DIP2B, DPY19L1, EDAR, ERBB4, ETNK1, FAM114A1, FAM8A1, FBXW2, FNDC3A, FRMD4A, FZD5, GPRASP2, HIPK2, HMGA2, INTS6, JPH1, KCTD21, KLF12, KLHDC10, LDB3, LMTK3, MED1, MSR1, MTCH2, MTR, MYCBP, MYO1D, N4BP2, NDFIP1, NETO1, NFIB, NIPAL3, NOTCH2, NSL1, NUFIP2, PAPD5, PARVA, PDE4D, PDYN, PELI2, PGM3, PHIP, PI15, PKP1, PRDM10, PRDM15, PRKAA1, PRKAA2, PRLR, QKI, RANBP9, RAPGEF6, RIBC1, RSI, RSU1, SALL4, SAMD12, SH2D4B, SMAD3, SNX30, STT3A, TAF4, TGOLN2, TMEM215, TMEM26, TRAM2, TXLNA, UTP23, VAMP1, VAMP4, WDFY1, WWC2, YKT6, ZFH3, ZFP28, ZFX, ZNF24

IV) significantly DEMs in L1 and L2, respectively, when compared with S1 and S2. A total of 133 significantly DEMs with 50 up-regulations and 83 down-regulations; Tables II and V) were identified in N1, when compared with n1. As shown in Fig. 1 and Table VI, 45 DEMs (33 up-regulators and 12 down-regulators) identified from L1 vs. S1 were included in the list of DEMs identified from L2 and S2 comparison. More importantly, 7 up-regulated miRNAs and 7 down-regulated miRNAs identified from the territory of sIUGR larger twins vs. sIUGR smaller twins (L1 vs. S1 and L2 vs. S2) were not included in the list of DEMs identified from N1 and n1 (Figs. 1B and 2). These 14 DEMs may be associated with the pathology of sIUGR, and then subjected to target

gene analysis, pathway analysis and miRNA-pathway analysis.

Pathway analysis. The potential target genes of the above 14 DEMs were then searched by using bioinformatic algorithms such as MiRanda and TargetScan. There are 712 and 929 target genes for up-regulated and down-regulated DEMs, respectively, and listed in Table VII.

To find out the significant pathway associated with the target genes, pathway analysis was performed according to the KEGG database. The results showed that 49 and 101 significant pathways were associated with the up-regulated and down-regulated DEMs, respectively ($P < 0.05$; Table VIII

Table VIII. Pathway analysis based on miRNA-targeted genes.

Regulation	Name	Diffgene count	Gene count	Enrichment	P-value	FDR
Up-regulated	Pathways in cancer	23	327	4.3892	1.822E-08	2.670E-06
	TGF-beta signaling pathway	12	81	9.2448	2.697E-08	2.670E-06
	MAPK signaling pathway	20	260	4.8002	4.116E-08	2.716E-06
	Hippo signaling pathway	15	156	6.0002	1.526E-07	7.555E-06
	Endocytosis	16	204	4.8943	9.288E-07	3.678E-05
	HTLV-I infection	18	268	4.1912	1.689E-06	5.371E-05
	Glutamatergic synapse	12	118	6.3460	1.899E-06	5.371E-05
	Estrogen signaling pathway	10	100	6.2403	1.987E-05	4.917E-04
	Protein processing in endoplasmic reticulum	12	167	4.4840	7.169E-05	1.577E-03
	Neurotrophin signaling pathway	10	120	5.2002	1.001E-04	1.981E-03
	Transcriptional misregulation in cancer	12	180	4.1602	1.505E-04	2.709E-03
	Insulin secretion	8	87	5.7382	3.211E-04	5.297E-03
	Wnt signaling pathway	10	143	4.3638	4.435E-04	6.350E-03
	GnRH signaling pathway	8	92	5.4263	4.769E-04	6.350E-03
	Cytokine-cytokine receptor interaction	14	267	3.2720	4.811E-04	6.350E-03
	Adherens junction	7	73	5.9838	6.869E-04	8.495E-03
	Calcium signaling pathway	11	183	3.7510	7.844E-04	8.495E-03
	Gastric acid secretion	7	75	5.8242	8.141E-04	8.495E-03
	Regulation of actin cytoskeleton	12	215	3.4829	8.152E-04	8.495E-03
	Melanogenesis	8	101	4.9428	9.132E-04	9.040E-03
	Axon guidance	9	131	4.2872	1.105E-03	1.042E-02
	RNA transport	10	165	3.7820	1.418E-03	1.276E-02
	Ubiquitin mediated proteolysis	9	138	4.0697	1.623E-03	1.398E-02
	Cholinergic synapse	8	113	4.4179	1.956E-03	1.614E-02
	Glycosaminoglycan biosynthesis-heparan sulfate/heparin	4	24	10.4004	2.153E-03	1.705E-02
	Synaptic vesicle cycle	6	64	5.8502	2.259E-03	1.721E-02
	Salivary secretion	7	90	4.8535	2.493E-03	1.829E-02
	Morphine addiction	7	93	4.6970	3.034E-03	2.146E-02
	Pancreatic secretion	7	96	4.5502	3.664E-03	2.502E-02
	Melanoma	6	71	5.2735	3.928E-03	2.592E-02
	Chemokine signaling pathway	10	192	3.2501	4.581E-03	2.926E-02
	Cocaine addiction	5	50	6.2403	4.868E-03	3.012E-02
	PI3K-Akt signaling pathway	14	347	2.5177	6.421E-03	3.853E-02
	Focal adhesion	10	206	3.0293	7.726E-03	4.499E-02
	Gap junction	6	89	4.2069	1.252E-02	6.800E-02
	Prostate cancer	6	89	4.2069	1.252E-02	6.800E-02
	Colorectal cancer	5	62	5.0325	1.271E-02	6.800E-02
	Lysosome	7	122	3.5805	1.444E-02	7.524E-02
	Proteoglycans in cancer	10	227	2.7490	1.550E-02	7.868E-02
	Renal cell carcinoma	5	66	4.7275	1.666E-02	8.044E-02
	Pancreatic cancer	5	66	4.7275	1.666E-02	8.044E-02
	Circadian entrainment	6	97	3.8600	1.913E-02	9.018E-02
	Proximal tubule bicarbonate reclamation	3	23	8.1395	2.286E-02	1.053E-01
	Tight junction	7	134	3.2598	2.404E-02	1.082E-01
	Chronic myeloid leukemia	5	73	4.2741	2.556E-02	1.125E-01
	Endocrine and other factor-regulated	4	49	5.0941	3.133E-02	1.348E-01

Table VIII. Continued.

Regulation	Name	Diffgene count	Gene count	Enrichment	P-value	FDR
Down-regulated	calcium reabsorption					
	Cell adhesion molecules (CAMs)	7	146	2.9919	3.776E-02	1.591E-01
	Basal cell carcinoma	4	55	4.5384	4.679E-02	1.930E-01
	Other types of O-glycan biosynthesis	3	30	6.2403	4.825E-02	1.950E-01
	Neurotrophin signaling pathway	22	120	8.8478	2.651E-14	5.567E-12
	Proteoglycans in cancer	26	227	5.5277	9.364E-12	9.832E-10
	Axon guidance	19	131	6.9996	1.365E-10	8.205E-09
	Hepatitis B	20	148	6.5217	1.563E-10	8.205E-09
	MAPK signaling pathway	26	260	4.8261	2.037E-10	8.554E-09
	Renal cell carcinoma	13	66	9.5059	3.543E-09	1.240E-07
	PI3K-Akt signaling pathway	28	347	3.8942	5.365E-09	1.610E-07
	Pathways in cancer	27	327	3.9848	6.399E-09	1.680E-07
	Colorectal cancer	12	62	9.3408	1.948E-08	4.545E-07
	Regulation of actin cytoskeleton	21	215	4.7138	2.194E-08	4.607E-07
	TGF-beta signaling pathway	13	81	7.7455	4.888E-08	8.699E-07
	HTLV-I infection	23	268	4.1418	4.971E-08	8.699E-07
	Circadian entrainment	14	97	6.9655	5.551E-08	8.967E-07
	Melanogenesis	14	101	6.6896	9.433E-08	1.415E-06
	Chronic myeloid leukemia	12	73	7.9333	1.346E-07	1.884E-06
	mTOR signaling pathway	11	60	8.8478	1.547E-07	2.031E-06
	HIF-1 signaling pathway	14	106	6.3741	1.767E-07	2.183E-06
	Wnt signaling pathway	16	143	5.3998	2.141E-07	2.498E-06
	Endocytosis	19	204	4.4949	2.489E-07	2.751E-06
	Viral carcinogenesis	19	207	4.4297	3.140E-07	3.297E-06
	Cholinergic synapse	14	113	5.9792	4.010E-07	4.010E-06
	Amphetamine addiction	11	70	7.5838	8.093E-07	7.725E-06
	Insulin signaling pathway	15	140	5.1708	9.843E-07	8.987E-06
	ErbB signaling pathway	12	88	6.5810	1.132E-06	9.903E-06
	Prostate cancer	12	89	6.5071	1.284E-06	1.078E-05
	T cell receptor signaling pathway	13	108	5.8092	1.605E-06	1.296E-05
	Chemokine signaling pathway	17	192	4.2731	2.494E-06	1.940E-05
	Pancreatic cancer	10	66	7.3122	4.108E-06	3.081E-05
	Endometrial cancer	9	52	8.3528	4.485E-06	3.248E-05
	Circadian rhythm	7	31	10.8976	1.092E-05	7.645E-05
	GnRH signaling pathway	11	92	5.7703	1.330E-05	9.010E-05
	Dopaminergic synapse	13	131	4.7892	1.463E-05	9.601E-05
	Phosphatidylinositol signaling system	10	81	5.9581	2.766E-05	1.760E-04
	Estrogen signaling pathway	11	100	5.3087	3.017E-05	1.839E-04
	Glioma	9	65	6.6823	3.065E-05	1.839E-04
	Cocaine addiction	8	50	7.7217	3.277E-05	1.911E-04
	Insulin secretion	10	87	5.5472	5.269E-05	2.990E-04
	Apoptosis	10	88	5.4842	5.835E-05	3.225E-04
	Long-term potentiation	9	71	6.1176	6.393E-05	3.442E-04
	Acute myeloid leukemia	8	57	6.7734	8.877E-05	4.635E-04
	Hepatitis C	12	133	4.3544	9.049E-05	4.635E-04
	Hippo signaling pathway	13	156	4.0217	9.772E-05	4.886E-04
	Alcoholism	14	180	3.7536	1.046E-04	4.993E-04
	Transcriptional misregulation in cancer	14	180	3.7536	1.046E-04	4.993E-04
	Calcium signaling pathway	14	183	3.6921	1.256E-04	5.864E-04
	Tuberculosis	14	184	3.6720	1.334E-04	6.092E-04

Table VIII. Continued.

Regulation	Name	Diffgene count	Gene count	Enrichment	P-value	FDR
	Retrograde endocannabinoid signaling	10	103	4.6855	2.309E-04	1.032E-03
	Chagas disease (American trypanosomiasis)	10	105	4.5963	2.720E-04	1.190E-03
	GABAergic synapse	9	90	4.8261	4.275E-04	1.832E-03
	Non-small cell lung cancer	7	54	6.2560	4.865E-04	2.041E-03
	Osteoclast differentiation	11	135	3.9324	4.958E-04	2.041E-03
	Adherens junction	8	73	5.2888	5.398E-04	2.180E-03
	Morphine addiction	9	93	4.6704	5.510E-04	2.183E-03
	Fc gamma R-mediated phagocytosis	9	94	4.6207	5.983E-04	2.301E-03
	Ubiquitin mediated proteolysis	11	138	3.8469	6.026E-04	2.301E-03
	Gastric acid secretion	8	75	5.1478	6.534E-04	2.450E-03
	B cell receptor signaling pathway	8	76	5.0801	7.171E-04	2.616E-03
	Glutamatergic synapse	10	118	4.0899	7.226E-04	2.616E-03
	Protein processing in endoplasmic reticulum	12	167	3.4678	8.172E-04	2.909E-03
	Shigellosis	7	61	5.5381	1.057E-03	3.701E-03
	Cell cycle	10	124	3.8920	1.083E-03	3.728E-03
	Thyroid cancer	5	29	8.3208	1.187E-03	4.021E-03
	Hypertrophic cardiomyopathy (HCM)	8	85	4.5422	1.555E-03	5.183E-03
	Progesterone-mediated oocyte maturation	8	86	4.4894	1.684E-03	5.525E-03
	Jak-STAT signaling pathway	11	158	3.3599	1.942E-03	6.274E-03
	Measles	10	134	3.6015	2.013E-03	6.388E-03
	Endocrine and other factor-regulated calcium reabsorption	6	49	5.9095	2.038E-03	6.388E-03
	Oocyte meiosis	9	112	3.8781	2.224E-03	6.867E-03
	Salivary secretion	8	90	4.2898	2.288E-03	6.964E-03
	Dilated cardiomyopathy	8	91	4.2427	2.464E-03	7.392E-03
	RIG-I-like receptor signaling pathway	7	71	4.7581	2.697E-03	7.976E-03
	Legionellosis	6	55	5.2648	3.804E-03	1.110E-02
	Aldosterone-regulated sodium reabsorption	5	39	6.1873	4.853E-03	1.396E-02
	Influenza A	11	179	2.9657	5.421E-03	1.538E-02
	Dorso-ventral axis formation	4	24	8.0435	5.593E-03	1.566E-02
	Cytokine-cytokine receptor interaction	14	267	2.5305	6.010E-03	1.661E-02
	Huntington's disease	11	183	2.9009	6.465E-03	1.763E-02
	Inositol phosphate metabolism	6	61	4.7470	6.563E-03	1.767E-02
	Toll-like receptor signaling pathway	8	108	3.5749	7.514E-03	1.997E-02
	Herpes simplex infection	11	188	2.8238	7.997E-03	2.099E-02
	Vasopressin-regulated water reabsorption	5	45	5.3623	9.299E-03	2.411E-02
	Gap junction	7	89	3.7958	1.015E-02	2.600E-02
	Serotonergic synapse	8	114	3.3867	1.055E-02	2.656E-02
	VEGF signaling pathway	6	67	4.3219	1.063E-02	2.656E-02
	NF-kappa B signaling pathway	7	92	3.6720	1.224E-02	3.024E-02
	Notch signaling pathway	5	48	5.0272	1.239E-02	3.025E-02
	Fc epsilon RI signaling pathway	6	70	4.1366	1.325E-02	3.199E-02
	Lysine degradation	5	49	4.9246	1.356E-02	3.236E-02

Table VIII. Continued.

Regulation	Name	Diffgene count	Gene count	Enrichment	P-value	FDR
	Adipocytokine signaling pathway	6	71	4.0784	1.423E-02	3.320E-02
	Melanoma	6	71	4.0784	1.423E-02	3.320E-02
	Epstein-Barr virus infection	11	204	2.6023	1.498E-02	3.457E-02
	RNA degradation	6	72	4.0217	1.525E-02	3.482E-02
	Focal adhesion	11	206	2.5770	1.612E-02	3.640E-02
	Pertussis	6	75	3.8609	1.866E-02	4.125E-02
	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	6	75	3.8609	1.866E-02	4.125E-02
	Regulation of autophagy	4	34	5.6777	2.073E-02	4.534E-02
	SNARE interactions in vesicular transport	4	36	5.3623	2.549E-02	5.517E-02
	Bladder cancer	4	38	5.0801	3.091E-02	6.625E-02
	Natural killer cell mediated cytotoxicity	8	140	2.7578	3.597E-02	7.568E-02
	Small cell lung cancer	6	86	3.3670	3.604E-02	7.568E-02
	Salmonella infection	6	88	3.2905	4.013E-02	8.345E-02

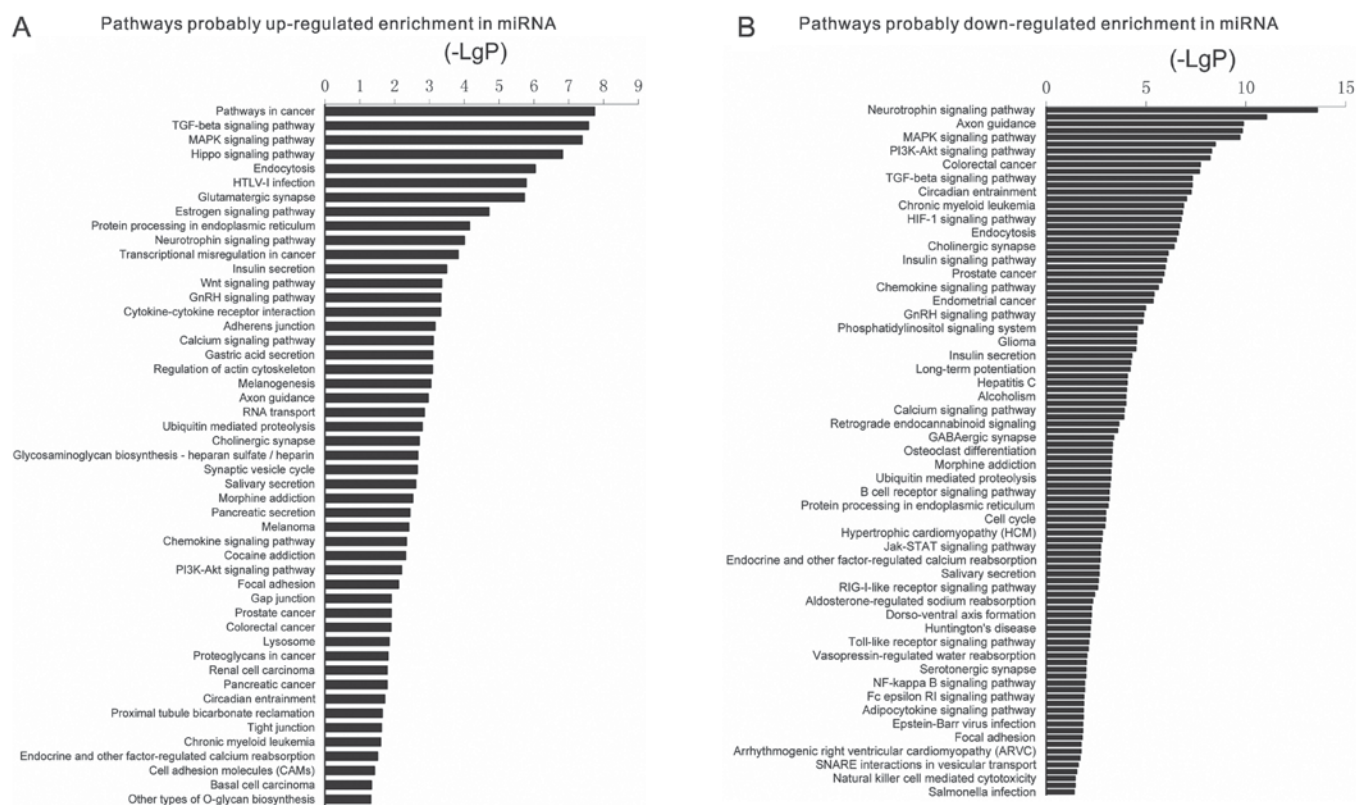


Figure 3. Pathway analysis based on miRNA-targeted genes. (A and B) The histogram of significant pathways targeted by up-regulated (A) and down-regulated miRNA (B) were shown. The vertical axis is the pathway category, and the horizontal axis represents -lg (P-value) of the pathways.

and Fig. 3). Signaling pathways associated with organ size, cell differentiation, cell proliferation and migration, such as transforming growth factor (TGF)- β , mitogen-activated protein kinase (MAPK), Hippo, PI3K-Akt, Wnt, mTOR, Jak/STAT, NF- κ B and Notch, were identified. These data

suggested the involvement of these 14 DEMs on the pathology of sIUGR.

miRNA-pathway network analysis. Based on the significantly regulated pathways, we further established miRNA-pathway

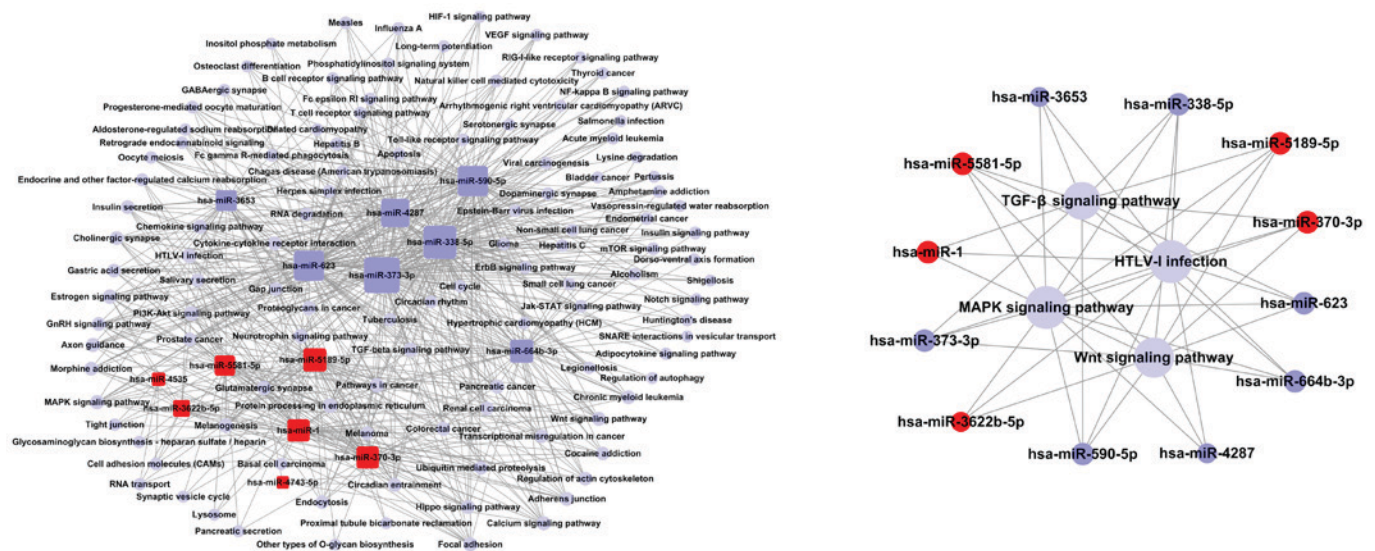


Figure 4. miRNA-pathway network. Red box nodes and blue box nodes represent up-regulated miRNA and down-regulated miRNA, respectively. Blue cycle nodes represent Pathway. Edges show the inhibitory effect of miRNA on Pathway. Left network included all the pathways. When the area of box or circle is larger, the degree of the miRNA or pathway is bigger. Right network extracted from the left network including the key miRNAs and pathways.

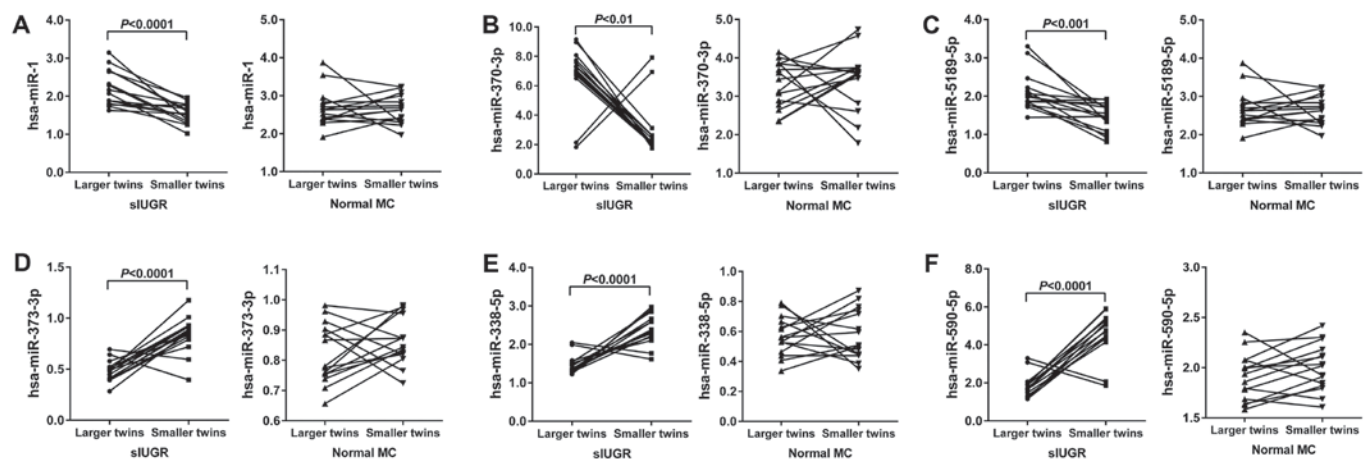


Figure 5. Expression of (A) has-miR-1, (B) has-miR-370-3p, (C) has-miR-5189-5p, (D) has-miR-373-3p, (E) has-miR-338-5p and (F) has-miR-590-5p in placenta tissues from sIUGR and normal NC by qRT-PCR analysis. n=15.

networks to screen the key regulatory functions and the key DEMs (Fig. 4). The top rated five miRNAs included has-miR-373-3p, has-miR-338-5p, has-miR-590-5p, has-miR-623 and has-miR-4287 (Table IX), all of which were down-regulated in placenta tissues supporting larger twins of sIUGR. The DEMs mainly play vital roles in various biological processes, including HTLV-I infection and signal transduction (TGF- β , MAPK and Wnt signaling pathways). These networks provided a large amount of information about the regulation of miRNAs in placenta tissues during the development of sIUGR.

Verification of miRNAs microarray with qRT-PCR. We chose three down-regulated miRNAs (has-miR-373-3p, has-miR-338-5p and has-miR-590-5p) and three up-regulated miRNAs (has-miR-1, has-miR-370-3p and has-miR-5189-5p) for the validation analysis. Our validation cohort included 15 cases with sIUGR [larger twin (L3-L17), smaller twin

(S3-S17)] and 15 cases with normal MC [larger twin (N2-N16) and smaller twin (n2-n16)]. The qRT-PCR results showed that the expression changes of these six miRNAs were in the same direction as determined by the miRNA microarray (Fig. 5).

Discussion

sIUGR MC twin gestations complicated by sIUGR are at high risk of perinatal complications. Recently, some studies have reported that miRNAs are associated with pregnancy-specific diseases (6). Although the pathophysiological insight of sIUGR has been substantially improved, there are few studies on miRNA profiles in the placentas complicated with sIUGR. In this microarray study, we evaluated differential placental miRNA expression in the territory of sIUGR larger twin than in that of corresponding smaller twin. We found 14 placenta miRNAs (7 up-regulated and

Table IX. The degrees of miRNA-Pathway-networks.

Rank	miRNAs	Degree	Feature
1	hsa-miR-373-3p	100	Down
2	hsa-miR-338-5p	88	Down
3	hsa-miR-590-5p	78	Down
4	hsa-miR-623	77	Down
5	hsa-miR-4287	69	Down
6	hsa-miR-5189-5p	48	Up
7	hsa-miR-664b-3p	48	Down
8	hsa-miR-1	44	Up
9	hsa-miR-370-3p	44	Up
10	hsa-miR-3653	39	Down
11	hsa-miR-5581-5p	36	Up
12	hsa-miR-3622b-5p	22	Up
13	hsa-miR-4535	7	Up
14	hsa-miR-4743-5p	4	Up

The degree of each miRNA was the number of pathways regulated by that miRNA.

7 down-regulated) specifically significantly differentially expressed among larger twins of sIUGR cases compared with smaller twins of sIUGR cases. Differentially expressed miRNAs included those that were previously associated with pregnancy-specific diseases, such as preterm delivery and preeclampsia (miR-338, miR-590-5p and miR-1) (24-26), and others that are novel in pregnancy-specific diseases (miR-373-3p, miR-623, miR-4287, miR-664b-3p, miR-3653, miR-5189-5p, miR-370-3p, miR-5581-5p, miR-3622b-5p, miR-4535 and miR-4743-5p). Several of these DEMs have been implicated in tumorigenesis of various types of tumors, such as miR-373-3p in breast, liver, gastric, esophageal, colon, prostate, pancreatic and lung cancer (27), miR-338-5p in colorectal (28) and liver cancer (29), miR-590-5p in cervical cancer (30), miR-623 in lung adenocarcinoma and miR-370-3p in glioma (31). Some of these DEMs have been identified in association with other human diseases. For example, miR-1 has been reported as a biomarker for predicting acute myocardial infarction (32). miR-4743 may serve as biomarker for the diagnosis of Major Depressive Disorder (MDD) (33).

Further, target genes of these DEMs were predicted and the pathway analysis was performed. The target genes are participated in diverse pathophysiological processes including cell organ size, cell differentiation, cell proliferation and cell migration, which may implicated in the pathogenesis of sIUGR. DEMs, including miR-373 (27), miR-338-5p (34), miR-590-5p (30,35,36), miR-623 (37) and miR-370-3p (31), have been reported involved in regulating the proliferation, migration and invasion of cancer cells, which was consistent with our findings. Further studies on the expression pattern and function of these target genes may advance our understanding of the implications of these DEMs in sIUGR pathogenesis. To reveal miRNA regulation of pathways, miRNA-pathway network was built. Of note, key miRNAs and pathways (TGF- β , MAPK and Wnt)

were identified (Fig. 4B). The TGF- β signaling pathway participates in diverse biological processes, including the formation of tissues and organs (38). miR-373 (39) and miR-590-5p (35) exerted their metastasis-inhibiting function via TGF- β signaling pathway. Wnt and MAPK signaling pathways are involved in the development of placenta (40). It has been shown that miR-370-3p (31) and miR-590-5p (36) suppressed the growth of glioma and liver cancer cells, respectively, by targeting Wnt/ β -catenin. miR-623 suppressed the invasion of lung adenocarcinoma cells through inactivating MAPK ERK/JNK (37). These results lay a foundation and provide ideas for future in-depth studies, particularly related to the 14 miRNAs specifically changed in sIUGR.

In summary, we have shown the differential placental miRNA expression associated with sIUGR. In addition, the results of the pathway analysis and miRNA-pathway network analysis represented comprehensive information on the molecular mechanisms of sIUGR from the point of miRNAs. Further experimental studies to evaluate biologic effects of identified miRNAs are warranted.

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