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 placenta 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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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 (TTSs), 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 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 sUeGR [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 sUeGR and other

Table I. Primer sequence for qRT-PCR.

| miRNA         | Primer sequence                      |
|---------------|--------------------------------------|
| has-miR-1     | 5'-CTCAACTGGTGTCGGAGTGCGAGTTGAGATGGGC-3' |
| has-miR-370-3p| 5'-CTCAACTGGTGTCGGAGTGCGAGTTGAGATGGGC-3' |
| has-miR-5189-5p| 5'-CTCAACTGGTGTCGGAGTGCGAGTTGAGATGGGC-3' |
| has-miR-373-3p| 5'-CTCAACTGGTGTCGGAGTGCGAGTTGAGATGGGC-3' |
| has-miR-338-5p| 5'-CTCAACTGGTGTCGGAGTGCGAGTTGAGATGGGC-3' |
| has-miR-590-5p| 5'-CTCAACTGGTGTCGGAGTGCGAGTTGAGATGGGC-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.
| 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              | MIMAT0000590 |
| 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               | MIMAT0000448 |
| 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              | MIMAT0000281 |
| hsa-miR-214-3p   | 2.0166378       | 1.1019519      | 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               | MIMAT0001498 |
| hsa-miR-3127-5p  | 7.3825746       | 2.884124       | chr2               | MIMAT0001499 |
| hsa-miR-323a-3p  | 25.762617       | 4.687207       | chr14              | MIMAT0000755 |
| hsa-miR-33b-3p   | 6.059397        | 2.5991743      | chr17              | MIMAT0000481 |
| 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  | 88.91365        | 6.474333       | chr1               | MIMAT00022967 |
| 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               | MIMAT00018110 |
| 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               | MIMAT00018191 |
| hsa-miR-3923     | 178.99112       | 7.483744       | chr3               | MIMAT00018198 |
| hsa-miR-409-3p   | 4.9062624       | 2.2946243      | chr14              | MIMAT00001639 |
| 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 |
| 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      | chr14             | 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              | MIMAT0021206 |
| 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         | 2.8765662      | chr16             | MIMAT0021120 |
| hsa-miR-532-3p      | 30.798903       | 4.944807       | chr5              | 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              | MIMAT00022275 |
| 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         | 2.9072953      | 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 |
| 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      | -2.2462209     | 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      | -4.238076       | -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.
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 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).

### Table III. Continued.

| Regulation name | Systematic name | FC (L1 vs. S1) | Log FC (L1 vs. S1) | Chromosome | 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      | -1.4343632     | chrX              | MIMAT0005417 |
| hsa-miR-4783-3p | -2.6541424      | -1.4082458     | chr2              | MIMAT0019047 |
| 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       | -2.535809      | 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 |

Figure 2. The 14 DEMs differentiated larger twins (L1 and L2) from smaller twins (S1 and S2). The hierarchical clustering analysis was shown.
| Regulation     | Systematic name | FC (L1 vs. S1) | Log FC (L1 vs. S1) | Chromosome | Accession no. |
|---------------|-----------------|----------------|-------------------|------------|--------------|
| Up-regulated  | hsa-miR-1       | 6.2799373      | 2.6507502         | chr18      | MIMAT0000416 |
|               | hsa-miR-101-3p  | 6.4381766      | 2.6866522         | chr1       | MIMAT0000999 |
|               | hsa-miR-1236-5p | 6.4806085      | 2.6961293         | chr6       | MIMAT0022945 |
|               | hsa-miR-1238-3p | 6.6554742      | 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       | MIMAT0007578 |
|               | hsa-miR-136-3p  | 29.46167       | 4.8807673         | chr14      | MIMAT0004606 |
|               | hsa-miR-136-5p  | 6.712153       | 2.7467756         | chr14      | MIMAT0004448 |
|               | 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-153-3p  | 80.835304      | 6.3369136         | chr14      | MIMAT000453  |
|               | hsa-miR-184     | 107.06464      | 6.742338          | chr15      | MIMAT0000454 |
|               | hsa-miR-191-3p  | 5.199818       | 2.3784611         | chr3       | MIMAT0000168 |
|               | 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       | MIMAT0001490 |
|               | 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       | MIMAT0001899 |
|               | hsa-miR-370     | 6.585063       | 2.7191973         | chr14      | MIMAT0000722 |
|               | hsa-miR-3911    | 136.38383      | 7.091529          | chr9       | MIMAT0018185 |
|               | hsa-miR-411-5p  | 6.842667       | 2.7745888         | chr14      | MIMAT0003329 |
|               | hsa-miR-4252    | 6.649911       | 2.733335          | chr1       | MIMAT0001686 |
|               | hsa-miR-4257    | 29.410418      | 4.8782554         | chr1       | MIMAT0001687 |
|               | hsa-miR-4274    | 6.356329       | 2.7128882         | chr4       | MIMAT0001690 |
|               | hsa-miR-4280    | 6.1641645      | 2.6239054         | chr5       | MIMAT0001691 |
|               | hsa-miR-4314    | 6.209586       | 2.6344972         | chr17      | MIMAT0001688 |
|               | hsa-miR-4317    | 29.65745       | 4.8903227         | chr18      | MIMAT0001682 |
|               | hsa-miR-4322    | 114.98171      | 6.8452606         | chr19      | MIMAT0001683 |
|               | hsa-miR-4327    | 168.50354      | 7.396635          | chr21      | MIMAT0001689 |
|               | hsa-miR-4428    | 6.98875        | 2.8050344         | chr1       | MIMAT0001894 |
|               | hsa-miR-4443    | 2.4926476      | 1.3176789         | chr3       | MIMAT0001896 |
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  | 8.59344             | 2.55911        | chr12             | MIMAT0019031 |
| hsa-miR-4513  | 131.67114           | 7.0407953      | chr15             | MIMAT0019050 |
| hsa-miR-4522  | 111.41926           | 6.7998584      | chr17             | MIMAT0019060 |
| hsa-miR-4535  | 84.8799             | 6.407351       | chr22             | MIMAT0019075 |
| hsa-miR-4539  | 156.92206           | 7.2930043      | 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  | 112.73174           | 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-501-3p  | 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            | 3.6404048      | 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           | 6.0868087      | chr22             | MIMAT0003337 |
| hsa-miR-671-5p | 116.28812          | 6.86156        | chr7              | MIMAT0003880 |
| hsa-miR-6722-3p | 35.948414         | 5.167856       | chr9              | MIMAT0025854 |
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 to the differential expression of the genes. The enrichment of signaling pathways was analyzed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (16). The results showed that the enrichment of the following pathways correlated with the differential expression of miRNAs: MAPK signaling pathway, PI3K-Akt signaling pathway, and Wnt signaling pathway. It was found that miRNAs were involved in these pathways, suggesting their potential role in the pathogenesis of S-IUGR.
Table V. DEMs (N1 vs. n1).

| Regulation name | FC (L1 vs. S1) | Log FC (L1 vs. S1) | Chromosome 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 MIMAT0000073        |
| hsa-miR-19a-3p  | 5.336239       | 2.4158232          | chr4 MIMAT0015006        |
| hsa-miR-3138    | 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      | 2.468195           | chr19 MIMAT0019074       |
| hsa-miR-382-5p  | 2.0238345      | 1.0170913          | chr14 MIMAT0000737       |
| hsa-miR-4257    | 2.8030267      | 1.4869859          | chr1 MIMAT0016878        |
| hsa-miR-4299    | 2.322742       | 1.2158289          | chr11 MIMAT0016851       |
| hsa-miR-4324    | 2.557805       | 1.3547796          | chr11 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    | 3.1487607      | 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 | 13.624151      | 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 MIMAT003221         |
| hsa-miR-6087    | 2.4719765      | 1.305665           | chrX MIMAT0023712        |
| hsa-miR-6127    | 2.003823       | 1.0027552          | chr1 MIMAT0024610        |
| hsa-miR-650     | 3.9777017      | 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 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        | -7.183927      | -2.8446252         | chr9       | MIMAT0000444          |
| hsa-miR-1281          | -2.6692638     | -1.4164419         | chr22      | MIMAT0005939          |
| hsa-miR-133b          | -6.7608595     | -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     | -2.892806          | chr11      | MIMAT0000452          |
| 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       | MIMAT0000454          |
| hsa-miR-184           | -2.7409573     | -1.4546798         | chr15      | MIMAT0000261          |
| hsa-miR-193a-3p       | -3.5614202     | -1.8324527         | chr17      | MIMAT0000459          |
| hsa-miR-193a-5p       | -7.283015      | -2.8645358         | chr17      | MIMAT0000461          |
| hsa-miR-1972          | -33.596        | -5.0702176         | chr16      | MIMAT0000947          |
| 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       | MIMAT0011116          |
| hsa-miR-218-5p        | -6.8879266     | -2.7840969         | chr4       | MIMAT0000275          |
| hsa-miR-222-3p        | -2.7792513     | -1.4746963         | chrX       | MIMAT0000279          |
| hsa-miR-249-3p        | -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       | MIMAT0004988          |
| hsa-miR-3127-5p       | -2.7899294     | -1.4802287         | chr2       | MIMAT0004990          |
| 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      | MIMAT0006685          |
| hsa-miR-362-3p        | -6.8308253     | -2.77206           | chr11      | MIMAT0004683          |
| hsa-miR-3620-5p       | -3.059833      | -1.6135237         | chr1       | MIMAT0022967          |
| hsa-miR-3660          | -7.1529465     | -2.835377          | 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    | -6.700465          | 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          |
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^(-ΔΔCt) 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 V. Continued.

| Regulation name | Systematic name | FC (L1 vs. S1) | Log FC (L1 vs. S1) | Chromosome | 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-651b-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 |
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The expression of 2,578 miRNAs were examined. miRNAs with Fold Change $\geq 2.0$, and P-value $< 0.05$ (t Student test) were defined 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 VI. DEMs (L1 vs. S1 and L2 vs. S2).

| Regulation | Systematic_name | FC (L1 vs. S1) | FC (L2 vs. S2) | Chromosome | accession no. |
|------------|-----------------|---------------|---------------|------------|---------------|
| Up-regulated | hsa-miR-1 | 6.5372915 | 6.2799373 | chr18 | MIMAT0000416 |
| | hsa-miR-101-3p | 7.129267 | 6.4381766 | chr1 | MIMAT0000999 |
| | 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 | 6.166797 | 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 | chr18 | MIMAT0019075 |
| | hsa-miR-4539 | 91.96671 | 156.92066 | 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 | 6.476723 | 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 | 6.427126 | 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 | MIMAT0001805 |
| | 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 |
Table VII. Target genes of potential target genes of 14 DEMs.

| Regulation | Systematic name | Target genes |
|------------|-----------------|--------------|
| Up-regulated hsa-miR-1 | ABCA1, ABHD2, A1B2, AB2L, ACER2, ADAM12, ADAR, AKAP11, AMOT, AMOTL2, ANKI1B, ANRDR29, ANKRD34B, ANO1, ANP32B, ANXA4, AP3D1, API5, ARF3, ARHGEF18, ARID2, ASH2L, ASPH, BCL11A, BDNF, BET1, BLCAP, BMP18B, BOLL, BSCL2, BSN, BZRAP1, C1RL, CAGE1, CALN1, CAPRIN1, CASK, CDC42, CEBPBZ, CHM, CLCN3, CLTC, CNN3, COIL, COLA43, CPEBI, CREB5, CREM, DDX5, DHX15, Dicer1, DLG4, DNAJC5, E2F5, EHM2T, EIF1AX, EIF4E, EML3, EPB41L4B, ETS1, FAM107B, FAM126A, FAM134A, FAM155A, FAM168B, FAM46C, FAM63B, FAM91A, FBXL14, FBXL20, FBXO22, FND3CA, FOXP1, FRS2, FZD4, GABRB2, GAS2LI, GCH1, GDF6, GJA1, GLCCI1, GLIS2, GMFB, GNPTAB, HACE1, HIAT1, HIGD1A, HMBX1, HMGN1, HNRNPK, HRNRNU, HOOK1, HOXB4, HSP90BI, HSPDI, JARID2, KCN2J, KCTD10, KDM5C, KDSR, KIAA1462, KTN1, LARP4, LPS1, LIN7C, LPPR4, LRC1H, LRRCA8, MAGI2, MAP3K1, MATR3, MEISI, MEOX2, MET, MGT4A4, MIER1, MMD2, MMP8, MON2, MXD1, NABI, NAMPT, NBEA, NCOA3, NDRG3, NET1, NFA5, NR3C1, NR4A3, NRP1, NUP50, NUX2, OBP7L, OBPL3, OTX2, PABPC4L, PAX6, PAX7, PDE7A, PDGFA, PDK1L1, PFKFB2, PHAX, PHIP, PHLD1A, PKD2, PLEKHO2, POGK, PPB1, PREX1, PRIC285, PRKRR1, PTPLAD1, PTPT2, PTPTK, RAD43, RARB, RNFI38, RNFI41, RNFI65, RNFI23, RSN1L1, RUNX1, SEC22C, SEC23B, SEC63, SELT, SFRP1, SH3PD2B, SH3TC2, SLC10A7, SLC16A6, SLC25A22, SLC25A30, SLC25A36, SLC29A3, SLC35B4, SLC35F1, SLC37A3, SLC39A1, SLC8A2, SEDAB1, SEDAB2, SEDAB3, SEDAB4, SMAP1, SNED1, SNX13, SNX2, SOX9, SRED1, SS18, ST2, STC2, STX12, SULF1, TAGLN1, TEMED5, TMEM135, TMEM178, TMSB4X, TNKS2, TNP1, TNC6B, TNS3, TPRP, TRAPPC3, TRHDE, TRIM2, TRIM9, UBE2H, UBE4A, UBR5, UTRN, VAMP2, WIPF2, WNK3, WSCD2, YPEL2, ZBTB41, ZC3HAV1, ZF9P1, ZNF148, ZNF236, ZNF652, ZZZ3 |
| hsa-miR-3622b-5p | ANKRD52, ATRNL1, BEND4, CADM4, CBX5, C CDC34, CCDC97, CNKSR2, COL5A3, CPNE5, DCX, DVL3, EDEM3, EXT1L3, FAM126B, FAM20B, FBXL20, FKBP5, FOXP3, GRIK2, HUWE1, KCTD20, KIAA0317, KIAA1239, KLF12, LARP1, LORL, LOXL4, LPPR2, MAP3K3, MBOAT2, MIB1, MUM1LI, MYOID, NDRG3, NTRK2, NUCKSI, NUP98, PAX6, PDE7B, PHF20L1, PHF21A, PTP4A1, PVR1L, PXT1, QKI, RIKMLA, SH3C2T, SLC1A2, SNTB2, SPG3A, SSH2, STAG2, TBC1D4, TCF20, TRIM46, TRIM66, TSGA10, TSPAN11, VPS53, ZBTB7B |
| hsa-miR-4535 | APBA1, CHD6, CLDN19, DNAJB12, EEF1A2, FKBP4, MAT2A, MYH7B, NDST1, PARVA, PTCDI, RIBC1, SCNB2, SPIN3, SPOPL, TUB |
| hsa-miR-370-3p | ACG4, ABR, ACCN4, ACX1, ACTR1A, ACVR2B, ADCY5, AFF1, ANGEL1, ANKH, ANRDR29, ARCN1, ARF3, ASB10, ATP11A, ATP12A, ATXNL3, BAG4, BMF, BSN, C1QTNF6, CCDC64, CCL21, CDC42EP4, CFL1, CFLAR, CHD2, CHRNA7, CIT, CNGBI, CRLFI, CYB5B, CYP2U1, DES, DGCR14, DHX35, DMRTB1, DNAJB1, DNAJC11, DND1, EML1, ENAH, ENOX2, FAM102A, FAM123B, FAM164C, FAM168B, FBLN5, FBXO46, FGFI1, FGF7, FOSL2, FOXI1, GADD45B, GSG1L, HDAC4, HEMI1, HHIP1L, HIF1AN, HRNRNPUL2, HPS5, HSPA12A, HTRA4, IKZF4, INO80, IPPK, IKD, JMY, KIAA1120, KIAA1239, KLF12, LARP1, LORL, LOXL4, LPPR2, MAP3K3, MBOAT2, MIB1, MUM1LI, MYOID, NDRG3, NTRK2, NUCKSI, NUP98, PAX6, PDE7B, PHF20L1, PHF21A, PTP4A1, PVR1L, PXT1, QKI, RIKMLA, SH3C2T, SLC1A2, SNTB2, SPG3A, SSH2, STAG2, TBC1D4, TCF20, TRIM46, TRIM66, TSGA10, TSPAN11, VPS53, ZBTB7B |
| | ABCG4, ABR, ACCN4, ACX1, ACTR1A, ACVR2B, ADCY5, AFF1, ANGEL1, ANKH, ANRDR29, ARCN1, ARF3, ASB10, ATP11A, ATP12A, ATXNL3, BAG4, BMF, BSN, C1QTNF6, CCDC64, CCL21, CDC42EP4, CFL1, CFLAR, CHD2, CHRNA7, CIT, CNGBI, CRLFI, CYB5B, CYP2U1, DES, DGCR14, DHX35, DMRTB1, DNAJB1, DNAJC11, DND1, EML1, ENAH, ENOX2, FAM102A, FAM123B, FAM164C, FAM168B, FBLN5, FBXO46, FGFI1, FGF7, FOSL2, FOXI1, GADD45B, GSG1L, HDAC4, HEMI1, HHIP1L, HIF1AN, HRNRNPUL2, HPS5, HSPA12A, HTRA4, IKZF4, INO80, IPPK, IKD, JMY, KIAA1120, KIAA1239, KLF12, LARP1, LORL, LOXL4, LPPR2, MAP3K3, MBOAT2, MIB1, MUM1LI, MYOID, NDRG3, NTRK2, NUCKSI, NUP98, PAX6, PDE7B, PHF20L1, PHF21A, PTP4A1, PVR1L, PXT1, QKI, RIKMLA, SH3C2T, SLC1A2, SNTB2, SPG3A, SSH2, STAG2, TBC1D4, TCF20, TRIM46, TRIM66, TSGA10, TSPAN11, VPS53, ZBTB7B |
**Table VII. Continued.**

| Regulation       | Systematic name | Target genes                                                                 |
|------------------|-----------------|-------------------------------------------------------------------------------|
| **hsa-miR-5189-5p** |                 | ZCCHC24, ZDHHC5, ZMYND11, ZNF148, ZNF185, ZNF37A, ZNF605, ZNF704 AAK1, ACVR1B, ACVR2A, ADAT2, ADRBK1, AGBL4, AH11, AKT2, ANKS6, ARHGAP19, ARID4A, ARRDC3, ASTN1, ASXL1, ATG4B, ATOH8, ATP2B2, ATP6V0D1, ATP8A1, BAIAP2L2, BAK1, BMP8B, BMPR2, BRAP, BRD4, BTRC, CA5B, CACNAII1, CACNB3, CALM3, CBEA2T2, CCEB1, CCDC69, CCDC76, CD300LG, CDH24, CHL1, CNP, CPLX2, CSM2D, CSRNP1, CYP26B1, DACT3, DCBLD2, DCHS1, DEND1A, DIISL2, DNAJC5G, DTX1, DUOX1, EEFSEC, ELF2F, FAM105B, FAM120C, FAM155B, FAM53C, FBXL19, FBXL20, FBOX33, FBOX41, FGD3, FGF4, FOXL3, FOXP1, GGT5, GLG1, GLUL, GNA12, GRIN1, GRIP2, GRK6, HEG1, HM13, HOXA13, IGFB1, KATNB1, KCTD15, KIF21B, EN1A, LCNL1, LHFP4L, LHX6, LHX8, LMAN2, LRRM3, LYPLA2, LTR1, M6PR, MAP3K3, MARK51, MCTSI, MDGA1, MECPP, MLL6, MTP11, MMP9, MYO1D, NAGS, NATRL, NAV1, NDRG4, NFYA, NIPSNAP1, NOL3, NPTX1, NRP1, NUA1, NUP43, OBFC1, OFD1, OBP7L, PBX1, PCDH11X, PFH15, PIGA, PLEKH3M, PODXL, POLDIP2, POLR2F, PPIL6, PMEI, PRMT2, PROSC, PTOV1, RABI1IP1F, RABI1IFP4, RABI1IFP5, RAB22A, RC3H1, REM1, RFX1, RHBDL3, RIMS3, RNF157, RNMTL1, RUNX3, SEC4L1, SEMA3G, SENP5, SETBP1, SH3PD2B, SHANK2, SHANK3, SLC17A5, SLC23A2, SLC26A9, SLC30A6, SLC38A3, SLC6A4, SMARC1, SNCA, SNX27, SRF, SRRMI1, SS18L1, ST3GAL3, ST7L, STAC3, STK4, STX1B, SV39H1, SV2A, SYNO, SYT9, TBCID13, TCF7L1, TFA2A, THR8, TLN2, TMEM79, TRIM10, TRIM16, TRIM44, TRIM9, TSG101, TSPAN18, TR2, TBK1, USH1G, USP54, VAMP2, VCPPI1, VPS39, WBSCR17, WDR73, WDTC1, WTAP, XYLTL1, YEATS2, ZBTB7B, ZERI, ZHX3, ZNF67, ZNF1F |
| **hsa-miR-4743-5p** |                 | AKT1S1, ARL3, GRIN1, HIC1, HNDC, OLFM2A, SCRT2, ZDHHC8 | APNLR, ATP6V1A, ATP8A1, BRD4, BTEB1, C3, CAPD1, CAPN1, CCDC62, CCL2, CDON, CHRN2B, CLDN2, CLIC4, CPEB2, CR2, CSN1D1, CTNND2, DCLK2, DRP2, FA2F, FAM13A, FGA, FND5C, FOXP1, GAAI, GMEBI, GRO2, HIF3A, HNRNP13, IFOF2, ILR4, IPO7, ITPKB, KCPK3, KPN4, LAMC1, LHES1, LIPH, MMP19, MTHFR, MYOSA, NACCI, NCOA3, NEDD4L1, NT1, NWD1, PARP16, PHF8, PHOSPHO1, PLBC3, PNKD, RALGPS1, RECQL4, RIMKLA, RMNDS5A, RNFL69, SH3PD2A, SHROOM4, SLC26A9, SYNRG3, SYT11, TBRG1, TGFBSR1, THS7A, TPS311, TPM3, TRIM47, TUB, UABP2L, UBXN7, UNICL, VEGF1, ZKSCAN2, ZNF304, ZNF576, ZNF608, ZNF629, ZHDCC | hsa-miR-5581-5p | APLNR, ATP6V1A, BRD4, BTEB1, C3, CAPD1, CAPN1, CCDC62, CCL2, CDON, CHRN2B, CLDN2, CLIC4, CPEB2, CR2, CSN1D1, CTNND2, DCLK2, DRP2, FA2F, FAM13A, FGA, FND5C, FOXP1, GAAI, GMEBI, GRIN1, HIF3A, HNRNP13, IFOF2, ILR4, IPO7, ITPKB, KCPK3, KPN4, LAMC1, LHES1, LIPH, MMP19, MTHFR, MYOSA, NACCI, NCOA3, NEDD4L1, NT1, NWD1, PARP16, PHF8, PHOSPHO1, PLBC3, PNKD, RALGPS1, RECQL4, RIMKLA, RMNDS5A, RNFL69, SH3PD2A, SHROOM4, SLC26A9, SYNRG3, SYT11, TBRG1, TGFBSR1, THS7A, TPS311, TPM3, TRIM47, TUB, UABP2L, UBXN7, UNICL, VEGF1, ZKSCAN2, ZNF304, ZNF576, ZNF608, ZNF629, ZHDCC |
| **Down-regulated hsa-miR-373-3p** |                 | A2LD1, AAK1, ABCA1, ABHD3, AB12, ABL2, ACBD5, ACVR1C, ADAM9, ADAMTS18, AFF2, AGAP2, AHNK, AKAP5, AKT1P, ANKRD15C, ANKRD50, ANKRD52, ANO6, APIM1, APBB2, ARHGAP30, ARHGIF10, ARHGIF18, ARHGIF3, ARHGIF7, ARID4A, ARID4B, ARL4A, ASAP1, ASB1, ASIB1, ASHIL, ATP2B2, ATXN1, BAHD1, BAMB1, BCA1, BCI1A, BCI1B, BCL1L1, BCL2, BMP2R, BCM2, BNIP3L, BRMSIL, BRWD1, BSCL2, BTBD7, BVC, CAMSAPI, CAMTA1, CASC4, CC2D1A, CCDC88A, CCND1, CCND2, CDC44, CDC25A, CDC40, CDC7, CFL2, CHD9, CLIP4, CNOI, CNOT6, CORIN, CREBI, CRK, CROT, CXCL12, CXCL14, CYB561D1, CYB8, CYBR1, CYB2, DD15H, DHH2, DEND15B, DERL2, DGKE, DLR1, DLMQ5A, DMTF1, DNAJ4A2, DNAJ4C7, DPP3, DPP8, DPP8L5, DYNLT3, EDNRB, EGLN1, EIF4B, ELAVL2, ENDOD1, EPS2A, EPS2A, EPS57, ERT1, ERT1B, EZH1, FATM102B, FAM17A, FAM18B2, FAM40B, FAM46C, FBX, FBXO10, FBXO41, FG6D, G6D, G6D, GALNT10, GALNT3, GATA2D2, GATC, GDA, GLIS3, GLS, GNB5, GNG12, GNPD2A, GOLGA1, GRP12, GRP137C, GRP180, GUCY1A3, HAUSH, HDB4, HEGL, HIP1, HIKP3, HK1, HLF, HMGBX3, HNL, HNRNPUL2, HOOK3, HIPBP3, IGDC3, IKZ2F2, IL28RA, IL8, INO80D, IPO7, IQSEC1, IRAK2, IRAK4, IRF2, IRF9, ISMI, ITGB8, |
| Systematic name | Target genes |
|-----------------|--------------|
| JUB, KDM2A, KIAA0226, KIAA0240, KIAA0513, KIAA1522, KIAA1549, KIAA1737, KIF3B, KLF12, KLF13, KLF3, KHLH28, KREME1, KSR2, LEFI, LEFTY1, LEFTY2, LHX6, LHX8, LF, LMO3, LRT1, LUC7L2, LYPD6, LYM2, LYSMD3, LYST, MAML1, MAP3K1, MAP3K14, MAP3K2, MB2B, MBNL2, MBNL3, MCCI, MCL1, MDM4, MEC2P, MEDI3L, MFAPI3L, MIB1, MICAL3, MKNK2, MKRN1, MLL, MLL3, MLLT6, MNT, MRPS25, MSL1, MTH2, MTF1, MTRM3, MTSU1, MYO1D, NEAPLED, NCOA3, NCOA7, NECTAPI, NEK9, NFATC3, NFI8, NFYA, NHLRC2, NHLRC3, NNAT, NPS3, NR2C1, NR2C2, OCR1L, ODF2, OPCML, ORMDL3, OSBPL5, OSM1, OTUD7B, PAFAH2, PAG1, PAK2, PAM, PAN3, PARP8, PBX3, PCDH7, PCGF5, PDCD4, PDLIM5, PFN2, PGBD5, PHACTR4, PHC3, PHF6, PHKA1, PHYHIPL, PIP4K2A, PKD2, PKN2, PLAG1, PLCLI, POFUT1, POLK, POU6F1, PPRA, PPARGC1B, PPR10, PPR19A, PPP6C, PRDM16, PRDM8, PRKACB, PRMT6, PRRT2, PRRX1, PSD3, PSEN1, PTGDR, PTPDC1, RAB1A, RAB1B, RAB2A, RABEP1, RAD18, RAD23B, RALGDS, RAPGEF2, RAPGEF5, RAPGEFL1, RASSF2, RBL1, RBMS2, RDX, RELA, RELLI, RGL1, RGMA, RHOC, RIMKLA, RNF180, RNF26, RNF38, RNF6, RORA, RPS6KA2, RAGD, RSBNI, RSBNL1, RSFI, RSR2, RUNX2, RYR2, SAMD12, SAR1B, SASH1, SBF1, SCD5, SCN2A, SCN5A, SCRT2, SDC1, SETBP1, SETD7, SHCBI, SIK1, SIPAIL3, SLC14A1, SLC16A2, SLC16A9, SLC35EI, SLC38A1, SLC39A6, SLC46A3, SLC6A9, SMARC2C, SNRK, SNTB2, SNX30, SNX5, SNX9, SORI, SPREDI, SSI9L1, SXX2IP, ST5GSL5, ST5SIA2, STX6, SUSD4, SYAP1, SYDE1, SYNPO2, TANC2, TAOK2, TAPT1, TARDBP, TBC1L, TCEB3, TET2, TET3, TGFBR2, TIA1M, TMCC1, TMEM100, TMTC2, TMUB2, TNRC18, TNRC6B, TNRC6C, TNS1, TOX, TRAPPCC2, TRHDE, TRIM2, TRIM44, TRIM66, TRPS1, TRPV6, TSEN3, TSH3, TTCH, TTPA, TUSC2, UBAH3B, UBE2B, UBE2D1, UBE2Q2, UBE2R2, UBE2W, UBN1, UBN2, UHRFI1, UHRF1B1, ULKI, UNK, UNKL, UPF3A, USP24, USP42, USP46, USP53, VSX1, WDR26, WDR37, WDR45, WEE1, WIPF3, YTHDF3, ZBTB11, ZBTB41, ZBTB43, ZBTB44, ZBTB47, ZBTB7A, ZCCHC24, ZDHHC8, ZDHHC9, ZFP91, ZFYVE26, ZKSCAN1, ZMYND1, ZNF148, ZNF2, ZNF235, ZNF259, ZNF362, ZNF385A, ZNF436, ZNF473, ZNF512B, ZNF518A, ZNF566, ZNF597, ZNF692, ZNF6962, ZNF697, ZNF6981 |
| hsa-miR-4287 | AKT2, AP3M2, APLN, ASTN1, ATG9A, BAIHDI, BHLHE41, BSCDC, BTG2, CALBI, CAMK2A, CAMK2B, CCDC113, CECR6, COL17A1, CRTC2, DDXX3, DDXX5, 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, ORA13, OSBP, PDE1B, PHF23, PIK4A2, PIK3CB2, PMP1, POI3, POLD3, RAB1B, RGL1, RIPKI, ROBO2, SGCC, SGTB, SH3BP2, SH3RF2, TIGD3, TIMM17B, TOX2, UBN2, UBN2, ZNF48, ZNRF3, CREB1, CRK, CROT, CXCL12, CXCL14, CYB561D, CYB7, CYBRD1, CYPR2B, DDHD1, DEND5B, DERR2, DGKE, DIRC2, DLG2, DMTF1, DNAJA2, DNAJC27, DPP3, DPP8, DYPS5L, DYNLT3, EDNRP, EGLN1, EIF4B, ELAVL2, ENDODI, EPHA2, EPHA5, EPHA7, EROIB, EZHI, FAM102B, FAM117A, FAM18B2, FAM40B, FAM46C, FBXL4, FBX010, FBX041, FGD4, FGD5, FLTI, FNM3, FOXX2, FOXO3, FRMD4A, FRMD4B, FYCO1, FZD6, GAB2, GALT1, GALNT3, GATAD2B, GATC, GDA, GLIS3, GLS, GN5B, GNG12, GNPD2A, GOLGA1, GPR12, GPR137C, GPR180, GUCY1A3, HAUS8, HDAC4, HEG1, HIP1, HIPK3, HIK1, HLF, HMGBX3, HNI, HNRPUL2, HOOK3, HPBP3, IGDC3, IKZF2, IL22RA, IL8, INOS0D, IPO7, IQSE1, IRAK2, IRAK4, IRF2, IRF9, ISMI1, ITGB8, JUB, KDM2A, KIAA0226, KIAA0240, KIAA0513, KIAA1522, KIAA1549, KIAA1737, KIF3B, KLF12, KLF13, KLF3, KHLH28, KREME1, KSR2, LEFI, LEFTY1, LEFTY2, LHX6, LHX8, LF, LMO3, LRT1, LUC7L2, LYPD6, LYM2, LYSMD3, LYST, MAML1 |
### Table VII. Continued.

| Regulation | Systematic name | Target genes |
|------------|-----------------|--------------|
|            | MAPIB, MAP3K1, MAP3K14, MAP3K2, MDB2, MBNL2, MBNL3, MCCD1, MCL1, MDM4, MECP2, MED13L, MFAP3L, MBI1, 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, NPS3, NR2C1, NR2C2, OCRl, ODF2, OPCML, ORMDL3, OSBPL5, OST1, OTUD7B, PAFAH2, PAG1, PAK2, PAM, PAN3, PARP8, PBX3, PCDH7, PCGF5, PDCD4, PDLIM5, PFN2, PGBD5, PHACTR4, PHC3, PHF6, PHKA1, PHYHPL, PIPI4K2A, PKD2, PKN2, PLG1, PLC1, POFUT1, POLK, POU6F1, PPARA, PPARGC1B, PPPIR10, PPPIR19A, PPPIR6, PRDM16, PRDM8, PRKACB, PRMT6, PRRT2, PRRX1, PSD3, PSEN1, PTGDR, PTPDC1, RAB11A, RAB11FIP1, RAB11FIP5, RAB22A, RABEP1, RAB18, RAD23B, RALGDS, RAPGEF2, RAPGEF5, RAPGEFL1, RASSF2, RBL1, RBMS2, RDX, RELA, RELL1, RGL1, RGM1, RHOC, RIMKLA, RNF180, RNF216, RNF38, RNF6, RORA, RPS6KA2, RRAGD, RSN1, RSNB1, RSNIL, RSF1, RSRC2, RUNX2, RYR2, SAMD12, SAR1B, SASH1, SBFI, SCD5, SCN2A, SCN5A, SCRT2, SDC1, SETBP1, SETD7, SHCBPI, SIK1, SIPA1L3, SLC14A1, SLC16A12, SLC16A9, SLC35E1, SLC38A1, SLC39A6, SLC46A3, SLC6A9, SMARC2C, SNRK, SNTEB, SNX30, SNX5, SNX9, SOS1, SPRED1, SSR1, SSR2, ST3GAL5, ST8SIA2, STX16, SVE20H1, SYAI, SYDI1, SYNC, SYNPO2, TANC2, TAO2, TAP1, TARDBP, TCBL, TCEB3, TET2, TET3, TGFBR2, TIAMI, TMC1C, TMEM100, TMCT2, TMU1B, TNRC18, TNRC6B, TNRC6C, TNS1, TOX, TRACPC2, TRHDE, TRIM2, TRIM44, TRIM66, TRP51, TRPV6, TSEN34, TSHZ3, TTC9, TTPAL, TUSC1, UBASH3B, UBE2B, UBE2J1, UBE2Q2, UBE2R2, UBE2W, UBI1, UBN2, UHRF1, UHRF1BP1, ULP1, UNK, UNKL, UPF3A, USP24, USP46, USP53, VSX1, WDR26, WDR37, WDR45, WEE1, WIF2, YTHDF3, ZBTB11, ZBTB41, ZBTB43, ZBTB47, ZCCHC2, ZDHHC8, ZDHHC9, ZFPI1, ZFYVE26, ZKSCAN1, ZMYND11, ZNF148, ZNF2, ZNF236, ZNF25, ZNF362, ZNF385A, ZNF436, ZNF473, ZNF512B, ZNF518A, ZNF566, ZNF597, ZNF682, ZNF682, ZNF1X |
| hsa-miR-338-5p | AAK1, ADAMTS17, ADARB2, AEBP2, AMMECR1, APPL1, ARFGAP3, ARID2, ARNT, ATAD1, ATF7, ATP2C1, ATRX, AUTS2, B4GALT6, BAZIB, BCL11B, BCL2L1, BTG3, CADM3, CALM3, CAST, CCDC140, CCNT2, CD25, CD28, CD29, CDK5R1, CDY1L2, CHST12, CLIC4, CLTC, CNR1, CNTN4, CPEPB4, CPNE3, CREB3L1, CRIM1, CSNK1G1, CUL3, DKG, DICER1, DIAT, DLX5, DNLAC6, DNM3, DMYK, EML1, EP300, EPAS1, EPHA7, ERRFI1, EXOC5, FAM126A, FAM135B, FAM177A1, FAM182, FMLN2, FOXL3, FUT9, GATA2, GREM2, GRIA4, GRM7, GTF3C2, GUCY1A3, HCN1, HDAC9, HIF1A, HIPK2, HSPA12A, IKZF1, IMACT, INO80D, IRE2, JMDIC, KAL1, KDM5B, KIAA1024, KIAA1467, KLF11, KLHL14, KLHL6, KLRQ1, KRAS, LM04, LRPI, MACF1, MBNL1, MBNL2, MCTS1, MEF2C, MIPO1, MLK2, MLL4, MLLT4, MN1, MON2, MPPED2, NCK2, NCOA3, NDFIP1, NPS4, NRP1, NUDT4, NUFIP2, OCAD1, ONCUT2, PARD6B, PCDH17, PCDH20, PCGF5, PCNXL, PEL11, PHC3, PHIP, PKN2, PLAGL2, PLEKHA5, PPARGC1A, PPPIB, PPPIR5A, PRDM10, PRLR, PTCH1, PTKS1, R3HDM2, RAB14, RABIA, RAB22A, RAB6B, RAP2C, RAPGEF5, RAPH1, ROC1, RICTOR, RND3, RNF138, RORA, SAMD12, SN01, SEC68, SEMA6A, SERTAD3, SERTDH, SERT2, SERT3, SKP1, SLC4A7, SLIT1, SLMAP, SNTB1, SOX6, SPAST, SPOC, SSX2IP, STAG2, SUB1, SYNCRIP, SYPL1, TAF4, TANC2, TARDBP, TDX2, TCEG1L1, TEADI1, TET2, TLK1, TRA2B, TRAF3, TRPM7, TSH23, UBE2N, UBR2, USP25, WASF1, WDFY3, WCC3, ZBTB44, ZFANS5, ZNF29 |
| hsa-miR-623 | AAK1, ACSM2A, ADARB2, AGPAT4, ALPL, AP3M2, APPL1, ATG9A, BAHD1, CAConAC, CAMK2B, CDC117, CDCC3, CELSR3, CLUAP1, CORO2A, CRTC2, CXCL12, DCLK1, DCLK3, DSEL, ECE1, EGFLAM, |
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, and listed in Table VII.
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   |
| Regulation                                      | Name                              | Diffgene count | Gene count | Enrichment | P-value    | FDR         |
|------------------------------------------------|-----------------------------------|----------------|------------|------------|------------|-------------|
| 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  |             |
| Down-regulated                                |                                   |                |            |            |            |             |
| 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   |
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-κB 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 network analysis. Based on the significantly regulated pathways, we further established miRNA-pathway network analysis.
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networks to screen the key regulatory functions and the key DEMs (Fig. 4). The top rated five miRNAs included hsa-miR-373-3p, hsa-miR-338-5p, hsa-miR-590-5p, hsa-miR-623 and hsa-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

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.
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 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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