Preeclampsia down-regulates microRNAs in fetal endothelial cells: Roles of miR-29a/c-3p in endothelial function

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Short title: PE down-regulates fetal endothelial microRNAs

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Supplemental Detailed Methods

Isolation and characterization of HUVECs

HUVECs were freshly isolated from umbilical veins immediately after cesarean-section delivery from NT and PE pregnancies using an enzymatic method as described (1-3). To avoid the potential impact caused by the long term *in vitro* culture, cells were purified using Dynabeads® CD31 Endothelial Cell (Life Technologies, Carlsbad, CA) after 16 hr of culture under 37 ℃ and 5% CO_2 in Endothelial Cell Medium (ECM; ScienCell Research Laboratories, Carlsbad, CA), which consisted of ECM basal medium (ECM-b) supplemented with 5% fetal bovine serum, 1% endothelial cell growth supplement, and 1% penicillin/streptomycin solution.

DiI-Ac-LDL uptake assay

The purity of HUVECs was examined as previously described (1, 2). Cells were incubated with Acetylated Low Density Lipoprotein labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiI-Ac-LDL, 10μg/mL, Biomedical Technologies, Stoughton, MA) for 4 hr at 37°C, fixed in 4% formaldehyde, and examined under a phase-contrast and fluorescent microscope. Images were taken under a Nikon TE2000U inverted microscope connected to a Spot Insight QE CCD camera using the SPOT advanced image analysis software. Cells exhibiting uptake of LDL were counted. Cells incubated without DiI-Ac-LDL were served as negative controls.

Human miRNome PCR array analysis

Screening of differentially expressed miRNAs between P0 HUVECs isolated from NT and PE pregnancies was performed using the miScript human miRNome PCR Array (Qiagen), which contained a total of 1008 mature miRNAs Total RNA samples were reverse transcribed into cDNAs using a miScript II RT Kit (Qiagen). A total of 750 ng total RNA sample was used in each reverse transcription reaction. Diluted cDNA corresponding with 0.6 ng of original total RNA was

utilized as the template in each RT-qPCR reaction of miScript human miRNome PCR Array. The miRNAs expression data were normalized to the external control (miRTC, Qiagen) along with five internal control miRNAs (SNORD61, SNORD68, SNORD72, SNORD95, and SNORD96A) and further analyzed using the miScript miRNA PCR Array Data Analysis Tools (Qiagen) to determine the DE-miRNAs between NT and PE groups. Three individual cell preparations were analyzed for each group.

Real-time qPCR (RT-qPCR) analysis

For parallel analysis of miRNAs of interest, 400ng small RNA fragment enriched total RNA isolated from each sample was reverse transcribed into cDNA using a miScript II RT Kit (Qiagen, CA). Diluted cDNA corresponding to 1 ng of original total RNA was utilized as the template in each RT-qPCR reaction. Each miRNA of interest was performed using commercially available miRNA miScript Primer Assays in Supplemental Table S1 (Qiagen), miScript SYBR Green PCR Kit (Qiagen), and StepOne^{Plus} qPCR system (Life Technologies). The RT-qPCR data were normalized to the external control (miRTC, Qiagen) along with internal control miRNAs (SNORD68, SNORD95, and SNORD96A) using the Biogazelle qBase^{Plus} (ver. 2.0) software (4). The normalized RT-qPCR data were then further analyzed using the $2^{-\Delta\Delta CT}$ method (4-6) to determine the relative abundance. Efficiency of all target and control miRNA assays were between 90% and 110%. Six individual cell preparations were analyzed for each group. Three technical repeats were performed for each target genes per sample.

Knockdown of miRNAs

Due to the limited amount of P0 HUVECs (usually only 3×10^5-1×10^6 per preparation), we used passages 3-4 (P3-4) HUVECs preparations pooled from 5 NT individuals as described(1, 2) in the miRNA knockdown study. Knockdown of each target miRNA was performed using miScript miRNA Inhibitor (Qiagen, refer as miRNA-(i)) which is chemically synthesized and modified single-strand RNA that specifically bind and inhibit target miRNA (7, 8).

P3-4 HUVECs at 40-50% confluence were transfected with miScript miRNA Inhibitors (Qiagen) that specifically target human miR-29a-3p (Cat.# MIN0000086), miR-29c-3p (Cat.# MIN0000681), and miScript Inhibitor Negative Control (Cat.# 1027272) at 0, 50, or 100 nM using the HiPerFect Transfection Reagent (Qiagen) for 6, 24, 48, and 72 hr (7). Cells transfected with the miScript Inhibitor Negative Control were used as negative controls (NC). Cells treated with only the HiPerFect Transfection Reagent were used as the vehicle controls (9). RT-qPCR (see above) was used to verify the efficiency of miRNA knockdown.

The dose and time of all VEGFA and FGF2 treatments are determined based on previous reports from our own lab (1, 3, 10) and others (8, 11-13).

Western blotting analysis

Western blot analysis was performed as described (3, 10, 14, 15). In brief, cells were lysed in lysis buffer [50 mM HEPES, 0.1 M NaCl, 10 mM EDTA, 4 mM sodium pyrophosphate, 10 mM sodium fluoride, 2 mM sodium orthovanadate (pH 7.5), 1 mM phenyl methyl sulfonyl fluoride, 1% Triton X-100, 5 μg/ml leupeptin, 5 μg/ml aprotinin] followed by sonication. The lysates were centrifuged at 13,000 g for 10 min. Protein concentrations of the supernatants were determined with bovine serum albumin (BSA; fraction V; Sigma) as standards. The protein samples (20 μg) were separated on SDS-PAGE gels and electrically transferred to polyvinylidene difluoride membranes. The membranes were immunoblotted with the antibody against each different target (Supplemental Table S2). Proteins were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientifics, MA) followed by exposure to chemiluminescence films. The immunoreactive signals of the bands on the films were recorded using densitometry and analyzed by Image J software. Total (T)-AKT1 and T-ERK1/2 levels were normalized to GAPDH. Phosphorylated (P)-AKT1 and P-ERK1/2 levels were first normalized to GAPDH and then proportioned to corresponding normalized T-AKT1 and T-ERK1/2, respectively.

Scratch healing assay

Cell migration was assessed by scratch healing assay as described (11, 12). HUVECs seeded in 24-well plates were treated with Vehicle, NC, and miRNA-(i) and cultured for 24-30h to allow cells reach confluence. The confluent cell monolayer was wounded by manually scraping the cells with a 200ul pipette tip. The cells were immediately treated with ECM-b (as control), VEGFA (10 ng/ml), and FGF2 (10 ng/ml). Cell migration into the wound area was monitored after 0 and 20h of treatments. For each scratch, five images were photographed under a 4X objective lens, which represent 80% of the length of the scratch. Images were photographed immediately after scratching (0 hr) and after 20 hr of ECM-b, VEGFA and FGF2 treatment. Sizes of the wound area (mm^2) were calculated using the Image J image analysis software.

Cell proliferation assay

Cell proliferation was assessed by methyl thiazolyl tetrazolium (MTT) assay using the MTT Assay Kit (Cayman Chemical, Ann Arbor, MI) as described (16). After treating with Vehicle, NC, and miRNA-(i) for 24 hr, cells seeded in 96-well plates (5000 cells/well) were serum-starved in ECM-b for 8 hr, and treated with ECM-b (as control), ECM, VEGFA (10 and 100 ng/ml), or FGF2 (10 and 100ng/ml) for 42 hr. Cells were then incubated with MTT, and absorbance at 580nm was measured using a Synergy HT Multi-Detection Microplate Reader (Bio-Tek Instruments, Winooski, VT).

Cell monolayer integrity assay

Cell monolayer integrity of HUVECs was determined using the ECIS Zθ+ 96-well array station (Applied BioPhysics, Troy, NY) using 96-well plates (96W10E+) (8, 13). Cells seeded at 2×10^4 per well were treated with Vehicle, NC, and miRNA-(i) and cultured for 30h to allow cells reach confluence. The confluent cells were treated with ECM-b (as control), ECM, VEGFA (100 ng/ml), or FGF2 (100 ng/ml) (4 wells per treatment). ECIS was used to real-time measure the electrical resistance over the monolayer of endothelial cells (indicating of the monolayer integrity). Electrical resistance was constantly monitored and recorded up to 25 hr after treatment.

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Supplemental Table S1. List of miRNA Primer Assays used in miRNA RT-qPCR.

	Mature miRNA ID	RT-qPCR Primer Assay type	Vendor	Catalog Number
1	hsa-miR-101-5p	Primer Assay for miRNA of interest	Qiagen	MS00008379
2	hsa-miR-1293	Primer Assay for miRNA of interest	Qiagen	MS00014539
3	hsa-miR-146a-5p	Primer Assay for miRNA of interest	Qiagen	MS00003535
4	hsa-miR-1537-3p	Primer Assay for miRNA of interest	Qiagen	MS00016471
5	hsa-miR-192-5p	Primer Assay for miRNA of interest	Qiagen	MS00003689
6	hsa-miR-2116-5p	Primer Assay for miRNA of interest	Qiagen	MS00031598
7	hsa-miR-219a-1-3p	Primer Assay for miRNA of interest	Qiagen	MS00009128
8	hsa-miR-29a-3p	Primer Assay for miRNA of interest	Qiagen	MS00003262
9	hsa-miR-29b-3p	Primer Assay for miRNA of interest	Qiagen	MS00006566
10	hsa-miR-29c-3p	Primer Assay for miRNA of interest	Qiagen	MS00003269
11	hsa-miR-3186-5p	Primer Assay for miRNA of interest	Qiagen	MS00020944
12	hsa-miR-32-5p	Primer Assay for miRNA of interest	Qiagen	MS00003297
13	hsa-miR-337-3p	Primer Assay for miRNA of interest	Qiagen	MS00023457
14	hsa-miR-3691-5p	Primer Assay for miRNA of interest	Qiagen	MS00007651
15	hsa-miR-369-5p	Primer Assay for miRNA of interest	Qiagen	MS00006860
16	hsa-miR-374c-5p	Primer Assay for miRNA of interest	Qiagen	MS00023492
17	hsa-miR-3924	Primer Assay for miRNA of interest	Qiagen	MS00031843
18	hsa-miR-4288	Primer Assay for miRNA of interest	Qiagen	MS00021385
19	hsa-miR-4317	Primer Assay for miRNA of interest	Qiagen	MS00021588
20	hsa-miR-551b-3p	Primer Assay for miRNA of interest	Qiagen	MS00004627
21	hsa-miR-598-3p	Primer Assay for miRNA of interest	Qiagen	MS00010290

22	hsa-miR-617	Primer Assay for miRNA of interest	Qiagen	MS00005082
23	hsa-miR-744-3p	Primer Assay for miRNA of interest	Qiagen	MS00010556
24	MIRTC	Primer Assay for external control	Qiagen	MS00000001
25	Hs_SNORD68	Primer Assay for endogenous control (small nucleolar RNA)	Qiagen	MS00033712
26	Hs_SNORD68	Primer Assay for endogenous control (small nucleolar RNA)	Qiagen	MS00033719
27	Hs_SNORD68	Primer Assay for endogenous control (small nucleolar RNA)	Qiagen	MS00033726
28	Hs_SNORD68	Primer Assay for endogenous control (small nucleolar RNA)	Qiagen	MS00033733

Supplemental Table S2. List of antibodies used for Western-blot analyses.

	Antibody	Vendor	Catalog Number
1	T-AKT1	Cell Signaling Technology	9272
2	T-ERK1/2	Cell Signaling Technology	9102
3	P-ERK1/2 (Thr202/Tyr204)	Cell Signaling Technology	9101
4	P-AKT (Ser473)	Cell Signaling Technology	9271
5	GAPDH	Abnova	H00002597-M01

Supplemental Table S3. Positively detected miRNAs in P0-HUVECs and DE-miRNAs between PE and NT P0-HUVECs.

miRNAs positively detected in P0-HUVECs*	DE-miRNAs† in PE vs. NT P0-HUVECs
hsa-miR-101-5p	hsa-miR-101-5p
hsa-miR-1293	hsa-miR-1293
hsa-miR-146a-5p	hsa-miR-146a-5p
hsa-miR-1537-3p	hsa-miR-1537-3p
hsa-miR-192-5p	hsa-miR-192-5p
hsa-miR-2116-5p	hsa-miR-2116-5p
hsa-miR-219a-1-3p	hsa-miR-219a-1-3p
hsa-miR-29a-3p	hsa-miR-29a-3p
hsa-miR-29b-3p	hsa-miR-29b-3p
hsa-miR-29c-3p	hsa-miR-29c-3p
hsa-miR-3186-5p	hsa-miR-3186-5p
hsa-miR-32-5p	hsa-miR-32-5p
hsa-miR-337-3p	hsa-miR-337-3p
hsa-miR-3691-5p	hsa-miR-3691-5p
hsa-miR-369-5p	hsa-miR-369-5p
hsa-miR-374c-5p	hsa-miR-374c-5p
hsa-miR-3924	hsa-miR-3924
hsa-miR-4288	hsa-miR-4288
hsa-miR-4317	hsa-miR-4317
hsa-miR-551b-3p	hsa-miR-551b-3p
hsa-miR-598-3p	hsa-miR-598-3p
hsa-miR-617	hsa-miR-617
hsa-miR-744-3p	hsa-miR-744-3p
hsa-miR-100-3p	
hsa-miR-100-5p	
hsa-miR-101-3p	
hsa-miR-103a-2-5p	
hsa-miR-103a-3p	
hsa-miR-103b	
hsa-miR-105-3p	
hsa-miR-105-5p	
hsa-miR-106a-3p	
hsa-miR-106b-3p	
hsa-miR-106b-5p	
hsa-miR-107	
hsa-miR-10a-3p	
hsa-miR-10a-5p	
hsa-miR-10b-3p	
hsa-miR-10b-5p	
hsa-miR-1178-3p	
hsa-miR-1179	
hsa-miR-1180-3p	

hsa-miR-1182
hsa-miR-1183
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hsa-miR-1185-5p
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hsa-miR-302d-3p
hsa-miR-302d-5p
hsa-miR-302e
hsa-miR-302f
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hsa-miR-575
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hsa-miR-7-2-3p
hsa-miR-744-5p
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hsa-miR-767-3p
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hsa-miR-889-3p
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hsa-miR-891a-5p
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hsa-miR-892b
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hsa-miR-92a-3p
hsa-miR-92b-5p
hsa-miR-93-3p
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hsa-miR-93-5p
hsa-miR-936
hsa-miR-937-3p
hsa-miR-938
hsa-miR-9-3p
hsa-miR-941
hsa-miR-942-5p
hsa-miR-943
hsa-miR-95-3p
hsa-miR-9-5p
hsa-miR-96-3p
hsa-miR-96-5p
hsa-miR-98-5p
hsa-miR-99a-3p
hsa-miR-99a-5p
hsa-miR-99b-3p
hsa-miR-99b-5p

*Positively detected: Ct value <35 in the human miRNome PCR Array analysis between P0-HUVECs from PE and NT

†DE-miRNAs: miRNAs with fold change>|2| and P<0.05 in the human miRNome PCR Array analysis between PE and NT P0-HUVECs.

Supplemental Table S4. PE-induced DE-miRNAs associated KEGG pathways.

KEGG pathway	Associated PE-induced DE-miRNAs	# of Associated PE-induced DE-miRNAs in P0-HUVECs	FDR adjusted p-value
ECM-receptor interaction (hsa04512)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-146a-5p hsa-miR-744-3p hsa-miR-4288 hsa-miR-1293 hsa-miR-337-3p hsa-miR-101-5p	9	3.93E-45
Lysine degradation (hsa00310)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-146a-5p hsa-miR-337-3p hsa-miR-551b-3p hsa-miR-192-5p hsa-miR-744-3p hsa-miR-4288 hsa-miR-101-5p hsa-miR-1293	11	2.63E-09
Protein digestion and absorption (hsa04974)	hsa-miR-744-3p hsa-miR-617 hsa-miR-4288 hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-146a-5p hsa-miR-1293 hsa-miR-337-3p hsa-miR-219a-1-3p hsa-miR-101-5p	11	1.10E-05
Proteoglycans in cancer (hsa05205)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-337-3p hsa-miR-146a-5p hsa-miR-192-5p hsa-miR-219a-1-3p	13	1.74E-05

	hsa-miR-551b-3p hsa-miR-4288 hsa-miR-1293 hsa-miR-744-3p hsa-miR-101-5p hsa-miR-617		
Amoebiasis (hsa05146)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-1293 hsa-miR-337-3p hsa-miR-146a-5p	7	2.39E-05
Signaling pathways regulating pluripotency of stem cells (hsa04550)	hsa-miR-146a-5p hsa-miR-101-5p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-337-3p hsa-miR-4288 hsa-miR-219a-1-3p hsa-miR-369-5p hsa-miR-744-3p hsa-miR-192-5p	11	7.64E-05
Glioma (hsa05214)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-744-3p hsa-miR-219a-1-3p hsa-miR-337-3p hsa-miR-146a-5p hsa-miR-617	8	2.62E-04
Focal adhesion (hsa04510)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-1293 hsa-miR-337-3p hsa-miR-744-3p hsa-miR-4288 hsa-miR-219a-1-3p hsa-miR-192-5p hsa-miR-101-5p hsa-miR-146a-5p	12	2.34E-03

	hsa-miR-617		
FoxO signaling pathway (hsa04068)	hsa-miR-4288 hsa-miR-101-5p hsa-miR-617 hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-1293 hsa-miR-337-3p hsa-miR-219a-1-3p hsa-miR-146a-5p hsa-miR-744-3p hsa-miR-3186-5p	12	2.51E-03
Thyroid hormone synthesis (hsa04918)	hsa-miR-146a-5p hsa-miR-192-5p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-1293	7	3.89E-03
Colorectal cancer (hsa05210)	hsa-miR-219a-1-3p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-337-3p hsa-miR-146a-5p hsa-miR-192-5p hsa-miR-744-3p hsa-miR-617	10	7.77E-03
Circadian rhythm (hsa04710)	hsa-miR-617 hsa-miR-4288 hsa-miR-146a-5p hsa-miR-337-3p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p	7	9.89E-03
TGF-beta signaling pathway (hsa04350)	hsa-miR-4288 hsa-miR-744-3p hsa-miR-337-3p hsa-miR-617 hsa-miR-369-5p	10	9.89E-03

	hsa-miR-1293 hsa-miR-219a-1-3p hsa-miR-146a-5p hsa-miR-29c-3p hsa-miR-101-5p		
Melanoma (hsa05218)	hsa-miR-337-3p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-219a-1-3p hsa-miR-744-3p hsa-miR-4288 hsa-miR-146a-5p hsa-miR-617	9	1.30E-02
Pancreatic cancer (hsa05212)	hsa-miR-4288 hsa-miR-146a-5p hsa-miR-617 hsa-miR-744-3p hsa-miR-219a-1-3p hsa-miR-337-3p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p	9	1.31E-02
Estrogen signaling pathway (hsa04915)	hsa-miR-29a-3p hsa-miR-192-5p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-744-3p hsa-miR-337-3p hsa-miR-146a-5p hsa-miR-219a-1-3p hsa-miR-1293	10	1.53E-02
Acute myeloid leukemia (hsa05221)	hsa-miR-744-3p hsa-miR-337-3p hsa-miR-146a-5p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-1293 hsa-miR-617	9	1.65E-02

Neurotrophin signaling pathway (hsa04722)	hsa-miR-29a-3p hsa-miR-192-5p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-337-3p hsa-miR-4288 hsa-miR-744-3p hsa-miR-146a-5p hsa-miR-219a-1-3p hsa-miR-617 hsa-miR-101-5p	11	1.98E-02
Platelet activation (hsa04611)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-146a-5p hsa-miR-101-5p hsa-miR-337-3p hsa-miR-1293 hsa-miR-219a-1-3p hsa-miR-4288 hsa-miR-192-5p	10	2.51E-02
Thyroid hormone signaling pathway (hsa04919)	hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-1293 hsa-miR-337-3p hsa-miR-146a-5p hsa-miR-101-5p hsa-miR-192-5p hsa-miR-744-3p	10	2.51E-02
ErbB signaling pathway (hsa04012)	hsa-miR-146a-5p hsa-miR-551b-3p hsa-miR-337-3p hsa-miR-1293 hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-192-5p hsa-miR-744-3p hsa-miR-4288 hsa-miR-617	11	2.51E-02
PI3K-Akt signaling	hsa-miR-29a-3p	12	2.51E-02

pathway (hsa04151)	hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-4288 hsa-miR-3186-5p hsa-miR-1293 hsa-miR-337-3p hsa-miR-192-5p hsa-miR-744-3p hsa-miR-146a-5p hsa-miR-219a-1-3p hsa-miR-598-3p		
Small cell lung cancer (hsa05222)	hsa-miR-146a-5p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-744-3p hsa-miR-337-3p hsa-miR-4288 hsa-miR-192-5p	8	3.87E-02
Non-small cell lung cancer (hsa05223)	hsa-miR-337-3p hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-744-3p hsa-miR-146a-5p hsa-miR-617	7	3.98E-02
AMPK signaling pathway (hsa04152)	hsa-miR-617 hsa-miR-29a-3p hsa-miR-29c-3p hsa-miR-29b-3p hsa-miR-598-3p hsa-miR-4288 hsa-miR-219a-1-3p hsa-miR-744-3p hsa-miR-192-5p hsa-miR-3186-5p hsa-miR-337-3p hsa-miR-1293	12	4.99E-02

Supplemental Table S5. Target gene of miR-29a-3p and miR-29c-3p in fibroblast growth factor receptor (FGFR) signaling pathway (GO0008543).

Official Gene Symbol	Target genes of miR-29a-3p in the FGFR signaling pathway	Target genes of miR-29c-3p in the FGFR signaling pathway
PHLPP2	PHLPP2	PHLPP2
GSK3B	GSK3B	GSK3B
NDST1	NDST1	NDST1
NRAS	NRAS	NRAS
CALM3	CALM3	CALM3
AGO3	AGO3	AGO3
FRS2	FRS2	FRS2
RPS27A	RPS27A	RPS27A
UBC	UBC	UBC
TNRC6B	TNRC6B	TNRC6B
RICTOR	RICTOR	RICTOR
AGO1	AGO1	
PDGFB	PDGFB	
PIK3R1	PIK3R1	PIK3R1
SOS1	SOS1	SOS1
FOXO3	FOXO3	FOXO3
CDKN1A	CDKN1A	CDKN1A
ITPR3	ITPR3	
TRIM71	TRIM71	
PTEN	PTEN	PTEN
RAB14	RAB14	
MDM2	MDM2	MDM2
PDGFA	PDGFA	
TNRC6A		TNRC6A