Supplementary Data

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were cultured from the umbilical cord which was separated from the placenta. The blood in the umbilical vein was washed away using 1 × HEPES solution. 0.1% collagenase type I (Sigma, USA) was subsequently infused into the umbilical vein, which was digested for 15 min at 37°C. The solution containing the endothelial cells was rinsed with an appropriate amount of 1 × HEPES solution. This entire operation was performed with sterile techniques and was completed within 3 h. The cells obtained from the solution were cultured in an endothelial cell medium (ECM, ScienCell, USA) supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin, and 1% endothelial cell growth supplement (ScienCell, USA) at 37°C in a humidified atmosphere with 5% CO_2 and 95% O_2. Culture medium was refreshed every 2–3 days. HUVEC passages from three to six were used for this study.

Endothelial Microparticle Isolation

Endothelial microparticles (EMPs) were isolated from cell culture medium by sequential centrifugation. First, medium of HUVECs was centrifuged at 800 ×g for 15 min and 12,500 g for 5 min at 4°C to remove cell debris and apoptotic bodies (1–5 μm), and the microparticle-enriched medium was collected. Second, the supernatant was further centrifuged at 4°C at 20,500 g for 2.5 h to pellet down MPs smaller than 1 μm.

Electron Microscopy

EMPs isolated from the supernatant of HUVECs were adsorbed onto formvar /carbon-coated copper mesh grids, rinsed in filtered phosphate-buffered saline (PBS), and then fixed in 1% glutaraldehyde at 4°C for 1 h and stained with 1% uranyl acetate for 10 min. All transmission electron micrographs were obtained using transmission electron microscope (FEI Tecnai Spirit) at 120 kV.

Flow Cytometry

The number of EMPs was analyzed on a flow cytometer (BD, USA). The EMP concentration was assessed by comparison to TruCount beads added to each sample. The EMP gate was defined as particles from 0.1 to 1 μm in size using calibration beads (Invitrogen). The presence of phosphatidylserine at the surface of MPs was assessed using FITC-conjugated Annexin V (Miltenyi). To identify the EMPs (Annexin V+CD31), EMPs were stained with anti-CD31-phycoerythrin. Annexin V-FITC with EDTA and isotype-matched antibodies served as negative controls.

Migration Assay and Tube Formation Assay

For scratch migration assay, A 200 μl pipette tip was used to create a scratch on cell monolayer. The plate was washed once with PBS followed by replacement of ECM. After incubation at 37°C, 5% CO_2 for 12 h, migration was quantified by measuring the cell-free area.

For transwell chamber migration assays, 5×10^4 cells which were suspended in 600 μl serum free and plated onto a gelatin-coated 8.0 μm pore size polycarbonate membrane in 24-well plates (Corning, NY). The lower chamber contained ECM with 10% FBS and 20 ng/ml vascular endothelial growth factor. After incubation at 37°C, 5% CO_2 for 18 h, the lower chambers were washed with PBS, and cells were fixed with 4% paraformaldehyde, followed by 0.1% crystal violet staining. The migrated cells were photographed for counting.

For tube formation assay, 200 μl Matrigel matrix (BD Biosciences, USA) was added to 48-well plates and the gel was formed at 37°C in 5% CO_2. Following gel solidification for 30 min, 50 μl of HUVEC suspension (4×10^5/ml) was added to each well and the cell culture medium was compensated to 1 ml. Tube formation was observed using an inverted microscope, and the average percentage of tube number of tube-like capillary measured in random 10 photographic fields (% control) was described to evaluate the activity.

Real-Time Polymerase Chain Reaction

For quantification of miRNAs, TaqMan MicroRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied Biosystems, USA) were used. Real-time polymerase chain reaction (RT-PCR) reactions were preincubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. MiR-19b levels in HUVECs were normalized to U6 snRNA or RNU6B. Values in EMPs were normalized to the spiked-in *Caenorhabditis elegans* miRNAs, cel-miR-39 (10 fmol/sample; Qiagen, Inc.).

For transcript relative quantification, ImProm-IITM Transcription System and GoTaq 2-Step RT-quantitative PCR System (Promega, USA) were used. RT-PCR was performed using the primers of transforming growth factor-β2 (*TGFβ2*, 5'-AAATGGAGGCCCAGAAAGAT-3'; 5'-ACTTGACTGCACCGTTGTTG-3') and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 5'-GAGTCAACGGATTTGGTCGT-3'; 5'-GACAAGCTTCCCGTTCTCAG-3'). Amplifications were preincubated at 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Values were normalized to GAPDH.

Western Blot Analysis

HUVECs were lysed in a RIPA lysis buffer (50 mM Tris HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1.2% Triton-X-114, 1 mM NaF, 200 mM NaVO4) with protease inhibitor (Roche Diagnostics) and centrifuged at 12,000 g for 10 min at 4°C. The supernatants were collected, and the protein concentration was determined with the bicinchoninic acid protein assay kit (Pierce Biotechnology Inc., USA). 20–30 μg proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel and electrophoretically transferred to PVDF membranes (Millipore, USA). The membranes were probed against TGFβ2 (Abcam, USA) and reprobed against GAPDH (Santa Cruz Biotechnology, USA) as a loading control.

Luciferase Plasmid Construct

The human *TGFβ2* 3'UTR segment was obtained by PCR amplification. Primer pairs were designed to amplify a segment of 1306 bp, which contained the predicted miR-19b target site within the *TGFβ2* 3'UTR (*TGFβ2*-WT-F: 5'-GACTAGTAATGGAATATTTGCAGTTTCACCT-3' and *TGFβ2*-WT-R: 5'-ATGGTACTGATAGGAAAAAGACATCCACATAT-3'). PCR products were ligated into the multiple cloning site of the luciferase reporter plasmid pMIR-REPORT™ (Applied Biosystems, USA) digested by SacI and PmeI. The correct orientation of the insert was determined by sequencing. To generate mutations in the predicted target site of miR-19b, seven nucleotides located in the seed region of miR-19b were mutated through PCR amplification (*TGFβ2*-MUT-F: 5'-AGAGAGATGAAACGTGCATGCTTTGGCTTTCT GGTTCTA-3' and *TGFβ2*-MUT-R: 5'-AAAGCATGCACGTTTCATCTCTCTGCATTTTTTC TGGTCGGC-3'). Sequencing was performed to verify the mutations of the seven nucleotides.