Supplementary material

Title: Anti-microbial peptide SR-0379 stimulates human endothelial progenitor cell-mediated repair of peripheral artery diseases

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Running Title: Peptide-enhanced cell therapy

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Supplementary Materials and Method

Western blotting

Cells were washed twice with HBSS and then lysed in lysis buffer (20 mm Tris-HCl, 1 mm EGTA, 1 mm EDTA, 10 mm NaCl, 0.1 mm phenylmethylsulfonyl fluoride, 1 mm Na3VO4, 30 mm sodium pyrophosphate, 25 mm β-glycerol phosphate, 1% Triton X-100, pH 7.4). The cell lysates were centrifuged for 15 min at 4 °C, and the supernatants were used for western blotting. The lysates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membranes, and then stained with 0.1% Ponceau S solution (Sigma-Aldrich, St Louis, MO, USA) to ensure equal loading of the samples. After being blocked with 5% non-fat milk for 30 min, the membranes were incubated with primary antibodies overnight, and the bound antibodies were visualized with horseradish peroxidase-conjugated secondary antibodies using the enhanced chemiluminescence western blotting system (Amersham Biosciences, Piscataway, NJ, USA).

MTT assay

The EPCs were seeded in 24-well plates at a density of 20000 cells/well after digestion. After the cells were adhered, EPCs were media changed with low FBS (0.5%) in EBM-2 media. EPCs were treated SR-0379 to 0.1 μg/mL to 100 μg/mL in a dose-dependent manner. At given time points after treatment of SR-0379 (0 h, 24 h and 48 h), the cells were incubated growth media with 50 μL of MTT (5 mg/mL) at 37 °C for a further 2 h. Then, the medium was removed and the precipitated formazan was dissolved in 200 μL of dimethyl sulfoxide
(DMSO). The absorbance at 562 nm was detected under a microplate spectrophotometer (Tecan, Sunrise). Four replicates were set up for each experiment.
Supplementary Figure 1. Activation of the FAK-AKT pathway by SR-0379 in EPCs.
Effects of SR-0379 on phosphorylated FAK (Tyr397) and phosphorylated Akt (Ser473) as determined by Western blot. The EPCs were treated with SR-0379 (10 μg/ml) for 0, 5, 15 and 30 minutes.
Supplementary Figure 2. Cell viability assay of SR-0379 in EPCs. Cytotoxic effect of SR-0379 as determined by MTT assay on EPCs. The EPCs were treated by dose-dependent on SR-0379 (0.1, 1, 10 and 100μg/ml) and FGF (200ng/ml). Cell viability was measured by MTT assays at 0, 24 and 48 hours post SR-0379 treatment. Data represent mean ± S.D. (n = 4 for each group). *, P<0.05 vs. control.