Supplementary Materials and Methods

Angiogenic Gene Expression by Real-time PCR

Total RNA was isolated using the TRIzol Reagent (Invitrogen) and reverse transcribed using Omniscript RT (Qiagen). cDNA was synthesized from 1 μg of total RNA using the Omniscript RT (Qiagen) kit. Real-time PCR (ABI-PRISM 7900HT) was then performed using ABI SYBR green or Taqman Gene Expression Assays (for eNOS only). All SYBR green PCR primers were designed using ABI Primer Express Software (Table S1). ACTB (β-Actin) was employed for analysis of relative expression.

Angiogenic Protein Secretion

Conditioned medium was obtained following culture in serum-free medium (EBM-2) for 12 hours and stored at -80°C until used for specific protein quantification by ELISA (R&D Systems).

Western Blot

100 μg of total protein was separated by SDS-PAGE and electrically transferred to a nitrocellulose membrane. The membrane was blocked for 1 hr at room temperature with TBS-T buffer (20 mM TrisHCl, 150 mM NaCl, 0.1% Tween-20, pH 7.5) containing 5% skimmed milk, and then incubated overnight at 4ºC with a mouse anti-human eNOS monoclonal antibody (1:5000 diluted in blocking buffer, BD). The second day the membrane was washed 3 times with TBS-T buffer and incubated for 1 hr with a HRP-conjugated anti-mouse secondary antibody (1:5000 diluted in blocking buffer). The membrane was washed 3 times and the specific band

was developed using an ECL Western blotting detection kit (Amersham) and visualized with the VersaDoc Imaging System (BioRad).

Chemotactic Migration Assay

CACs were serum-starved for 1 hour, detached from culture dishes, pelleted and resuspended in EBM-2 + 0.5% BSA ($5x10^5$ cells/mL). 500 μL of this cell suspension ($2.5x10^5$ cells) was placed within cell culture inserts (Becton Dickinson, 8μm pore size) and the inserts placed within 24-well companion plates containing 500 μL of EBM-2 + 0.5% BSA with either $VEGF_{165}$ (50 ng/mL) or human SDF-1 (100 ng/mL). After four hours at 37°C, the inserts were removed, and the cells bound to the underside of the membrane were fixed and stained using the DiffQuick staining kit (Sigma). The cells bound to bottom of the membrane were visualized using an inverted light microscope (Nikon Eclipse TS100). Five random fields were photographed per membrane and the number of cells per high-power field (HPF) counted in a blinded manner.

Adhesion to Activated Endothelial Cells

HUVECs (~90% confluent) were activated by incubation with TNF-α (5 ng/mL) for 4 hours in 4-well chamber slides. CMTMR-labeled CACs ($1x10^5$) were then cultured over HUVECs in regular CAC medium for 3 hours to allow for adhesion. Following two PBS washes, all remaining cells were fixed with cold 2% paraformaldehyde pH 7.4. Three random fields per chamber were visualized by confocal microscopy, using both brightfield and fluorescent channels (for CMTMR detection). The mean number of adherent CACs was then quantified in a blinded manner.