SUPPLEMENTARY MATERIAL

Supplementary Methods

Recombinant Adenoviruses

Ad.SCF was constructed using the AdEasy XL Adenoviral Vector System (Stratagene). The full length of SCF membrane isoform cDNA was cloned into the pShuttle-IRES-hrGFP-1 vector. The linearized shuttle vector was recombined in Escherichia coli strain BJ5183 with AdEasy, a serotype 5 first-generation adenoviral backbone. The recombinant viral backbones were transformed into 293 cells and grown at large scale. Adenoviruses were purified by double cesium chloride gradient. Ad-EGFP-β-gal (Ad.β-gal) adenovirus was used as a control. To confirm the predicted viability of recombinant viruses, in-vitro transfections of cultured cardiomyocytes were conducted for 8 hours at a multiplicity of infection 50 for Ad.β-gal and 100 for Ad.SCF for an additional 24 to 48 hours. A total of $1.5x10^{12}$ viral particles were injected per animal which was the equivalent of $1x10^{11}$ plaque forming units.

Cardiac function measurements

The pigs were prepared in the same manner as the MI creation. Under propofol anesthesia, percutaneous punctures were performed to obtain arterial and venous accesses. A 7-Fr Swan-Ganz catheter (Edwards-Lifesciences LLC, Irvine, CA USA) was advanced through the venous sheath up into the pulmonary artery, where cardiac output was measured by several

injections of a saline bolus until 3 or more stable measurements were obtained. A 7-Fr, 12-electrode, dual-field conductance catheter (Millar Instruments, Houston, TX) was advanced into the LV for assessment of LV pressure-volume relationships. Subsequently, an 11-Fr balloon catheter was advanced to the inferior vena cava for preload alterations. Data analysis was performed using iox2 (Emka Technologies, Falls Church, VA). The conductance catheter gain factor α was calculated as the ratio of conductance derived cardiac output to that measured by thermodilution. Parallel conductance was adjusted using the end-diastolic volume obtained from 3DE. Following the pressure-volume relationship measurement, left ventriculography was performed and analyzed as previously described (1).

A Philips ie-33 ultrasound system (Philips Medical Systems, Andover, MA, USA) was used to acquire echocardiographic data with a multi-frequency imaging transducer. Complete Doppler trans-thoracic echocardiographic studies were performed. Images were recorded during end-expiratory breath-hold in the standard LV apical view. 3DE datasets were acquired from 4 to 7 consecutive cardiac cycles in R-wave-triggered mode. Post-acquisition image analyses were performed offline using the Q-lab application (Phillips Medical Systems) by a single blinded investigator. LV volumes were calculated using 3D full-volume algorithms with semi-automated border detection. Analyses were performed for 2 different sequences and the average of 2 measurements was used for final data. The 3DE data were also used to evaluate changes in wall motion as flows. Wall motion score indices were

assigned depending on the segmental EF. A score of either 1 (normal regional EF>55%), 2 (hypokinesia: EF=33-55%), 3 (akinesia (EF=0-25%), or 4 (dyskinesia EF<0%) was given to each of the 17 segments and averaged.

Histology

At the end of the study, pigs were euthanized under deep anesthesia. Hearts were explanted and sectioned into 6 slices along the long axis. To quantify the infarction size by digital planimetry, 5 slices of heart tissue were immersed in 1% triphenyl tetrazolium chloride. The remaining slice was used for histological and protein analysis, and was divided into sections. Each sample was preserved in three different sub-samples respectively for histology, biodistribution, and immunohistochemistry: formalin, snap frozen, and snap frozen in optimum cutting temperature (OCT) compound. Frozen samples were sectioned into 3 layers: epicardium, myocardum, endocardium.

Western blotting

Cardiac tissue was minced and subsequently homogenized in radioimmunoprecipitation assay buffer containing a protease inhibitor cocktail (Sigma-Aldrich). Protein extracts (10 μg) were separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad), and probed with following antibodies specific for SCF (Cell signaling),phospho-histone H3 (Cell

signaling), and proliferation cell nuclear antigen (PCNA) (Cell signaling). Peroxidase-conjugated secondary antibodies (Sigma-Aldrich) and GAPDH (Sigma-Aldrich) protein loading control were also employed. Blots were developed with Super Signal West Pico (Pierce). Protein band densities were quantified by using quantity Image J software (NIH).

Immunohistochemistry

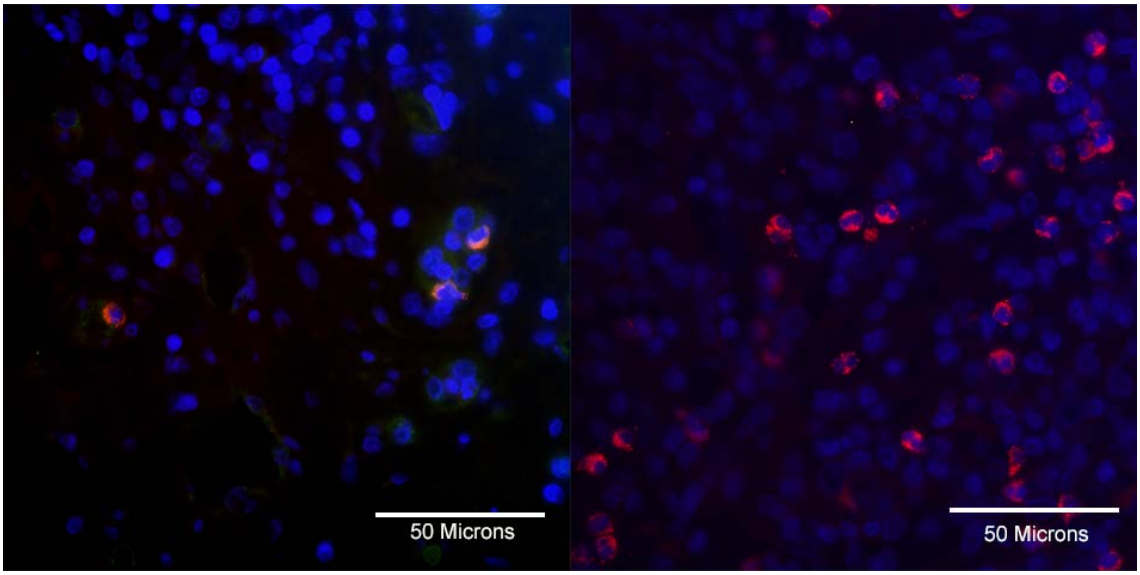
OCT-embedded blocks of myocardium were sectioned (8 μm) and mounted on positively charged microscope slides. These sections were fixed with 4% paraformaldehyde and treated with the antigen specific primary antibodies, followed by fluorescently labeled secondary antibodies specific for the primary antibody. Images were acquired using Zeiss LSM510 META confocal microscope. The heart sections from 1 week post gene transfer were treated with CD117, CD45, α-sarcomeric actin, PCNA, and VEGFR2 specific antibodies to evaluate their expression. Apoptosis rate was also assessed in the sections 1 week after SCF therapy using TUNEL technique by direct immunofluorescence with the ApopTag Red in situ apoptosis detection kit (Chemicon International Inc, Temecula, CA). Tissue sections were counterstained with Hoechst 33258 (1 μg/ml) (Sigma, St. Louis, MO). To quantify apoptosis, ten randomly selected microscopic fields per section were examined in the infarct border area of each section. The percentages of apoptotic cells were determined by counting the

TUNEL-positive nuclei relative to the total number of nuclei. Angiogenesis was assessed at 3Mo by measuring the number of smooth-muscle covered vessels (vessels/mm^2) in the myocardial sections of infarct border area using a double staining technique for anti-α-SMA antibody and CD31 (both from Abcam, Cambridge, MA). Vasculogenesis was also evaluated by counting the density of capillaries (capillaries/mm^2) in the infarct border area stained with isolectin IB4 (Sigma, St. Louis, MO). Only vessels <10 μm in diameter were taken into account to exclude venules and small arterioles.

Coronary flow measurement

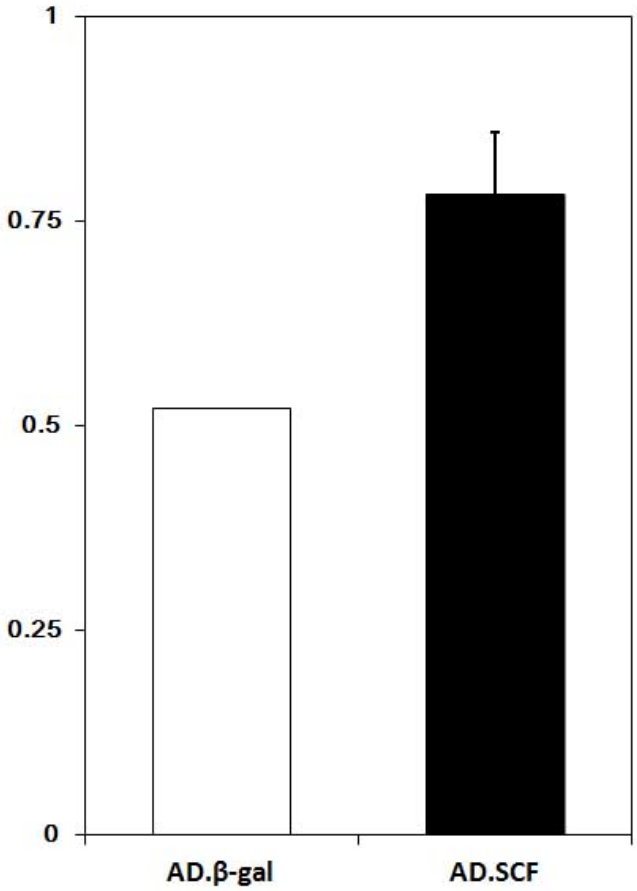
After hemodynamic measurement, a guiding catheter was advanced and a coronary angiography was performed to confirm the status of coronary arteries. In two of Ad.SCF and one of Ad.β-gal pigs, regional coronary flows in border area and remote area were assessed. Regional coronary flow was measured as published previously(2). In brief, 2 x 10^7 polystyrene fluorescent microspheres (15 μm; Interactive Medical Technologies, Irvine, CA) were injected into the left ventricle (LV). Reference blood was withdrawn from a femoral artery sheath using a specialized pump for 2 min at a rate of 2.9 ml/min (Harvard Apparatus, Holliston, MA). Distribution of fluorescent microspheres in the border zone and remote zone was quantified by flow cytometric analysis (Interactive Medical Technologies) and the numbers were compared.

Supplementary Figure1. Co-staining of c-kit and VEGFR2 in the infarct border zone 1 week after the gene transfer. Only a few c-kit^{+}/VEGFR2^{+} cells are found in the infarct border and majority of c-kit^{+} cells were negative for VEGFR2. Blue; DAPI-stained nuclei, red; c-kit, green; VEGFR2



Supplementary Figure2. Regional coronary flow measured by microspheres.

Relative coronary flow of infarct border area to remote area were compared (Ad.SCF n=2, Ad.β-gal n=1). The pigs treated with Ad.SCF had a higher relative coronary flow compared to the pig treated with Ad.β-gal.



Supplementary References

1. Ishikawa K, Chemaly ER, Tilemann L et al. Assessing left ventricular systolic dysfunction after myocardial infarction: are ejection fraction and dP/dt(max) complementary or redundant? Am J Physiol Heart Circ Physiol 2012;302:H1423-8.
2. Ishikawa K, Ladage D, Takewa Y et al. Development of a preclinical model of ischemic cardiomyopathy in swine. Am J Physiol Heart Circ Physiol 2011;301:H530-7.