SUPPLEMENTAL MATERIAL – Takehara et al.

Methods

Isolation and culture of Sca-1-positive cardiac progenitor cells (CPCs) in detail

Hearts from 8-10-week-old male C57Bl/6 mice were washed with cold phosphate-buffered saline (PBS) to remove blood cells, followed by removal of aortic and pulmonary vessels. Dissected hearts were minced, treated twice with 0.2% type II collagenase and 0.01% DNase I (Worthington Biochemical Corp, Lakewood, NJ, USA) for 20 min at 37°C. Cells were passed through 70- and 40-µm filters to remove debris and size-fractionated in a 30%–70% Percoll gradient to remove mature cardiomyocytes and obtain single-cell suspensions. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture (DMEM/F-12; Gibco/Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin, and recombinant mouse basic fibroblast growth factor (40 ng/mL; R&D Systems, Minneapolis, MN, USA) at 37°C and 5% CO_2. Expanded cells were cloned and Sca-1-positive CPCs were isolated by magnetic-activated cell sorting (Miltenyi Biotec, San Diego, CA, USA).

Flow cytometry analysis

Cells were detached with 0.2% TrypLE (Invitrogen, Carlsbad, CA, USA) and resuspended in DMEM with 10% FBS. After centrifugation at 1500 rpm for 5 min, cells were washed with PBS and 1×10^5 cells were resuspended in a 100-µL solution of PBS containing 1% bovine serum albumin (BSA) and 2 mM ethylenediaminetetraacetic acid (FACS solution). Cells were then incubated with 1 µL fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibody (CD11b-FITC, CD29-FITC, CD45-FITC, vascular cell adhesion molecule-FITC, CD105-FITC,

CD90-FITC, CD31-FITC or c-kit-PE; BD BioLab, Franklin Lakes, NJ, USA) in the dark at 4°C for 10 min. After washing twice with FACS solution, cells were resuspended in 400 µL FACS solution and analyzed on a FACSCalibur instrument (BD Biosciences).

Induction of cardiac differentiation in CPCs

CPCs, control-CPCS, and APE1-CPCs were seeded in a Matrigel Matrix Growth Factor-Reduced coated dish (BD Biosciences) and cultured for 14 days in Roswell Park Memorial Institute medium containing 2% B27 supplement (Gibco/Life Technologies) and penicillin/streptomycin. Cardiac differentiation was induced by adding activin A (100 ng/mL) and bone morphogenetic protein 4 (10 ng/mL) to the culture medium on day 1 and days 2–5, respectively.

Isolation and culture of rat neonatal ventricular myocytes (NRVMs)

Hearts from neonatal rats were washed with cold PBS to remove blood cells, and large vessels and atria were removed. The tissue was minced and treated four times with 0.1% type II collagenase for 10 min at 37°C. Cells were size-fractionated in a 45%–65% Percoll gradient to obtain a pure cardiomyocyte population. Cells (NRVMs) were purified (80-90%) by pre-plate method twice to remove the cardiac fibroblasts. Cells were seeded on collagen-coated two-chambered slides (1.5×10^5 cells/19×19mm cover slide well.) in DMEM/F-12 supplemented with 5% fetal calf serum and penicillin/streptomycin at 37°C and 5% CO_2. The following day, the culture medium was replaced with DMEM/F-12 supplemented with 0.1% BSA, 30 mM HEPES (pH 7.5), and 1× insulin-transferrin-selenium (Gibco/Life Technologies). NRVMs were co-cultured with 5.0×10^4 control-CPCs or APE1-CPCs.

Echocardiography

Mice were divided into groups using a random number table after the surgery and transthoracic echocardiography was performed to evaluate heart function 1, 7, and 28 days after cell transplantation using a Vevo 660 system (VisualSonics, Toronto, Canada). B- and M-mode images of hearts were recorded from the parasternal short axis view. Intraventricular septum and posterior wall thickness as well as left ventricular end-diastolic and end-systolic dimensions (LVDd and LVDs, respectively) were measured from the average of two short axis images at the mid-portion level. Indices of LV systolic function, including LV fractional shortening (LVFS) and LV ejection fraction (LVEF) were calculated with the following formulae: LVFS = [(LVDd − LVDs)/LVDd] × 100%; and LVEF = [(LVEDV−LVESV)/LVEDV] ×100%, where LVEDV and LVESV are LV end-diastolic and -systolic diameters, respectively, and $V = 7D^3/(2.4 + D)$.

Endothelial Tubing Assay

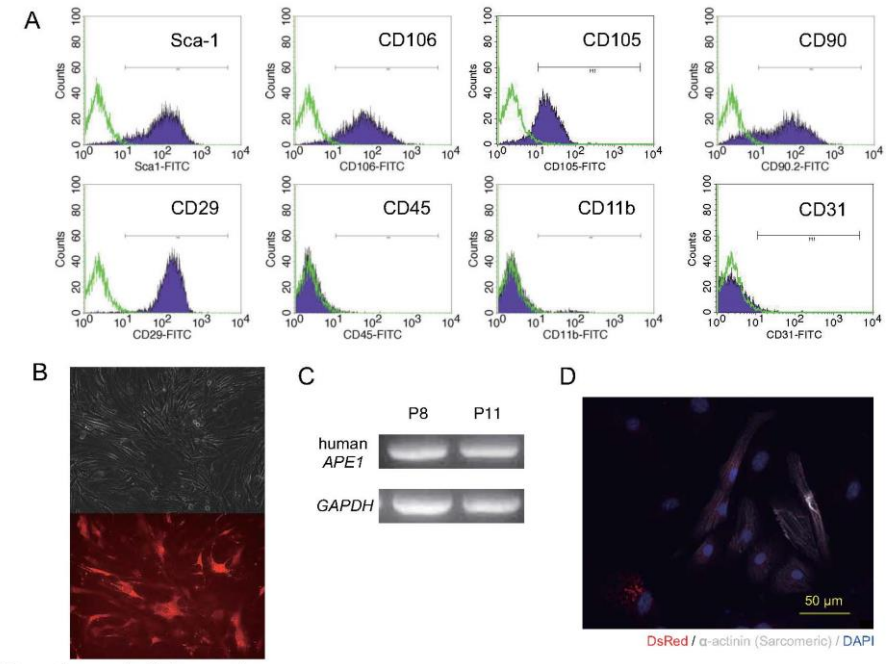
HUVECs were cultured in EBM-2 (Lonza, Basel, Switzerland) medium with 10% FBS, penicillin/straeptomycin, and vascular endothelial growth factor (10 ng/mL; Peprotech, Rocky Hill, NJ, USA). BD Matrigel Matrix Growth Factor Reduced (BD Biosciences) was added (45µL) to each well of a 96-well plate and incubated for 1 h at 37℃. HUVECs were suspended by supernatant medium (EBM-2 medium with 2% FBS) of Ct-CPC or APE1-CPC and reseeded on Matrigel-coated 96 well cell culture plate (1.0×10^4 cells/well). Cells were incubated for O/N at 37 °C and viewed using a microscope. Total tubing length was calculated using Image-J software (National Institutes of Health, Bethesda, MD, USA).

Angiogenesis ELISA assay

Control-CPCs and APE1-CPCs were grown in 12-well cell culture plate. The culture medium of confluent CPCs was replaced to 700 µL of serum free media with 200µM of hydrogen

peroxide. After a 4 h incubation, the mouse VEGF and FGF basic concentration in each culture supernatant was determined using ELISA Kit (Abcam, Cambridge, UK). The level of fluorescence was calculated with a Multidkan^{TM} FC Microplate Photometer (Thermo Fisher Scientific).

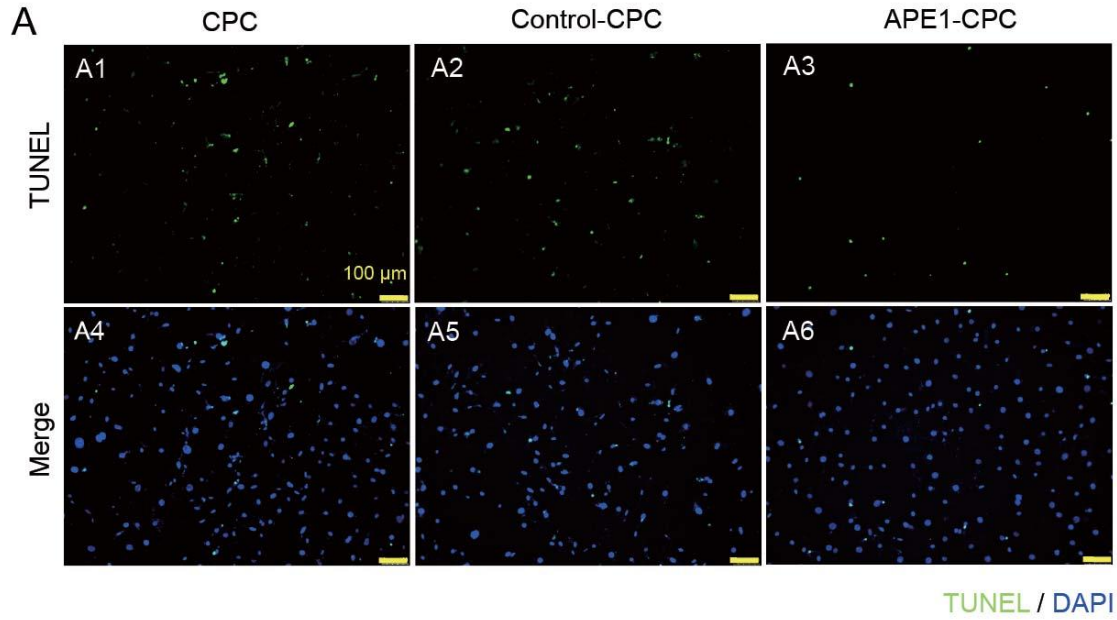
Supplemental Figures



Supplemental Figure 1

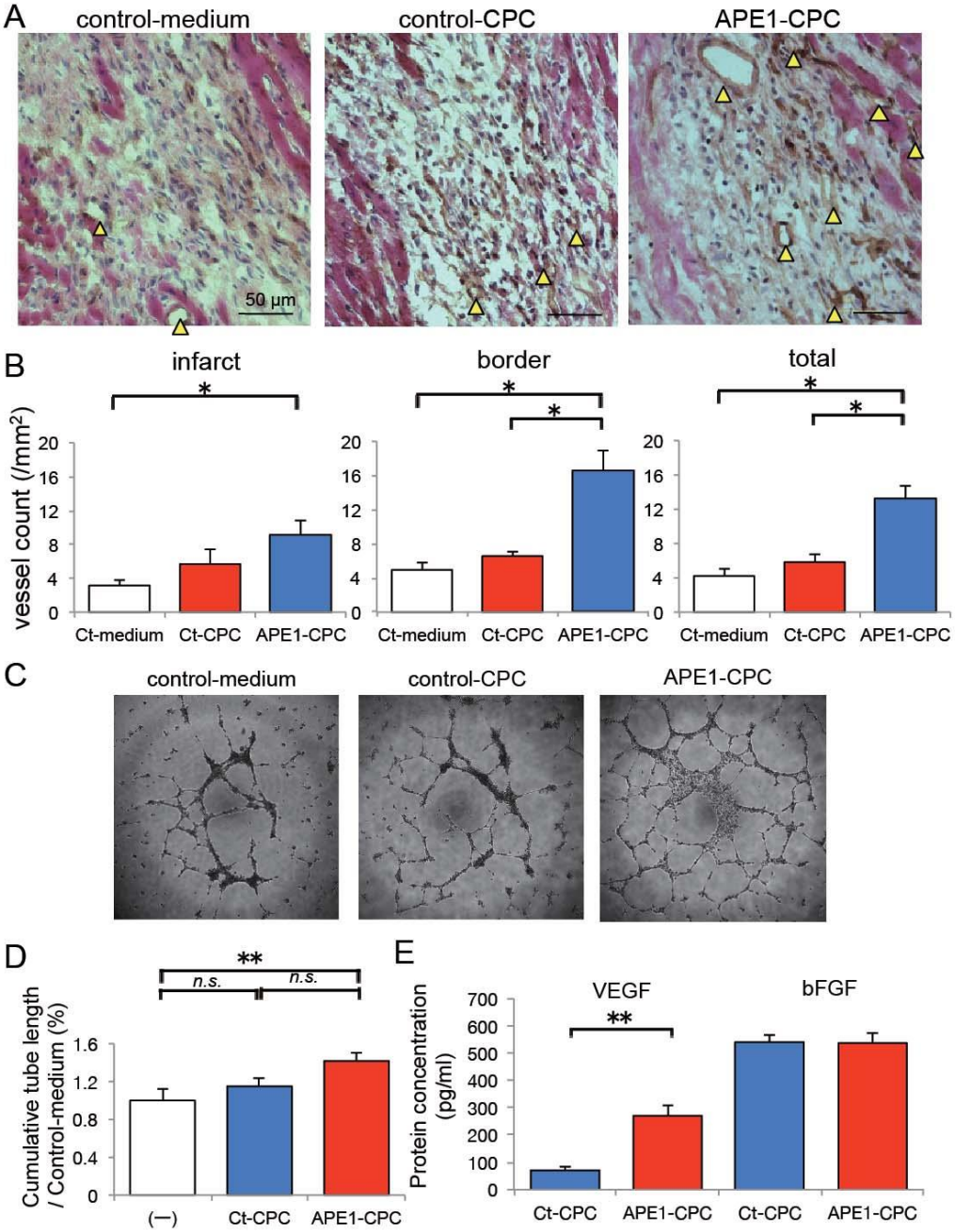
Figure S1. Characteristics of Sca-1-positive CPCs and APE1 overexpressing CPCs

A, Analysis of cell surface marker expression in Sca1-positive CPCs. Sorted cells were positive for Sca-1 (94.3%), cluster of differentiation (CD) 29 (99.4%), CD90 (59.9%), CD105 (79.7%), and vascular cell adhesion molecule (94.8%) and negative for CD11b (3.28%), CD31 (2.1%), CD45 (1.26%), and c-kit (3.07%). **B**, Micrographs of DsRed-expressing CPCs isolated by flow cytometry. CPCs were labeled with DsRed [red]. Image magnification = 40×, **C**, Exogenous human APE1 levels at passage 8 and passage 11 as determined by RT-PCR. **D**, Micrographs of CPCs and NRVMs after 7 days of co-culturing. CPCs were labeled with DsRed [red]. Cardiomyocytes were labeled with an antibody against cardiac α-sarcomeric actinin [white]. Nuclei were stained with 4', 6-diamidino-2-phenylindole [blue]. Undifferentiated CPCs = red. Differentiated CPCs = merged image of cardiac α-sarcomeric actinin [white] and DsRed [red].



Supplemental Figure 2

FigureS2. H_2O_2-induced the ROS production and apoptosis in CPCs. Representative images of TUNEL-positive cells (green; A1–A3); nuclei were stained with 4', 6-diamidino-2-phenylindole (blue; A4–A6). CPC; CPC transferred without any genes, control-CPC; CPC transferred with DsRed gene, APE1-CPC; CPC transferred with APE1-DsRed gene.



Supplemental Figure 3

Figure S3. Vascularization in host ischemic heart 7 days post-MI.

A, Images of cardiac tissue sections (hematoxylin and eosin staining) labeled for CD31 expression (brown) in the ischemic area 7 days post-surgery. Yellow arrowheads indicate CD31-positive vessels (capillary structure with brown [3, 3-Diaminobenzidine: DAB substrate] staining). Image magnification = 20×, **B**, Number of CD31-positive vessels in the total ischemic area and border and infarct areas (n = 6 per group). **C,** Representable image of a tubule formation assay by exposure of CPC conditioned-medium in vitro. Image magnification = 4×, **D,** Cumulative tube length of HUVEC in control medium, control- and APE1-CPC supernatant (n=7 per group). **E,** Angiogenesis ELISA assay in control-CPC and APE1-CPC (n=6 respectively). VEGF; vascular endothelial growth factor, bFGF; basic fibroblast growth factor, blank bar; control(Ct)-medium, blue bar; control(Ct)-CPC, red bar; APE1-CPC, *$p < 0.05$, **$p < 0.01$.

Supplemental Table

Table.S1 protein array analysis in control-CPC vs APE1-CPC

protein/actin	control-CPC	APE1-CPC	APE1-CPC/control-CPC
TAK1	3.60	4.46	1.24
Bad	1.64	1.39	0.85
Akt	1.45	1.45	1.00
ERK	1.54	1.38	0.90
p38MAPK	1.15	1.13	0.98
Ikbα	1.81	1.70	0.94
PARP	N/A	N/A	N/A
IkBα phos	1.06	1.06	1.00
HSP27	1.18	1.26	1.07
Smad2	1.15	1.18	1.03
p53	N/A	N/A	N/A
SAPK/JNK	1.42	1.32	0.93
Casp3	N/A	N/A	N/A
Casp7	N/A	N/A	N/A
Chk1	1.18	1.14	0.97
Chk2	1.13	1.07	0.94
eIF2α	N/A	N/A	N/A
Survivin	N/A	N/A	N/A