ONLINE DATA SUPPLEMENT

Materials and Methods

Nuclear Transfer and Embryo Culture. Cloned 129/Sv-*ROSA26*::*lacZ* fetuses were produced by piezo-actuated microinjection (Prime Tech, Japan) essentially as described previously[1,2]. Nucleus donor cells were isolated from primary cultures derived from tail tip biopsies of 8-week-old 129/Sv-*ROSA26*:*lacZ* males and cultured at 37°C in 5% (v/v) CO_2 in humidified air in gelatin-coated 3.5cm^2 flasks for 10-14 days in Dulbecco's modified ES medium (DMEM; GIBCO) supplemented with 15% (v/v) fetal calf serum (FCS). Prior to use, cells were dissociated by trypsinization; the reaction was quenched by the addition of DMEM prior to washing in phosphate buffered saline (PBS). A 1-3 μl aliquot of the resultant nucleus donor cell suspension was mixed with a 10-20 μl drop of HEPES-buffered CZB[3] containing polyvinylpyrrolidone (M_r 360,000) and nuclei injected into enucleated B6D2F1 oocytes within 1 hour of mixing. Approximately 1 hour after the final injection, nuclear transfer oocytes were activated by exposure to $SrCl_2$ for one hour after which incubation was in KSOM (Specialty Media, NJ) lacking $SrCl_2$ at 37°C in 5% (v/v) CO_2 in humidified air[2]. Cleaved (2-cell) embryos were transferred the next day (E1.5) to the oviducts of pseudopregnant CD1 surrogate mothers. Cloned fetuses recovered at 11 to 13 days of gestation were used as source of liver cells.

Isolation of c-kit Positive Liver Cells. On two separate occasions cloned embryos were obtained: in the first instance a group of four embryos of 12-13 days gestation and in the second, two of 11 and 13 days gestation. Isolated livers were mechanically disaggregated by passage through a 40 μm cell strainer (Becton Dickinson). A total of 1.67 x 10^7 nucleated cells were obtained from the first group, and 5.8 x 10^6 from the second. Cells were incubated with phycoerythrin-conjugated anti-c-kit antibody (BD Pharmingen), and sorted on a MoFlow cell sorter (Dako Cytomation). In the first study, 5

x 10^5 c-kit positive cells were obtained (see Figure 1 in the text), and in the second, 1.95 x 10^5 c-kit positive cells. Cells were suspended in 1 ml PBS with 10% FCS at 4°C.

Myocardial Infarction and Cell Implantation. Under ketamine (100 mg/kg) and acepromazine (1.2 mg/kg) anesthesia, the left ventricle of 129/Sv male mice was exposed and the left anterior descending coronary artery was ligated near its origin[4,5]. Four-six hours later, cloned c-kit positive cells were injected near the border zone. Control animals consisted of infarcted and sham-operated mice injected with saline. BrdU was given in the drinking water, 1 mg/ml, throughout the period of investigation. Mice were killed at 30 days after surgery or sham-operation. Hemodynamic measurements were obtained in 9, 10 and 8 sham-operated, infarcted-untreated and infarcted-treated female mice, respectively. Corresponding n values in all other determinations were 9, 10 and 10. Protocols were approved by New York Medical College.

Echocardiography and Hemodynamics. Echocardiography was performed in conscious mice at 15 and 29 days after surgery using a Sequoia 256c (Acuson) equipped with a 13-MHz linear transducer (15L8). The anterior chest area was shaved and two-dimensional (2D) images and M-mode tracings were recorded from the parasternal short axis view at the level of papillary muscles. From M-mode tracings, anatomical parameters in diastole and systole were obtained. Ejection fraction (EF) was derived from left ventricular (LV) cross sectional area in 2D short axis view[6]: EF=[(LVDA-LVSA)/LVDA]*100 where LVDA and LVSA correspond to LV areas in diastole and in systole. Mice were anesthetized with chloral hydrate (400 mg/kg body weight, i.p.) and a microtip pressure transducer (SPR-671, Millar) connected to a chart recorder was advanced into the LV and cavitary pressures and + and - dP/dt in the closed-chest preparation were obtained[7,8].

Cardiac Anatomy. At sacrifice, the abdominal aorta was cannulated, the heart was arrested in diastole with $CdCl_2$ and the myocardium was perfused with 10% formalin. LV chamber was filled with fixative at a pressure equal to the in vivo measured end-diastolic pressure[7,8] LV intracavitary axis was measured and three transverse slices from the base,

mid-region and apex were embedded in paraffin. The mid-section was used to measure LV thickness, chamber diameter and volume[7,8].

Infarct Size and Myocyte Volume and Number. The volume of regenerating myocardium was determined by measuring in each of three sections the fractional area occupied by the restored tissue within the infarcted-scarred myocardium. In a similar manner, the relative volumes of spared and infarcted myocardium were evaluated. These measurements, in combination with the total volume of LV and septum (weight divided by the specific gravity of muscle tissue, 1.06 g/ml), allowed us to compute the absolute volumes of formed, unaffected and infarcted tissue in each animal. Additionally, the volume of a total of 2,138 myocytes was measured by confocal microscopy in the repairing portion of the wall of mice treated with cloned c-kit positive cells[7,8]. Tissue sections were stained with cardiac myosin and laminin antibodies and propidium iodide (PI). Only longitudinally oriented cells with centrally located nuclei were included. The length and diameter across the nucleus were collected in each myocyte to compute cell volume, assuming a cylindrical shape[9]. Myocytes were divided in classes and the number of myocytes in each class was calculated from the quotient of total myocyte class volume and average cell volume. Myocyte cell volume and number in the spared myocardium of infarcted and sham-operated mice were measured by nuclear counts in transverse sections complemented by the estimation of nuclear length in longitudinal sections. These primary determinations, in combination with the aggregate volume of myocytes in the spared portion of the ventricle, allowed us to compute the total number of nuclei. Since in the mouse, 94%, 5.0% and 1.0% of myocytes are binucleated, mononucleated and multinucleated, the total number of cells was computed[7,10]. Thus, the number of myocytes lost from the LV and septum was obtained and infarct size was determined[8,10]. Numbers of arteriole and capillary profiles were counted and values were corrected for vessel obliquity to obtain their number per unit area and their length density per unit volume of myocardium.

Immunocytochemistry. Sections were incubated with β-gal (Rockland), BrdU (Roche) or Ki67 (Novocastra) antibody. Myocytes were recognized with cardiac myosin (Chemicon), α-sarcomeric actin, α-actinin, desmin, connexin 43, N-cadherin (from Sigma) and troponin I (Santa Cruz) antibodies[4,5,8]. Endothelial cells were identified with a factor VIII antibody and *Griffonia simplicifolia* lectin and smooth muscle cells with α-smooth muscle actin antibody (Sigma). Scar was detected by collagen type I and type III antibodies (Santa Cruz). Erythrocytes were recognized by TER-119 antibody (Pharmingen). Nuclei were stained with PI. The fractions of nuclei labeled by BrdU and Ki67 were obtained by confocal microscopy. Myocyte nuclei sampled in treated mice for BrdU and Ki67 labeling were 1,898 and 3,038, respectively. For DNA content, 1,500 myocyte and 1,400 lymphocyte nuclei were measured by confocal microscopy.

Statistics. All samples were coded and the code was broken at the end of the experiments. Results are mean±SD. Significance was determined by the Student's t test and Bonferroni method. $P < 0.05$ was considered significant.

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