Bone Marrow-Derived Cell Therapy Stimulates Endogenous Cardiomyocyte Progenitors and Promotes Cardiac Repair

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Inventory of Supplemental Information

Figure S1
- Flow cytometry data with cell surface marker expression of isolated mesenchymal stem cells and c-kit+ cells. Representative quality control data.
- Supplemental to Figure 1 and 2.

Figure S2
- Analysis of endogenous progenitor activity in the right ventricle after myocardial infarction. There is evidence of progenitor activity in the right ventricle after myocardial infarction 8 wks after MI, but no evidence of increased activity in the hearts of mice administered c-kit+ cells.
- Analysis of early (2 wks post-MI) endogenous progenitor activity after MI. The c-kit+ cell mediated regenerative effect is not yet evident at 2wks post-MI, suggesting that the c-kit+ cell therapy leads to a late boost in endogenous regeneration.
- Supplemental to Figure 1.

Figure S3
- Analysis of BrdU+ cardiomyocytes after MI and cell therapy, as a function of GFP/β-galactosidase expression. C-kit+ cell therapy leads to an increase in BrdU+ cardiomyocytes compared to vehicle, yet the majority of BrdU+ cardiomyocytes are GFP- consistent with progenitor proliferation and differentiation.
- Analysis of cardiomyocyte cross-sectional area as a function of GFP/β-galactosidase expression, demonstrated equivalent size in the uninjured heart but hypertrophy in preexisting GFP+ cardiomyocytes with injury. This supports the concept that there are no baseline differences between the GFP+ and β-galactosidase+ cardiomyocyte populations.
- Supplemental to Figure 1.

Figure S4
- Infarct size data suggesting no inter-group differences in the size of the experimental myocardial infarctions.
- Complete hemodynamic data for c-kit+ cell and mesenchymal stem cell treated mice, compared to control.
- Supplemental to Figure 2.
Figure S5

- Echocardiographic data demonstrating no discernable evidence of cardiotoxicity with the 4-OH-tamoxifen protocol. Important control for cardiac function data derived from mice treated with 4-OH-tamoxifen.
- Supplemental to Figure 2.
Figure S1. Cell surface marker profile of administered cells.
A. Bone-marrow c-kit+ cells administered after myocardial infarction (see Figure 1 and 2). Confirmation by flow cytometry of c-kit expression of cells isolated by immunomagnetic cell sorting. Conjugated isotope control (red) used in all experiments.
B. Bone-marrow derived mesenchymal stem cells administered after myocardial infarction (see Figure 2). Representative flow cytometry panel. Panel was reconfirmed within 1 passage of cell administration for all experiments. Conjugated isotope control (red) used in all experiments.
Figure S2. Right ventricular endogenous progenitor activity 8wks post-MI and MI borderzone progenitor activity 2wk post-MI (see Figure 1). A. Immunohistochemical staining for GFP and β-galactosidase in sham, MI and c-kit+ cell injected mice. Scale bar=100 μm. B. Bar graph depicting the percentage of GFP+ cardiomyocytes 2 wks after myocardial infarction or sham surgery. Data expressed as mean ± SEM. C. Bar graph depicting the percentage of β-galactosidase+ cardiomyocytes 2 wks after myocardial infarction or sham surgery. Data expressed as mean ± SEM. D. Bar graph depicting the percentage of GFP+ cardiomyocytes in the right ventricle after either sham surgery, myocardial infarction, or myocardial infarction with either MSC or c-kit+ cell therapy. Data expressed as mean ± SEM. E. Bar graph depicting the percentage of β-galactosidase+ cardiomyocytes in the right ventricle after either sham surgery, myocardial infarction, or myocardial infarction with either MSC or c-kit+ cell therapy. Data expressed as mean ± SEM.
Figure S3. After MI the β-galactosidase+ pool demonstrates characteristics consistent with a higher frequency of both smaller and BrdU+ cardiomyocytes (see Figure 1). A. BrdU+/troponin+ cardiomyocytes after either administration of c-kit+ cells or vehicle control. In both groups, the majority of BrdU+ cardiomyocytes are GFP- consistent with proliferation and differentiations of GFP- precursors. Data expressed as mean ± SEM. B. Cross-sectional area of β-galactosidase+ versus GFP+ cardiomyocytes. In the absence of injury (sham surgery) there is no difference in the size of the two populations. After MI, in all groups, there the GFP+ cardiomyocytes are significantly larger than β-galactosidase+ cardiomyocytes. Data expressed as mean ± SEM.
**Figure S4.** Infarct size and hemodynamic data 8 weeks after sham surgery or myocardial infarction and treatment with vehicle control, c-kit+ cells, or mesenchymal stem cells (see Figure 2). A. Infarct size determined by measurement of scar in serial trichrome stained sections at multiple ventricular levels. Infarct size between all MI groups was similar. Data expressed as mean ± SEM. B. Table with hemodynamic data derived from blinded terminal cardiac catheterization performed 8 weeks after MI. *p<0.05. Data expressed as mean ± SEM.
Figure S5. Reversible cardiotoxicity resulting from high-dose tamoxifen and 4-OH-tamoxifen, but not from a 14-day low dose protocol. High-dose experiment represented in the left-hand column. Mice were treated with oil (control), tamoxifen, or 4-OH-tamoxifen (80mg/kg/day) for 5 days (see Figure 2). Echocardiograms were performed at day 0, day 5, and day 19.

*p<0.05. Low-dose experiment represented in the right-hand column. Echocardiograms were performed at the completion of the 14-day dosing protocol (20mg/kg/day). No significant differences were noted in echocardiographic parameters of ventricular remodeling or function. EDD=end diastolic dimension; ESD=end systolic dimension; FS=fractional shortening. Data expressed as mean ± SEM.