

# Stem Cell Repair in Ischemic Heart Disease: An Experimental Model

Donald Orlic

*National Human Genome Research Institute/NIH, Bethesda, MD, USA*

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## Abstract

Bone marrow stem cells (BMSC) from adult mice are now believed to generate non-hematopoietic cell types. This newly defined property is referred to as stem cell plasticity. We tested the potential of lineage negative c-kit positive (Lin<sup>-</sup> c-kit<sup>+</sup>), GFP<sup>+</sup> BMSC to differentiate into cardiac myocytes in myocardial infarcts produced by ligation of the left coronary artery. At 9 days post-transplant the hearts showed a band of developing GFP<sup>+</sup> myocytes within the damaged myocardium. These GFP<sup>+</sup> myocytes were positive for cardiac specific myosin and early expressed transcription factors. Endothelial cells and smooth muscle cells also developed from the donor bone marrow cells. Left ventricular end diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP) were improved. Lin<sup>-</sup>c-kit<sup>-</sup> cells did not regenerate myocardium. We next tested the ability of cytokine-mobilized BMSC to regenerate myocardium. Nuclei in regenerating cardiomyocytes were positive for Csx/Nkx 2.5, GATA-4 and MEF2. Cytoplasmic proteins included desmin, nestin and connexin 43. Regenerating arterioles consisted of endothelial cells and smooth muscle cells positive for Ki67, and flk1. These regenerating vessels contained circulating TER119 positive red blood cells. Repair of infarcted myocardium resulted in improved heart function and survival. At day 27 after cytokine treatment and surgery, 11 of 15 mice survived compared with 9 of 52 non-treated mice. Left ventricular ejection fraction in infarcted hearts in cytokine-treated mice was 48%, 62% and 114% higher than the ejection fraction in non-treated mice at 9, 16 and 26 days following coronary artery occlusion. These findings demonstrate that circulating autologous stem cells traffic to the ischemic, infarcted myocardium and undergo differentiation into cardiomyocytes and vascular structures. We conclude that adult BMSC have the potential for repair in acute, ischemic heart disease.

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Stem cells that emerge during late embryonic and fetal development are thought to be restricted to the production of tissue specific cell types. This is considered to be the result of gene expression patterns that are imprinted and although adult stem cells continue to proliferate and self-renew their developmental ability is limited to the tissue in which they reside. But we are beginning to ask if that is so? We will examine this dogma in light of recent remarkable data that indicate adult stem cells retain a high degree of developmental plasticity. If this challenge to the dogma of adult stem cell commitment is sustained, we are about to enter a revolutionary period in the field of stem cell biology.

The bone marrow stem cells (BMSCs) of adult mice are believed to have the potential to generate non-hematopoietic cell types. This newly discovered ability of stem cells is referred to as plasticity and is the focus of

numerous, diverse investigations. Data in a series of recent remarkable reports indicate that adult BMSC retain the capacity to produce cells of unrelated tissues. This emerging concept of stem cell plasticity has been demonstrated in several murine models that show tissues of all three germ layers can be derived from adult BMSC. These include skeletal muscle (Gussoni et al., 1999 *Nature* 401:390-394), hepatocytes (Lagasse et al., *Nature Medicine* 2000, 6:1229-1234), neural cells (Brazelton et al., 2000, *Science* 290:1775-1779; Mezey et al., 2000, *Science* 290:1779-1782), vascular endothelium (Kocker et al., 2001, *Nature Medicine* 7:430-436) and epithelium of skin and several internal organs (Krause et al., 2001, *Cell* 105:369-377). Plasticity was established by the identification of Y-positive transplanted male BMSC or specific surface markers. Acquired function was determined in these studies by the onset of tissue

specific protein synthesis in Y-positive cells.

We have investigated the potential of BMSCs to differentiate into cardiac myocytes in myocardium that has been damaged by ischemia. Infarcts were produced in the left ventricle of adult female mice by ligation of the left coronary artery (LCA). The infarcts were treated with Lin<sup>-</sup> c-kit<sup>+</sup> cells isolated from bone marrow from adult male transgenic mice that expressed enhanced green fluorescent protein (eGFP). Two injections, of  $0.15 \times 10^5$  to  $1 \times 10^5$  cells in a volume of 2.5  $\mu$ L, were administered into the healthy myocardium adjacent to the infarcted region at 5 hours after LCA occlusion. At 9 days after transplantation the hearts showed a band of eGFP-positive, Y-chromosome positive myocytes within the damaged myocardium. These eGFP-positive myocytes were also positive for cardiac myosin and sarcomeric  $\alpha$ -actin. In cell-proliferation assays the developing myocytes were positive for BrdU and Ki67. Several myocyte-specific proteins, including the transcription factors GATA-4, MEF2, and Csx/Nkx2.5, and a gap junction/intercalated disc component, connexin 43, were observed. Endothelial cells and smooth muscle cells in the developing capillaries and small arterioles were positive for eGFP and Y chromosome, indicating their origin from the donor male bone marrow cells. They were positive for factor VIII and smooth muscle  $\alpha$ -actin, respectively. We did not find evidence of myocardial repair when as many as  $5 \times 10^5$  Lin<sup>-</sup> c-kit<sup>-</sup> cells were transplanted. Just prior to sacrificing the mice the left ventricular end diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP) were 30% to 40% greater than those in the hearts of mice that had been transplanted with Lin<sup>-</sup> c-kit<sup>-</sup> bone marrow cells.

In a second series of experiments we investigated the ability of BMSCs that had been mobilized by several injections of stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) to traffic to the infarcted myocardium and promote repair. At 27 days after cytokine treatment and the induction of an infarct

by coronary artery occlusion the cytokine-mobilized BMSCs had regenerated a new band of myocardium in the left ventricle. The nuclei of the regenerating cardiomyocytes were positive for Csx/Nkx2.5, GATA-4, and MEF2. The cytoplasmic proteins included desmin, nestin, and connexin 43. Regenerating arterioles consisted of endothelial cells and smooth muscle cells that were positive for Ki67 and flk1. These regenerating vessels contained circulating TER119+ red blood cells.

The repair of infarcted myocardium by cytokine-mobilized BMSCs resulted in improved heart function and greater survival. The left ventricular ejection fraction in the cytokine-treated mice was 48%, 62%, and 114% higher than that in the nontreated mice at 9, 16, and 26 days, respectively, after coronary artery occlusion. At day 27 after cytokine treatment and surgery 11 of 15 (73%) treated mice were alive but only 9 of 52 (17%) nontreated mice survived. These findings extend our initial results on myocardial repair using transplanted BMSCs. They show that circulating autologous stem cells traffic to the ischemic myocardium and undergo differentiation into cardiomyocytes and vascular structures. We conclude that adult BMSCs have the potential for repair in acute ischemic heart disease.

## References

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