

# Mesenchymal lineage precursor cells induce vascular network formation in ischemic myocardium

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## SUMMARY

Mesenchymal lineage precursors can be reproducibly isolated from adult mammalian bone marrow and grown in culture. Immunoselection with monoclonal antibodies against STRO-1 and vascular-cell-adhesion molecule 1 (VCAM1/CD106) prior to expansion results in a 1,000-fold enrichment of mesenchymal precursors compared to standard isolation techniques. Intramyocardial injection of human STRO-1-selected precursors in an athymic rat model of acute myocardial infarction results in induction of vascular network formation and arteriogenesis coupled with global functional cardiac recovery.

**KEYWORDS** cardiac myogenesis, cardiac neovascularization, mesenchymal precursor cells, STRO-1, VCAM1

## INTRODUCTION

In the prenatal period, definitive vascular network formation to sustain embryonic organogenesis is dependent on influx of mesenchymal lineage cells from the dorsal aorta and neural crest<sup>1-3</sup> to form the vascular supporting mural cells such as vascular smooth muscle cells and pericytes. Mesenchymal lineage precursor cells have been identified to be present in the postnatal mammalian bone marrow, and can be reproducibly isolated and grown in culture by prospective immunoselection.<sup>4-7</sup> These cells are anatomically located in perivascular niches in the bone marrow and throughout the body, and demonstrate phenotypic and genetic identity to vascular pericytes.<sup>8</sup>

Freshly isolated multipotent human adult bone marrow mesenchymal lineage stem cells have been extensively characterized for a long list of surface markers.<sup>4-7</sup> The combined use of monoclonal antibodies against the antigens STRO-1 and VCAM1/CD106 results in up to 1,000-fold enrichment of mesenchymal precursors capable of giving rise to colony-forming units of the fibroblastoid type relative to their incidence in unseparated bone marrow.<sup>7</sup> At a clonal level, cells positive for STRO-1<sup>bright</sup> and vascular-cell-adhesion molecule 1 (VCAM1) demonstrate multipotential capability, differentiating to smooth muscle, bone, cartilage, and adipose tissue. Since vascular network formation supports endogenous cardiac regenerative capacity and long-term survival of cardiomyocyte precursors,<sup>9,10</sup> we investigated whether mesenchymal lineage precursors could enhance both cardiac neovascularization and myogenesis. Here we show that injection of immunoselected human adult bone marrow mesenchymal precursor cells expressing a STRO<sup>bright</sup> VCAM1-positive phenotype results in induction of arteriogenesis in various tissues, including ischemic myocardium, and in sustained improvement in both global systolic and diastolic parameters of cardiac function.

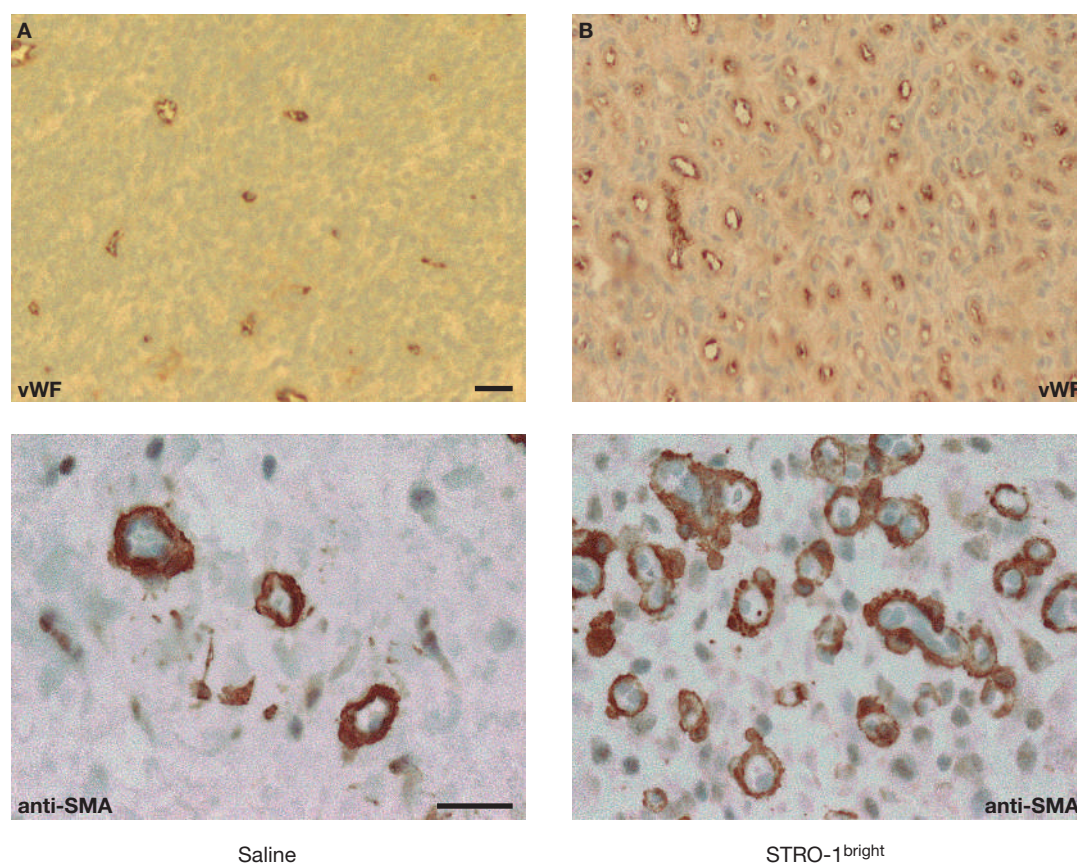
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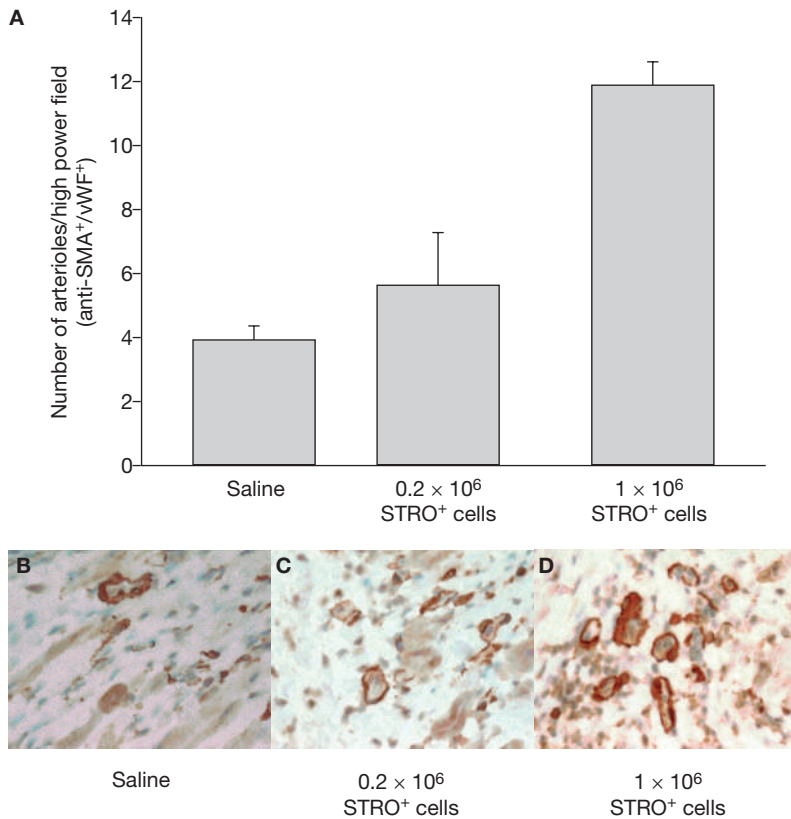


**Figure 1** Induction of tumor neovascularization (angiogenesis and arteriogenesis) by human STRO-1<sup>bright</sup> cells. Athymic nude rats were irradiated with 6 Gy for 5 min to remove residual natural killer function, then injected subcutaneously in the flank with  $1 \times 10^6$  rat glioblastoma cells. Two weeks after implantation, the glioblastoma tumors were directly injected with either (A) saline or (B)  $0.5 \times 10^6$  STRO-1<sup>bright</sup> cells, and animals were killed 7 days later. In consecutive sections of the tumor tissue stained by the immunoperoxidase method using monoclonal antibodies directed, respectively, against von Willebrand's factor (vWF) and anti-smooth muscle actin (anti-SMA), animals injected with STRO-1<sup>bright</sup> cells demonstrated significantly greater numbers of capillaries and arterioles (defined, respectively, by vWF staining alone and combined expression of vWF and anti-SMA) than animals injected with saline.

## METHODS AND RESULTS

Human mesenchymal precursor cells were isolated to greater than 99% purity from donor bone marrow by dual immunoselection using monoclonal antibodies against STRO-1 and VCAM1. Following *ex vivo* cellular culture and expansion for over six passages, phenotypic analysis of the cells demonstrated continued high-level expression of STRO-1 by 25–40% of the cultured cells, and these cells were immunoselected a second time using STRO-1 monoclonal antibody. To evaluate whether these cells were capable of inducing vascular network formation *in vivo*,  $0.5 \times 10^6$  culture-expanded STRO-1<sup>bright</sup> cells were directly injected into a rat glioblastoma tumor that produced high amounts of vascular endothelial growth factor

and had been placed 2 weeks earlier in the subcutaneous tissue of an athymic nude rat (Figure 1). Seven days later, the animals were killed and the glioblastoma tissue was evaluated by immunohistochemistry for evidence of vascular network formation. As shown in Figure 1, human mesenchymal precursor cells persisted in large numbers at 2 weeks in rat tissue, as defined by a monoclonal antibody with specificity for human mitochondrial structures, and were found adjacent to and surrounding vascular structures. Marked induction of arteriogenesis was noted at the sites of mesenchymal precursor cell injection, as defined by over eightfold greater numbers of large-caliber vessels (20–100  $\mu\text{m}$  diameter) dually staining with monoclonal antibodies



**Figure 2** Dose-dependent effect of STRO-1<sup>bright</sup> cells on myocardial neovascularization. To examine whether induction of angiogenesis and arteriogenesis could be extended to other tissues, and was associated with biological significance, cultured progeny of STRO-1-selected cells were injected by direct intramyocardial injection into the peri-infarct regions of the ischemic hearts in athymic nude rats who had undergone ligation of the left anterior descending coronary artery 2 days earlier. Animals injected with  $1 \times 10^6$  STRO-1<sup>bright</sup> cells (**D**) demonstrated threefold greater numbers of arterioles at the peri-infarct region than animals injected with saline (**B**;  $P < 0.01$ ). In contrast, animals injected with only  $0.2 \times 10^6$  STRO-1<sup>bright</sup> cells (**C**), delivered in a total of  $1 \times 10^6$  unfractionated cultured progeny of STRO-1-selected cells, induced only 50% greater numbers of arterioles at the peri-infarct region than saline ( $P < 0.05$ ), indicating that STRO-1<sup>bright</sup> cells have a dose-dependent effect on arteriolar induction in the ischemic heart. Anti-SMA, anti-smooth-muscle-actin; vWF, von Willebrand's factor.

against the endothelial marker von Willebrand's factor (vWF) and  $\alpha$ -smooth-muscle-actin ( $\alpha$ -SMA), compared with saline-treated controls. Arteriogenesis was limited to the injection site and was not observed distal to the site.

To investigate whether similar induction of arteriogenesis could occur in the acutely ischemic myocardium, culture-expanded STRO-1<sup>bright</sup> cells ( $0.2 \times 10^6$  cells or  $1 \times 10^6$  cells),  $1 \times 10^6$  bone marrow cells depleted of STRO-1<sup>bright</sup> cells, or saline was injected into the myocardium of

athymic nude rats 48 h after ligation of the left anterior descending coronary artery. Arterioles were quantitated by identifying structures coexpressing an outer layer of  $\alpha$ -SMA-positive cells and an inner layer of vWF-positive cells. As shown in Figure 2, animals injected with STRO-1<sup>bright</sup> cells demonstrated a dose-dependent increase in arteriolar induction in the ischemic heart, with the highest dose inducing threefold greater numbers of arterioles than saline-treated controls ( $P < 0.01$ ).

Finally, cardiac function at 2 and 6 weeks was assessed by echocardiography (Figure 3) and measurement of hemodynamic parameters, and correlated with cardiac histology and immunohistochemistry. Animals receiving  $1 \times 10^6$  STRO-1<sup>bright</sup> cells demonstrated mean improvement in ejection fraction of 50% and 75% at 2 and 6 weeks, respectively, relative to baseline values 2 days after ligation of the left anterior descending coronary artery. In stark contrast, saline-treated animals showed only 5% mean improvement in ejection fraction by 6 weeks ( $P < 0.01$ ), and animals treated with STRO-1-depleted bone marrow mononuclear cells demonstrated no difference from those receiving saline. Injection of  $1 \times 10^6$  STRO-1<sup>bright</sup> cells resulted in similar dramatic improvement in fractional shortening (mean improvement of 70% and 90% at 2 and 6 weeks, respectively). STRO-1-depleted bone marrow mononuclear cells again had no effect, while modest improvement was seen after injection of  $0.2 \times 10^6$  STRO-1<sup>bright</sup> cells. Finally, injection of  $1 \times 10^6$  STRO-1<sup>bright</sup> cells resulted in significant improvement in left ventricular compliance compared with saline-treated controls.

## DISCUSSION

In this study, we have shown that intramyocardial injection of human STRO-1<sup>bright</sup> mesenchymal precursor cells results in dose-dependent induction of arteriogenesis and vascular network formation in various tissues, including ischemic myocardium. In the mature vascular system, the endothelium is supported by mural cells, with the smallest capillaries partially covered by solitary pericytes, and arteries and veins surrounded by single or multiple layers of vascular smooth muscle cells. Pericytes coexpress  $\alpha$ -SMA and STRO-1 surface markers, consistent with a shared lineage identity with stromal/mesenchymal progenitors.<sup>8</sup>



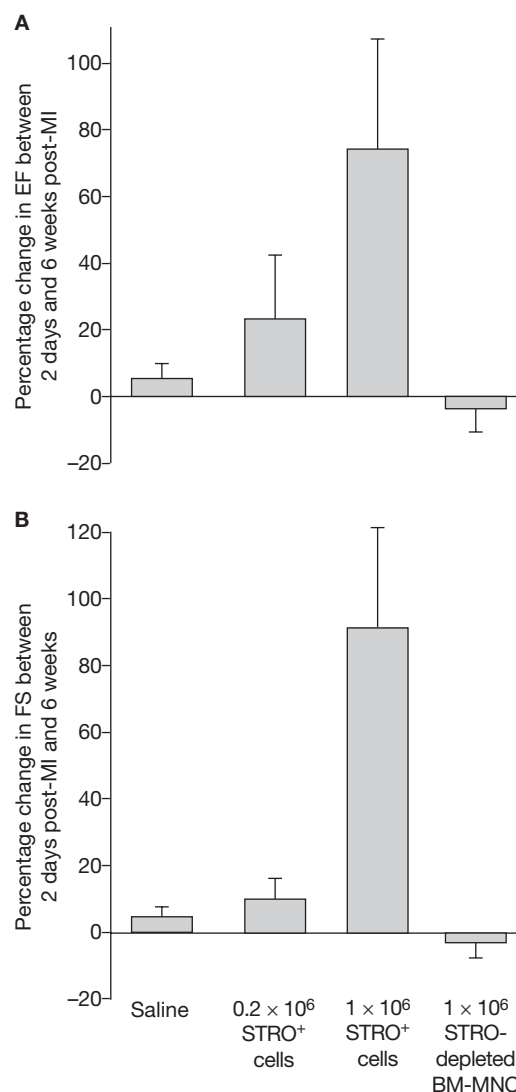
The perivascular *in vivo* location of human mesenchymal lineage precursors, together with their coexpression of markers of both endothelial and smooth muscle lineage cells and their multipotential capabilities, raise the intriguing possibility that mesenchymal lineage precursors may be true progenitors of the vascular tree.

The intimate proximity of human perivascular mesenchymal lineage precursors to vascular endothelium suggests that each cell type influences the biology of the other. Migration of mesenchymal lineage precursors and formation of a pericyte coating in physical continuity with the nascent vascular network is dependent on production of endothelial growth factor and platelet-derived growth factor  $\beta$  by nascent endothelial tubes.<sup>9</sup> Conversely, maintenance of vessel integrity, stabilization, and prevention of vessel pruning is dependent on pericyte coating of the microvessel.<sup>9</sup>

A major limitation to successful cellular therapy in animal models of myocardial damage has been the inability of the introduced donor cells to survive in their host environment because of the lack of a sufficient vascular supply. Recent studies have shown that development of thin-walled capillaries in ischemic myocardium following transplantation of hematopoietic lineage endothelial precursors enhances survival of endogenous cardiomyocytes.<sup>10,11</sup> Moreover, transplanting cultured embryonic cardiomyocytes that incorporate vascular structures *in vivo* results in significantly greater cell survival and protection against apoptosis.<sup>12</sup> Finally, in situations where transplanted cardiomyocyte precursors contained an admixture of cells also giving rise to vascular structures, survival and function of the newly formed cardiomyocytes have been significantly augmented.<sup>13</sup>

In the present study, we showed that implantation of STRO-1<sup>bright</sup> mesenchymal precursors into the acutely ischemic myocardium was accompanied not only by arteriogenesis, but also by sustained improvement in both global systolic and global diastolic parameters of cardiac function. While not directly examined, these results suggest a direct effect on regeneration of endogenous, mature cardiomyocytes and/or on survival or differentiation of cardiomyocyte precursors. Alternatively, both the functional improvement witnessed and the regeneration of cardiomyocytes may depend on paracrine secretion rather than direct

cell-to-cell interactions. While assessment of global function using echocardiography is somewhat limited, further studies using invasive hemodynamic measures may shed additional light on the mechanism of systolic and diastolic recovery.



**Figure 3** STRO-1<sup>bright</sup>-dependent myocardial neovascularization results in global improvement of parameters of myocardial function. Injection of  $0.2 \times 10^6$  and  $1 \times 10^6$  STRO-1<sup>bright</sup> cells resulted in dose-dependent improvement in (A) ejection fraction (EF) and (B) fractional shortening (FS) at 2 and 6 weeks, as measured by echocardiography performed and analyzed by a technician blinded to the treatments, compared with saline-treated animals ( $P < 0.01$ ) and animals treated with STRO-1-depleted fresh bone marrow mononuclear cells ( $P < 0.01$ ). BM-MNC, bone marrow mononuclear cells; MI, myocardial infarction.

**Competing interests**

The authors declared they have no competing interests.

**CONCLUSION**

Cellular therapies for the treatment of ischemic cardiomyopathy will probably need to address two interdependent processes: first, a renewable source of proliferating, functional cardiomyocytes, and, secondly, the development of a network of capillaries and larger-size blood vessels for supply of oxygen and nutrients to both the chronically ischemic, endogenous myocardium and to the newly implanted cardiomyocytes. To achieve these end points, a common cellular source for regenerating cardiomyocytes, vascular structures, and supporting cells such as pericytes and smooth muscle cells would be ideal. The mesenchymal lineage precursor cell may be an appropriate candidate for such a cellular source.

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