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Direct delivery of syngeneic and allogeneic large-scale expanded multipotent adult progenitor cells improves cardiac function after myocardial infarct

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Background

Multipotent adult progenitor cells (MAPC) comprise interesting candidates for myocardial regeneration because of a broad differentiation ability and immune privilege. We aimed to compare the improvement of cardiac function by syngeneic and allogeneic MAPC produced on a large scale using a platform optimized from MAPC research protocols.

Methods

Myocardial infarction was induced in Lewis rats by direct left anterior descending ligation followed immediately by direct injection into the infarct border zone of either Sprague–Dawley or Lewis MAPC from large-scale expansions. Echocardiography was performed to evaluate improvement in cardiac function, and immunohistochemistry was performed to identify MAPC within the infarct zone.

Results

Significant increases were observed in functional performance in animals transplanted with expanded MAPC compared with saline controls, with no significant differences between the syngeneic and allogeneic groups. Immunostaining demonstrated significant engraftment of expanded MAPC at 1 day after acute myocardial infarction, with $< 10\%$ of either syngeneic or allogeneic cells remaining at 6 weeks. At this point there was no evidence of myocardial regeneration. However, a significant increase in vascular density within the infarct zone in MAPC-transplanted animals was observed, and MAPC were found to produce high levels of VEGF in culture.

Discussion

These findings support a model in which delivery of expanded MAPC following acute myocardial infarction results in improvement in cardiac function because of paracrine effects resulting in vascular density increases, as well as potentially other trophic effects, supporting newly injured cardiac myocytes. Thus transplantation with MAPC may represent a promising therapeutic strategy with application in the stimulation of neovascularization in ischemic heart disease.

Keywords

cell transplantation, MAPC, myocardial infarct, neo-angiogenesis, VEGF.

Introduction

In response to myocardial infarction (MI), the heart employs naturally occurring, but inefficient, stem cell-based reparative mechanisms for repair and regeneration of damaged myocardial tissue [1–3]. The development of stem cell-based therapies is currently receiving great attention for treatment of degenerative heart ailments [4–7]. It is widely hypothesized that regeneration of infarcted myocardium may be enhanced by stem cell transplantation, provided that this treatment takes

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place within a window of a few days after the ischemic insult [3,8,9]. One approach to augment this naturally occurring system is to deliver stem cells to the newly injured myocardium in an attempt to augment myocardial regeneration and/or trophic support of the myocardium. While significant controversy exists regarding whether there is direct differentiation or transdifferentiation of engrafted HSC, mesenchymal stromal cells (MSC) or other types of adult stem cells towards a cardiac myocyte phenotype in newly infarcted myocardium, there is little controversy regarding whether this approach results in improved myocardial function [10–16].

Consequently the field of cell-based cardiac repair strategy is expanding at a fast pace to test and optimize the utility of an array of cell types, including MSC, skeletal myoblast, endothelial progenitors, resident cardiac stem cells and embryonic stem cells [9,12,15,17,18]. Multipotent adult progenitor cells (MAPC) were first described by Jiang *et al.* [19] and various related stem cell cultures have subsequently been reported, some of which use comparable conditions for isolation and expansion [20]. MAPC were originally reported as rodent adherent BM cells exhibiting a broad lineage differentiation capacity for all three primitive germ layers *in vitro* and *in vivo* [19]. MAPC have also demonstrated a wide-ranging replicative capacity and expression of low levels of transcription factors associated with pluripotency in embryonic stem cells, such as Pouf5 (also Oct4). When injected into NOD/SCID mice, MAPC did not develop into teratomas [21].

MAPC thus represent a unique class of adult stem cells that emulate the broad biologic plasticity characteristic of ES cells, while maintaining characteristics that make adult stem cells appealing for clinical application. One of these attractive characteristics is the capacity for long-term expansion *in vitro*, similar to ES cells and a property not found in other adult stem cell types, and this provides a key component in cell expansion strategies to use these cells for clinical or research use. Another critical characteristic when considering MAPC for use in patients with acute MI (AMI) is immune privilege, allowing allogeneic cells to be used safely. Independent studies on the immunologic properties of MAPC produced using culture protocols adapted for large-scale expansion have shown that these cells are immunoprivileged and present strong immunosuppressive properties [22], overall supportive of the concept that expanded MAPC constitute a valuable cell-based therapy for tissue repair following inflammatory damage. Thus, in view of the potential clinical utility of MAPC as an alternative source for cellular and gene therapies for heart disease, we studied the fate of allogeneic and syngeneic adult stem cell populations, acquired by large-scale expansions of Lewis and Sprague–Dawley MAPC lines, after transplantation into fully immunocompetent adult recipients without immunosuppression.

Methods

LAD ligation

All experimental animal procedures were performed under protocols approved by the Animal Research Committee of the Cleveland Clinic Foundation (Cleveland, Ohio, USA). Acute anterior wall MI was induced in 150–175-g male Lewis rats anesthetized with an i.p. injection of a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg), ventilated with room air at 80 breaths/min (RSP1002; Kent Scientific Corp., Torrington, CT, USA), by sternotomy and surgical ligation of the left anterior descending (LAD) artery.

Echocardiography

2D- and M-mode echocardiography was performed at baseline and at 2 weeks and 6 weeks after LAD ligation and MAPC transplantation, using a 15-MHz linear array transducer interfaced with a Sequoia C256 (Acuson, Malvern, PA, USA), as described previously [2,23]. At each time point six separate measurements were recorded for each test animal, after which three randomly selected images were analyzed by an independent observer who was blinded to the treatment arm. The shortening fraction (SF) was calculated from M-mode recordings using the formula $[(\mathrm{LVEDD} - \mathrm{LVESD})/\mathrm{LVEDD} \times 100]$, where LVEDD is the left ventricular end diastolic dimension and LVESD is the left ventricular end systolic dimension. For standardization, these dimensions were measured between the anterior wall and posterior wall from the short axis view just below the level of the papillary muscle.

MAPC isolation, expansion and validation

Adult BM-adherent stem cells were isolated according to the MAPC procedures established by the laboratory of Dr Verfaillie at the University of Minnesota (Minneapolis, MN, USA) [19,21]. In order to limit variability and facilitate *in vivo* application, MAPC-expansion protocols were optimized to enable banking and production of rat