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PROSPECT

Virtual Reality of Stem Cell Transplantation to Repair Injured Myocardium

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Abstract The search for the fountain of youth continues into the 21st century with hopes that embryonic or hematopoietic stem cells (SC) will repair injured tissues in the heart, lungs, pancreas, muscles, nerves, liver, or skin. This commentary focuses on the potential of SC for inducing cardiac regeneration after myocardial injury, the barriers to SC treatment that need to be overcome for ensuring successful cardiac repair, and the experimental approaches that can be applied to the problem. J. Cell. Biochem. 95: 869–874, 2005. © 2005 Wiley-Liss, Inc.

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Hematopoietic stem cells (SC) reportedly have the ability to transdifferentiate into epithelial cells [Krause et al., 2001; Badiavas et al., 2003a; Badiavas and Falanga, 2003b], cardiac myocytes [Hamill et al., 1981; Klug et al., 1996; Li et al., 1996; Mar et al., 1997; Scorsin et al., 1997; Tomita et al., 1999; Wang et al., 2000; Orlic et al., 2001a,b; Yeh et al., 2003; Madeddu et al., 2004; Tomita et al., 2004], liver cells [Petersen et al., 1999; Lagasse et al., 2000; Theise et al., 2000a,b], bone [Becker et al., 1999], lung cells [Aliotta et al., 2004], neurons [Brazelton et al., 2000], and skeletal myocytes [Gussoni et al., 1999]. This response, with few exceptions, is seen only in response to injury. Despite SC offering a promise for tissue regeneration, important challenges continue to confront investigators seeking to apply SC transplantation for cardiac and organ repair including: (1) identifying the best regenerative SC type (embryonic, hematopoietic, or mesenchymal) for repairing injury; (2) identifying potential facilitator cells that may augment or modulate the functions of SC; (3) improving SC homing to the "niche" in the specific tissue through exploitation of existing receptor-ligand interactions; (4) identifying novel tissue-specific injury antigens that may function as appropriate receptors for SC; and (5) identifying SC growth factors and their signaling pathways that may induce SC proliferation and/or differentiation.

Early studies showed that green fluorescent protein (GFP)-marked stem cells traffic to myocardial infarcts and repair injured myocardium in mice [Orlic et al., 2001a,b]. In other studies, myocytes were found to develop from mesenchymal bone marrow cells [Makino et al., 1999], and when mesenchymal stem cells were expanded ex vivo and injected into areas of myocardial injury, evidence of myocardial repair was observed [Kamihata et al., 2001]. Similarly, embryonic SC injected post-infarction were

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observed to produce long-term improvement in cardiac function over a period of 32 weeks [Min et al., 2003]. Infarction mobilized bone marrow derived cells were also shown to accumulate in the peri-infarction zone of the myocardium as both differentiated cardiomyocytes and endothelial cells [Jackson et al., 2001]. Consistent with the aforementioned preclinical studies, clinical success for improving cardiac function has been observed with intracoronary injection of human bone marrow mononuclear cells (BMC) in clinical trials [Strauer et al., 2002; Tse et al., 2003; Wollert et al., 2004], with purified CD34+ cells [Nagamine et al., 2004] or CD133+ cells (AC133 cells) injected into the myocardium of patients after MIs [Stamm et al., 2003], and with intramyocardial injection of skeletal myoblasts [Smits et al., 2003].

Evidence of clinical efficacy of human SC to repair MIs stands in striking contrast to results from a number of experimental studies in which genetically modified murine SC, used for tracking the development of SC into myocytes or arteriolar cells, failed to show engraftment or improvement in cardiac function [Balsam et al., 2004; Murry et al., 2004; Nygren et al., 2004]. One possible explanation for the disparate results is that the latter studies used genetically modified SC that may have increased risk for immunogenic clearance. Observations from gene therapy studies in children with severe combined immunodeficiency disease show that *NeoR* gene marked cells induce immune responses directed at the *NeoR* gene product [Young et al., 1994]. This mechanism may, in part, explain the lack of engraftment of syngeneic murine cells genetically labeled with GFP [Balsam et al., 2004] or transfected with the β-galactosidase reporter gene driven by a cardiac-specific β-myosin heavy chain promoter [Murry et al., 2004]. Alternatively, engraftment of the genetically modified cells could have occurred but may have been eliminated before transdifferentiation or fusion took place.

In contrast, most preclinical studies show that unmodified syngeneic SC can engraft and persist for weeks to months in the injured cardiac tissue albeit with variable functional recovery. Furthermore, marrow stromal cells (MSC) from C57BL6 mice appear to induce a unique immunologic tolerance that permits them to engraft and create stable chimerism in the xenogeneic environment of Lewis rats [Saito et al., 2002]. Interestingly, immunosuppression of T cell-associated anti-donor responses may be mediated by factors secreted by MSC [Bartholomew et al., 2002].

Successful transdifferentation of SC into myocytes and/or arteriolar cells may be a function of several factors including the precursor frequency of SC, the type of SC that homes to the injury and the expression of factors upregulated during injury that may serve to enhance homing of SC to the myocardium. To date, investigations of myocardial repair have fallen into the following categories: (1) the transplant of bone marrow, mesenchymal stem cells, fetal cardiac myocytes, and myocytes into mice or rats [Hamill et al., 1981; Klug et al., 1996; Li et al., 1996; Mar et al., 1997; Scorsin et al., 1997; Tomita et al., 1999; Wang et al., 2000; Orlic et al., 2001b]; (2) the transplant of human SC into immunodeficient mice with results suggesting that transdifferentiation into various cardiomyocytic cells that facilitate repair does occur [Yeh et al., 2003; Madeddu et al., 2004; Tomita et al., 2004; Zhang et al., 2004]; and (3) clinical studies using directly injected unseparated bone marrow [Strauer et al., 2002; Tse et al., 2003], separated populations containing hematopoietic SC or CD34+ purified cells [Stamm et al., 2003; Kang et al., 2004], or myoblasts [Smits et al., 2003] with results supporting a clinical benefit. In one study, improvement was also observed in patients given intracoronary infusion of granulocyte-colony stimulating factor (G-CSF)-primed peripheral blood stem cells (PBSC) and G-CSF injections; however, the G-CSF administration was associated with an unexpectedly high rate of in-stent restenosis [Kang et al., 2004].

The electrophysiologic, structural, and contractile properties of fetal cardiac myocytes that enable functional integration into the myocardium suggest that fetal cardiac myocytes can repair myocardial injury [Atkins et al., 1999]. Although many tissue receptors and their cell-expressed ligands have been identified, the mechanism (s) for how SC home to specific organs is unknown. The ability of either unstimulated or G-CSF primed hematopoietic SC to traffic to MIs is likely dependent upon the expression of injury receptors or factors, such as stromal cell-derived factor-1 (SDF-1), that are upregulated after injury of cardiac tissue [Abbott et al., 2004]. Once SC arrive and bind to SDF-1 via their CXCR receptors [Lapidot

and Kollet, 2002], SC may need to interact with other tissue factors to trigger differentiation. Obviously, the SC need to home, bind, and persist in large enough numbers in a milieu that supports growth, proliferation, and differentiation. One major limitation to achieving effective tissue regeneration by SC, therefore, may be the low frequency with which stem cell precursors localize to and persist in the infarct zone.

In order to deliver high numbers of SC to MIs while avoiding the clinical risks of bone marrow harvest or intracardiac injections, we have adapted a new strategy that involves directly targeting SC to the infarct. Borrowing from our experience in retargeting killer T cells to cancer using bispecific antibody (BiAb) technology [Sen et al., 2001], we reasoned that the same strategy could be used to target SC to MIs. Preclinical studies evaluating the efficacy of monoclonal antibodies directed at target adhesion molecules and their inhibition of myocardial damage after acute ischemic injury have explored: VCAM-1 [Kalawski et al., 1998], ICAM-1 [Hartman et al., 1995; Hawkins et al., 1996; Gumina et al., 1997; Ishibashi et al., 1999; Sun et al., 2001], P-Selectin [Hawkins et al., 1996; Gumina et al., 1997; Nagashima et al., 1998], E-Selectin [Ma et al., 1993], Mo1/CD18 [Simpson et al., 1988; Aversano et al., 1995], TNFα receptors 1 and 2 [Irwin et al., 1999], vascular adhesion protein-1 (VAP-1) [Jaakkola et al., 2000], and angiotensin receptors [Yang et al., 1998].

In our first study, we selected anti-VCAM-1 to target the MI and an anti-c-kit to target stem cells. A BiAb, anti-c-kit, and anti-VCAM-1, was produced by chemically heteroconjugating anti-mouse c-kit to anti-mouse VCAM-1 [Lum et al., 2004b]. Lin-Sca+ cells from bone marrow suspensions of C57BL/6 mice were produced by depleting the lineage positive cells using a cocktail containing rat anti-Ter119, B220, MAC-1, GR-1, Lyt-2, and L3T4 monoclonal antibodies. For these studies, the cells were first sorted for Sca+ cells using a high speed MoFlo sorter and then armed with chemically heteroconjugated anti-mouse c-kit $\times$ anti-mouse VCAM-1 BiAb. The c-kit $\times$ anti-VCAM-1 BiAb-armed lin-Sca+ cells or unarmed control lin-Sca+ cells labeled with carboxyfluorescein diacetate succinimidyl diester (CFSE) dye were injected into mice via the jugular vein 24 h after the left anterior descending arteries (LAD) of the mice had been ligated. The mice were euthanized 24 h after the IV injection, the MIs were sectioned, and the infarct area from the mouse that received 100,000 armed lin-Sca+ cell showed markedly increased numbers of CFSE+ cells compared to the infarcts of the control mouse that had received the same number of unarmed lin-Sca+ cells [Lum et al., 2004b]. Subsequently, we generated a BiAb that would target an organ specific injury protein, myosin light chain (MLC), on injured myocardium [Mair et al., 1994; Lyn et al., 2000] by producing anti-human CD45 $\times$ anti-MLC (MLCBi).

To test the hypothesis that BiAbs can target SC to MIs, we armed human G-CSF human primed PBSC or CD34+ cells with MLCBi and infused them into nude rats in the absence of growth factors and tracked the human SC and their progeny using anti-human HLA-Class I antibodies. Our goal was to limit the variables associated with growth factor administration or immune rejection [Grinnemo et al., 2004] that may inhibit engraftment and confound interpretation of the results. PBSC or CD34+ cells purified by Isolex column, armed with MLCBi, and infused 24 h after a 17 min transient ligation of the LAD in nude rats led to remarkable numbers of armed and only a few unarmed cells present at 48 h in the infarct zone [Christman et al., 2004; Lum et al., 2004a]. No SC were seen in the non-infarcted areas of the rats. After 5 weeks, BiAb-mediated targeting of human SC to injured myocardium not only increased the numbers of SC arriving at the injury but also increased the number of SC-derived cardiomyocytes in rat hearts as evidenced by in situ immunofluorescent detection of cells co-expressing human Class I and troponin I [Lum et al., 2004a]. These data are consistent with those reported for the GFP-bone marrow cells that differentiated into cells expressing ANP, MHCa, Tn1, and connexin 43 suggesting various stages of cardiogenic differentiation by BMC [Tomita et al., 2004]. The persistence of human Class I and disappearance of human CD45+ cells in the MI areas suggest that there was preferential engraftment of somatic cells in the MIs. Moreover, these findings correlated with significant improvement in cardiac function of rats that received armed CD34+ cells compared to those that received unarmed CD34+ cells by echocardiogram evaluations [Lee et al., 2004].

Obviously, these results raise a host of questions. Although it is clear that murine or human stem cells can develop into myocytes or endothelial cells and contribute to functional recovery in the injured myocardium, it is not clear whether they can establish functional electromechanical couplings. Furthermore, the cell(s) that contribute to short- or long-term recovery remain unknown. Carefully controlled laboratory studies as well as well-designed clinical trials are needed to answer these questions. Our approach, however, provides a novel platform from which basic, preclinical, and clinical investigations can be launched to explore the fate and function of transplanted human SC.

BiAb-mediated targeting of SC to injured tissue increases the number of SC that arrive and persist in the injured tissue thereby optimizing the conditions required for performing kinetic studies to characterize the biochemical and biosynthetic changes that occur in the donor cells during the process of engraftment and differentiation. With increased numbers of SC targeted to the MI, dose-response relationships as well as quantitative histological, morphological, functional analyses can be addressed in both acute and chronic preclinical models, and the mechanism(s) for cardiac repair can be elucidated. Specifically, if there are enough cells to track, a more accurate assessment of the frequency of fusion and/or transdifferentiation can be performed. A recent study suggests that both fusion and transdifferentiation are involved in myocyte repair or new myocyte development whereas new blood vessels are derived from transdifferentiation of the donor SC [Zhang et al., 2004]. Targeting of SC to MIs may have a major clinical impact not only as a non-invasive therapy for repairing injured myocardium but also for targeting SC to repair other tissues. This approach, however, needs to be confirmed before it can be translated to clinical applications.

In conclusion, taken together, these proof-of-principle studies provide a strong rationale for utilizing BiAb technology to enhance SC targeting to MIs as well as other tissues. In addition to immediate implications for stem cell research, these studies have broader implications for the development of human clinical trials that seek to circumvent inadequate stem cell recovery or inadequate localization to injury sites for organ repair.

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