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Human Cardiosphere-Derived Cells From Advanced Heart Failure Patients Exhibit Augmented Functional Potency in Myocardial Repair

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Objectives This study sought to compare the regenerative potency of cells derived from healthy and diseased human hearts.

Background Results from pre-clinical studies and the CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) trial support the notion that cardiosphere-derived cells (CDCs) from normal and recently infarcted hearts are capable of regenerating healthy heart tissue after myocardial infarction (MI). It is unknown whether CDCs derived from advanced heart failure (HF) patients retain the same regenerative potency.

Methods In a mouse model of acute MI, we compared the regenerative potential and functional benefits of CDCs derived from 3 groups: 1) non-failing (NF) donor: healthy donor hearts post-transplantation; 2) MI: patients who had an MI 9 to 35 days before biopsy; and 3) HF: advanced cardiomyopathy tissue explanted at cardiac transplantation.

Results Cell growth and phenotype were identical in all 3 groups. Injection of HF CDCs led to the greatest therapeutic benefit in mice, with the highest left ventricular ejection fraction, thickest infarct wall, most viable tissue, and least scar 3 weeks after treatment. In vitro assays revealed that HF CDCs secreted higher levels of stromal cell-derived factor (SDF)-1, which may contribute to the cells' augmented resistance to oxidative stress, enhanced angiogenesis, and improved myocyte survival. Histological analysis indicated that HF CDCs engrafted better, recruited more endogenous stem cells, and induced greater angiogenesis and cardiomyocyte cell-cycle re-entry. CDC-secreted SDF-1 levels correlated with decreases in scar mass over time in CADUCEUS patients treated with autologous CDCs.

Conclusions CDCs from advanced HF patients exhibit augmented potency in ameliorating ventricular dysfunction post-MI, possibly through SDF-1–mediated mechanisms. (J Am Coll Cardiol HF 2014;2:49–61) © 2014 by the American College of Cardiology Foundation

Extensive pre-clinical studies of cardiosphere-derived cells (CDCs) have recently culminated in the first-in-human CADUCEUS (CArdiosphere-Derived aUtologous stem

See page 62

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Abbreviations and Acronyms

CDC = cardiosphere-derived cell

ELISA = enzyme-linked immunosorbent assay

HF = heart failure

HNA = human-specific nuclear antigen

LVEF = left ventricular ejection fraction

MI = myocardial infarction

NF = non-failing

NRCM = neonatal rat cardiomyocyte

NYHA = New York Heart Association

RT-PCR = reverse transcription–polymerase chain reaction

SDF = stromal cell-derived factor

TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling

CElls to reverse ventricULar dySfunction) trial (1). CDCs are intrinsic to the heart (2), express a distinctive profile of antigens (>98% $CD105^+$, <0.5% $CD45^+$) (3,4), and promote cardiac regeneration after ischemic injury. In animal models of myocardial infarction (MI), CDCs temporarily engraft (5–8) and exert strong bystander effects leading to the recruitment of endogenous stem cells (5,6,9), attenuation of apoptosis in the host myocardium (3,6,9,10), stimulation of cardiomyocyte cell-cycle re-entry (3,6,11,12), promotion of angiogenesis (5,6), and production of long-lasting functional benefits (2,4–6,9,10,13–18).

So far, CDCs have been derived from nominally healthy (post-transplantation donor hearts) or moderately dysfunctional (post-MI) hearts. It is unknown whether CDCs from end-stage heart failure (HF) patients retain comparable therapeutic potential. Also, no previous study has performed direct head-to-head comparison of CDCs (or any other heart-derived cells) from patients with varying severities of cardiac dysfunction. Here, we compared the in vitro properties and in vivo regenerative potential of CDCs derived from non-failing (NF) donor, acute MI, and failing heart tissues. We further evaluated potential roles for various secreted growth factors in product potency, and correlated the levels of each of these factors with structural remodeling in CDC-treated CADUCEUS patients.

Methods

A detailed description of the methods can be found in the Online Appendix.

Donor comorbidity and study design. Patient characteristics from the 3 groups are shown in Table 1. NF donor CDCs were derived from endomyocardial biopsies of donor hearts after transplantation. The hearts had been exposed to various regimens of immunosuppressive drugs but were otherwise healthy and free of cardiomyopathy. MI CDCs were derived from endomyocardial biopsies of acute MI patients enrolled in the CADUCEUS trial (harvested 9 to 35 days post-MI). Most of these patients were New York Heart Association (NYHA) functional class I, and the remaining were class II. HF CDCs were derived from myocardial samples of failing hearts from heart transplant or ventricular assist device recipients. All HF patients were NYHA functional class IV, with various types of cardiomyopathy.

CDCs were derived as described (3,4) (n = 6 donors for each group). Passage 2 cells were used for all studies. Expression of surface markers was assessed by flow cytometry. Enzyme-linked immunosorbent assay (ELISA) and reverse transcription–polymerase chain reaction (RT-PCR) were performed to measure secreted factors and expression of key genes, respectively. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed to measure cell apoptosis under oxidative stress. Endothelial cell tube formation assay was conducted to evaluate angiogenesis. A total of 100,000 cells were intramyocardially injected into the border zone of SCID mice immediately after myocardial infarction (left anterior descending coronary artery ligation). Echocardiography was performed at baseline and 3 weeks afterwards to measure left ventricular ejection fraction (LVEF). Afterwards, animals were euthanized and histology was performed to evaluate cell engraftment, differentiation, and stimulation of endogenous repair. Comparison between groups was conducted by 1-way analysis of variance followed by post-hoc Bonferroni test.

Results

CDC morphology, growth, and phenotype from the 3 donor groups. With a 3-stage processing protocol (explants, cardiospheres, and replating) (4), CDCs could be readily derived and expanded from all groups including HF tissues. Cell morphologies at all stages were identical for all groups (Fig. 1A). As a measure of cell growth and proliferation, population doublings over time and averaged doubling times were calculated (Fig. 1B). No differences were found among the 3 groups, indicating that the generation and expansion of CDCs was not affected by the diseases in question.

Flow cytometry was performed to characterize the antigenic profile of CDCs from the different patient groups. As per the product release criteria for the CADUCEUS trial (1), CDCs are consistently positive for CD105 (a TGF-β receptor subunit) and negative for CD45 (a pan-hematopoietic marker) (Fig. 1D). A small fraction (<10%) of CDCs expresses the stem cell marker c-kit (CD117) in all groups (Fig. 1D). Another very small fraction of CDCs expresses DDR2, a cardiac fibroblast marker (Fig. 1D), in all groups. In sum, the antigenic profiles of CDCs from different patient groups are comparable.

Therapeutic benefits in mice with myocardial infarction. 2,3,5-triphenyltetrazolium chloride (TTC) staining revealed superior myocardial tissue viability in hearts that had been injected with HF CDCs (Fig. 2A, red bar in the graph). Trends suggest that NF donor CDC or MI CDC therapies may also reduce acute injury as compared with Control injections, but the differences are not statistically significant. TUNEL staining revealed decreases of apoptosis ($TUNEL^+$ nuclei) in all 3 cell-injected groups, with the greatest protection seen in the HF CDC group (Fig. 2B). These data manifest a cardioprotective effect of CDCs in acute MI that is augmented in HF CDCs.