HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2018 July 07.

Published in final edited form as:

Circ Res. 2017 July 07; 121(2): 95–97. doi:10.1161/CIRCRESAHA.117.311293.

Cardiac Cell Therapy 3.0: The Beginning of the End or The End of the Beginning?

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Keywords

Cardiac Stem/Progenitors; Mesenchymal Stem/Progenitor Cells; Endothelial Progenitors; Cell Therapy

Cardiac cell therapy (CCT) holds great promise as a regenerative medicine approach for the treatment of cardiovascular diseases (CVDs)[1]. The first generation of CCTs tested various adult cell types, including skeletal myoblasts, bone marrow (BM)-derived mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs). More recently, the advent of induced pluripotent stem cells (PSCs) led to the much-anticipated second generation of CCTs with *bona fide*, PSC-derived CPCs and cardiomyocytes[1]. The bad news is that, so far, both adult and PSC-based CCTs have failed to meet their promise of directly remuscularizing and repairing the heart to a therapeutically meaningful extent[2, 3]. The good news is that some cell types clearly demonstrate encouraging results in terms of efficacy and safety and, more importantly, reveal a previously underestimated key role of CCT: to indirectly promote repair by regulating mechanisms of endogenous cardiac regeneration in the host[1, 4].

Understandably, the increasingly high burden of CVDs, coupled with the limited efficacy seen in both adult and PSC-based CCTs, and incomplete mechanistic understanding of adult human heart regeneration, have fueled disappointment, skepticism and polarized the field[5]. This schism has been particularly apparent in the area of adult CCTs, which also faces a current crisis of scientific distrust[5]. However, the interpretation that a possible stumble in research progress is proof that CCT is "broken", would be unscientific. As Daniel Wegner noted "…*tipping the balance toward skepticism can eradicate ideas faster than we can generate them. Eventually, we arrive at a vacuous chasm, with no theory standing and no idea left without serious wounds*"[6].

Under this prism, it is worth exploring how the field of adult CCTs fares, compared to other regenerative medicine approaches. PSC-based CCTs offer perhaps the strongest argument against adult CCTs, due to their unsurpassed ability to proliferate and differentiate into

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DISCLOSURES.

Dr. Hatzistergos discloses a relationship with Vestion Inc. that includes equity. Vestion did not contribute funding to this study.

cardiomyocytes[5]. The idea that such a trait is the premise of CCT is based on experiments in zebrafish and newborn mice, both of which retain full capacity to regenerate a resected heart, possibly via cardiomyocyte amplification-based remuscularization mechanisms[1]. However, experiments in more clinically relevant CVD models indicate that PSC-based direct remuscularization approaches exert effects that are more “cosmetic” than “regenerative” in nature, since cardiomyocyte engraftment is not accompanied by scar resorption and regeneration[3, 4]. Moreover, both adult and PSC-based CCTs produce comparable improvements in cardiac function[7], likely indirectly, via paracrine stimulation of endogenous repair mechanisms in the host[4]. Similarly, although gene-editing approaches offer hope for elucidating the genetic basis of CVDs, their potential application as regenerative therapy is currently limited. In addition to the technical challenges with safely and efficiently gene-editing billions of cardiomyocytes *in-vivo*, CVDs are molecularly complex, rather than of monogenic etiology[8]. Likewise, the molecular mechanisms of cardiomyogenesis entail precise, spatiotemporal modulation of multiple signaling gradients in both cardiomyogenic and non-cardiomyogenic cells, and therefore the possibility of developing cell-free, drug-based approaches to recapitulate such complex and dynamic processes *in-vivo* is currently limited[1].

In this issue of *Circulation Research*, Monsanto *et al.*[9] lend support to a promising strategy to address the limitations of cardiac regenerative approaches by engineering combinatorial CCTs. This idea stands on two pillars: (i) no single cell population can produce all cell types that make up the human heart; and (ii) both cardiomyogenic and non-cardiomyogenic cells are essential for heart development and repair. Thus, engineering adult and/or PSC-derived cell combinations with complementary roles may more efficiently regulate endogenous regenerative pathways, compared to conventional CCT (Figure)[1]. For example, the observation that BM-MSC therapy stimulates endogenous CPCs[10, 11] led to the idea of combining the two adult cell types for greater, synergistic effects. Indeed, this hypothesis has produced encouraging results in several large and small-animal studies of CVD[1], and is currently in a phase II, randomized, placebo-controlled trial in ischemic cardiomyopathy patients (NCT02501811). Similarly, the combination of human PSC-derived cardiomyocytes with vascular cells[12] or MSCs[13] produces further improvements in heart repair compared to cardiomyocytes alone, likely due to enhanced stimulation of endogenous repair mechanisms. The new method by Monsanto *et al.*, to derive three distinct cardiac stem cell types from within the adult human heart, could potentially foster such applications[9].

Using the cell-surface receptor cKit, both as a positive and negative selection marker, the authors devised a strategy to purify concurrently MSCs, CPCs, and endothelial progenitor cells (EPCs) from adult heart biopsies obtained during cardiac surgery[9]. MSCs are the most abundant derivative, comprising ~90–95% of the cardiac stem cell pool, and are purified as the $CD105^{+}/CD90^{+}$ fraction of cKit-negative cardiac cells. Consistent with previous reports[14], cardiac MSCs exhibit a fibroblastoid morphology, produce colony-forming units-fibroblast (CFU-Fs), and exhibit multilineage differentiation into adipocytes, chondrocytes, and osteocytes[9]. However, compared to BM-MSCs, cardiac MSCs exhibit slow *in-vitro* growth kinetics and express cardiac lineage-markers, such as GATA4 and smooth muscle actin. Immunologically, expression of major histocompatibility complexes (MHC) class I and II, and co-stimulatory molecules CD80 and CD86, are similar to BM-MSCs, but cardiac

MSCs express higher levels of the co-stimulatory molecule CD40. It is, therefore, unclear whether cardiac MSCs are as immunoprivileged as BM-MSCs. Such differences, however, are not surprising since mouse studies indicate distinct identities for BM and cardiac MSCs, with the latter possibly representing postnatal epicardial progenitors[14].

The use of cKit as a CPC marker has been controversial[5]. Recent studies identify at least 2 distinct cell types expressing *cKit* in the heart: a rare, cardiomyogenic cell likely of neural crest lineage, and a more abundant vasculogenic cell, possibly of mesodermal lineage[15]. The work by Monsanto *et al.* further supports these findings. Positive selection for cKit yields two stem cell types with distinct immunophenotypic and gene-expression profiles[9]. $cKit^+$ EPCs are morphologically round and committed to vascular fates, as indicated by high angiogenic potential in a Matrigel-based ex-vivo angiogenesis assay and expression of CD133 and PECAM1. $cKit^+$ CPCs exhibit spindle-like morphology and a more myogenic profile, as indicated by lack of PECAM1 and relatively higher expression of GATA4 and smooth muscle actin. However, whether CPCs retain cardiomyogenic capacity is not demonstrated. Importantly, gene-expression profiling reveals striking differences between the 3 cardiac stem cell types in cytokines and extracellular matrix genes, such as SDF1, NRG1, FGF2, TIMP1 and MMP1.

The study by Monsanto and colleagues is an important advance in cardiac regenerative medicine. First, it is a bold demonstration of cellular plasticity retained in the human heart, regardless of age, gender or health condition. Stem cells were isolated from patients up to 84-years old and suffering from a range of diseases, including diabetes and coronary artery disease. Second, the ability to isolate 3 stem cell types from a single heart biopsy allows us to gain insight into the cellular composition in the adult human heart and the potential role of these unique cell types in CVD and regeneration. Since ~70% of human heart cells are non-cardiomyocytes, thorough research of their nature should be at the forefront of cardiac regenerative medicine[1]. For example, Monsanto *et al.* noted that some cultures failed to yield all 3 stem cell types, a finding which merits further investigation for any potential relationship to disease mechanisms[9]. Last, the method of Monsanto *et al.* enables the isolation and expansion of therapeutic volumes of cardiac MSCs, CPCs, and EPCs from a single biopsy with 80–90% success (~100 million cells of each type could be manufactured in ~10 passages). Such technology is expected to be important for engineering combinatorial CCTs, using adult and/or PSC-based combinations[1, 12, 13], in a manner that effectively eliminates barriers to endogenous cardiac regeneration and may eventually lead to a much-needed scientific breakthrough for the treatment of CVDs.

Acknowledgments

FUNDING SOURCES.

AV is supported by a National Eye Institute (NEI) T32 Training Grant 4T32EY023194-04.

References

1. Golpanian S, Wolf A, Hatzistergos KE, Hare JM. Rebuilding the Damaged Heart: Mesenchymal Stem Cells, Cell-Based Therapy, and Engineered Heart Tissue. Physiol Rev. 2016; 96:1127–68. [PubMed: 27335447]

2. Nguyen PK, Rhee JW, Wu JC. Adult Stem Cell Therapy and Heart Failure, 2000 to 2016: A Systematic Review. JAMA Cardiol. 2016; 1:831–841. [PubMed: 27557438]

3. Shiba Y, Gomibuchi T, Seto T, Wada Y, Ichimura H, Tanaka Y, Ogasawara T, Okada K, Shiba N, Sakamoto K, Ido D, Shiina T, Ohkura M, Nakai J, Uno N, Kazuki Y, Oshimura M, Minami I, Ikeda U. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. Nature. 2016; 538:388–391. [PubMed: 27723741]

4. Ong SG, Huber BC, Lee WH, Kodo K, Ebert AD, Ma Y, Nguyen PK, Diecke S, Chen WY, Wu JC. Microfluidic Single-Cell Analysis of Transplanted Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes After Acute Myocardial Infarction. Circulation. 2015; 132:762–71. [PubMed: 26304668]

5. A futile cycle in cell therapy. Nat Biotechnol. 2017; 35:291. [PubMed: 28398319]

6. Wegner DM. The Premature Demise of the Solo Experiment. Personality and Social Psychology Bulletin. 1992; 18:504–508.

7. Fernandes S, Chong JJ, Paige SL, Iwata M, Torok-Storb B, Keller G, Reinecke H, Murry CE. Comparison of Human Embryonic Stem Cell-Derived Cardiomyocytes, Cardiovascular Progenitors, and Bone Marrow Mononuclear Cells for Cardiac Repair. Stem Cell Reports. 2015; 5:753–62. [PubMed: 26607951]

8. Strong A, Musunuru K. Genome editing in cardiovascular diseases. Nat Rev Cardiol. 2017; 14:11–20. [PubMed: 27609628]

9. Monsanto MM, White KS, Kim T, Wang BJ, Fisher K, Ilves K, Khalafalla FG, Casillas A, Broughton K, Mohsin S, Dembitsky WP, Sussman MA. Concurrent Isolation of Three Distinct Cardiac Stem Cell Populations from a Single Human Heart Biopsy. Circ Res. 2017

10. Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE. Bone Marrow Mesenchymal Stem Cells Stimulate Cardiac Stem Cell Proliferation and DifferentiationNovelty and Significance. Circulation research. 2010; 107:913–922. [PubMed: 20671238]

11. Hatzistergos KE, Saur D, Seidler B, Balkan W, Breton M, Valasaki K, Takeuchi LM, Landin AM, Khan A, Hare JM. Stimulatory Effects of MSCs on cKit+ Cardiac Stem Cells Are Mediated by SDF1/CXCR4 and SCF/cKit Signaling Pathways. Circ Res. 2016

12. Ye L, Chang YH, Xiong Q, Zhang P, Zhang L, Somasundaram P, Lepley M, Swingen C, Su L, Wendel JS, Guo J, Jang A, Rosenbush D, Greder L, Dutton JR, Zhang J, Kamp TJ, Kaufman DS, Ge Y, Zhang J. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. Cell Stem Cell. 2014; 15:750–61. [PubMed: 25479750]

13. Puymirat E, Geha R, Tomescot A, Bellamy V, Larghero J, Trinquart L, Bruneval P, Desnos M, Hagege A, Puceat M, Menasche P. Can mesenchymal stem cells induce tolerance to cotransplanted human embryonic stem cells? Molecular therapy : the journal of the American Society of Gene Therapy. 2009; 17:176–82. [PubMed: 18841094]

14. Chong JJ, Chandrakanthan V, Xaymardan M, Asli NS, Li J, Ahmed I, Heffernan C, Menon MK, Scarlett CJ, Rashidianfar A, Biben C, Zoellner H, Colvin EK, Pimanda JE, Biankin AV, Zhou B, Pu WT, Prall OW, Harvey RP. Adult cardiac-resident MSC-like stem cells with a proepicardial origin. Cell Stem Cell. 2011; 9:527–40. [PubMed: 22136928]

15. Hatzistergos KE, Takeuchi LM, Saur D, Seidler B, Dymecki SM, Mai JJ, White IA, Balkan W, Kanashiro-Takeuchi RM, Schally AV, Hare JM. cKit+ cardiac progenitors of neural crest origin. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:13051–6. [PubMed: 26438843]

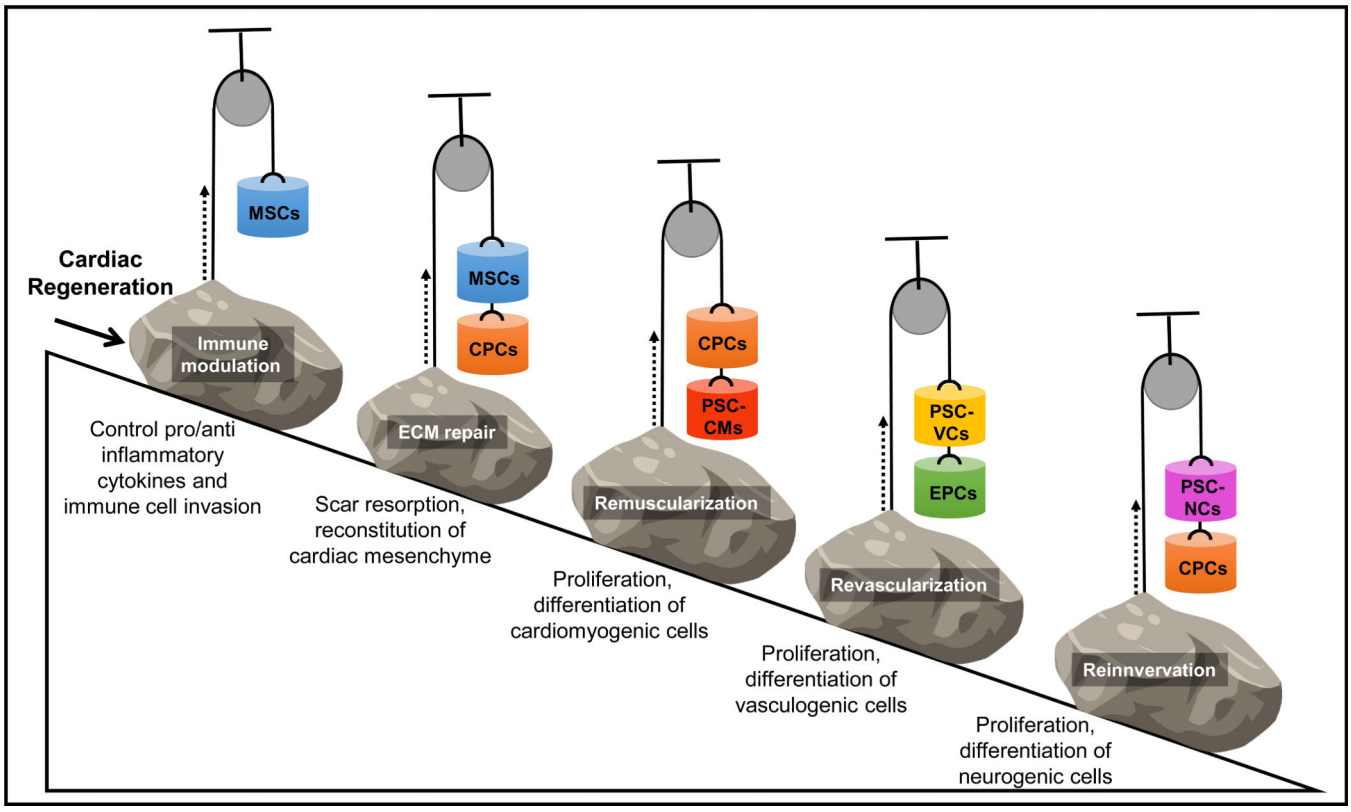

FIGURE. Combinatorial CCTs for heart regeneration

Although “regeneration“ and “remuscularization” are thought of as synonymous in cardiac regenerative medicine, adult and PSC-based CCT trials unveil regenerative barriers unlikely to be circumvented by remuscularization alone. Synergism between complementary cell types, in the form of combinatorial CCTs, is a promising strategy for therapeutically targeting endogenous cardiac regeneration roadblocks. MSCs, mesenchymal stem cells; CPCs, cardiac progenitors; ECM, extracellular matrix; CM, cardiomyogenic cells; VC, vasculogenic cells; NC, neurogenic cells.