Cardioprotective repair through stem cell-based cardiopoiesis

Atta Behfar* and Andre Terzic

Marriott Heart Disease Research Program, Division of Cardiovascular Diseases, Departments of Medicine, Molecular Pharmacology and Experimental Therapeutics, and Medical Genetics, Mayo Clinic, Rochester, MN, USA

Running title: Cardiopoiesis and cardioprotection

*Address for correspondence: Mayo Clinic, 200 First Street SW, Rochester, MN 55905 (E-mail: behfar.atta@mayo.edu)
ABSTRACT

Ischemic heart disease continues to progress at pandemic levels despite current preventive and therapeutic interventions. Recent advances in stem cell biology have provided the impetus for a paradigm shift in treatment options, potentially transforming palliative care into curative therapy. Although delivery of stem cells in clinical trials has resulted in a modest functional improvement of myocardial performance in the setting of infarction, ongoing efforts at the bench and bedside are taking place to increase stem cell propensity for engraftment and homing into diseased myocardium. The newest opportunity has arisen with the delivery of stem cells guided to execute the cardiac program. Here, we examine the recent application of genomic and proteomic technology to decipher the process of cardiopoiesis, and recruit cardiopoietic stem cells for cardioprotection and safe myocardial repair.

Key words: Myocardial Infarction, Cardiopoiesis, Genomics, Heart, Proteomics, Repair, Regenerative Medicine, Stem cells
Stem cells: From biology to therapy

Ischemic heart disease is a leading cause of morbidity and mortality worldwide (9, 40). State-of-the-art acute care includes catheterization or intervention with thrombolytic therapy within three hours of a coronary event (2). Despite efforts to avoid permanent injury in the setting of myocardial infarction, even most efficiently managed cases reveal heart muscle damage (24). As such, ischemic heart disease continues to be a major epidemic associated with poor prognosis (40). Thus, extensive efforts are ongoing to establish effective therapeutic modalities that would improve both quality of life and survival in this patient population. Two strategies have been identified that have the potential of added benefit to the current therapeutic armamentarium. The first focuses on enhancing the heart’s intrinsic capacity to protect itself against injury, and the second strives to create an environment within the cardiac parenchyma that will result in repair of the damaged myocardium (7, 14, 38).

Stem cell biology has emerged as critical in driving these two goals towards clinical translation with recent trials demonstrating functional benefit to the myocardium following delivery of various stem cell types and enhanced cardioprotection with established engraftment (18, 36). Evidence of stem cell differentiation and successful implantation into diseased myocardium came with delivery of bone marrow derived stem cells demonstrating remuscularization and revascularization (3, 32, 37). While ejection fraction benefit has been demonstrated (37), the inter-trial variability in outcome has raised the question of the uniformity in the therapeutic efficacy of implanted stem cells. Despite initial demonstration of adult stem cell contribution to the myocardium following transplantation (21, 32), a recognized limitation for adult stem cell use is the inter-patient variability potentially impeding uniform benefit following delivery. This warrants development of a technology capable to standardize cardiogenic priming of patient-derived cells prior to transplantation (7).
Concomitantly, embryonic stem cell-based myocardial repair has also shown promise in the setting of ischemic injury with evidence of improved myocardial performance and stable functional recovery (8, 17, 20, 27). Despite embryonic stem cell efficacy in the repair of damaged myocardium, transplantation of pluripotent cells harbors a risk of teratogenic transformation dependent on the cell dose delivered (5, 8, 16, 28). In this regard, the risk for tumorigenic outcome hinges on the capacity of the host myocardial microenvironment to secrete cardiogenic growth factors that guide implanted pluripotent cells towards the cardiac program (5, 8). Titration of tumorigenic transformation indicates little risk, of this otherwise significant side effect, with delivery of <1,000 cells per milligram of heart tissue, yet precipitous tumor formation as the cell number exceeds this threshold level (4, 5, 28). The latent risk of tumorigenic transformation has created an impediment for further translation of embryonic stem cell therapeutics into practice, necessitating the development of a protocol for cardiogenic priming to limit the reliance on the cardiac host environment.

As such, a systematic dissection of the stem cell profile has been pursued at the embryonic (5, 8) and adult (7, 14) stem cell levels to delineate the molecular basis for stem cell-based cardiogenesis within the host myocardium. The need to uniformly enhance cardiogenic priming of adult stem cells or hone the unguided aptitude of pluripotent embryonic stem cells was recently addressed by a strategy that stimulates naïve stem cells of adult or embryonic origin, guiding them towards the cardiac fate (5, 6). Consequently, this effort has led to the identification of a novel cellular phenotype, the cardiopoietic stem cell, both from adult and embryonic sources, with the definitive capacity to generate de novo heart muscle for repair (6).

**Cardiopoietic optimization**

Cardiopoietic specification was based on the emulation of natural cardiac differentiation within the embryo, which occurs within the mesoderm under the
cardioinductive influence of the neighboring endoderm (16, 29, 31, 38). The cardioinductive potential of the endoderm was found enhanced by the cytokine TNFα, allowing dissection of a combination of factors sufficient to drive naïve stem cells towards the cardiac program (5). With high throughput genomic and proteomic screening, cardiogenic instructive signals were deciphered and found efficacious in inducing the expression and nuclear translocation of cardiac transcription factors such as Nkx2.5, MEF2C, GATA4 in treated stem cells (5, 8, 15, 33, 39), indicative of definitive cardiac commitment and lineage specification. Identified proteins within the primed endodermal secretome clustered into networks downstream of TNFα and included potent cardiogenic factors, such as members of the TGFβ and FGF families (25, 26) whose synergistic influence secured commitment to the cardiac program, recapitulating the influence of the natural embryonic environment. This approach recruited the cardiopoietic cell phenotype, found fully capable of repairing host myocardium without the variability of adult stem cells or the tumorigenic side effect of embryonic stem cells (5).

**Cardioprotective repair**

Even in the setting of safe infarction repair, patients with existing coronary artery disease continue to remain at high risk for new cardiac events. Indeed, cardiac rehabilitation measures are often taken to reduce cholesterol levels in an effort to stabilize atherosclerosis and prevent future ischemic events (1, 12). Yet, new myocardium generated with stem cell therapy will continue to remain at risk for injury. As such, recent efforts aim to achieve repair with stem cell-derived myocytes that would be resistant to injury through optimization of their capacity for cardioprotection (41).

The notion of cardioprotection was first proposed in 1772 as William Heberden read a letter to the Royal College of Physicians describing a patient’s ability to exercise...
to the point of angina, and then continue exertion with little or no symptoms. This phenomenon came to be known as walk-through or warm-up angina. Little evidence for the underlying mechanism of this phenomenon existed until discovery of cardioprotective pathways, including the metabolism-sensing ATP-sensitive K⁺ (K<sub>ATP</sub>) channel implicated in securing protection in both health and disease (10, 11, 22, 23). This capacity of the heart to protect itself from injury during a “fight-or-flight” response, ischemia or in failure (23, 42, 43) may also serve as the key to securing enhanced protection in cell therapy. As such, recent studies have assessed mitochondrial and energetic maturation in the differentiating embryonic stem cell (13) providing great insight into the metabolic foundation supporting cardiac differentiation. Future studies attempting to guide cardiopoiesis in an environment of hypoxia may successfully forge, during differentiation, an optimization of the cardioprotective cellular response to a hostile environment such that the transplanted cells will be able to thrive despite the metabolic stress of the ischemic milieu (19).

**Concluding remarks**

Stem cells, though recognized for their great potential in tissue repair, fall into two categories. The adult stem cells have very limited capacity for repair when delivered in the naïve form, whereas embryonic stem cells demonstrate high capacity for repair but due to their continued proliferative propensity could deteriorate into teratomas following transplantation. Both of these findings reflect on the evolutionary reality that naïve stem cells are not designed for an exogenous therapeutic purpose. Furthermore, such non-optimized cells have been delivered into a tissue microenvironment that has not been fully explored (30, 34, 35), making cellular adaptation during the engraftment process difficult to interpret. The advances in strategies utilized to generate, from naïve stem cell sources, cell phenotypes that are designed and optimized to fulfill a therapeutic purpose open new avenues for
translation of stem cell biology. Focusing on the myocardium, the strategy to overcome stem cell limitations was founded on a new function for TNFα in promoting stem cell cardiogenesis both in vitro and within the context of the diseased myocardium. Molecular dissection of the pro-cardiogenic action of TNFα has shed new insight into the identity of a host of cardiac growth factors capable of recruiting cardiac pre-determined phenotypes, such as the cardiopoietic stem cell (5). As such understanding of the molecular and genetic composition of stem cells during differentiation, and the host microenvironment that they are transplanted into, allows the capture of progenitor cells with a heightened aptitude for directed repair of the organ of interest.
ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health, Marriott Heart Disease Research Program, Marriott Foundation, and Ted Nash Long Life Foundation. AB is supported by Mayo Clinic Clinician-Investigator Program.
References


18. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, and Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med* 11: 367-368, 2005.


