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GUIDED STEM CELL CARDIOPOIESIS: DISCOVERY AND TRANSLATION

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Abstract

Over 1,000 patients have participated worldwide in clinical trials exploring the therapeutic value of bone marrow-derived cells in ischemic heart disease. Meta-analysis evaluation of this global effort indicates that adult stem cell therapy is in general safe, but yields a rather modest level of improvement in cardiac function and structural remodeling in the setting of acute myocardial infarction or chronic heart failure. Although promising, the potential of translating adult stem cell-based therapy from bench to bedside has yet to be fully realized. Inter-trial and inter-patient variability contribute to disparity in the regenerative potential of transplanted stem cells with unpredictable efficacy on follow-up. Strategies that mimic the natural embryonic program for uniform recruitment of cardiogenic progenitors from adult sources are currently tested to secure consistent outcome. Guided cardiopoiesis has been implemented with mesenchymal stem cells obtained from the bone marrow of healthy volunteers, using a cocktail of secreted proteins that recapitulate components of the endodermal secretome critical for cardiogenic induction of the embryonic mesoderm. With appropriate validation of this newly derived cardiopoietic phenotype, the next generation of trials should achieve demonstrable benefit across patient populations.

1. Introduction

Ischemic heart disease is a leading cause of mortality and morbidity that accounts annually for 20 million deaths globally. Despite advances in medical therapy and increased access to interventional strategies, the prognosis of patients with ischemic cardiomyopathy remains poor. The emerging recognition that stem cells can uniquely differentiate into specified cellular phenotypes that produce beneficial outcome when transplanted into diseased heart, often beyond that achieved with current standards of care, offers a new therapeutic paradigm in cardiovascular medicine. Recent clinical trials underscore cell-based therapy as an attainable and safe strategy for reparative outcome, yet intense validation and optimization is required to ensure maximal benefit in practice [1–5].

Over one thousand patients have enrolled in clinical trials to assess safety and efficacy of adult stem cells in ischemic heart disease [6]. Initial studies with skeletal muscle-derived myoblasts and circulating progenitor cells have progressed to the use of bone marrow derived stem cells

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tested in patients presenting with acute myocardial infarction or heart failure in the setting of ischemic heart disease [6–9]. Collectively, these early studies indicate that stem cell transplantation is associated with favorable outcome and limited post-infarct maladaptive remodeling, implicating induction of cardioprotective and vasculogenic factors in mechanisms of repair [6]. Paracrine secretion, transdifferentiation and/or myocardial fusion, along with the critical role of the cardiac stem cell niche, have been proposed as contributors that increase viable cardiomyocyte content following stem cell transplantation [10,11]. It is still uncertain, however, whether implanted somatic stem cells reliably contribute to long-term regeneration, as clinical trials show various degrees of efficacy on follow-up [6,12]. Indeed, inter-trial and interpatient variability, along with disparity in the regenerative potential of transplanted stem cells, contribute to uncertain efficacy [12].

Differentiation of adult bone marrow-derived stem cells into specialized cell types can, in principle, be fostered by injection into blastocysts to secure ectopic exposure to the embryonic program [13]. Alternatively, application of DNA methyltransferase inhibitors, such as 5-azacytidine, has been used to induce cardiogenic outcome after derivation of stem cells from bone marrow [14]. Although effective, this approach results in cellular apoptosis and exit from cell cycle [15]. It has been recently suggested that patient-derived stem cells can be induced through natural means to secure cardiogenic uniformity while maintaining proliferative capacity for reliable clinical application [16,17]. Indeed, the need to achieve cardiogenic priming was successfully addressed by establishing a strategy that stimulates naïve stem cells, guiding them towards a cardiac fate [18–20].

2. Guided cardiopoiesis

Cardiopoiesis defines the process of definitive engagement of pluripotent or multipotent stem cells into the cardiac differentiation program [18]. The foundation for achieving guided cardiopoiesis stems from the feasibility of deriving a cardiac progenitor population through mimicry of natural cardiogenic signaling (Fig. 1). In the developing embryo, instructive guidance from the ventral endoderm secures cardiac program induction within the neighboring antero-lateral mesoderm [21–27]. Recently, enhancement of the cardioinductive potential of the endoderm by the stress cytokine TNF α enabled dissection of a combination of factors sufficient to drive naïve stem cells towards the cardiac program [19,28]. With high throughput screening, cardiogenic instructive signals were deciphered and found to be efficacious in inducing expression and nuclear translocation of cardiac transcription factors including Nkx2.5, MEF2C, GATA4 [19,20,28,29], indicative of definitive cardiac commitment and lineage specification. Accordingly, cardiopoietic guidance emulates natural cardiac differentiation, and can be recapitulated through stem cell stimulation with TGF- β 1, BMP-2/4, FGF-2/4, IL-6, IGF-1/2, VEGF-A, EGF, and activin-A [19]. Induction with single recombinant factors is typically insufficient to initiate nuclear import of cardiac transcription factors, a critical step to definitively engage differentiating stem cells into the cardiac program [19]. Rather, the synergy of factors used as a recombinant cocktail regimen is necessary to induce nuclear translocation of Nkx2.5 by day 2 of differentiation, and of later cardiac transcription factors MEF-2C and GATA4 by day 4, indicative of definitive cardiac commitment [19]. Systematic dissection of stem cell differentiation was then pursued to map the molecular underpinnings for stem cell-based cardiogenesis [19,20]. This effort led to identification of a novel cell phenotype, the cardiopoietic stem cell, that possesses definitive capacity to generate *de novo* heart muscle for repair [16–19]. *En route* to achieving sarcomerogenesis, cardiopoietic stem cells are an “intermediate” progenitor phenotype distinguished by nuclear translocation of cardiac transcription factors [19]. The recruited cardiopoietic population can be enriched using a dual interface Percoll gradient to separate sarcomere-rich high density cardiomyocytes from a lower density sarcomere-poor cardiopoietic phenotype [19]. Specifically, when day 4 cardiopoietic stem cells at 10,000 cells/ml are continuously cultured in monolayer in the

presence of a cardiogenic cocktail, sarcomeric differentiation can be achieved by day 7 in ~10% of cells, by day 9 in ~30% of cells, and by day 12 in ~65% of cells [19]. Electron microscopy has visualized the transitional state of cardiopoietic cells, which relinquish a phenotype of high nucleus-to-cytosol ratio typical of a pluripotent/multipotent stem cell with acquisition of a progressively mature cardiac structure [19]. The cardiopoietic cell population preserved mitotic activity, a remnant property of the embryonic source, and acquired contact inhibition properties with execution of the cardiac program [19].

3. Transcriptomic signature of cardiopoiesis

Concerted molecular changes are anticipated to drive distinct cellular fates, such as cardiopoiesis, and generation of rich bodies of transcriptomic data must be resolved to pinpoint molecules and pathways integral to phenotype determination. To this end, novel high throughput tools have enriched modern biological analyses, with concepts such as massively parallel signature sequencing and network theory providing added value through contextualization of the large volume of data established by traditional reductionist approaches [30]. A paradigm often employed to deconstruct complex lineage-specification processes has centered on embryonic stem cell malleability.

Embryonic stem cells harbor incipient differentiation programs concealed by a complex pluripotential background conferring a multiplicity of cell fates. This intricate genetic context has been addressed by major advances that have expanded current understanding of lineage specification. Recently, delineation of a transcriptional hierarchy provided insights into a pluripotency-supportive functional gene network [31]. Furthermore, these transcriptional underpinnings sustain concomitant proteomic frameworks for collective thematic maintenance of a developmental repertoire [32]. The interplay among genomic, transcriptomic and proteomic strata emphasizes how pluripotency is a dynamically orchestrated systems biology process amenable to nonstochastic bioinformatic dissection for unbiased, integrated resolution [33].

Pertinent to safe heart repair [34], embryonic stem cells require unequivocal cardiac commitment, i.e., cardiopoietic induction, a process which facilitates cardiac program engagement. Recently, a systems biology approach was applied to track cardiopoietic transcriptional reorganization through undifferentiated and differentiating embryonic stem cells, pro-cardiac progenitors, and cardiomyocyte progeny along the differentiation continuum that prioritizes cardiogenesis out of a pluripotent, embryonic stem cell parental background [20].

Extraction of a signature precursor sub-transcriptome facilitates ontological dissection [35]. In this way, components of DNA replication, recombination and repair machinery, cell cycling, cancer mechanisms, and RNA post-translational modifications were identified as downregulated during cardiopoietic progression [20]. Simultaneously, cardiovascular development, cell-to-cell signaling, cell development and cell movement were upregulated functional themes that enhanced pluripotential confinement [20]. Collectively, these gene ontology rearrangements engaged a switch that specified and restricted lineage development from an incipient transcriptional program, with further bioinformatic integration of genomic and ontological data revealing hierarchical categorization of canonical signaling cascades within discrete phases of cardiopoiesis [20,36].

Transcriptional changes, followed through a series of cardiopoietic stages, highlight functionally salient gene relationships and demonstrate guidance of phenotype elaboration coordinated by gene expression dynamics that synergistically operate through functionally reticulated interactions [20]. Implementation of network analysis, to identify topological features of the interconnected cardiopoietic framework, revealed integrin, WNT/ β -catenin,

TGF β and VEGF pathways as necessary molecular anchors for functional robustness in this embryonic stem cell derivation paradigm. Control of differentiation by these network nodes was validated upon selective pharmacological manipulation of these bioinformatically predicted cascades, and provided quantifiable measures of enhanced or impeded cardiomyocyte yield [20].

Non-stochastic reorganization and streamlining of the transcriptional landscape in response to defined signaling axes precedes differentiation and cellular fate selection in embryonic stem cells [36]. Cardiopoietic cells represent a cytotype in which essential cardiogenic elements are elevated beyond pluripotential criticality, thus recapitulating necessary conditions to efficiently derive extraembryonic cardiomyocytes for downstream feasibility [19,20].

Gene expression profile resolution allows targeted deconvolution of developmental programs buried within a pluripotential genomic context. This expedites codification of critical molecular elements responsible for phenotype selection, and the present example illustrates how bioinformatic dissection provides a tangible molecular prognostic of cardiopoiesis, as well as the opportunity to refine necessary cardioinductive prerequisites. These advantages significantly favor procurement of rationally guided, efficient cytotypes for clinical application.

4. Proteomic dissection of cardioinduction

Integrating genomic with proteomic analyses yields complementary datasets offering comprehensive profiling of cell differentiation [37], as highlighted through recent international initiatives emphasizing the value of applying multidimensional technologies to resolve mechanisms of stem cell self-renewal and differentiation [38,39]. Studies on early stages of stem cell differentiation validate the utility of systems-based proteomic approaches, in concert with genomics, to expand our understanding of developmental processes [40–42]. A case in point is leukemia inhibitory factor (LIF) which, when present in conjunction with FGF-4 and fibronectin, was found to stimulate embryonic stem cell differentiation, unmasking a previously unrecognized function beyond a traditional role in maintaining pluripotency [42]. Moreover, multivariate analysis of differentiation and proliferation signaling pathways did not identify unique signaling activities as critical for specific stages of differentiation, but rather predicted multiple signaling cascades working in concert [42].

Proteomic methodologies are increasingly employed to map definitive lineage specification from a pluripotent state. Initial proteomic profiling of distinct stem cell lines [e.g., 43–45] has been extended to discriminate proteomic variations between cytotypes within and between species [46,47]. Proteomic applications are also beginning to focus on various aspects of discrete lineage specification. For example, undifferentiated Royan B1 embryonic stem cells were compared to corresponding embryoid body-derived, as well as neonatal, cardiomyocytes indicating source-independent similarity between cardiac progeny [48]. Proteomic-based comparison of CGR8 embryonic stem cells, derived cardiopoietic cells, and adult myocardium indicated that cardiac progenitors demonstrate properties of a primordial stage of lineage-specification [49]. Recently, extant proteomic differences between H1 and HES2 human embryonic stem cell lines were profiled to quantify differing cardiogenic propensities [50]. Moreover, a 2-D gel electrophoresis proteomic approach with independent validation by shotgun 2-D LC-MS/MS, identified proteins secreted from cardiogenically primed *versus* unprimed endoderm. This approach mapped a TNF α -primed endodermal secretome, from which a definitive set of 48 unique secretome proteins was deduced by iterative bioinformatic screening using algorithms for detection of canonical and non-canonical indices of secretion [28].

In order to integrate paracrine effectors guiding stem cell cardiopoiesis, acquired proteomic data were further interpreted through network analysis [19,28]. In conjunction with respective expression level changes, protein interaction network analysis revealed a TNF α -centric secretome network with a scale-free, hierarchical architecture (Figure 2). Cardiovascular development was the primary predicted function associated with the resolved network [28]. This was verified through application of primed secretome on embryonic stem cells which potentiated cardiac commitment and sarcomerogenesis, whereas exclusion of TNF α from network generation demoted primary ranking of cardiovascular development [28]. Functional inhibition of primary network hubs, TNF α and TGF β , negated pro-cardiogenic effects. Specifically, stimulation of embryoid bodies with primed secretome tripled beating activity, whereas pharmacological inhibition with infliximab or latency associated peptide reduced cardiogenesis. Activity of TNF α and TGF β is thus critical to potentiating effects of primed over unprimed secretome, validating functional cooperativity of the derived cardioinductive network [28].

5. Nuclear transport machinery remodels for cardiopoiesis

A critical step in cardiogenic engagement of stem cells is nuclear translocation of cardiac transcription factors to engage the cardiopoietic stem cell phenotype. Nuclear transport plays a vital role in tissue specificity [51]. Nuclear pores are gatekeepers of nucleocytoplasmic exchange, and dynamic changes in nuclear transport factor expression and pore microanatomy were recently determined for cardiac commitment of embryonic stem cells [29]. Global genomic down-regulation of subtranscriptomes during differentiation translated into targeted streamlining of the nucleocytoplasmic transport machinery, namely nucleoporins, importins, exportins, transportins and Ran-related nuclear transport genes [29]. Establishment of the cardiac molecular phenotype was associated with increased nuclear pore density. At nanoscale resolution, individual nuclear pores exhibited conformational changes with expansion of pore diameter and augmented probability of conduit occupancy [29]. Thus, embryonic stem cells undergo active remodeling of nuclear transport infrastructure to support nuclear translocation of cardiac transcription factors and engagement of cardiac differentiation.

6. Energy metabolism maturation supports cardiopoiesis

Coordination of genetic circuits with developmental bioenergetics is critical in phenotype specification. The metabolic mechanism that drives cardiac transformation includes a switch from anaerobic glycolytic metabolism, sufficient for embryonic stem cell homeostasis, into more efficient mitochondrial oxidative metabolism to secure cardiopoietic energetic needs, and ultimately functional excitation-contraction coupling [52]. This energetic switch was programmed by rearrangements in the metabolic transcriptome involving genes that encode components of glycolysis, fatty acid oxidation, the Krebs cycle, and the electron transport chain. Changes in copy number of mitochondrial fusion and fission regulators were associated with mitochondrial maturation and network expansion, providing an energetic continuum to supply nascent sarcomeres [52]. Disruption in respiratory chain function prevented mitochondrial organization and compromised energetic infrastructure, causing deficient sarcomerogenesis and contractile malfunction. Thus, evolution of a mitochondrial system and establishment of oxidative metabolism are prerequisites for cardiac differentiation, identifying mitochondria-dependent energetic circuits as mediators of *de novo* cardiogenesis and heart regeneration.

7. Applied cardiopoiesis

Therapeutic *in vivo* utility of embryonic stem cell-derived cardiopoietic cells was demonstrated after direct transplantation into infarcted heart [19]. Engrafted cardiopoietic cells integrated within host myocardium for functional repair [19]. Achieving full translation would require

adoption of embryonic stem cell-derived technology to secure adult stem cell induction (Fig. 1). Subpopulations of circulating bone marrow derived stem cells are likely to be innately primed for cardiogenesis, but insufficient for achieving full repair. Cardioinductive factor identification is thus necessary to uniformly prime adult stem cell populations for cardiogenesis, while maintaining proliferative capacity for scaled-up production [16,17]. Therefore, endodermal secretome components – deciphered by subtractive genomics and proteomics methodologies [19,20,28] – have been modified to derive a cardiopoietic population from human mesenchymal stem cells through mimicry of the embryonic cardiogenic program utilizing recombinant inductive factors [18].

A mandate for standardization of mesenchymal stem cell expansion, and establishment of release criteria of expanded cell populations, has been issued to maximize safety and effectiveness of clinical transplantation [53]. Culture-based expansion offers the opportunity to produce, *en masse*, a large pool of stem cells, and derive progeny honed for lineage-specification [54]. In this regard, biomarker selection of fate-specified precursors enables targeted selection of cardiopoietic lineages [55,56]. Derivation and characterization of specialized mesenchymal stem cell subpopulations, and their selective application to specific disease conditions is an emerging strategy for enhanced therapeutic outcome. Appropriate efficacy validation and quality-control release of cellular phenotypes [57,58], should achieve next generation inter-trial consistency with demonstrable translation benefits for patients [59, 60].

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Figure 1.

Decoding natural cardiogenic cues has led to the establishment of “cocktail-based” lineage-specification of naïve stem cells towards tissue-specific priming to yield uniform specialized cardiopoietic progenitors effective in achieving successful repair. In this way, tumor propensity associated with pluripotent embryonic stem cells can be nullified, while the low cardiogenic efficacy of adult stem cells can be enhanced. Successful energy metabolism maturation and metamorphosis of the nuclear transport machinery are integral in securing effective cardiopoiesis.

Figure 2.

Endodermal secretome protein interaction network. Secretome proteins submitted to Ingenuity Pathways Analysis as focus nodes (blue squares) integrate within a 105 protein composite network. Nodes were designated by their UniProt gene names (<http://us.expasy.org/sprot/>), with focus nodes shaded in accordance with extent of cardiogenic-induced expression level change (legend), and network members deduced through interactions with focus nodes designated by yellow circles. Network clustering coefficient distribution (clustering coefficient, $C[k]$ versus degree, k , lower left), indicated that connectivity within protein neighborhoods followed a power law distribution ($R^2 = 0.9417$) consistent with a hierarchical architecture, typical of biological networks. Network degree distribution also followed a power law distribution, indicative of scale-free, non-stochastic topography.