Cardiogenesis and the Complex Biology of Regenerative Cardiovascular Medicine

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REVIEW

Cardiogenesis and the Complex Biology of Regenerative Cardiovascular Medicine

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The heart is a complex organ system composed of a highly diverse set of muscle and nonmuscle cells. Understanding the pathways that drive the formation, migration, and assembly of these cells into the heart muscle tissue, the pacemaker and conduction system, and the coronary vasculature is a central problem in developmental biology. Efforts to unravel the biological complexity of in vivo cardiogenesis have identified a family of closely related multipotent cardiac progenitor cells. These progenitors must respond to non–cell-autonomous signaling cues to expand, differentiate, and ultimately integrate into the three-dimensional heart structures. Coupling tissue-engineering technologies with patient-specific cardiac progenitor biology holds great promise for the development of human cell models of human disease and may lay the foundation for novel approaches in regenerative cardiovascular medicine.

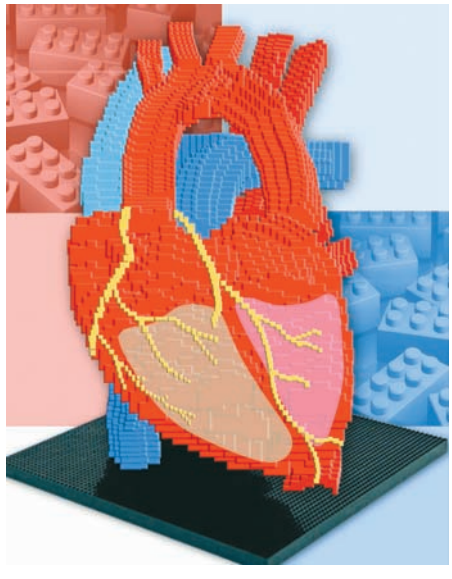

Fig. 1.

"There is always an easy solution to every human problem—neat, plausible and wrong."
—H. L. Mencken (*1*)

The view of the heart as muscular pump has dominated cardiovascular science and medicine for over a century. However, the heart is clearly more than muscle, with a panoply of diverse cardiac and smooth muscle, valvular, pacemaker, and endothelial cell types with discrete contractile, electrical, and vascular roles (Figs. 1 and 2). To form a fully functional heart organ system, a set of embryonic precursor cells must give rise to these distinct cell types, which must ultimately assemble and align within specific heart compartments to form ventricular chambers, coronary arteries, and the conduction system. In this regard, recent studies have identified a novel set of multipotent heart progenitors that can give rise to many of the major cell types in the heart. At the same time, a host of clinical studies in regenerative cardiovascular medicine have attempted to reverse heart muscle failure by augmenting the amount of functional human cardiac muscle via transplantation of a diverse group of adult progenitor cell types (*2*–*8*). The concept itself is relatively simple: Progenitor cells isolated from outside the heart are transplanted into the adult heart, with the hope that they will eventually expand and integrate into the intact myocardial tissue and thereby improve cardiac function. Unfortunately, to date, the results have largely been ambiguous, marginal, or negative, suggesting that simply transplanting adult nonheart progenitors and other muscle cells into a failing heart will not necessarily lead to substantial, long-term clinical improvement (*9*). The lessons from these clinical studies and parallel work in animal models (*10*–*18*) point to the need to account for the biological complexity of in vivo embryonic cardiogenesis. How is the diversity of heart cells generated? Is there a "master" heart progenitor that can give rise to the major muscle and nonmuscle cell lineages, and, if so, how is information conveyed for the precise differentiation of daugh-

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ter cell types? What controls the expansion of progenitors and their downstream differentiated progeny into discrete tissue compartments? How are they assembled into three-dimensional structures that are required for coordination of mechanical work, electrical signal propagation, and uniform delivery of blood flow? Can we reconstruct this complexity ex vivo by combining recent advances in stem cell biology, developmental biology, and tissue bioengineering? This brief review will highlight how recent advances in our understanding of the biological complexity of cardiogenesis itself are beginning to point to novel approaches for regenerative cardiovascular medicine.

Generation of Diverse Heart Cell Lineages from Multipotent Progenitors

Mammalian cardiogenesis requires the generation of a highly diversified set of both muscle and nonmuscle cell types, including atrial and ventricular cardiomyocytes; conduction system and pacemaker cells; and smooth muscle, endothelial, valvular, and endocardial cells (*19*–*21*). The formation of these various cardiovascular cell lineages in distinct heart and vascular compartments has its basis in the existence of a closely related set of multipotent progenitors in the early embryonic heart field (*22*, *23*), which can be divided into the primary heart field and secondary heart field (SHF) lineages (Fig. 2) (*19*, *24*–*26*). The primary heart field arises from the anterior lateral mesoderm and forms a group of cardiovascular precursors that form a cardiac crescent in the early embryo. Later in development, these cells coalesce into the linear heart tube and ultimately give rise to the left ventricle of the mature four-chambered mammalian heart. The SHF is derived from a population of cells in the pharyngeal and splanchnic mesoderm, which migrate into the developing heart and give rise to the right ventricle, the outflow tract, and portions of the inflow tract. Recent work has now also identified a set of multipotent epicardial progenitor cells that contribute to the atrial and ventricular myocardium, coronary smooth muscle, and cardiac fibroblasts (*27*, *28*). To date it has not been possible to isolate and characterize the developmental potential of purified populations of primary heart field progenitors because of an absence of molecular markers unique to that field. In contrast, lineage tracing experiments have demonstrated that most of the early SHF myocardial, smooth muscle, and endothelial cells can be traced to multipotent heart progenitors that express the LIM–homeodomain transcription factor Islet1 [Isl1 (Fig. 2)] (*22*, *26*, *29*). Clonal assays with purified Isl1 progenitors from embryos or embryonic stem (ES) cells have also documented the ability of a single SHF progenitor to give rise to the above three cardiac lineages (*22*, *23*, *30*). In a parallel series of experiments, the cardiac-specific Nkx2.5 enhancer has been used to isolate bipotential cardiac progenitors from murine embryos as well as murine ES cells (*20*). Similarly, the mesoderm marker Brachyury T, in combination with the cell surface marker Flk1, was used to enrich for a population of multipotent cardiac progenitors from human and murine ES cells (*30*, *31*). Unlike the Isl1 progenitors, however, the Nkx2.5 progenitors and the Flk1 progenitors likely represent a heterogeneous mix of primary and secondary heart field progenitors. It is unclear whether cardiac regeneration of the left ventricle, a primary heart field–derived structure, will require purified populations of primary heart field progenitors.

Non–Cell-Autonomous Cues Control Lineage Specification and Expansion of Cardiac Progenitor Populations

Cardiac progenitor cells, which originate in the primary and secondary heart fields, are subject to a rapidly changing environment because of cell migration and changes in the three-dimensional architecture of the primitive heart. Developing cardiac progenitor populations are therefore susceptible to temporally and spatially modulated variations of non–cell-autonomous signaling molecules that originate from neighboring endothelial, endocardial, and other mesodermally derived cells in the primitive embryonic heart. These cues work in concert to control lineage commitment and expansion of the progenitor population. The development of the SHF appears to be critically dependent on bone morphogenic protein (BMP) signaling. BMP 2, 4, 6, and 7 have overlapping patterns of expression with Isl1, and conditional deletion of type I BMP receptor Bmpr1a in Isl1 progenitor results in multiple defects of SHF-derived structures (*32*). Similarly, fibroblast growth factor (FGF) signaling plays a key role in the formation and subsequent maturation of the SHF. FGF8 null mutations, for example, result in embryonic lethality at gastrulation; hypomorphic mutations result in multiple cardiovascular defects affecting primarily SHF derivatives, including the outflow tract (*33*). Furthermore, disruption of FGF signaling within the SHF by conditional inactivation of the FGF receptors FGFr1 or FGFr2 results in outflow tract defects associated with failure of extracellular matrix secretion as well as failure in BMP and transforming growth factor (TGF)–β signaling (*34*). Ablation of the FGF adaptor molecule Frs2α in outflow tract progenitor cells also inhibits their expansion and results in outflow tract hypoplasia (*35*).

During the later stages of cardiogenesis, the robust expansion of cardiogenic cells is critical, particularly for the ventricular chamber. The increase in muscle mass is required to generate sufficient mechanical work to maintain the demands for blood flow in the exponentially growing embryo. In the outflow tract, this expansion has to

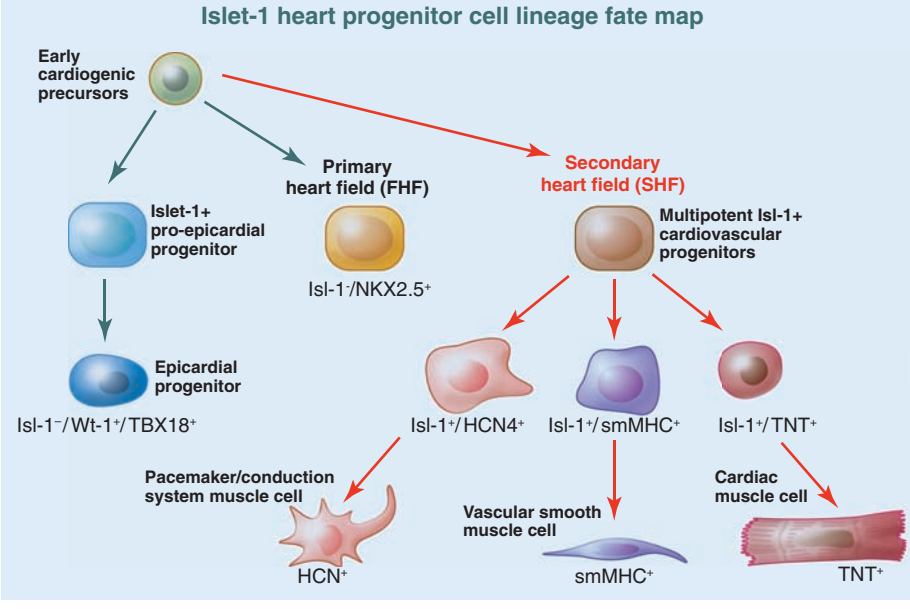

Fig. 2. Multipotent heart progenitors in the Isl1 lineage. Early mesoderm-derived cardiac precursors give rise to progenitors in the first and second heart fields (FHF and SHF, respectively). The LIM–homeodomain transcription factor Isl1 marks a multipotent cardiovascular progenitor that can give rise to myocardium, conduction system, smooth muscle cells, and endothelial lineages. The developmental potential of FHF progenitors is not well established because of an absence of specific molecular markers for that field and an inability to isolate purified progenitor populations of that lineage. Recent work (*48*) has also identified a third multipotent set of epicardial progenitors that appears to arise from a very early Isl1 precursors and to express the transcription factors Tbx18 or Wt1. TNT indicates troponin T; MHC, myosin heavy chain.

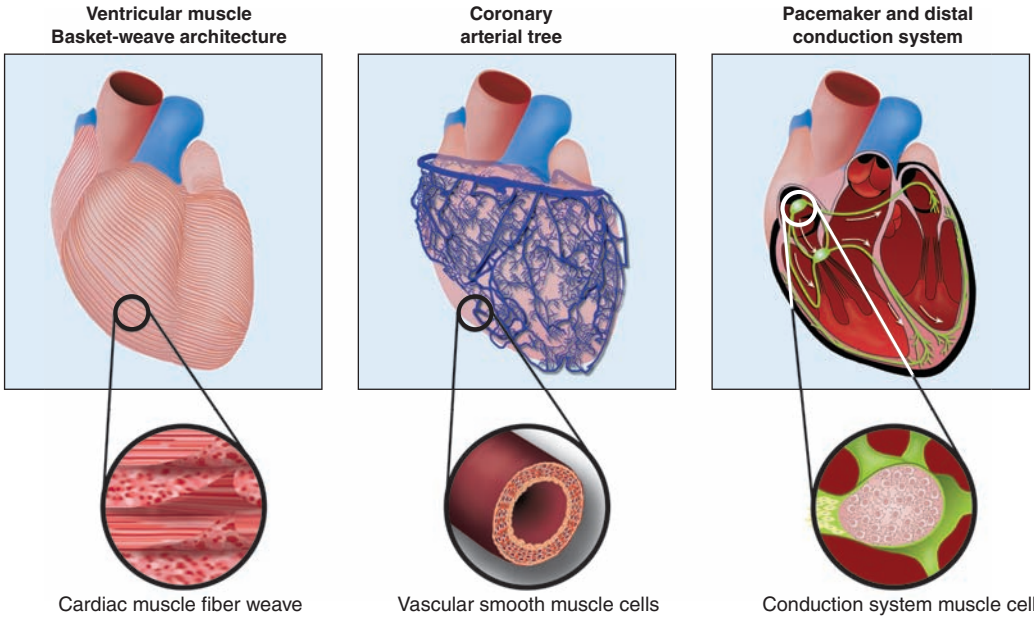

Fig. 3. Three-dimensional structure of ventricular muscle basket weave, coronary arterial tree, and pacemaker and conduction system. One of the central challenges of cell-based therapy for regenerating specific heart components is guiding transplanted cells into a functional syncytium with the existing three-dimensional architecture. Transplanted cells must make functional connections with neighboring specialized heart cells to result in a net gain of global function. Transplanted myogenic progenitors, for example, must align with and integrate into the existing ventricular muscle basket weave to allow synchronous contraction and relaxation of graft and host myocardium. Integration of pacemaker and conduction system progenitors into the appropriate tissue type is necessary to generate a biological pacemaker and avoid cardiac arrhythmia. For example, having a transplanted heart muscle progenitor integrate into the conduction system might have arrythmogenic consequences, as would the introduction of cells with independent pacemaker potential in the heart. Similarly, cell-based therapies to promote coronary collateral formation or neo-arteriogenesis require functional integration of transplanted cells with the host coronary arterial tree.

be carefully controlled to insure the proper rotation and positioning of the aorta and pulmonary artery as they form a junction with the heart itself. The precise positioning of the aorta over the left ventricular chamber and the pulmonary artery over the right ventricular chamber is one of the most critical steps in cardiogenesis. Defects in this process are the most common cause of human congenital heart disease, and a growing body of evidence suggests that the Isl1-derived heart progenitors play a pivotal role. Although relatively little is known regarding how special cues control cell proliferation, recent advances from several laboratories have suggested that Wnt/β-catenin pathways appear to play a critical role in the expansion of Isl1 cardiac progenitors and their differentiated progeny in the right ventricle and the outflow tract. Gain-of-function mutations of β-catenin in Isl1 progenitor lineages result in a massive expansion of the progenitor pool in vivo (*36*–*38*). Thus, defining the non–cell-autonomous cues that regulate in vivo cardiogenesis will be of critical importance to understanding normal development and generating the cell populations necessary for regenerative cardiovascular medicine. To that end, various combinations of FGF, BMP, and Wnt signaling molecules have been used to promote cardiogenesis and expand cardiac progenitor populations in murine and human embryonic model systems (*30*, *31*, *37*).

Three-Dimensional Organizational Structure of Ventricular Muscle, Vascular Smooth Muscle, and Conduction System Muscle

Perhaps one of the most unexplored areas of cardiogenesis has been in understanding how differentiated cell types are assembled into the specific three-dimensional structures of the mature heart (Fig. 3). With regard to ventricular myocardium, a precise linear alignment of cardiac myocytes in alternating layers of muscle fibers forms a basket weave of muscle tissue and leads to a muscle fiber alignment designed to propel the blood forward through the outflow tract. This tissue alignment is evident at multiple levels, from the microscopic scale of sarcomere assembly up to the three-dimensional structure of the ventricular chamber (Fig. 4). In the failing heart, this cardiac muscle fiber linear alignment can be highly disorganized, with fibers emanating at angles within the same muscle cell layer, as well as be disrupted by tissue fibrosis. Given this loss of fiber organization, it is increasingly likely that the transplantation of cardiac muscle progenitors or their differentiated progeny, in the absence of cues to drive their appropriate linear alignment with the native heart tissue, may not result in a substantive improvement in global heart function. Toward this goal, recent work suggests that coupling of myocytes to adjacent cells, tissues, and extracellular matrix results in external cues that shape ventricular myocytes architecture. These appear to be similar to the navigational cues that guide cell migrations and the formation of laminar layers that wrap around the ventricular cavities in the developed heart (*39*, *40*). Whether these guidance cues are maintained in the mature heart is an important unanswered question because therapies based on the direct introduction of cardiogenic cells into the failing heart assume that transplanted cells will maintain this ability for intramyocardial pathfinding, differentiation, and functional integration into the host tissue (*41*).

Establishment and maintenance of blood flow through the epicardial coronary arteries and through the microcirculation, the small vessels that intercalate through the heart muscle itself, are critical for normal cardiac function and the prevention of heart failure (*42*, *43*). The smooth muscle and endothelium of the coronary arterial tree are assembled into a labyrinth that effectively intercalates the entire myocardium, and relatively little is known about the guidance cues that drive this assembly (Fig. 3).

Similarly, the electrical conduction system has a characteristic three-dimensional structure where the heart beat is initiated in the sinoatrial node and travels through a series of modified conduction system muscle cells to carry the electrical impulse throughout the heart (Fig. 3). Understanding the pathways governing the normal development of these structures will also be critical in the application of biologically targeted approaches for cardiac regeneration.

The Road Ahead: Engineering Humanized Organ Model Systems

To unravel the biological complexity of embryonic cardiogenesis and its growing intersection with cardiovascular regenerative biology, novel model systems will be required in which the individual variables of progenitor cell type, extracellular matrix cues, two- and three-dimensional structure, and tissue function can be analyzed for their effects on the global physiological function

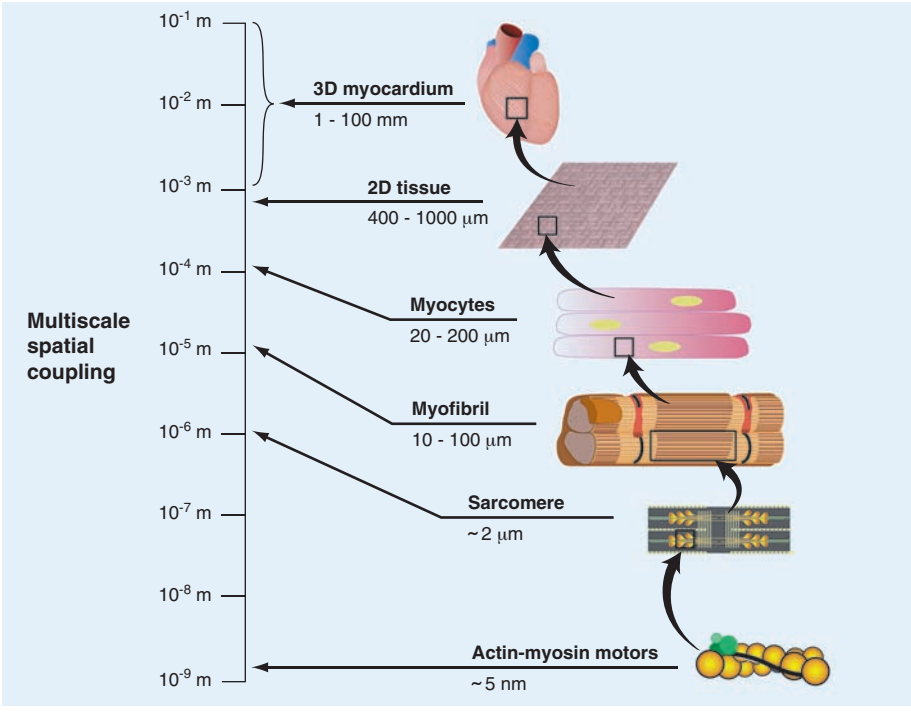

Fig. 4. Scaling of ventricular muscle. The assembly of ventricular muscle represents a scaling problem that spans several orders of spatial magnitude from the alignment of actin-myosin complexes within a sarcomere, their alignment in myofibrils, the organization of myofibrils in a myocyte, and the coupling between myocytes in anisotropic, laminar muscle. 2D and 3D indicate two- and three-dimensional, respectively.

of an intact organ. Whereas reconstructing the milieu of the entire multicellular heart will be difficult, recent advances suggest the possibility of engineering specific heart parts that correspond to the ventricular muscle, conduction and pacemaker systems, and aspects of vascular smooth muscle tissue (*44*–*46*).

To that end, one of the central challenges of cell-based therapy for the treatment of heart failure is the identification of the optimal cell type to drive robust cardiac myogenesis. The ideal cell type would be completely committed to the myogenic cell fate and yet maintain the capacity to expand in vivo or in vitro. In addition, it will be important for the cell type of interest to be able to differentiate into functional, force-generating myocardial tissue. To date, although a number of laboratories have identified multipotent cardiogenic precursors from ex vivo and in vitro sources in both mouse and human, no one has identified such a committed expandable myogenic precursor from a renewable source of cells. In addition to the identification of the appropriate cell type(s) for tissue regeneration, it will be important to define the three-dimensional structure to guide cell growth and differentiation. A complete reconstitution of cardiac muscle from a decellularized heart matrix has recently been shown, indicating that there may be specific matrix cues for the assembly of heart progenitors and their downstream differentiated progeny (*47*).

Coupling tissue engineering technologies with state-of-the-art protocols for the generation of ES cell–derived heart progenitor–based systems holds great promise for a new era of cardiovascular regenerative medicine. The development of physiological assays for contractility, conduction, and mechanical work with patient-specific heart progenitors may allow the generation of heart muscle that harbors specific genetic backgrounds, facilitating the direct functional analysis of the role of specific genetic variations in human populations, as well as novel approaches for drug screening and development. In this regard, the rapid advances in the generation of inducible pluripotent stem (iPS) cells from somatic cells, such as skin cells, will clearly increase the value of having access to carefully phenotyped patient tissue. In the future, the development of patient-specific heart tissues from iPS cells might serve as prototypes for replacement parts that could be introduced in situ into diseased heart tissue components via advances in delivery device technology, ultimately offering an alternative to direct cell transplantation into the injured or disease myocardium. This may, in fact, mark a new era in which developmental biology and regenerative medicine converge to create human models of cardiovascular disease (*34*).

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