Published in final edited form as:

Annu Rev Cell Dev Biol. 2010 ; 26: 667–687. doi:10.1146/annurev-cellbio-100109-103948.

Stem Cell Models of Cardiac Development and Disease

Kiran Musunuru, MD, PhD, MPH, Ibrahim J. Domian, MD, PhD, and Kenneth R. Chien, MD, PhD

Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA; Harvard Stem Cell Institute, Cambridge, MA

Kiran Musunuru: kiranmusunuru@gmail.com; Ibrahim J. Domian: idomian@partners.org

Abstract

The last few years have witnessed remarkable advances in stem cell biology and human genetics, and we have arrived at an era where patient-specific cell and tissue models are now practical. The recent identification of cardiovascular progenitor cells, as well as the identification of genetic variants underlying congenital heart disorders and adult disease, opens the door to the development of human models of human cardiovascular disease. We review the current understanding of the contribution of progenitor cells to cardiogenesis, and we outline how pluripotent stem cells can be applied for the modeling of cardiovascular disorders of genetic origin. A key challenge will be to implement these models in an efficient manner to develop a molecular understanding of how genes lead to disease and to screen for genes and drugs that modify the disease process.

Keywords

cardiovascular disease; congenital heart disease; cardiac development; stem cells; genetics

I. Introduction: Cardiovascular stem cells and the genetics of cardiovascular disease

The development of the heart is one of the earliest and most essential steps during vertebrate embryonic development. Over a century of work has resulted in a clear understanding of the morphogenic events that result in the four-chambered heart during normal mammalian cardiovascular development. More recently, studies over the past decade have uncovered many of the key genes and molecular pathways that govern cellular differentiation in mouse model systems. Despite these advances in uncovering the molecular basis of cardiac patterning and cellular differentiation, the developmental logic that accounts for the generation of the diverse cell lineages in the heart (atrial, ventricular, pacemaker, vascular smooth muscle, and endothelial), as well as the principles that underlie their assembly into discrete compartments and structures, remain poorly understood. In particular, relatively little is known about the mechanisms of human cardiogenesis, a point that is crucial for understanding the roots of congenital heart disease, as well as the many forms of juvenile and adult heart disease that arise due to defects in later steps of cardiovascular development. Given the distinct differences in size and complexity of the human heart, understanding the points of divergence with mouse model systems is a major challenge.

In this regard, recent studies from a number of laboratories have uncovered a series of early heart progenitors that underlie mammalian cardiac development. The ability to identify and isolate these progenitors in both embryos and in pluripotent stem cell model systems such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have reshaped our thinking about mammalian cardiac development in general, and human cardiogenesis in particular. This review will highlight recent advances that have resulted in a convergence of the fields of cardiac development, stem cell biology, and human genetics. Recent work has resulted in the identification of a family of human cardiac progenitor cells marked by the LIM homeodomain transcription factor *Isl1* (*Isl1*). We will here probe the utility of these cells in evaluating the function of human genetic variations linked to congenital and adult heart disease as well as risk factors for heart disease.

II. Discovery of multipotent heart progenitors

The heart is composed of diverse cell types: cardiac muscle, smooth muscle, conduction system, endothelial, valvular, and interstitial mesenchymal fibroblasts. An increasing body of evidence suggests that this diversity is generated to a large extent by the presence of multipotent cardiovascular progenitors located in the early heart fields (Farrell et al. 1999, Meilhac et al. 2004, Buckingham et al. 2005, Chien et al. 2008, Martin-Puig et al. 2008, Wu et al. 2008, Dyer & Kirby 2009). In this regard, *Isl1*⁺ progenitors in the second heart field differentiate into several cardiac lineages and give rise to over two thirds of the cells in the heart including most of the conduction system, proximal coronary arterial tree, atrial and right ventricular chambers, and outflow tract myocardium (Figure 1; Cai et al. 2003, Laugwitz et al. 2005, Moretti et al. 2006, Sun et al. 2007). Multipotent *Isl1*⁺ cardiovascular progenitors capable of giving rise to cardiac myocytes, smooth muscle, and endothelial cells have been identified in both mouse and humans, indicating that the initial steps in the generation of this diversity are due to cell fate decisions at the single cell level (Moretti et al. 2006). The availability of this well-defined assay system has led to the discovery of a number of key signaling pathways that drive specific steps in the renewal and differentiation of the *Isl1* heart lineages, including a Wnt/ β -catenin renewal system for the multipotent *Isl1*⁺ progenitors (Kwon et al. 2007, Qyang et al. 2007).

Relatively less is known about the first heart field precursors, which give rise to the left ventricular chamber (Buckingham et al. 2005). This largely reflects the lack of specific markers for this cell population. Recent studies have employed two-color embryo and embryonic stem cell systems, and a combination of positive and negative fluorescence-activated cell sorting (FACS) screening, to document the presence of distinct first and second heart field progenitors in the murine embryo that have signature transcriptional profiles with distinct patterns of both coding and non-coding transcripts (Domian et al. 2009). Since the most common cause of human heart failure occurs due to the loss of functional muscle cells in the left ventricular chamber, it will be particularly relevant to determine whether progenitors in the first heart field persist into late adulthood. Conditional lineage tracing should be invaluable in assessing their potential for mobilization and/or regeneration of cardiac muscle following heart injury.

With the ability to purify first and second heart field progenitors, it is now possible to dissect the pathways that drive the conversion of multipotent heart progenitors to specific differentiated cell types at the earliest stages of cardiogenesis (Cai et al. 2003, Laugwitz et al. 2005, Kattman et al. 2006, Moretti et al. 2006, Wu et al. 2006, Prall et al. 2007, Smart et al. 2007, Yang et al. 2008, Bu et al. 2009, Domian et al. 2009). In the murine embryonic heart, a rare transient intermediate can be found, which co-expresses *Isl1* and Troponin T, in the ventricular outflow tract. FACS has recently allowed the purification of this intermediate from murine embryos and embryonic stem cells, documenting that it represents a committed

ventricular progenitor in the Isl1 lineage. The onset of expression of differentiation markers is concomitant with the downregulation of the progenitor marker Isl1. Remarkably, these ventricular progenitors can undergo limited expansion and maintain their ability to form fully functional ventricular muscle cells, suggesting that they might play a pivotal role in ventricular myogenesis during the early stages of cardiogenesis. Understanding the intrinsic checkpoints that ultimately limit the proliferative capacity of these committed ventricular progenitors will be critical in unlocking their full potential as models of human cardiac muscle disease. In addition, it will be of interest to determine if a similar set of committed intermediates also underlie the generation of other key components that are known to be derived from Isl1 lineages, such as vascular (endothelial and smooth muscle) and pacemaker lineages. Ultimately, it should be possible to construct a well-characterized cardiogenic cell fate map, which charts the steps that guide the formation and renewal of the growing family of Isl1 and other multipotent heart progenitors, and their subsequent conversion to specific differentiated cardiovascular cell types (Figure 2).

III. Stem cell paradigm for human cardiogenesis

To date, most of our understanding of the molecular and cellular basis for cardiogenesis has been based on studies of murine cardiovascular development. The program that governs cardiac development in humans, however, differs significantly from the murine program and results in an organ that is over 10,000 times larger than its mouse counterpart. Murine cardiogenesis is largely completed within 48 hours, while human cardiogenesis occurs over several weeks. The question arises as to the basis of this divergence. One paradigm might be related to replication of already differentiated cells, leading to the increase in muscle and other tissue compartments, while an alternative might represent a “stem cell paradigm” where the increase in cell number is due to expansion of a progenitor pool which then differentiate in to functional progeny.

Recent studies now support the stem cell model as a central paradigm underling human cardiogenesis (Bu et al. 2009). Late during human cardiac development, a diverse family of ISL1+ cells co-express cardiac myocyte, smooth muscle, or endothelial lineage markers. The co-expression of ISL1 with differentiation markers suggests that these cardiac progenitors are undergoing active conversion from a pluripotent mother cell to the distinct lineage-restricted daughter cells of the mature heart. The ISL1 progenitors are particularly concentrated in the developing outflow tract that gives rise to the aorta, pulmonary artery, and portions of the ventricular chambers. Direct evidence that human ISL1 cardiac progenitors are multipotent has now also been provided by the purification of these cells from human ES cells in vitro differentiation (Bu et al. 2009). Taken together, these studies suggest that human cardiovascular development follows a stem cell paradigm where a limited number of lineage-restricted progenitors proliferate to generate the requisite number of diverse cell types necessary for the extended period of human cardiovascular development. This model would account for the difference in size between the human heart and its murine counterpart.

The availability of well-characterized human heart progenitor cells will allow a direct examination of the pathways that drive the earliest stages of human cardiogenesis, as well as allow the development of human models of human congenital heart disease. In that regard, the largest concentration and diversity of human ISL1 heart progenitors are localized to the outflow tract, a hotspot for most forms of life-threatening human congenital heart disease. It is therefore of great interest to determine if at least a subset of human congenital heart disorders represents “stem cell” disease, where defects in cell fate decisions of ISL1 heart progenitors result in the defects in cell growth and expansion that accompany many forms of congenital heart disease. Intriguingly, many of the known, rare monogenic forms of human

congenital heart disease have been localized in genes that are known to play pivotal roles in cell fate decisions of the ISL1 heart progenitor family (see below).

IV. Human models of human cardiovascular disease

Limitations of animal models

Much of what is known about the molecular pathways that lead to human cardiovascular disorders has come from studying animal models, particularly genetically modified mouse models. Although these approaches have been useful in the past, important differences exist between rodent and human cells as well as between normal and diseased human cells.

For example, mice diverge considerably from humans with regard to lipoprotein metabolism as well as their susceptibility to disorders of lipid metabolism. In particular, mice lack the enzyme cholesteryl ester transfer protein (CETP), which is responsible for the transfer of cholesterol from HDL to LDL particles. Furthermore, mice preferentially produce a truncated form of apolipoprotein B—apoB-48—in liver by RNA editing of the *apoB* mRNA, whereas in humans only the full-length form—apoB-100—is produced in liver; apoB-100 but not apoB-48 is used for hepatic synthesis of functional lipoprotein particles secreted into the bloodstream. As a consequence of these differences, wild-type mice have very low levels of plasma LDL-C compared to humans and are resistant to the development of coronary atherosclerosis even when placed on high fat, high cholesterol diets. Although genetic modification of mice to boost LDL-C levels does allow for formation of aortic atherosclerotic plaque, the coronary vasculature is largely spared, and it is thus extremely challenging to reproduce the heart attack phenotype in mice (without the simple expedient of surgically ligating a coronary artery).

Difficulties also emerge from the considerable differences between the human and mouse genomes. Although there are some cases where it is possible to “translate” genetic discoveries from humans to mice—e.g., a genetic study may identify a nonsense mutation in a gene as being responsible for human disease, and so a knockout mouse for that gene may be expected to phenocopy the disease—in most circumstances there are no direct correlates for human genetic variants such as single nucleotide polymorphisms or copy number variants in mice. This problem will take on increasing relevance in light of recent genome-wide association studies (GWAS) identifying large numbers of disease-associated non-coding DNA variants that lack orthology in mice and other non-human models. It is therefore imperative to replicate relevant features of human cardiovascular physiology and in the context of the human genome. Recent advances in stem cell and cardiovascular progenitor biology now raise the possibility of generating human models of human cardiovascular development, physiology, and disease.

Genetic modification of human ES cells

Gene targeting allows for the introduction of precise genetic modifications into cells, offering the potential to generate cells and tissues that harbor genetic variants specific to individual patients. Potential modifications include “knockout” of genes, “knockin” of missense mutations into genes or reporters into gene loci, and “chromosome engineering” entailing the deletion of a portion of a chromosome. Traditionally, homologous recombination has been the method of choice for gene targeting (Figure 3). Although homologous recombination in mouse ES cells has become routine, significant differences between mouse ES cells and human ES cells contrive to make genetic modification of the latter far more challenging (Thomas & Capecchi 1987). Cloning and expansion of human ES cell lines from single cells is inefficient (Amit et al. 2000) and electroporation conditions typically used to introduce DNA targeting plasmids into mouse ES cells are suboptimal for

human cells (Eiges et al. 2001). At the time of this writing there have been only a few published studies in which successful homologous recombination in human ES cells was undertaken, with recombination rates typically on the order of 0.1%–0.2% (Zwaka & Thomson 2003, Urbach et al. 2004, Costa et al. 2007, Irion et al. 2007, Lombardo et al. 2007, Davis et al. 2008, Di Domenico et al. 2008, Bu et al. 2010).

An alternative technology promises to significantly enhance our ability to genetically modify human ES cells. Introducing a double-stranded break in genomic DNA at the site of a desired alteration has been shown to greatly improve the efficiency of homologous recombination (to rates exceeding 20%; Porteus & Baltimore 2003, Porteus & Carroll 2005). Zinc finger proteins can now be designed to specifically bind to sites defined by 18 DNA bases or more, enough to ensure uniqueness in the genome. Addition of endonuclease moieties to these zinc finger proteins can thereby introduce double-stranded breaks at desired sites in the genome. When used in combination with traditional gene targeting constructs, zinc finger proteins have been successfully used to target a variety of genes in human cells, including iPS cells (Maeder et al. 2008, Zou et al. 2009). More recently, zinc finger technology has been used to engineer chromosome deletions and translocations in human cells (Brunet et al. 2009, Lee et al. 2010).

Although homologous recombination in differentiated cells (as opposed to pluripotent ES cells) is theoretically possible with traditional techniques, the success rate has been very poor to date. Accordingly, efforts to obtain genetically modified somatic cell types have centered on developing recombinant ES cell lines and then subjecting them to differentiation protocols to derive specific cell types of interest (e.g., neurons, cardiomyocytes, hepatocytes). This approach has the added advantage of establishing a renewable source of differentiated cells for study, since pluripotent ES cells can in principle be propagated and expanded indefinitely. Zinc finger technology offers a potentially useful alternative in that it promotes genetic modification at high efficiency even in cultured immortalized cell lines. Thus, one can envision developing recombinant cultured lines that, while not differentiated, nevertheless retain some properties of the somatic cell types of interest (e.g., hepatoma cell lines used as models for liver tissue).

Generation of disease-specific iPS cells lines

A parallel approach to generating “patient-specific” cells and tissues has recently emerged in the last few years. Yamanaka and colleagues demonstrated that the exogenous expression of four proteins in human somatic cells—c-Myc, Klf4, Oct4, and Sox2—is sufficient to induce pluripotency in the cells (Figure 3; Takahashi et al. 2007, Yu et al. 2007, Park et al. 2008b). Although iPS cells are not perfectly equivalent to human ES cells (Takahashi et al. 2007), they retain important properties of ES cells such as the capacity for long-term propagation and expansion and the ability to differentiate into all human germ layers and, by extension, all somatic cell types. Thus, it is now feasible to obtain easily accessible somatic cells from patients—from skin biopsies, hair follicles, or whole blood—and derive cultured iPS lines that are genetically matched to the patients. This has successfully been done for a variety of human diseases, including type 1 diabetes mellitus, muscular dystrophies, Parkinson disease, Huntington disease, and Down syndrome (Park et al. 2008a).

iPS cell lines offer several advantages over recombinant human ES lines. If the genetic variant linked to the disease is difficult to generate with homologous recombination—e.g., a chromosomal deletion or translocation—then finding a patient with the genetic alteration and obtaining somatic cells for generation of an iPS cell line may be the quicker approach. In some cases, the genetic variant responsible for the disease may not be known, and iPS technology will allow for study of the disease despite this lack of knowledge. For complex human diseases such as coronary artery disease, a single genetic variant introduced into

control human ES cells may not be sufficient to fully manifest the disease phenotype. In contrast, an iPS cell line derived from a patient with the disease would have the appropriate genetic background and may therefore be more likely to display the relevant disease phenotype at the cellular level.

One important consideration in adapting iPS models for the study of human disease is the identification of appropriate control cell lines derived from appropriate control patient populations. This consideration takes on particular importance when the genetic variant under study is not associated with a large phenotypic effect. Comparing cellular phenotypes of disease specific iPS cell lines to well matched controls would still be confounded by differences in the genetic backgrounds of the two cell lines. This would be akin to comparing a knockout mouse of one strain with a wild-type mouse of an entirely different strain. In this regard, genetic modification of human ES cells to introduce the disease variant offers a built-in control—the parental ES cell line, which would be genetically matched with the recombinant cell line except for the disease variant. A highly rigorous experimental strategy that would combine the advantages of both approaches might start with the generation of a patient-specific iPS cell line, followed by the genetic modification of the iPS cell line to “cure” it of the pathogenetic DNA variant and thereby provide an appropriate control cell line.

Isolation and expansion of human cardiovascular progenitors

The ability to generate, expand, and purify functional cardiac cells will be critical to developing pluripotent stem cell models of human cardiovascular disease (Figure 4; Chien 2008). A useful intermediate step will be to isolate committed “progenitor” cells with the potential to expand as well as differentiate into multiple cell types. Such progenitor cells offer the possible advantage of being more likely than pluripotent cells to yield homogeneous populations of differentiated cells for analysis.

In fact, studies in mouse embryos confirm that multipotent Isl1+ cardiac progenitor cells differentiate into the major cardiac cell types including cardiac myocytes, smooth muscle, and endothelial cells (Cai et al. 2003, Laugwitz et al. 2005, Kattman et al. 2006, Moretti et al. 2006). In order to determine if human cardiogenesis proceeds in a similar fashion, lineage tracing experiments were performed with genetically engineered human ES cells (Bu et al. 2009). The gene encoding the Cre recombinase was introduced into the ISL1 locus by homologous recombination and a red fluorescent protein (RFP) reporter cassette was introduced into these same cell lines by stable transfection. Expression of the Cre recombinase results in the excision of a transcriptional stop and the expression of the red fluorescent RFP. Thus, cells in the ISL1 lineage were irreversibly marked as red, allowing for their purification (Bu et al. 2009). As expected, FACS purified RFP+ cells expressed significantly increased levels of the cardiac markers ISL1 and NKX2-5 compared to unlabeled control cells. Further, clones derived from the marked cells displayed expression of the markers Troponin T, SMTN, and PECAM1, markers for cardiomyocytes, smooth muscle cells, and endothelial cells respectively. Thus, human ES derived Isl1+ progenitors, like their murine counterparts, are multipotent and capable of differentiating into the major cardiac lineages.

Although lineage tracing and isolation of progenitor cells are useful in establishing the identity and multipotency of the cells, protocols to reliably expand and differentiate the cells are necessary for their practical use as models for disease. In the case of ISL1+ cardiac progenitor cells, the use of Wnt3a-secreting feeder mouse embryonic fibroblast cells allowed for the expanded growth of the progenitor cells while still maintaining *ISL1* expression (Bu et al. 2009). Thus, it should be possible to scale up isolation and production of cardiac progenitor cells so that large numbers of differentiated cells can be ultimately

obtained. However, efficient methods to separate the desired differentiated cell type (e.g., cardiomyocytes) from unwanted cells (e.g., smooth muscle cells, endothelial cells, and the parental progenitor cells) are mandatory.

Such methods could take several forms. An optimized differentiation protocol that utilizes experimentally verified culture conditions could in principle restrict differentiation from a multipotent progenitor cell population to a single desired cell lineage. However, even the most efficacious protocol is unlikely to achieve 100% differentiation. As a result contamination with other cell types may confound downstream analyses. Such a protocol would find best use as a means to improve the yield of differentiated cells in combination with one of the alternative strategies described below.

Gene targeting to place a selectable marker (whether a fluorescent marker or an antibiotic resistance gene) under the transcriptional control of a promoter of a gene specific for the desired cell type would allow for purification of differentiated cells by either FACS or selection with antibiotics (Meyer et al. 2000, Kouskoff et al. 2005, Moretti et al. 2006, Wu et al. 2006). However, the technical challenge inherent in gene targeting in human ES cells (as discussed above) make this a less attractive option, particularly in the context of using patient-specific iPS cells, for which each individual cell line would have to be targeted.

A similar approach that skirts this technical barrier would be to use a selectable marker delivered via a viral transgene, e.g., a lentivirus encoding the cell-specific promoter and marker. Virus would be administered at high enough titer to ensure infection of most of the expanded progenitor cell population. There would be heterogeneity among the cells, both with regard to the number of viral genomes integrated per cells and the genomic locations of integration (some of which might disrupt cellular genes), but in aggregate this should not affect downstream analyses of diseased vs. control cells unless the cells under study represent single or few clonal populations.

Another approach would avoid the insertion of genes into cells and instead use defined, cell-specific surface antigens (Kouskoff et al. 2005, Kattman et al. 2006). This would allow for the use of antibodies and FACS to isolate the desired cell type. However, this strategy requires knowledge of appropriate surface antigens, and in most cases such knowledge is lacking; indeed, in some cell types, highly specific surface antigens may not even exist.

Stem cell models of cardiovascular physiology

An example of successful terminal differentiation of pluripotent stem cells into a desired tissue type is the generation of functional heart muscle comprising organized ventricular cardiomyocytes. In order to recapitulate embryonic cardiac development, markers specific for progenitor cells giving rise to the first and second heart fields were used—a two-colored transgenic fluorescent reporter system, with a green protein driven by the cardiac-specific *Nkx2-5* enhancer and a red protein driven by a *Isl1*-dependent enhancer (Domian et al. 2009). Mouse ES cells harboring both transgenes were differentiated via embryoid bodies and yielded discrete populations of cells that were marked solely by green, solely by red, or by both colors. Appropriately, green cells expressed *Nkx2-5*, red cells expressed *Isl1*, and cells that were solely green expressed *Nkx2-5* and *Tbx5* but not *Isl1*, consistent with gene expression patterns in the first heart field and second heart field, respectively.

The FACS-isolated, distinct groups of progenitor cells were then expanded and seeded into muscular thin films with extracellular matrix elements that were patterned to facilitate the formation of longitudinal muscle fibers (Domian et al. 2009). Whereas single-colored progenitor cells failed to give rise to contractile muscle—they gave rise to a mix of cardiomyocytes and smooth muscle cells—double-colored progenitor cells yielded

spontaneously beating muscular thin films that were force-generating and could be paced by external stimulation. The higher propensity of double-labeled progenitor cells to differentiate into cardiac myocytes is consistent with their *in vivo* restriction to the myocytes of the right ventricle and outflow tract. The identification of these committed ventricular progenitors provides a template for the development of efficient protocols to produce cardiac muscle cells. These cell types could then be combined with tissue engineering technologies to generate contractile-force two-dimensional and, ultimately, three-dimensional muscle (Ott et al. 2008, Domian et al. 2009, Stevens et al. 2009). Although it remains to be demonstrated that such protocols will be equally effective for human pluripotent stem cells as for mouse cells, the technology opens the door for the engineering of patient-specific cardiac muscle samples that recapitulate key aspects of cardiac physiology.

Generation of non-cardiac cells for the study of cardiovascular disease

Although cardiac cell types are of obvious relevance to cardiovascular disease, particularly congenital heart disease, other cell types are key to the understanding other types of cardiovascular disease. For example, dyslipidemia can result from perturbations of lipoprotein metabolism centered at the liver; conversely, therapies targeting enzyme activity in the liver (such as HMG-CoA reductase inhibitors) can cause favorable changes in the plasma lipid profile that reduce the risk of MI. Thus, human hepatocytes can provide a model system in which to study molecular pathways that are important modulators of cardiovascular risk.

With liver having an embryonic origin quite different from heart (endoderm vs. mesoderm), the protocols to derive hepatocytes from pluripotent stem cells are quite different from those discussed above for cardiac cell types. Nonetheless, the same general considerations for the generation, expansion, and purification of differentiated cells are pertinent. As with cardiac cells, the development of robust, efficient methods to convert pluripotent stem cells into functional liver cells remains a work in progress (Cai et al. 2007, Agarwal et al. 2008, Shiraki et al. 2008, Si-Tayeb et al. 2010). In one recently published hepatic differentiation protocol, a series of culture conditions were used to advance human cells through various stages of a pathway thought to reflect normal embryological development—pluripotent stem cell, definitive endoderm, specific hepatic precursor (hepatoblast), immature hepatocyte, and mature hepatocyte—with each stage characterized by the expression of specific gene markers, with up to 85% of the final cells displaying hepatic markers (Seguin et al. 2008). Whether these cells truly recapitulate all features of hepatocytes, or merely reflect specific, limited aspects of hepatic function, is under active investigation. It may emerge that different protocols can be tailored to yield liver-like cells appropriate for different functional assays. The generation of endodermal progenitor cells (analogous to cardiac progenitor cells) has been reported (Seguin et al. 2008), though it is not yet clear whether these cells will be of practical use in generating large numbers of hepatocytes.

Besides liver, other non-cardiac tissues of relevance to cardiovascular disease include vascular endothelium, vascular smooth muscle, adipose, skeletal muscle, and pancreas. Methods to reliably produce these cell types from pluripotent stem cells are under development.

V. Genetics of cardiovascular disease

Most forms of cardiovascular disease are now appreciated to at least partly arise from genetic variants, whether chromosomal abnormalities, rare DNA variants, or common DNA variants (Table 1). As the genetic bases of various disorders are better understood,

knowledge of the responsible variants will greatly enhance the ability to develop faithful cell-based models of the disorders.

Congenital heart disease

Occurring in about 1% of newborns, developmental defects of the heart can manifest in a variety of ways, some with mild physiological consequences, some life-threatening. Many congenital heart disorders are now appreciated to have genetic causes in some patients: atrial and ventricular septal defects, Ebstein's anomaly of the tricuspid valve, double outlet right ventricle, transposition of the great arteries, truncus arteriosus, patent ductus arteriosus, tetralogy of Fallot, aortic coarctation, aortic stenosis, pulmonic stenosis, hypoplastic left heart syndrome, and bicuspid aortic valve (Bruneau 2008). Not surprisingly, some of the genes implicated in these conditions are key players in cardiac development.

Among the first identified genes underlying congenital heart disorders (Table 1) was the *TBX5* transcription factor (T-box protein 5), linked to the autosomal dominant Holt-Oram syndrome that features atrial and ventricular septal defects as well as conduction system defects. Nonsense or missense mutations in *TBX5* were identified in both families and sporadic cases of the syndrome (Basson et al. 1997, Li et al. 1997b). Neonatal mice heterozygous for a deleted allele of *Tbx5* exhibited atrial septal defects, ventricular septal defects, and mild cardiac conduction abnormalities, consistent with Holt-Oram syndrome and confirming that *TBX5* plays an important role in cardiac septation into four chambers during development (Bruneau et al. 2001).

Other T-box proteins also appear to be responsible for cardiac abnormalities in humans. For example, *TBX20* has been found to harbor mutations in patients with septal defects, ventricular dilation, and valvular abnormalities (Kirk et al. 2007). Experiments with a mouse model of DiGeorge syndrome (also called velo-cardio-facial syndrome), which in humans results from a deletion on chromosome 22q11 and is characterized by congenital conotruncal abnormalities in the heart, suggested that *TBX1* in the 22q11 locus is partially if not fully responsible for the cardiac phenotype (Lindsay et al. 2001, Merscher et al. 2001). Subsequent investigations identified rare variants in *TBX1* in patients with the syndrome but lacking the 22q11 deletion, providing further evidence of the gene's pathogenetic role (Yagi et al. 2003).

Genes that interact with *TBX5* in transcription factor pathways have also been implicated in congenital heart disorders. Analysis of families with inherited atrial septal defects with or without ventricular septal defects identified mutations in *GATA4*; one of these mutations impaired *GATA4*'s ability to physically interact with *TBX5* (Garg et al. 2003). This observation suggests that the two genes cooperate in regulating cardiac septation. Similarly, mutations in *NKX2-5* were discovered as the cause of autosomal dominant atrial septal defects in some families (Schott et al. 1998). Subsequent studies have established that *NKX2-5* and *TBX5* are direct interactors that synergistically induce cardiac development (Bruneau et al. 2001, Hiroi et al. 2001).

NKX2-5 is of particular note because perturbation of its function can manifest as a variety of different phenotypes. Besides septal defects, mutations in *NKX2-5* have been identified in patients with cardiomyopathy, outflow tract defects, tetralogy of Fallot, hypoplastic left heart, and associated arrhythmias (Benson et al. 1999, Goldmuntz et al. 2001, Gutierrez-Roelens et al. 2002, Elliott et al. 2003). Global and conditional knockout mouse models confirm that *NKX2-5* has important functions in multiple cardiac cell lineages (Lyons et al. 1995, Tanaka et al. 1999, Pashmforoush et al. 2004), consistent with the finding that *NKX2-5*⁺ progenitor cells have the capacity to form cardiomyocytes, conduction system cells, and smooth muscle cells (Wu et al. 2006).

Although *ISL1* has not yet been found to harbor disease-causing variants in humans, *ISL1* has been shown to coordinately regulate at least two genes, *GATA4* and *TBX20*, that are associated with congenital heart disease (Dodou et al. 2004, Takeuchi et al. 2005). Future medical sequencing efforts should shed light on *ISL1*'s role in human cardiovascular disease.

Just as one gene can give rise to a variety of congenital heart disorders, some disorders arise from mutations in a variety of genes. As discussed, abnormalities in cardiac septation are linked to *NKX2-5*, *TBX1*, *TBX5*, *TBX20*, and *GATA4*. tetralogy of Fallot, which comprises pulmonary stenosis, overriding aorta, ventricular septal defect, and right ventricular hypertrophy, afflicts patients with mutations in many of the same genes—*NKX2-5*, *TBX1*, *TBX5*, *GATA4*—as well as additional genes—*NOTCH1*, *NOTCH2*, and *JAG1*—and chromosomal abnormalities—DiGeorge syndrome, Down syndrome (Basson et al. 1997, Schott et al. 1998, Eldadah et al. 2001, Goldmuntz et al. 2001, Yagi et al. 2003, Garg et al. 2005, McDaniell et al. 2006, Tomita-Mitchell et al. 2007, Greenway et al. 2009). Recent whole-genome genetic analyses of hundreds of sporadic cases of tetralogy of Fallot identified copy number variants, i.e., deletions or duplications of chromosomal loci, at ten sites across the genome, including two loci harboring the *NOTCH1* and *JAG1* genes (Greenway et al. 2009). The most frequent copy number variant (in about 1% of cases) was at chromosome 1q21, defining a region spanning seven genes, none of which have previously been implicated in congenital heart disease or cardiac development.

Undoubtedly, many sporadic cases of congenital heart disease are driven by unknown genetic abnormalities or variants and the interaction of these with environmental stressors. As more genes involved in cardiac development are fully characterized, it seems likely that next-generation sequencing technologies (e.g., whole exome sequencing) will identify variants in these genes or chromosomal abnormalities impinging on these genes that are responsible for these cases.

Adult Cardiovascular Disease

Contrasting with the rarity of congenital heart disease in newborns, cardiovascular disease is highly prevalent in adults. Notably, coronary artery disease (CAD) leading to myocardial infarction (MI), is the single leading cause of morbidity and mortality in the developed world, with rapidly rising incidence in the developing world as well. Although there are important environmental risk factors underlying MI, such as cigarette smoking and air pollution, there is a strong genetic component to the disease particularly in earl-onset MI (< 55 years in men, < 65 years in women) for which there is a large degree of heritability, as high as 63% documented in one study (Nora et al. 1980).

In the last few years, GWAS of CAD and MI have been successful in characterizing genetic loci with robust associations with the clinical phenotypes. By studying thousands of individuals with a disease or a trait of interest, GWAS are best suited to the identification of common genetic variants that typically have modest effects on phenotype. At the time of this writing, GWAS have identified 13 loci associated with CAD and MI (Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007, Erdmann et al. 2009, Gudbjartsson et al. 2009, Myocardial Infarction Genetics Consortium et al. 2009, Tregouet et al. 2009). In all studies, a locus on chromosome 9p21 has emerged as the most strongly associated with the clinical phenotypes in the human genome, conferring up to a 40% reduction in risk of MI in individuals with protective DNA variants in the locus (compared to those with risk-conferring DNA variants). These DNA variants are not associated with any of the traditional cardiovascular risk factors, such as blood lipid concentrations, blood pressure, or diabetes mellitus. Thus, it appears the association signal at the 9p21 locus represents a novel risk pathway. Subsequent studies have established an association of the 9p21 locus with a variety

of vascular phenotypes such as abdominal aortic aneurysm, intracranial aneurysm, and peripheral arterial disease, suggesting that the novel pathway involves vascular tissue (Helgadottir et al. 2008).

Among the 12 other MI-associated loci, five are also associated with blood lipid concentrations. Four are strongly linked to low-density lipoprotein cholesterol (LDL-C) and harbor the genes *LDLR* (encoding the LDL receptor), *PCSK9*, *HNF1A*, and *SORT1*. The first three were known to be linked to lipid metabolism and/or metabolic disease prior to the GWAS era; indeed, each of the three had already been characterized as having rare variants that cause Mendelian (monogenic) disorders. *LPA* encodes apolipoprotein(a), the primary constituent of Lp(a), a specialized lipoprotein. Notably, LDL-C and Lp(a) are well-established, strong epidemiological risk factors for MI, and therapy that reduces blood LDL-C levels (statin medications) is the mainstay of MI prevention.

Prior to the GWAS era, considerable effort had been devoted to identifying Mendelian causes of coronary disease and MI. In virtually all cases, rare variants in lipid-associated genes have emerged from these studies. Through their seminal work in the 1970s and 1980s, Brown and Goldstein and colleagues identified mutations in *LDLR* in patients with autosomal dominant hypercholesterolemia, who are prone to early-onset MI (Goldstein & Brown 2009). Subsequent work characterized mutations in the *APOB* (encoding apolipoprotein B, or apoB) and *PCSK9* genes as alternative causes of autosomal dominant hypercholesterolemia (Rader et al. 2003). Other Mendelian lipid disorders that predispose to MI—autosomal recessive hypercholesterolemia, sitosterolemia, and primary hypoalphalipoproteinemia—have been linked to specific rare variants in genes involved in lipoprotein metabolism—*LDLRAP1*, *ABCG5/ABCG8*, and *APOA1* (encoding apolipoprotein A-I, or apoA-I), respectively. Thus, the understanding of the genetic basis of dyslipidemia has proven to be highly relevant to understanding the genetic basis of MI.

Mendelian mutations have also been identified for a number of other cardiovascular conditions (Seidman & Seidman 2001). Most notably, mutations in a variety of sarcomere proteins have been shown to be responsible for inherited hypertrophic cardiomyopathy. Dilated cardiomyopathy can arise not only from mutations in sarcomeric proteins but also in cytoskeletal sarcoglycans, intermediate filament proteins, and nuclear envelope proteins (Seidman & Seidman 2001). Arrhythmias can also have a genetic predisposition; for example, long QT syndrome is caused by mutations in any of at least seven different genes, most of which encode components of cardiac ion channels (Shah et al. 2005). GWAS of cardiomyopathy, arrhythmias, and other cardiovascular disorders will undoubtedly identify many more casual genes. These advances highlight the importance of developing human based in vitro model systems to address the functional significance of such disease associations.

VI. The way forward

The use of patient-specific cells to understand disease is something of a hallowed tradition in cardiovascular medicine, this being the approach used by Brown and Goldstein and colleagues in the 1970s to identify the cause of autosomal dominant hypercholesterolemia (Goldstein & Brown 2009). Although it was well appreciated at that time that the liver and intestines were central sites for lipid metabolism, they recognized that it was not feasible to use samples of these organs from hypercholesterolemic patients to study disease pathophysiology. They therefore chose skin-derived fibroblasts as their model system in the hope that the primary genetic defect would manifest in these cells, as was the case. The cells were defective in LDL uptake, which successively led to the discovery of the LDL receptor,

the phenomenon of receptor-mediated endocytosis, and the identification of disease-causing, loss-of-function variants in the *LDLR* gene.

Although this example stands out as one in which the use of pluripotent cells was unnecessary, it is clear that the ability to generate patient-specific pluripotent stem cells and use these for the generation of tissue represents an unprecedented opportunity to create human models of human cardiovascular disease. As knowledge of genetic variants underlying various conditions grows, it will be possible to generate a wide array of diseased cell lines, each harboring a different mutation or chromosomal abnormality, either via homologous recombination of ES cells or derivation of patient-specific iPS cells. Of particular interest will be genes already implicated in disease—the *TBX* gene family, *NKX2-5*, *GATA4*, *NOTCH* genes, and *JAG1*—as well as amplifications and deletions of well-characterized loci such as chromosomes 1q21 and 2q11. There will also be the opportunity to study the function of genes that are known to be important for cardiac development but in which no disease-causing mutations have yet been identified, such as *ISL1*, using homologous recombination.

Another particular advantage offered by iPS technology is that it will allow for cells to be derived from patients with congenital disease in whom the casual genetic variants are unknown. The phenotypic consequences of the variants can be studied at the cellular level despite this lack of knowledge; it may even be possible to work backwards to discover the variants. Furthermore, one can envision banking a large panel of cell lines taken from patients with a shared disorder (ranging from specific congenital disorders such as the tetralogy of Fallot to specific cardiovascular risk factors such as dyslipidemia) recruited without regard to the etiology. Comparing the phenotypic changes across the full panel could reveal a dysregulated molecular pathway or pathways common to all patients with these disorders, yielding insights into core aspects of the disease pathophysiology and making it possible to develop a single therapeutic strategy to target the common pathway(s). This may thereby all the development of novel platforms for drug discovery and development, regardless of the cause of the disease.

Although the ability to recapitulate cardiac organogenesis in vitro does not yet exist, future advances in tissue engineering may allow for essential aspects of development to be modeled. For the time being, the most productive readout may be gene expression and proteomic comparisons of diseased vs. normal cell lines. Differences in these profiles should yield important clues about molecular pathways that lie downstream of the mutant proteins that are responsible for disease. Moreover, these profiles can be in and of themselves treated as endpoints against which to gauge the efficacy of therapies. For example, screens could be performed to identify drugs that reverse changes in gene expression observed in a disease model compared to a normal model; any drug that fully or largely normalized the gene expression profile would be a credible candidate to take forward in further preclinical and clinical trials. Although such drugs would probably not be used *in utero*, they may find application as adjuncts to surgery in infants and children to ameliorate congenital heart defects and prevent long-term pathophysiological sequelae.

The ability to create functional cardiac muscle tissue would permit studies of conditions besides congenital heart disease. For example, the phenotypic effects of mutations in sarcomere genes that are responsible for hypertrophic cardiomyopathy could be analyzed by comparing muscle models with and without individual mutations; readouts might include gene expression, cell morphology, and sarcomere integrity. Moreover, these muscle models could be employed for drug screening aimed at fixing the phenotypic abnormalities. Similarly, alterations in genes responsible for long QT syndrome or other arrhythmogenic conditions could be modeled, with channel currents and other electrophysiological

parameters being the phenotypes of interest. The arrhythmia models could be used to identify drugs that normalize channel function. A particularly attractive aspect of this work would be the ability to use iPS-derived muscle with a mutation private to an individual patient and screen for an agent effective for that patient—true personalized medicine at work.

The same strategies will be relevant for the use of non-cardiac cell models to study cardiovascular disease. For example, pluripotent stem cells harboring either rare DNA variants (identified in family-based studies) or common DNA variants (identified in GWAS) linked to blood lipid levels could be used to derive liver-like cells that recapitulate key aspects of lipid metabolism: synthesis, processing, and secretion of lipoprotein components such as apoB and apoA-I, as well as uptake and degradation of LDL and other lipoprotein particles. These cells could thus be used to interrogate the effects of the DNA variants on lipoprotein phenotypes. Similarly, the effects of DNA variants identified in GWAS for coronary artery disease and MI, such as those harbored by the novel chromosome 9p21 locus, could potentially be modeled in pluripotent stem cells differentiated into relevant tissues, such as vascular endothelium and vascular smooth muscle. Gene expression profiling of the differentiated cells should reveal the downstream targets of regulatory elements harbored in the 9p21 locus and yield insights into the novel molecular pathways by which the locus affect the pathogenesis of MI, perhaps resulting in the identification of new drug candidates.

The last few years have witnessed remarkable advances in stem cell biology and human genetics, and we have arrived at an era where patient-specific cell and tissue models are now practical. In this review we have outlined how pluripotent stem cells can be applied for the modeling of cardiovascular disorders of genetic origin. The key challenge now is to implement these models in an efficient manner to develop a molecular understanding of how genes lead to disease and to screen for genes and drugs that modify the disease process. We anticipate that such studies will ultimately be of considerable benefit to the large number of patients worldwide who suffer from cardiovascular disease.

LITERATURE CITED

- Agarwal S, Holton KL, Lanza R. Efficient differentiation of functional hepatocytes from human embryonic stem cells. *Stem Cells*. 2008; 26:1117–27. [PubMed: 18292207]
- Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, et al. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol*. 2000; 227:271–8. [PubMed: 11071754]
- Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, et al. Mutations in human TBX5 cause limb and cardiac malformation in Holt-Oram syndrome. *Nat Genet*. 1997; 15:30–5. [PubMed: 8988165]
- Benson DW, Silberbach GM, Kavanaugh-McHugh A, Cottrill C, Zhang Y, et al. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. *J Clin Invest*. 1999; 104:1567–73. [PubMed: 10587520]
- Bruneau BG. The developmental genetics of congenital heart disease. *Nature*. 2008; 451:943–8. [PubMed: 18288184]
- Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, et al. A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell*. 2001; 106:709–21. [PubMed: 11572777]
- Brunet E, Simsek D, Tomishima M, DeKolver R, Choi VM, et al. Chromosomal translocations induced at specified loci in human stem cells. *Proc Natl Acad Sci U S A*. 2009; 106:10620–5. [PubMed: 19549848]
- Bu L, Gao X, Jiang X, Chien KR, Wang Z. Targeted conditional gene knockout in human embryonic stem cells. *Cell Res*. 2010 in the press.

- Bu L, Jiang X, Martin-Puig S, Caron L, Zhu S, et al. Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature*. 2009; 460:113–7. [PubMed: 19571884]
- Buckingham M, Meilhac S, Zaffran S. Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet*. 2005; 6:826–35. [PubMed: 16304598]
- Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, et al. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell*. 2003; 5:877–89. [PubMed: 14667410]
- Cai J, Zhao Y, Liu Y, Ye F, Song Z, et al. Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology*. 2007; 45:1229–39. [PubMed: 17464996]
- Chien KR. Regenerative medicine and human models of human disease. *Nature*. 2008; 453:302–5. [PubMed: 18480810]
- Chien KR, Domian IJ, Parker KK. Cardiogenesis and the complex biology of regenerative cardiovascular medicine. *Science*. 2008; 322:1494–7. [PubMed: 19056974]
- Costa M, Dottori M, Sourris K, Jamshidi P, Hatzistavrou T, et al. A method for genetic modification of human embryonic stem cells using electroporation. *Nat Protoc*. 2007; 2:792–6. [PubMed: 17446878]
- Davis RP, Ng ES, Costa M, Mossman AK, Sourris K, et al. Targeting a GFP reporter gene to the MIXL1 locus of human embryonic stem cells identifies human primitive streak-like cells and enables isolation of primitive hematopoietic precursors. *Blood*. 2008; 111:1876–84. [PubMed: 18032708]
- Di Domenico AI, Christodoulou I, Pells SC, McWhir J, Thomson AJ. Sequential genetic modification of the hprt locus in human ESCs combining gene targeting and recombinase-mediated cassette exchange. *Cloning Stem Cells*. 2008; 10:217–30. [PubMed: 18386992]
- Dodou E, Verzi MP, Anderson JP, Xu SM, Black BL. Mef2c is a direct transcriptional target of ISL1 and GATA factors in the anterior heart field during mouse embryonic development. *Development*. 2004; 131:3931–42. [PubMed: 15253934]
- Domian IJ, Chiravuri M, van der Meer P, Feinberg AW, Shi X, et al. Generation of functional ventricular heart muscle from mouse ventricular progenitor cells. *Science*. 2009; 326:426–9. [PubMed: 19833966]
- Dyer LA, Kirby ML. The role of secondary heart field in cardiac development. *Dev Biol*. 2009; 336:137–44. [PubMed: 19835857]
- Eiges R, Schuldiner M, Drukker M, Yanuka O, Itskovitz-Eldor J, Benvenisty N. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. *Curr Biol*. 2001; 11:514–8. [PubMed: 11413002]
- Eldadah ZA, Hamosh A, Biery NJ, Montgomery RA, Duke M, et al. Familial tetralogy of Fallot caused by mutation in the jagged1 gene. *Hum Mol Genet*. 2001; 10:163–9. [PubMed: 11152664]
- Elliott DA, Kirk EP, Yeoh T, Chandar S, McKenzie F, et al. Cardiac homeobox gene NKX2-5 mutations and congenital heart disease: associations with atrial septal defect and hypoplastic left heart syndrome. *J Am Coll Cardiol*. 2003; 41:2072–6. [PubMed: 12798584]
- Erdmann J, Grosshennig A, Braund PS, Konig IR, Hengstenberg C, et al. New susceptibility locus for coronary artery disease on chromosome 3q22.3. *Nat Genet*. 2009; 41:280–2. [PubMed: 19198612]
- Farrell M, Waldo K, Li YX, Kirby ML. A novel role for cardiac neural crest in heart development. *Trends Cardiovasc Med*. 1999; 9:214–20. [PubMed: 10881754]
- Garg V, Kathiriya IS, Barnes R, Schluterman MK, King IN, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature*. 2003; 424:443–7. [PubMed: 12845333]
- Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, et al. Mutations in NOTCH1 cause aortic valve disease. *Nature*. 2005; 437:270–4. [PubMed: 16025100]
- Goldmuntz E, Geiger E, Benson DW. NKX2.5 mutations in patients with tetralogy of fallot. *Circulation*. 2001; 104:2565–8. [PubMed: 11714651]
- Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol*. 2009; 29:431–8. [PubMed: 19299327]

- Greenway SC, Pereira AC, Lin JC, DePalma SR, Israel SJ, et al. De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. *Nat Genet.* 2009; 41:931–5. [PubMed: 19597493]
- Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadóttir A, Sulem P, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet.* 2009; 41:342–7. [PubMed: 19198610]
- Gutierrez-Roelens I, Sluysmans T, Gewillig M, Devriendt K, Vikkula M. Progressive AV-block and anomalous venous return among cardiac anomalies associated with two novel missense mutations in the CSX/NKX2-5 gene. *Hum Mutat.* 2002; 20:75–6. [PubMed: 12112663]
- Helgadóttir A, Thorleifsson G, Magnusson KP, Gretarsdóttir S, Steinthorsdóttir V, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. *Nat Genet.* 2008; 40:217–24. [PubMed: 18176561]
- Helgadóttir A, Thorleifsson G, Manolescu A, Gretarsdóttir S, Blondal T, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science.* 2007; 316:1491–3. [PubMed: 17478679]
- Hirayama-Yamada K, Kamisago M, Akimoto K, Aotsuka H, Nakamura Y, et al. Phenotypes with GATA4 or NKX2.5 mutations in familial atrial septal defect. *Am J Med Genet A.* 2005; 135:47–52. [PubMed: 15810002]
- Hiroi Y, Kudoh S, Monzen K, Ikeda Y, Yazaki Y, et al. Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. *Nat Genet.* 2001; 28:276–80. [PubMed: 11431700]
- Ikeda Y, Hiroi Y, Hosoda T, Utsunomiya T, Matsuo S, et al. Novel point mutation in the cardiac transcription factor CSX/NKX2.5 associated with congenital heart disease. *Circ J.* 2002; 66:561–3. [PubMed: 12074273]
- Irion S, Luche H, Gadue P, Fehling HJ, Kennedy M, Keller G. Identification and targeting of the ROSA26 locus in human embryonic stem cells. *Nat Biotechnol.* 2007; 25:1477–82. [PubMed: 18037879]
- Kasahara H, Benson DW. Biochemical analyses of eight NKX2.5 homeodomain missense mutations causing atrioventricular block and cardiac anomalies. *Cardiovasc Res.* 2004; 64:40–51. [PubMed: 15364612]
- Kasahara H, Lee B, Schott JJ, Benson DW, Seidman JG, et al. Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease. *J Clin Invest.* 2000; 106:299–308. [PubMed: 10903346]
- Kattman SJ, Huber TL, Keller GM. Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. *Dev Cell.* 2006; 11:723–32. [PubMed: 17084363]
- Kirk EP, Sunde M, Costa MW, Rankin SA, Wolstein O, et al. Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. *Am J Hum Genet.* 2007; 81:280–91. [PubMed: 17668378]
- Kouskoff V, Lacaud G, Schwantz S, Fehling HJ, Keller G. Sequential development of hematopoietic and cardiac mesoderm during embryonic stem cell differentiation. *Proc Natl Acad Sci U S A.* 2005; 102:13170–5. [PubMed: 16141334]
- Krantz ID, Smith R, Colliton RP, Tinkel H, Zackai EH, et al. Jagged1 mutations in patients ascertained with isolated congenital heart defects. *Am J Med Genet.* 1999; 84:56–60. [PubMed: 10213047]
- Kwon C, Arnold J, Hsiao EC, Taketo MM, Conklin BR, Srivastava D. Canonical wnt signaling is a positive regulator of mammalian cardiac progenitors. *Proc Natl Acad Sci U S A.* 2007; 104:10894–9. [PubMed: 17576928]
- Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, et al. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature.* 2005; 433:647–53. [PubMed: 15703750]
- Lee HJ, Kim E, Kim JS. Targeted chromosomal deletions in human cells using zinc finger nucleases. *Genome Res.* 2010; 20:81–9. [PubMed: 19952142]
- Li L, Krantz ID, Deng Y, Genin A, Banta AB, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet.* 1997a; 16:243–51. [PubMed: 9207788]

- Li QY, Newbury-Ecob RA, Terrett JA, Wilson DI, Curtis AR, et al. Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachyury (T) gene family. *Nat Genet.* 1997; 15:21–9. [PubMed: 8988164]
- Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, et al. Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature.* 2001; 410:97–101. [PubMed: 11242049]
- Lombardo A, Genovese P, Beausejour CM, Colleoni S, Lee YL, et al. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nat Biotechnol.* 2007; 25:1298–306. [PubMed: 17965707]
- Lyons I, Parsons LM, Hartley L, Li R, Andrews JE, et al. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5. *Genes Dev.* 1995; 9:1654–66. [PubMed: 7628699]
- Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, et al. Rapid “open-source” engineering of customized zinc-finger nucleases for highly efficient gene modification. *Mol Cell.* 2008; 31:294–301. [PubMed: 18657511]
- Martin-Puig S, Wang Z, Chien KR. Lives of a heart cell: Tracing the origins of cardiac progenitors. *Cell Stem Cell.* 2008; 2:320–31. [PubMed: 18397752]
- McDaniell R, Warthen DM, Sanchez-Lara PA, Pai A, Krantz ID, et al. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. *Am J Hum Genet.* 2006; 79:169–73. [PubMed: 16773578]
- McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E. NKX2.5 mutations in patients with congenital heart disease. *J Am Coll Cardiol.* 2003; 42:1650–5. [PubMed: 14607454]
- McPherson R, Pertsemidis A, Kavaslar N, Stewart A, Roberts R, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science.* 2007; 316:1488–91. [PubMed: 17478681]
- Meilhac SM, Esner M, Kelly RG, Nicolas JF, Buckingham ME. The clonal origin of myocardial cells in different regions of the embryonic mouse heart. *Dev Cell.* 2004; 6:685–98. [PubMed: 15130493]
- Merscher S, Funke B, Epstein JA, Heyer J, Puech A, et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell.* 2001; 104:619–29. [PubMed: 11239417]
- Meyer N, Jaconi M, Landopoulou A, Fort P, Puceat M. A fluorescent reporter gene as a marker for ventricular specification in ES-derived cardiac cells. *FEBS Lett.* 2000; 478:151–8. [PubMed: 10922488]
- Moretti A, Caron L, Nakano A, Lam JT, Bernshausen A, et al. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell.* 2006; 127:1151–65. [PubMed: 17123592]
- Kathiresan S, Voight BF, Purcell S, Musunuru K, et al. Myocardial Infarction Genetics Consortium. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet.* 2009; 41:334–41. [PubMed: 19198609]
- Nora JJ, Lortscher RH, Spangler RD, Nora AH, Kimberling WJ. Genetic—epidemiologic study of early-onset ischemic heart disease. *Circulation.* 1980; 61:503–8. [PubMed: 7353240]
- Oda T, Elkahoul AG, Pike BL, Okajima K, Krantz ID, et al. Mutations in the human Jagged1 gene are responsible for alagille syndrome. *Nat Genet.* 1997; 16:235–42. [PubMed: 9207787]
- Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, et al. Perfusion-decellularized matrix: Using nature’s platform to engineer a bioartificial heart. *Nat Med.* 2008; 14:213–21. [PubMed: 18193059]
- Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, et al. Disease-specific induced pluripotent stem cells. *Cell.* 2008a; 134:877–86. [PubMed: 18691744]
- Park IH, Zhao R, West JA, Yabuuchi A, Huo H, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008b; 451:141–6. [PubMed: 18157115]
- Pashmforoush M, Lu JT, Chen H, Amand TS, Kondo R, et al. Nkx2-5 pathways and congenital heart disease; loss of ventricular myocyte lineage specification leads to progressive cardiomyopathy and complete heart block. *Cell.* 2004; 117:373–86. [PubMed: 15109497]

- Pehlivan T, Pober BR, Brueckner M, Garrett S, Slaugh R, et al. GATA4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease. *Am J Med Genet.* 1999; 83:201–6. [PubMed: 10096597]
- Porteus MH, Baltimore D. Chimeric nucleases stimulate gene targeting in human cells. *Science.* 2003; 300:763. [PubMed: 12730593]
- Porteus MH, Carroll D. Gene targeting using zinc finger nucleases. *Nat Biotechnol.* 2005; 23:967–73. [PubMed: 16082368]
- Prall OW, Menon MK, Solloway MJ, Watanabe Y, Zaffran S, et al. An Nkx2-5/Bmp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation. *Cell.* 2007; 128:947–59. [PubMed: 17350578]
- Qyang Y, Martin-Puig S, Chiravuri M, Chen S, Xu H, et al. The renewal and differentiation of Isl1+ cardiovascular progenitors are controlled by a Wnt/beta-catenin pathway. *Cell Stem Cell.* 2007; 1:165–79. [PubMed: 18371348]
- Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J Clin Invest.* 2003; 111:1795–803. [PubMed: 12813012]
- Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, et al. Genomewide association analysis of coronary artery disease. *N Engl J Med.* 2007; 357:443–53. [PubMed: 17634449]
- Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. *Science.* 1998; 281:108–11. [PubMed: 9651244]
- Seguin CA, Draper JS, Nagy A, Rossant J. Establishment of endoderm progenitors by SOX transcription factor expression in human embryonic stem cells. *Cell Stem Cell.* 2008; 3:182–95. [PubMed: 18682240]
- Seidman JG, Seidman C. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell.* 2001; 104:557–67. [PubMed: 11239412]
- Shah M, Akar FG, Tomaselli GF. Molecular basis of arrhythmias. *Circulation.* 2005; 112:2517–29. [PubMed: 16230503]
- Shiraki N, Umeda K, Sakashita N, Takeya M, Kume K, Kume S. Differentiation of mouse and human embryonic stem cells into hepatic lineages. *Genes Cells.* 2008; 13:731–46. [PubMed: 18513331]
- Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology.* 2010; 51:297–305. [PubMed: 19998274]
- Smart N, Risebro CA, Melville AA, Moses K, Schwartz RJ, et al. Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization. *Nature.* 2007; 445:177–82. [PubMed: 17108969]
- Stevens KR, Pabon L, Muskheli V, Murry CE. Scaffold-free human cardiac tissue patch created from embryonic stem cells. *Tissue Eng Part A.* 2009; 15:1211–22. [PubMed: 19063661]
- Sun Y, Liang X, Najafi N, Cass M, Lin L, et al. Islet 1 is expressed in distinct cardiovascular lineages, including pacemaker and coronary vascular cells. *Dev Biol.* 2007; 304:286–96. [PubMed: 17258700]
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007; 131:861–72. [PubMed: 18035408]
- Takeuchi JK, Mileikovsky M, Koshiba-Takeuchi K, Heidt AB, Mori AD, et al. Tbx20 dose-dependently regulates transcription factor networks required for mouse heart and motoneuron development. *Development.* 2005; 132:2463–74. [PubMed: 15843409]
- Tanaka M, Chen Z, Bartunkova S, Yamasaki N, Izumo S. The cardiac homeobox gene Csx/Nkx2.5 lies genetically upstream of multiple genes essential for heart development. *Development.* 1999; 126:1269–80. [PubMed: 10021345]
- Thomas KR, Capecchi MR. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell.* 1987; 51:503–12. [PubMed: 2822260]
- Tomita-Mitchell A, Maslen CL, Morris CD, Garg V, Goldmuntz E. GATA4 sequence variants in patients with congenital heart disease. *J Med Genet.* 2007; 44:779–83. [PubMed: 18055909]
- Tregouet DA, König IR, Erdmann J, Munteanu A, Braund PS, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. *Nat Genet.* 2009; 41:283–5. [PubMed: 19198611]

- Urbach A, Schuldiner M, Benvenisty N. Modeling for Lesch-Nyhan disease by gene targeting in human embryonic stem cells. *Stem Cells*. 2004; 22:635–41. [PubMed: 15277709]
- Watanabe Y, Benson DW, Yano S, Akagi T, Yoshino M, Murray JC. Two novel frameshift mutations in NKX2.5 result in novel features including visceral inversus and sinus venosus type ASD. *J Med Genet*. 2002; 39:807–11. [PubMed: 12414819]
- Wu SM, Chien KR, Mummery C. Origins and fates of cardiovascular progenitor cells. *Cell*. 2008; 132:537–43. [PubMed: 18295570]
- Wu SM, Fujiwara Y, Cibulsky SM, Clapham DE, Lien CL, et al. Developmental origin of a bipotential myocardial and smooth muscle cell precursor in the mammalian heart. *Cell*. 2006; 127:1137–50. [PubMed: 17123591]
- Yagi H, Furutani Y, Hamada H, Sasaki T, Asakawa S, et al. Role of TBX1 in human del22q11.2 syndrome. *Lancet*. 2003; 362:1366–73. [PubMed: 14585638]
- Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, et al. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature*. 2008; 453:524–8. [PubMed: 18432194]
- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–20. [PubMed: 18029452]
- Zou J, Maeder ML, Mali P, Pruett-Miller SM, Thibodeau-Beganny S, et al. Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell Stem Cell*. 2009; 5:97–110. [PubMed: 19540188]
- Zwaka TP, Thomson JA. Homologous recombination in human embryonic stem cells. *Nat Biotechnol*. 2003; 21:319–21. [PubMed: 12577066]

Figure 1.

Embryological origin of the mammalian heart. Early in cardiac development, cardiac progenitors from the first heart field (FHF) coalesce to form the cardiac crescent. Progenitors from the second heart field (SHF) localize anterior and medial to cells of the FHF. As development progresses, the heart tube forms and then loops to ultimately form the primitive chambers of the four chambered mammalian heart. Progenitors from the FHF most greatly contribute to the left ventricle, whereas progenitors from the SHF cells contribute the majority of the cells of the right ventricle and outflow tract. Progenitors from both heart fields contribute to the inflow tract. In mature heart, SHF cells contribute to the majority of the cells of the heart except for the left ventricular free wall., FHF cells contribute to the left ventricle, the ventricular septum, and both atria. Green = areas with FHF cells; red = areas with SHF cells; brown = areas with both FHF and SHF cells. Ao = aorta; CC = cardiac crescent; OT = outflow tract; IT = inflow tract; LA = left atrium; LV = left ventricle; HF = head fold; ML = midline; PA = pulmonary artery; RA = right atrium; RV = right ventricle.

Figure 2.

Proposed cell fate map for progenitor cells during development. Following gastrulation, multipotent cardiac progenitor cells responsible for populating the FHF and SHF emerge from cardiac mesoderm. Multipotent Isl1+ progenitors from the SHF give rise to endothelium, smooth muscle, and cardiac muscle; Isl1- progenitors appear to give rise to at least smooth muscle and cardiac muscle.

Figure 3.

Strategies for obtaining disease-specific human cardiomyocytes and other cell types. Disease-specific human embryonic stem (ES) cells can be derived via homologous recombination in normal ES cells to insert disease-associated mutations. Alternatively, patient-specific induced pluripotent stem (iPS) cells can be derived directly from patient-specific somatic cells (typically skin fibroblasts). Either type of disease-specific pluripotent stem cells can be differentiated into various cell types, e.g., into multipotent cardiac progenitors that can be further differentiated into cardiomyocytes, smooth muscle cells, or endothelial cells.

Figure 4.

Scheme for the derivation of disease-specific cardiomyocytes for use in phenotypic assays and drug screens. Disease-specific pluripotent stem cells will undergo *in vitro* differentiation and cardiac progenitors will be purified (via any of several techniques, including lineage tracing, directed differentiation, and antibody sorting). Purified populations of cardiac progenitors will then be expanded into larger numbers, followed by further differentiation into functional cardiomyocytes. Cardiomyocytes will then be used in various cellular assays, several examples of which are shown, to study and develop therapies for a variety of cardiovascular disorders, including cardiomyopathy, electrophysiological disorders, and congenital defects.

Table 1

Examples of genetic variants underlying cardiovascular disease.

Gene/locus	Disease	Frequency	References
Chromosomal abnormalities			
chr 22q11 (deletion)	DiGeorge syndrome	rare	Goldmuntz et al. 1998
chr 1q21 (deletion or amplification)	tetralogy of Fallot	rare	Greenway et al. 2009
Rare DNA variants			
<i>TBX5</i>	Holt-Oram syndrome	rare	Basson et al. 1997, Li et al. 1997b
<i>TBX20</i>	ASD, VSD, cardiomyopathy, valvular abnormalities	rare	Kirk et al. 2007
<i>TBX1</i>	DiGeorge syndrome	rare	Yagi et al. 2003
<i>GATA4</i>	ASD, VSD, tetralogy of Fallot	rare	Pehlivan et al. 1999, Garg et al. 2003, Hirayama-Yamada et al. 2005, Tomita-Mitchell et al. 2007
<i>NKX2-5</i>	ASD, VSD, cardiomyopathy, valvular abnormalities, outflow tract defects, tetralogy of Fallot, hypoplastic left heart, arrhythmias	rare	Schott et al. 1998, Benson et al. 1999, Kasahara et al. 2000, Goldmuntz et al. 2001, Gutierrez-Roelens et al. 2002, Ikeda et al. 2002, Watanabe et al. 2002, McElhinney et al. 2003, Kasahara and Benson 2004, Pashmforoush et al. 2004
<i>NOTCH1</i>	tetralogy of Fallot, valvular abnormalities	rare	Garg et al. 2005, Greenway et al. 2009
<i>NOTCH2</i>	Alagille syndrome, tetralogy of Fallot, valvular abnormalities	rare	McDaniell et al. 2006
<i>JAG1</i>	Alagille syndrome, tetralogy of Fallot, valvular abnormalities	rare	Li et al. 1997a, Oda et al. 1997, Krantz et al. 1999, Eldadah et al. 2001
<i>LDLR</i>	familial hypercholesterolemia, early-onset MI	rare	Goldstein & Brown 2009
Common DNA variants			
chr 9p21	early-onset MI	~50% (in European populations)	Myocardial Infarction Genetics Consortium et al. 2009
<i>LDLR</i>	increased LDL-C levels, early-onset MI	~75% (in European populations)	Myocardial Infarction Genetics Consortium et al. 2009

ASD = atrial septal defect; VSD = ventricular septal defect; MI = myocardial infarction