Induced pluripotent stem cell-derived cardiomyocytes as models for genetic cardiovascular disorders

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Purpose of review

The development of induced pluripotent stem cell (iPSC) technology has led to many advances in the areas of directed cell differentiation and characterization. New methods for generating iPSC-derived cardiomyocytes provide an invaluable resource for the study of certain cardiovascular disorders. This review highlights the current technology in this field, its application thus far to the study of genetic disorders of the RAS/MAPK pathway and long-QT syndrome (LQTS), and future directions for the field.

Recent findings

Enhanced methods increase the efficiency of generating and stringently purifying iPSC-derived cardiomyocytes. The use of cardiomyocytes derived from patients with LEOPARD syndrome and LQTS has shed light on the molecular mechanisms of disease and validated their use as reliable human disease models.

Summary

The use of iPSC-derived cardiomyocytes to study genetic cardiovascular disorders will enable a deeper and more applicable understanding of the molecular mechanisms of human disease, as well as improving our ability to achieve successful cell-based therapies. Methods to efficiently generate these cells are improving and provide promise for future applications of this technology.

Keywords

hypertrophic cardiomyopathy, induced pluripotent stem cells, LEOPARD syndrome, long-QT syndrome

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Introduction

The seminal discovery of a method to engineer human induced pluripotent stem cells (iPSCs) from terminally differentiated cells by Shinya Yamanaka's group [1] in 2007 paved the way for the development of iPSC-based models of human disease. For many years, stem cells have been recognized for their potential for regenerative therapies and as tools for deciphering the molecular pathways of disease. Although the use of embryonic stem cells (ESCs) is limited due to ethical and technical issues, iPSCs display properties of ESCs but are readily generated from patients. The broad utility of iPSCs is well illustrated in the study of genetic cardiovascular diseases, as the available human ESCs do not harbor the desired mutation, and relevant primary cells are difficult to obtain and propagate in culture (Table 1). Additionally, as the concept of cardiac stem cell transplantation advances, avoiding the problem of host–donor immunological incompatibility is strongly advantageous.

Generation and characterization of induced pluripotent stem cell-derived cardiomyocytes

Technologies to generate iPSCs and differentiate them into a variety of cell types have become increasingly sophisticated and have enabled the derivation of iPSC-derived cardiomyocytes with relatively high efficiencies.

Generation of induced pluripotent stem cells

Current iPSC technology is based on the overexpression of a cocktail of transcription factors that act as master regulators of pluripotency through the modification of epigenetic patterns and gene expression. The earliest experiments to generate iPSCs were performed by transducing somatic cells with retroviruses or lentiviruses [1,2], whereas newer methods such as inducible lentiviruses [3], adenoviruses [4], transposon-based systems [5], small molecules [6], and transfection of modified RNAs [7•] have been employed to avoid genomic integration and increase the efficiency of iPSC generation.

The ESC-like colonies that emerge must demonstrate promoter demethylation and activation of endogenous stemness transcription factors such as OCT4 and NANOG, and expression of pluripotency markers such as surface antigens TRA1-81, TRA1-60, and SSEA4. The gold standard to verify pluripotency of human iPSCs is the ability to differentiate them into cells or tissues derived from each of the three germ cell layers *in vitro* and *in vivo* [8].

Generation of induced pluripotent stem cell-derived cardiomyocytes

The ability to differentiate iPSCs into cardiomyocytes was advanced by the observation that three-dimensional ESC aggregates called embryoid bodies could mature into spontaneously contracting cells, which stained positive for cardiomyocyte-specific markers [9]. Since then, our knowledge about the mechanisms of early heart development and the undertaking of screens for small molecule enhancers of cardiogenesis have been applied to generate improved protocols, which increase the efficiency of producing iPSC-derived cardiomyocytes. Although earlier protocols relied on culturing embryoid bodies in media containing 20% fetal bovine serum [10], the use of three families of extracellular growth factors required for cardiac development in mammals – the bone morphogenic proteins (BMPs), the WNT proteins, and the fibroblast growth factors (FGFs) – have been shown to enhance cardiomyocyte formation from embryoid bodies to 40–50% after incubation in culture over a variety of days [11••] (Fig. 1). These growth factors can likely be used in synergy with small molecules such as ascorbic acid and sulfonyl hydrazones, which have been shown to increase in-vitro cardiogenesis from stem cell populations and to upregulate the expression of cardiac-specific genes [12•].

Key points

- Methods to generate and purify iPSC-derived cardiomyocytes provide a unique tool for investigating cardiac disease pathogenesis.
- iPSC-derived cardiomyocytes from patients with LEOPARD syndrome and LQTS recapitulate their respective clinical disease phenotypes and can be therapeutically modulated.
- Advances in iPSC-derived cardiomyocyte technology will lead to increased efficiency in their generation, enabling their use for dissecting disease pathogenesis, screening for novel drug treatments, and developing cell-based therapies.

Purification of induced pluripotent stem cell-derived cardiomyocytes

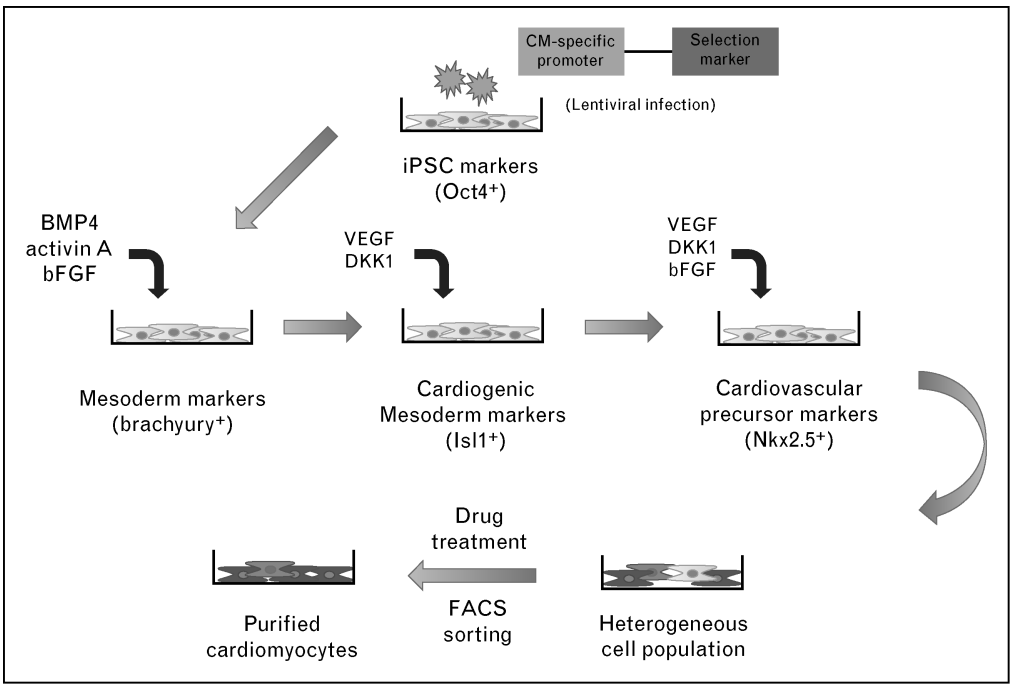
Although the mixed cell population resulting from embryoid bodies is adequate for some studies, nearly pure cardiomyocytes are needed for other potential uses such as gene expression analyses. The lack of a reliable cardiomyocyte cell surface marker makes this purification challenging, but several methods have been developed that enable cardiomyocyte identification and isolation. The most promising are the transgenic selection methods, which currently involve lentiviral infection of iPSCs with fluorescent or antibiotic resistance selection genes under the control of cardiac gene-specific promoters, such as human myosin light chain-2v and α-myosin heavy chain (αMHC) (Fig. 1). These transgenic methods allow efficient in-vitro monitoring of cardiomyocyte generation and yield a purity of more than 90% [13,14••]. The isolation of pure iPSC-derived cardiomyocytes is essential for their characterization *in vitro*, although the development of efficient nonviral purification methods would be optimal, and required for

Table 1 Advantages and disadvantages of iPSC-derived cardiomyocytes and alternative model systems

Model systems	Advantages	Disadvantages
Animal models	Ability to genetically manipulate whole organism; can perform invasive studies; can assay in-vivo function; availability of isogenic controls	Species-dependent differences in gene expression and regulation; variations in pathophysiological mechanisms, drug response
Primary human cardiomyocytes	Harbors human genetic background; may contain mutation of interest; provides mature cardiomyocyte morphology and function	Difficult to obtain; short propagation time in culture; only maintains physiological morphology and function in culture less than 48 h; difficulty of genetic manipulation
Human ESC-derived cardiomyocytes	Harbors human genetic background; increased ease of genetic manipulation; prolonged propagation in culture	Ethical issues; difficulty of ESC derivation; likely does not harbor mutation of interest; cardiomyocytes resemble immature or fetal state
Human iPSC-derived cardiomyocytes	Harbors human genetic background and mutation of interest; iPSCs relatively easily generated from patient samples; increased ease of genetic manipulation; prolonged propagation in culture	Decreased efficiency of cardiomyocyte generation; cardiomyocytes resemble immature or fetal state; lack of availability of isogenic controls

ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.

Figure 1 Generation and purification of induced pluripotent stem cell-derived cardiomyocytes



Successful derivation of purified iPSC-derived cardiomyocytes has been achieved through infection of iPSCs with a lentivirus encoding a selectable marker driven by a cardiomyocyte-specific promoter [14••]. Exposure to a cocktail of cytokines over a defined time-course efficiently induces cardiogenesis. TGFβ signaling, activated by BMP4 and Activin A, along with bFGF signaling, induces a population of cells expressing mesodermal markers. The addition of VEGF and DKK1, a selective inhibitor of the canonical WNT signaling pathway, further promotes cardiogenesis through induction of markers of cardiogenic mesoderm [11••]. Cardiovascular precursors develop into a heterogeneous cell population including endothelial cells, fibroblasts, smooth muscle cells, and cardiomyocytes. Purification of cardiomyocytes is achieved through drug selection or cell sorting. bFGF, basic fibroblast growth factor; DKK1, Dickkopf-related protein 1; FACS, fluorescence-activated cell sorting; iPSC, induced pluripotent stem cell; ISL1, Islet1; VEGF, vascular endothelial growth factor.

potential future therapeutic use. This technology will also enable the future isolation of cardiac lineage and chamber-specific cell types, which will greatly enhance our capacity to investigate normal and abnormal cardiac development.

Characterization of induced pluripotent stem cell-derived cardiomyocytes

The ability to use iPSC-derived cardiomyocytes for cell-based therapies or to investigate disease pathogenesis relies on the establishment of their similarity to functioning cardiomyocytes. Many studies have shown that cardiomyocytes generated from ESCs and iPSCs resemble immature fetal cardiomyocytes in their gene expression profile as well as their electrophysiological and structural properties. Gene expression analysis in a pure ESC-derived cardiomyocyte population demonstrated upregulation of cardiac-specific transcription factors and basic cardiac function genes, yet was accompanied by high levels of expression of several genes involved in cardiac differentiation not found in adult cardiomyocytes [15]. Ultrastructural examination of contracting ESC-derived embryoid bodies in culture revealed initial myofibril and sarcomere disorganization, with progressive evolution to a more organized structure and a concomitant increase in cell length and area. Importantly, these cells still lacked the development of the T tubule system after 60 days in culture, indicating a failure of complete maturation to an adult cardiomyocyte phenotype [16]. Similarly, investigation of ESC-derived and iPSC-derived cardiomyocyte excitation–contraction coupling revealed immature calcium handling and a negative force–frequency relationship when stimulated [17,18]. Despite these differences, studies have shown that iPSC-derived cardiomyocytes express functional Na^+, K^+, and L-type Ca^{2+} channels, and respond to β-adrenergic stimulation [19]. The maturity level of these cells is a potential confounding factor in modeling certain disease phenotypes. Methods to enhance the maturity of these cells to an adult state are currently under investigation.

Using induced pluripotent stem cell-derived cardiomyocytes to study LEOPARD syndrome

The iPSC-derived cardiomyocytes were first shown to recapitulate a cardiac disease phenotype as a model for disorders of the RAS/MAPK signaling pathway.

RAS/MAPK genetic disorders and their clinical phenotypes

The 'RASopathies' are a family of autosomal dominant developmental disorders caused by mutations in the RAS/MAPK pathway, which generally result in increased signaling. The RAS/MAPK pathway is integral to many aspects of cell migration, proliferation, differentiation, and survival, and thus genetic mutations in this pathway result in developmental defects affecting multiple organ systems, one of the most prominent being the cardiovascular system. Cardiovascular abnormalities are common in many RASopathies, including Noonan syndrome and its related disorder LEOPARD syndrome. Pulmonic stenosis is seen in 23% of patients with LEOPARD syndrome, and more than 50% of patients with Noonan syndrome, whereas hypertrophic cardiomyopathy (HCM) is observed in up to 80% of patients with LEOPARD syndrome compared with about 20% of patients with Noonan syndrome [20,21].

The most commonly occurring mutations causing Noonan syndrome and LEOPARD syndrome occur in *PTPN11*, which encodes the Src homology-2 (SH2) domain-containing nontransmembrane protein tyrosine phosphatase SHP2. Heterozygous missense mutations in *PTPN11* are observed in about 50% of Noonan syndrome cases and up to 90% of LEOPARD syndrome cases [22,23]. Noonan syndrome-associated *PTPN11* mutations result in a gain-of-function phenotype [22,24], whereas LEOPARD syndrome-associated *PTPN11* mutations have mixed gain-of-function and dominant-negative effects [25,26]. Despite their varying molecular effects, mutations causing Noonan syndrome and LEOPARD syndrome result in a very similar spectrum of clinical phenotypes. This phenomenon necessitates further investigation into the complex involvement of SHP2 in cell processes such as proliferation, migration, and differentiation, and its role in a wide variety of signaling events such as receptor tyrosine kinase, cytokine, and integrin-evoked extracellular-signal-regulated kinase (ERK) activation. Understanding how altered RAS pathway signaling engenders cardiac hypertrophy and the characterization of Noonan syndrome-associated and LEOPARD syndrome-associated hypertrophy is of great clinical importance.

Modeling LEOPARD syndrome-associated cardiac hypertrophy

Carvajal-Vergara *et al.* [27••] generated iPSCs from dermal fibroblasts derived from two LEOPARD syndrome patients harboring the T468M substitution in *PTPN11* using recombinant retroviruses encoding OCT4, SOX2, KLF4, and MYC. After exposure of iPSCs to a defined set of growth factors over a series of days, cardiac troponin T (cTNT) expression was detected in spontaneously beating embryoid bodies. The LEOPARD syndrome-derived cardiomyocytes displayed increased median cell surface area and sarcomeric organization compared with wild type (WT)-derived cardiomyocytes, consistent with a hypertrophic response. The authors also investigated the status of nuclear factor of activated T-cells (NFAT)c4 localization, as dephosphorylation of NFAT transcription factors by active calcineurin and subsequent NFAT nuclear localization has been implicated in the pathogenesis of cardiac hypertrophy. A higher proportion of LEOPARD syndrome cardiomyocytes displayed NFATc4 nuclear localization compared with WT, possibly implicating the calcineurin–NFAT pathway in the pathogenesis of LEOPARD syndrome hypertrophy. The LEOPARD syndrome iPSCs also had an increased abundance or phosphorylation of various signaling receptors and kinases, including EGFR, MEK1, and ERK1/2, consistent with increased activation of the RAS/MAPK pathway. bFGF-evoked ERK1/2 activation, however, was not observed in LEOPARD syndrome iPSCs. This study was the first to create a human iPSC-derived cardiomyocyte disease model, recapitulating a hypertrophic phenotype and altered RAS/MAPK signaling. This model validated the use of iPSC-derived cells to study the cardiac effects of human genetic disorders, an important advance as these iPSC-derived cardiomyocytes closely mimic the human disease state and are efficient laboratory tools (Table 1). Additionally, the ability to differentiate mutant iPSCs into a multitude of cell types enables investigation into the diverse manifestations of genetic syndromes.

Using induced pluripotent stem cell-derived cardiomyocytes to study long-QT syndrome

The iPSC-derived cardiomyocytes have also recently been used to further understand the mechanisms underlying long-QT syndrome (LQTS). This work is important not only in advancing our knowledge about the pathogenesis of LQTS, but also in laying the groundwork for the future use of iPSC-derived cardiomyocytes in electrophysiological studies, an important area of investigation of drug safety and pharmacological effects.

Long-QT syndrome

LQTS is a congenital cardiovascular disorder characterized by a prolonged QT interval, recurring syncopal episodes, and a tendency toward polymorphic ventricular tachycardia leading to sudden cardiac death [28]. Autosomal dominant mutations in several genes encoding cardiac ion channels have been identified as causative for this disorder, with the majority of cases due to mutations in *KCNQ1*, *KCNH2*, and *SCN5A*. These genes respectively encode the alpha subunits of the delayed rectifier potassium channel conducting the I_{KS} (slow) current, the I_{KR} (rapid) current, and the cardiac sodium channel. Mutations in *KCNQ1* are estimated to cause

about 50% of LQTS cases and underlie LQTS type 1 (LQT1), whereas mutations in *KCNH2* cause about 40% of LQTS cases and underlie LQTS type 2 (LQT2). Although it is widely accepted that *KCNQ1* and *KCNH2* mutations cause a reduction in their respective potassium currents, this has not yet been demonstrated in human cardiomyocytes, which have ion channel expression and electrophysiological properties that are distinct from animals typically used for disease modeling.

Modeling long-QT syndrome

Moretti *et al.* [29••] generated iPSCs from skin fibroblasts derived from two family members with the autosomal dominant missense mutation R190Q in *KCNQ1* using retroviruses encoding the transcription factors OCT3/4, SOX2, KLF4, and c-MYC. The iPSCs were subsequently differentiated into cardiomyocytes in differentiation media consisting of 20% fetal calf serum. Three distinct single-cell action potentials were generated resembling fetal atrial, ventricular, or nodal cardiomyocytes. The authors found that atrial and ventricular cardiomyocytes with the LQT1 mutation displayed prolonged action potentials with decreased repolarization velocities, as well as decreased action potential duration (APD) shortening in response to higher pacing frequencies compared with control cardiomyocytes. In controls, KCNQ1 was localized to the cell membrane as expected, whereas mutant KCNQ1 was intracellular and colocalized with the endoplasmic reticulum marker disulfide isomerase, a novel finding. In cotransfection experiments, the extent of WT KCNQ1 cell membrane localization was found to be dependent upon the amount of mutant KCNQ1 present, suggesting a dominant negative effect.

Electrophysiological analysis of the I_K current combined with I_{KS}-specific or I_{KR}-specific channel inhibitors revealed a specific reduction in I_{KS} in patient-derived cardiomyocytes. Additionally, the I_{KS} current in patient-derived cardiomyocytes required higher voltages for activation, displayed delayed rates of deactivation, and was unresponsive to adrenergic stimulation. Consistent with altered I_{KS} function, isoproterenol exposure resulted in a positive chronotropic effect with a shortened APD in control cardiomyocytes, whereas the mutant cells paradoxically displayed an increased APD with some cardiomyocytes developing early afterdepolarizations (EADs), illustrating their potential for arrhythmic activity. Importantly, these effects on patient-derived cardiomyocytes were mitigated by pretreatment with propranolol, a nonselective beta-blocker, which is clinically efficacious for LQT1.

A more recent publication by Itzhaki *et al.* [30••] addressed the effects of the A614V missense mutation in *KCNH2* in iPSC-derived cardiomyocytes from patients with LQT2. Similarly to Moretti *et al.*, the authors were able to recapitulate a prolonged APD in both atrial and ventricular cardiomyocytes along with delayed repolarization velocities. The use of an I_{KR}-specific inhibitor allowed the validation of a specific reduction in the I_{KR} current in LQT2-derived cardiomyocytes. In both single-cell analysis and microelectrode array mapping on multicellular clusters of cardiomyocytes, an increased incidence of EADs and subsequent premature beats was observed in the LQT2-derived cardiomyocytes. This disease phenotype was exploited to identify potentially corrective drugs not currently used clinically for LQTS. The authors found that the Ca^{2+} channel blocker, nifedipine, and the K_{ATP} channel opener, pinacidil, significantly reduced the APD in LQT2-derived cardiomyocytes, while completely eliminating all EADs and premature beats in single cells and multicellular clusters. The late Na^+ channel blocker, ranolazine, did not have an effect on ADP, yet still reduced EADs in LQT2-derived cardiomyocytes.

The studies by Moretti *et al.* and Itzhaki *et al.* recapitulated the electrophysiological characteristics of LQTS in iPSC-derived cardiomyocytes and provided insights into its pathogenesis. Notably, both LQT1 patients used in the LQT1 study were asymptomatic at the time of skin biopsy, yet cardiomyocytes derived from these patients displayed molecular manifestations of the disease *in vitro* that could be therapeutically modulated. Species-dependent differences in cardiac ion channel expression and global genetic regulation provide the rationale for the use of human cardiomyocytes that can be pharmacologically manipulated to study cardiac disease mechanisms and identify novel therapeutics.

Future directions and conclusion

In the study of both LEOPARD syndrome and LQTS, iPSC-derived cardiomyocytes have recapitulated the clinical disease phenotype *in vitro*. They are able to be therapeutically modulated and are a platform for the discovery of novel mechanisms of pathogenesis. The future of iPSC technology provides promising and exciting avenues for further elucidating the pathogenesis of complex disorders, developing cell-based therapies, and identifying novel therapeutic compounds.

Transdifferentiation of fibroblasts into cardiomyocytes

The process of creating iPSCs and their subsequent differentiation into cardiomyocytes is time-consuming and subjects the cells to multiple rounds of manipulation. Recently, Ieda *et al.* [31••] demonstrated for the first time the ability to differentiate mouse cardiac and dermal fibroblasts directly into cardiomyocytes. The authors used the viral transduction of a combination of three transcription factors (Gata4, Mef2c, and Tbx5), selected for their known central roles in early heart development,

to induce differentiation. The derived cardiomyocytes expressed cardiac-specific markers, spontaneously contracted in culture, and displayed a gene expression pattern similar to neonatal mouse cardiomyocytes. Injecting cardiac fibroblasts into mouse hearts just 1 day after viral transduction led to in-vivo differentiation into cardiomyocytes expressing α-actinin and αMHC. Another recent paper reported the successful generation of cardiomyocytes directly from mouse embryonic fibroblasts *in vitro* through the short-term overexpression of pluripotency factors followed by exposure to cardiogenic cytokines [32•]. It remains to be seen if transdifferentiation of fibroblasts directly into cardiomyocytes can occur in human cells, but this work lays the foundation for more efficient generation of cardiomyocytes with a decreased potential for tumor formation, and the possibility of reprogramming cells *in vivo*.

Identifying cardiac progenitor populations

Successful cardiac cell transplantation is dependent upon cell survival and proliferation *in vivo*, and the absence of tumor formation or an immunological response. The identification of a human cardiac progenitor cell population capable of giving rise to all three major cardiovascular lineages (cardiomyocytes, endothelial cells, smooth muscle cells) would be a major advance for the field of cardiac regeneration. Yang *et al.* [11••] identified an $FLK1^{low}$, $C\text{-}KIT^{neg}$ population derived from human ESCs that was able to differentiate into all three cardiovascular lineages *in vitro* and after transplantation, without evidence of teratoma formation. Another human ESC-derived progenitor population expressing ISL1 was identified by Bu *et al.* [33•] and has subsequently been derived from human iPSCs and been shown to repopulate the three cardiovascular lineages *in vitro* [34•]. iPSCs are a uniquely useful tool for the identification of progenitor populations, as analysis and manipulation along developmental timelines are possible, and provide a platform for autologous cardiac cell transplantation in the future.

Using induced pluripotent stem cell-derived cardiomyocytes for drug screening

Finally, the use of iPSC-derived cardiomyocytes for drug screening is an exceedingly valuable application for this technology, but investigation into whether these cells respond to drugs similarly to ESC-derived and adult cardiomyocytes is essential before this can become a reality. Yokoo *et al.* [35•] compared the effects of an array of cardioactive compounds on human iPSC-derived and ESC-derived cardiomyocytes. The drugs elicited similar responses in both cell populations in beating frequency and contractility measurements and mirrored their clinically observed effects. A larger number of compounds have been tested in ESC-derived cardiomyocytes, with most eliciting the expected responses but some producing no observable effect [36•]. It has been shown that, as iPSC-derived and ESC-derived cardiomyocytes mature in culture, their protein expression, structural organization, and ion channel functioning mature as well. Therefore, it is important to consider the phenotype of these derived cardiomyocytes as well as the appropriate therapeutic doses when undertaking a drug screen.

In sum, the applications for iPSC-derived cardiomyocytes are vast and provide us with the ability to enhance greatly our understanding of disease processes, as well as provide improved genotype-specific therapeutics to our patients.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 272).

1 Takahashi K, Tanabe K, Ohnuki M, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131:861–872.

2 Yu J, Vodyanik MA, Smuga-Otto K, *et al.* Induced pluripotent stem cell lines derived from human somatic cells. Science 2007; 318:1917–1920.

3 Wernig M, Lengner CJ, Hanna J, *et al.* A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. Nat Biotechnol 2008; 26:916–924.

4 Stadtfeld M, Nagaya M, Utikal J, *et al.* Induced pluripotent stem cells generated without viral integration. Science 2008; 322:945–949.

5 Woltjen K, Michael IP, Mohseni P, *et al.* piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009; 458:766–770.

6 Ichida JK, Blanchard J, Lam K, *et al.* A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell 2009; 5:491–503.

7 • Warren L, Manos PD, Ahfeldt T, *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 2010; 7:18–30.

This work describes the use of synthetic modified mRNAs to generate highly efficient iPSCs. This nonviral technology generates iPSCs with the highest efficiency to date.

8 Maherali N, Hochedlinger K. Guidelines and techniques for the generation of induced pluripotent stem cells. Cell Stem Cell 2008; 3:595–605.

9 Itskovitz-Eldor J, Schuldiner M, Karsenti D, *et al.* Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 2000; 6:88–95.

10 Police XUC, Hassanipour S, Gold MJ. Cardiac bodies: a novel culture method for enrichment of cardiomyocytes derived from human embryonic stem cells. Stem Cells Develop 2006; 15:631–639.

11 •• Yang L, Soonpaa MH, Adler ED, *et al.* Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. Nature 2008; 453:524–528.

This paper describes the generation of a highly efficient protocol to differentiate iPSCs into cardiomyocytes using growth factors and cytokines involved in cardiovascular development. The authors also identify a human $FLK1^{low}$, $C\text{-}KIT^{neg}$ progenitor population able to differentiate into the three cardiovascular lineages *in vitro* and *in vivo* without teratoma formation.

12 • Willems E, Bushway PJ, Mercola M. Natural and synthetic regulators of embryonic stem cell cardiogenesis. Pediatr Cardiol 2009; 30:635–642.

This review describes various small molecules shown to enhance cardiogenesis and cardiomyocyte maturation from stem cells in culture.

13 Huber I, Itzhaki I, Caspi O, *et al.* Identification and selection of cardiomyocytes during human embryonic stem cell differentiation. FASEB J 2007; 21:2551–2563.

14 Kita-Matsuo H, Barcova M, Prigozhina N, *et al.* Lentiviral vectors and protocols •• for creation of stable hESC lines for fluorescent tracking and drug resistance selection of cardiomyocytes. PLoS One 2009; 4:e5046.
This paper describes the creation of lentiviral constructs for the tandem selection of a pure population of stem cells and their derived cardiomyocytes.

15 Xu XQ, Soo SY, Sun W, Zweigerdt R. Global expression profile of highly enriched cardiomyocytes derived from human embryonic stem cells. Stem Cells 2009; 27:2163–2174.

16 Snir M, Kehat I, Gepstein A, *et al.* Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. Am J Physiol Heart Circ Physiol 2003; 285:H2355–H2363.

17 Binah O, Dolnikov K, Sadan O, *et al.* Functional and developmental properties of human embryonic stem cells-derived cardiomyocytes. J Electrocardiol 2007; 40 (Suppl 6):S192–S196.

18 Germanguz I, Sedan O, Zeevi-Levin N, *et al.* Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. J Cell Mol Med 2011; 15:38–51.

19 Tanaka T, Tohyama S, Murata M, *et al.* In vitro pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes. Biochem Biophys Res Commun 2009; 385:497–502.

20 Limongelli G, Pacileo G, Marino B, *et al.* Prevalence and clinical significance of cardiovascular abnormalities in patients with the LEOPARD syndrome. Am J Cardiol 2007; 100:736–741.

21 Sarkozy A, Digilio MC, Dallapiccola B. Leopard syndrome. Orphanet J Rare Dis 2008; 3:13.

22 Tartaglia M, Mehler EL, Goldberg R, *et al.* Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001; 29:465–468.

23 Digilio MC, Conti E, Sarkozy A, *et al.* Grouping of multiple-lentigines/LEOPARD and Noonan syndromes on the *PTPN11* gene. Am J Hum Genet 2002; 71:389–394.

24 Fragale A, Tartaglia M, Wu J, Gelb BD. Noonan syndrome-associated SHP2/PTPN11 mutants cause EGF-dependent prolonged GAB1 binding and sustained ERK2/MAPK1 activation. Hum Mutat 2004; 23:267–277.

25 Kontaridis MI, Swanson KD, David FS, *et al.* PTPN11 (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects. J Biol Chem 2006; 281:6785–6792.

26 Oishi K, Zhang H, Gault WJ, *et al.* Phosphatase-defective LEOPARD syndrome mutations in *PTPN11* gene have gain-of-function effects during Drosophila development. Hum Mol Genet 2008; 18:193–201.

27 Carvajal-Vergara X, Sevilla A, D'Souza SL, *et al.* Patient-specific induced •• pluripotent stem-cell-derived models of LEOPARD syndrome. Nature 2010; 465:808–812.
This paper is the first to use iPSC-derived cardiomyocytes as a disease model. The derived cardiomyocytes reproduced the LEOPARD syndrome clinical phenotype and underlying aberrant molecular signaling.

28 Crotti L, Celano G, Dagradi F, Schwartz PJ. Congenital long QT syndrome. Orphanet J Rare Dis 2008; 3:18.

29 Moretti A, Bellin M, Welling A, *et al.* Patient-specific induced pluripotent stem-•• cell models for long-QT syndrome. NEJM 2010; 363:1397–1409.
This paper is the first to generate and characterize iPSC-derived cardiomyocytes as a model for a cardiac electrophysiological disorder. The cells displayed altered electrophysiological properties yet were amenable to therapeutic modulation.

30 Itzhaki I, Maizels L, Huber I, *et al.* Modelling the long QT syndrome with •• induced pluripotent stem cells. Nature 2011 [Epub ahead of print].
This paper created the first iPSC-derived cardiomyocyte model for LQT2. The cells recapitulated the expected disease phenotype and were used as tools to identify potentially useful therapeutic compounds.

31 Ieda M, Fu JD, Delgado-Olguin P, *et al.* Direct reprogramming of fibroblasts •• into functional cardiomyocytes by defined factors. Cell 2010; 142:375–386.
This paper is the first to generate cardiomyocytes directly from somatic cells, avoiding a stem cell intermediate stage. While performed in mouse cells, the possibility of replication with human cells could eliminate many of the complications associated with generation of cardiomyocytes from stem cells.

32 Efe JA, Hilcove S, Kim J, *et al.* Conversion of mouse fibroblasts into cardi-• omyocytes using a direct reprogramming strategy. Nat Cell Biol 2011 [Epub ahead of print].
This paper uses short-term overexpression of pluripotency factors combined with exposure of fibroblasts to cardiogenic cytokines to induce their direct reprogramming to cardiomyocytes.

33 Bu L, Jiang X, Martin-Puig S, *et al.* Human ISL1 heart progenitors generate • diverse multipotent cardiovascular cell lineages. Nature 2009; 460:113–117.
This paper is the first to reveal the ability of human ESC-derived ISL1+ cardiovascular progenitors to differentiate into the three cardiovascular lineages *in vitro*.

34 Moretti A, Bellin M, Jung CB, *et al.* Mouse and human induced pluripotent • stem cells as a source for multipotent Isl1+ cardiovascular progenitors. FASEB J 2010; 24:700–711.
This paper demonstrates the ability of mouse and human iPSC-derived ISL1+ cardiovascular progenitors to differentiate into the three cardiovascular lineages *in vivo* without teratoma formation, an important advance for the field of cardiac regenerative medicine.

35 Yokoo N, Baba S, Kaichi S, *et al.* The effects of cardioactive drugs on • cardiomyocytes derived from human induced pluripotent stem cells. Biochem Biophys Res Commun 2009; 387:482–488.
This paper profiles the effects of an array of cardioactive drugs on contractility and beating frequency of iPSC-derived cardiomyocytes. The authors show a close correspondence between observed effects and known clinical effects, validating the use of these cells for drug testing and screening.

36 Dick E, Rajamohan D, Ronksley J, Denning C. Evaluating the utility of • cardiomyocytes from human pluripotent stem cells for drug screening. Biochem Soc Trans 2010; 38:1037–1045.
This paper reviews various studies on the electrophysiological effects of a range of cardioactive agents on human ESC-derived and iPSC-derived cardiomyocytes. Patch-clamp and field potential recordings reveal that most drugs produce their expected effects.