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Perspective From the Heart: The Potential of Human Pluripotent Stem Cell-Derived Cardiomyocytes

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ABSTRACT

Human pluripotent stem cells (hPSC) are self-renewing cells with the potential to differentiate into a variety of human cells. They hold great promise for regenerative medicine and serve as useful in vitro models for studying human biology. For the past few years, there is vast interest in applying these cells to advance cardiovascular medicine. Human cardiomyocytes can be readily generated from hPSC and they have been characterized extensively with regards to molecular and functional properties. They have been transplanted into animal models of cardiovascular diseases and also shown to be potentially useful reagents for drug discovery. Yet, despite great progress in this field, significant technical hurdles remain before these cells could be used clinically or for pharmaceutical research and development. Further research using novel approaches will be required to overcome these bottlenecks. J. Cell. Biochem. 114: 39–46, 2013.

KEY WORDS: HUMAN PLURIPOTENT STEM CELLS; CARDIOMYOCYTE; REGENERATIVE MEDICINE; DRUG DISCOVERY

When human embryonic stem cells (hESC) were first isolated near the end of last century, it was immediately apparent that they, being pluripotent and self-renewing, hold great promise for developing cell therapies for regenerative medicine [Thomson et al., 1998]. The excitement surrounding stem cell research persisted with the finding that differentiated somatic cells can be reprogrammed into an embryonic stem cell-like state, termed induced pluripotent stem cells (iPSC) [Takahashi et al., 2007]. The cardiomyocyte is one cell type that can readily be generated from these human pluripotent stem cells (hPSC) [Kehat et al., 2001; Xu et al., 2002; Zhang et al., 2009]. The potential to generate an inexhaustible supply of functional human cardiomyocytes from hPSC has propelled research into downstream applications including the development of cell therapy for restoring cardiac function and in vitro cellular models for testing pharmaceuticals (Fig. 1). However, significant technical bottlenecks exist, especially for the development of cell therapies. These include unreliable differentiation, lack of a robust scale up platform, and inability to generate cardiomyocytes of a defined subtype and mature phenotype. Transplantation of hPSC derived cardiomyocytes in small animal models of myocardial infarction has so far not resulted in significant long term benefit. Much more work is still necessary to resolve these issues before hPSC-derived cardiomyocytes (hPSC-CM) could be routinely used for therapeutic purpose or for preclinical drug testing.

DIFFERENTIATION OF hPSC TO CARDIOMYOCYTES

The efficient production of functional cardiomyocytes from hPSC is a prerequisite for developing cardiac drug screens, in vitro models of disease, and cell therapies. Current methods for differentiating hPSC towards cardiomyocytes include spontaneous differentiation by embryoid body (EB) formation, co-culture with endodermal cells, and directed differentiation with defined media and inducers.

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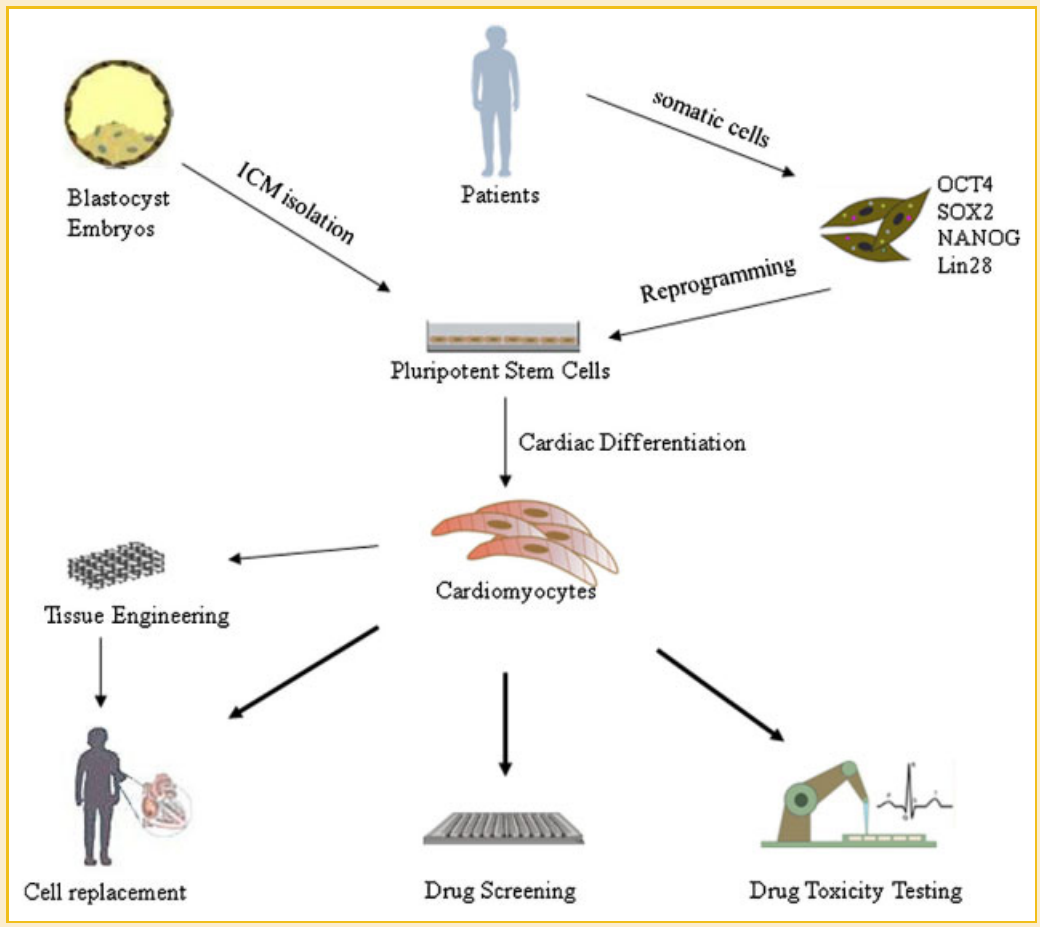

Fig. 1. Potential applications of human pluripotent stem cells derived cardiomyocytes (hPSC-CM) in regenerative medicine and drug development. hPSC are derived from human blastocyts (hESC) or by direct reprogramming of somatic cells using transcription factors (iPS technology) from patients. Cardiomyocytes derived from both hESC and iPSC could be transplanted directly in the heart or transplanted after tissue engineering. While cell replacement therapy is a long-term objective owing to significant technical hurdles, the more immediate application would be to use hPSC-CM as in vitro models for drug screening and safety pharmacology.

The most widely used method to differentiate hPSC is formation of three-dimensional aggregates called embryoid body (EB) in suspension. Similar to a developing embryo, an EB contains tissues of the three primary germ layers—endoderm, mesoderm, ectoderm. Over time, some of the mesodermal derivatives will spontaneously develop to cardiomyocytes, causing a fraction of the EBs to undergo rhythmic contraction. To generate EBs from hPSC, most protocols rely on treatment with collagenase or dispase, followed by mechanical disruption to remove the hPSC colonies from the feeder layer [Kehat et al., 2001; Xu et al., 2002]. Although the hanging drop and methylcellulose culture methods work for making EBs from mouse ES [Kurosawa, 2007], they have not been successfully applied to make hPSC EBs. These methods require dissociation of hPSC into single cells, and most hPSC do not survive such a procedure. Some groups tried to overcome this problem by forcing the dissociated hPSC to aggregate by using V-bottom plates [Ungrin et al., 2008] with the addition of a Rho-associated kinase (ROCK) inhibitor, which promotes survival of dissociated hPSC [Watanabe et al., 2007]. Notably, these methods could provide better control over EB size and uniformity, however they are not suitable for large scale production. Although differentiation in EBs is somewhat stochastic, addition of certain factors could direct differentiation towards the cardiac lineage. The additions of small molecules such as 5′-AZT and ascorbic acid have been reported to have a cardiomyogenic effect on hPSC [Yoon et al., 2006; Cao et al., 2012].

Another approach to differentiate hPSC into cardiomyocytes involves co-culturing with the mouse visceral endoderm-like cell line END2 in the serum free (SF) medium [Passier et al., 2005]. However, the END2 cells are derived from mouse EC (embryonal carcinoma), and cardiomyocytes generated using this method would contain xenogeneic components that are not desirable for clinical applications.

In order to develop a clinically-compliant cardiomyocyte differentiation method, Xu et al. systemically analyzed the cardiomyogenic activity of the END2 conditioned medium [Xu et al., 2008a]. The analysis revealed that insulin, which is present at high levels in KOSR (knockout serum replacement), has a striking inhibitory effect on cardiomyocytes differentiation. Conditioning the KOSR medium with END2 cells effectively depleted the insulin. In addition, the analysis identified a small molecule, prostacyclin (PGI2), as a metabolite produced by END2 cells. Addition of PGI2 to a fully synthetic, insulin-free medium resulted in cardiomyogenic activity

comparable to END2-conditioned media alone. Finally, addition of the p38 MAPK inhibitor SB203580 further enhanced cardiomyocyte generation. These findings represent a significant step towards developing a scalable production method for cardiomyocytes from hESC in suspension culture using reagents compatible with Good Manufacturing Practice (GMP) and clinical application [Xu et al., 2008a].

Alternate approach to generate cardiomyocytes involves "directed differentiation" of the hPSC without EB formation. Taking lessons from studying embryonic heart development, Laflamme et al. [2007] used a combination of Activin and BMP4 in serum free media to generate cultures containing >30% cardiomyocytes. Similarly, another group employed the same two factors in combination with FGF (fibroblast growth factor) and VEGF (vascular endothelial growth factor) to induce cardiac differentiation from mESC and hESC [Yang et al., 2008]. In this method, multipotent cardiovascular progenitors were identified based on sorting subpopulations of differentiating cells with respect to the surface markers KDR and c-kit. The sorted progenitors could differentiate into myocardial, endothelial, and smooth muscle cells. Further refinement of the method based on the additional cell surface marker PDGFRα generated cardiomyocytes with high efficiency [Kattman et al., 2011]. The optimized protocol was reported to generate >50% cardiomyocytes even without first sorting out the cardiovascular progenitors.

Given the many reported differentiation protocols for hPSC-CM, it is difficult to determine if any one protocol is better than another. Moreover, different hPSC lines may respond differently to different protocols. Indeed, the variability in the inherent propensity of different hESC lines to differentiate into cardiomyocytes has been reported [Osafune et al., 2008]. It may result from genetic and epigenetic difference between cell lines [Adewumi et al., 2007] as well as differences in hESCs culture practices between research groups. Burridge et al. reported that an optimized cardiac differentiation method was equally effective for several different hESC and iPSC cell lines. This protocol is based on EB formation from single cells by centrifugation in a V-shaped 96-well dish [Burridge et al., 2011]. This method was reported to yield on average 64–89% cardiac troponin I-positive cells from multiple hPSC lines. One most recent study reported that temporal modulation of Wnt signaling is sufficient for efficient hPSC cardiac induction. Sequential treatment of hPSCs with glycogen synthase kinase 3 inhibitors followed by β-catenin shRNA or chemical inhibitors of Wnt signaling produced a high yield of (up to 98%) functional cardiomyocytes from multiple hPSC lines under defined conditions [Lian et al., 2012]. It remains to be seen whether this protocol can be applied to a broader range of hPSC lines and reproduced in the many stem cell research labs.

MOLECULAR AND FUNCTIONAL PHENOTYPES OF hPSC-CM

When hPSC were differentiated as EBs, beating masses began to appear around days 10–14. hPSC differentiation in vitro apparently recapitulates the embryonic developmental programs in vivo with respect to the expression of lineage-specific markers, which could be monitored to track the differentiation process (Fig. 2). Upon differentiation initiation, the expression of key pluripotent markers like Oct-4 and Nanog were quickly down-regulated, followed by transient upregulation of mesodermal markers such as Tbra and Mesp1 [Xu et al., 2008a]. The expression of the early cardiomyocyte-specific transcription factor Nkx2.5, Gata4, Hand2, Tbx5, and Mef2 marked the beginning of cardiomyogenesis. By day 12, the cardiac-specific markers, hormonal A- and B-natriuretic peptides (ANP and BNP), and structural proteins alpha myosin heavy chain (α-MHC), myosin light chain 2a (MLC-2a), and tropomyocin were expressed. Comprehensive expression profile was captured by transcriptome analysis on nearly pure hESC-CM [Xu et al., 2009]. Immunostaining revealed that different subtypes of cardiomyocytes were present in hPSC derived population. The presence of atrial cardiomyocytes was

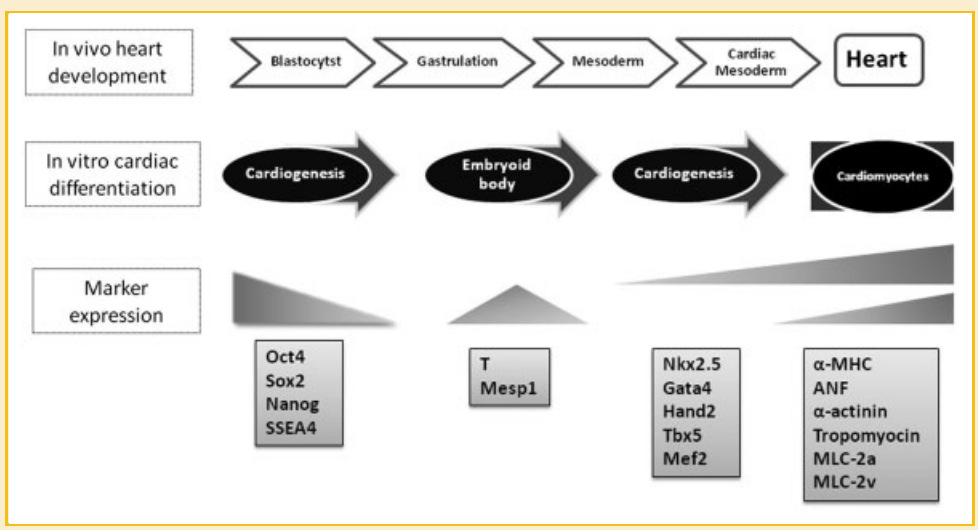

Fig. 2. Schematic of hPSC cardiac differentiation stages and associated markers. Differentiation of hPSC to cardiomyocytes in vitro showed temporal expression of essential markers reflecting that of the developing heart in the embryo.

indicated by expression of myosin light chain-2a (MLC-2a) while ventricular cardiomyocytes showed higher expression of MLC-2v [Xu et al., 2009].

The presence of different cardiomyocyte subtypes can also be demonstrated by electrophysiological analysis. When hESC-CM were evaluated by pipette-aided intracellular recording or patch-clamping, the results showed that the differentiated population contained a mixture of nodal, atrial and ventricular cells [He et al., 2003; Mummery et al., 2003]. Interestingly, the distribution of these different subtypes seems to vary depending on the method of differentiation used to generate the hPSC-CM. For instance, hPSC-CM generated by spontaneous differentiation via EB formation yielded a majority of ventricular cells, but also significant portion of nodal and atrial cells [He et al., 2003]. Regardless of the differentiation method, the hPSC derived cardiomyocytes exhibited a fetal phenotype [Xu et al., 2009], and displayed a relatively positive resting potential and low action potential upstroke velocity. By monitoring changes in membrane currents and in ion channel gene expression, Sartiani et al. [2007] showed that hPSC-CM in EBs matured over a period of 3 months in culture, but they never matured to a stage equivalent to typical adult ventricular myocytes.

SCALE-UP AND ENRICHMENT OF hPSC-CM

Application of hPSC-CM in cell therapy and pharmaceutical research requires their large scale production and purification. Although hPSC are essentially immortal, generating large batches of these cells remains a major challenge. Most large scale cell culture platforms have been developed for immortalized cell lines which can withstand trypsinization and growth from single cells. hPSC grow best as colonies on a feeder layer, conditions which are not suitable for most bioreactors. Development of feeder-free cultures and use of ROCK inhibitors have made hPSC growth somewhat amenable to these conditions, but efficient growth in suspension is still challenging. A recent report described the growth of hPSC as cell clusters in suspension [Steiner et al., 2010]. The cell clusters were "passaged" by trituration to break up large clusters into smaller ones. Three different hPSC lines were shown to be maintained in the undifferentiated state using this protocol for up to 10 weeks. The growth rate was not as high as hPSC on feeders mainly due to cell loss during passaging. After 20 weeks in culture, however, one of the hESC lines showed karyotypic abnormality. It remains to be determined whether these protocols could be transferred to large scale bioreactors.

Even the best differentiation protocol will not be able to direct all hPSC in a given culture to become cardiomyocytes. Yet, a population of homogenous and well-characterized hPSC-CM is imperative for downstream in vitro and in vivo applications. In particular, the use of hPSC-CM for cell therapy must be free of residual, undifferentiated cells that can give rise to teratomas after transplantation. In early report of hESC-CM generation, enrichment of these cells was performed by manual dissection of beating areas [Kehat et al., 2001]. Clearly, this is a rather crude method which cannot result in a highly enriched population. Reported protocols of enrichment include cell sorting based on surface markers CD166 [Rust et al., 2009], elastin microfibril interfacer 2 (EMILIN2) [Van Hoof et al., 2010], or uptake of a mitochondria staining dye tetramethylrhodamine methyl ester perchlorate [Hattori et al., 2010]. The levels of enrichment reported ranged from 60–99%. However, sorting of cardiomyocytes is a harsh procedure and the percentage of cells surviving the sort was not reported in these studies. Cell sorting is also a rather inefficient way to purify a large population of cardiomyocytes.

Arguably, the most efficient method of enriching hPSC-CM is to use a lineage selection strategy based on transgene expression. A DNA construct consisting of the cardiac-specific myosin heavy chain promoter driving the neomycin resistance gene (MHCneoR) was stably transfected into the mES cells [Klug et al., 1996]. After differentiation to generate mESC-CM, G418 (Geneticin) was applied to result in a highly pure population of cardiomyocytes. This lineage selection strategy was successfully applied to hESC to achieve a purity of >99% cardiomyocytes based on immunostaining of α-MHC and α-actinin [Xu et al., 2008b]. Compared to the other aforementioned enrichment methods, the lineage selection approach is also the most suitable for large scale hPSC-CM production. Although genetic modification of hPSC poses regulatory hurdles in the context of developing a cell therapy, integration of a well-characterized transgene in a "safe harbor" genomic site should minimize any safety concerns.

CELL THERAPY FOR HEART REGENERATION

Cardiovascular disease is a leading cause of mortality and morbidity world-wide. The potential to generate an unlimited supply of human cardiomyocytes from hPSC offers an exciting opportunity to develop regenerative therapies for the ailing heart. In addition, with the discovery of iPSC, the hope of developing autologous cell therapies has become closer to reality. So far, however, transplantation of hPSC-CM in animal models have only shown modest efficacy.

In a preliminary study, Laflamme et al. transplanted hESC-CM into the left ventricular wall of healthy athymic rats [Laflamme et al., 2005]. They transplanted hESC-CM that were enriched using Percoll gradient centrifugation and heat-shocked prior to transplantation to improve survival. Although there was extensive cell death in the grafts, the surviving cells engrafted well and were found to be predominantly positive for sarcomeric myosin heavy chain. These encouraging results led to a follow-up study where hESC-CM were evaluated in LAD (left anterior descending artery) ligated rats [Laflamme et al., 2007]. This time, heat-shock in combination with a pro-survival cocktail helped to promote engraftment and remuscularize a significant portion of the infarct zone. The transplanted hESC-CMs apparently elicited an angiogenic response and the graft showed a substantial degree of rat vessel growth. Interestingly, there were also a substantial number of human vessels, implying that endothelial cells or progenitors were present in the transplanted cells. Transthoracic echocardiography and magnetic resonance imaging at 4 weeks post-transplantation showed modest improvements in ventricular dilation and systolic wall thickening, but no significant improvement in left ventricular ejection fraction.

In another study, van Laake et al. [2007] transplanted 2 million differentiated, GFP-marked hESC-CM into infarcted NOD/SCID (Non-Obese Diabetic/Severe Combined Immunodeficiency) mouse. Histological analysis at 13 weeks post-transplant revealed long-term engraftment and survival of transplanted cells. Although the transplanted population initially consisted of only about 20–25% cardiomyocytes, there was a time-dependent enrichment and maturation of cardiomyocytes in the graft. MRI indicated functional improvement at 4 weeks post-MI for the transplanted group when compared to the non-transplanted group, but this difference was no longer significant when assessed at 3 months post-MI. Finally, a recent report showed that transplantation of hESC-CM in a rat chronic infarction model was unable to restore heart function and prevent adverse cardiac remodeling despite successful engraftment [Fernandes et al., 2010].

Instead of evaluating hESC-CM in MI models, other groups tested their potential use as biological pacemakers. Kehat et al. [2004] and Xue et al. [2005] studied the coupling of hESC-CM with cultured rat cardiomyocytes in culture. They showed that hESC-CMs are capable of actively pacing quiescent ventricular cardiomyocytes in vitro and ventricular myocardium in vivo. The mechanical contractions of the embryoid bodies were synchronized with the electrical activity in the rat tissues. Coupling between human and rat cells was further demonstrated by the presence of positive connexin-43 immunostaining at the cell junctions. When transplanted into a pig model of atrioventricular block, the hESC-CM paced the pig heart as shown by 3-dimensional electrophysiological mapping [Kehat et al., 2004]. These studies provided evidence for long-term electromechanical integration between host and donor tissues at several levels and demonstrated the therapeutic potential of hESC-CM as biological pacemakers.

A consistent observation in transplantation studies of hPSC-CM in the rodent models is the lack of significant long term benefit. Perhaps transplantation of cardiac progenitors [Blin et al., 2010] or engineered heart tissues [Zimmermann et al., 2006] might be more effective under such circumstance. Cardiac tissue engineering is a dynamic area of research that will surely garner more attention in the near future. A few research groups have constructed cardiac tissues from hESC-CM with or without scaffolds [Caspi et al., 2007; Stevens et al., 2009]. The addition of endothelial cells and fibroblasts helped to generate vascularized tissues. Assembly of hPSC-CM into tissue constructs might improve survival of the transplanted cells and facilitate integration [Tulloch et al., 2011]. Future transplant studies should be carried out on large animals. Unsuccessful coupling of donor to recipient cardiomyocytes could explain lack of efficacy in rodents despite successful tissue engraftment. Even if these transplant issues are resolved, the prospect of developing autologous cell therapy for cardiac disease is unlikely to happen in the near future unless great improvement in differentiation efficiency and scale up are made to lower production time and cost.

hPSC-CM FOR DRUG DISCOVERY

With the many technical and regulatory hurdles facing translation of hPSC-CM to the clinical setting, the more achievable application of these cells would be to use them as in vitro cellular models for the pharmaceutical industry, in areas of preclinical testing including target validation, drug screening, and safety pharmacology. One area of particular concern for pharmaceutical companies is assessing the risk of drug-induced cardiotoxicity for new compounds. Experience from previous drug development highlight the potential of many drugs to cause a potentially lethal form of arrhythmia termed Torsades de Pointes (TdP) [Fermini and Fossa, 2003]. The risk of developing Torsades is associated with the lengthening of the QT interval on the electrocardiogram, which reflects the duration of the ventricular action potential. Research into the pharmacology of QT prolonging compounds showed that most of them inhibited the hERG potassium channel [Hoffmann and Warner, 2006]. The hERG potassium current mediates the repolarization of the cardiac membrane potential, and its inhibition prolongs the action potential duration, thereby leading to arrhythmic events.

Currently most drug developers assess cardiotoxic risk of new drugs by first screening for inhibition of the human hERG channel expressed in a cell line. However, inhibition of hERG generates many false positives, since hERG inhibition does not always lead to QT lengthening. An example is verapamil, which blocks both the hERG channel as well as the L-type calcium channel [Hoffmann and Warner, 2006]. The dual effect of verapamil on both channels leads to minimal effect on the QT interval, and in some experimental systems, actually shortens it. A more predictive model is required to minimize generation of false positive hits given by the hERG channel screen. hPSC-CM could be one such model since it harbors nearly the full repertoire of channels found in the human heart. Because hPSC-CM showed spontaneous beating activity, the action potential duration could be measured by microelectrode arrays (MEA). Xu et al. [2009] have shown that compounds such as E-4031 and astemizole could prolong QT in a hPSC-CM preparation. Other labs have also reported the potential use of hPSC-CM on MEA for drug toxicity testing. Tanaka et al. [2009] showed that iPSC-CM responded to verapamil and E-4031. In their system, verapamil shortened the field potential duration while E-4031 lengthened it. Assessing QT prolongation alone, however, is also not fully predictive of TdP. Many compounds that lengthen QT in animals and in man do not lead to TdP [Redfern et al., 2003]. Conversely, some compounds that cause TdP do not prolong QT. In this regard, the hPSC-CM model could offer more information than merely QT prolongation assessment. For example, two studies showed that, in patch clamp recordings, application of E-4031 to hES-CM induced early after depolarizations (EAD), which could be a cellular substrate for initiating TdP [Caspi et al., 2009; Peng et al., 2010]. The latter study showed that hPSC-CM was more sensitive to quinidine and terfenadine when compared to the rabbit or canine Purkinje fiber assay. Furthermore, Jonsson et al. [2010] analyzed hPSC-CM by patch-clamping for additional parameters of proarrhythmic risks, including reverse use dependence and triangulation of the action potential. The hPSC-CM platform generated results comparable to those with the rabbit Purkinje fiber assay. Overall, the hPSC-CM platform offers significant advantages over conventional methods to assess QT liability. Despite these encouraging data, concerns remain that hPSC-CM showed fetal-like properties. In addition, differentiation usually results in a cell population that is a mixture

of cardiac subtypes. Better differentiation schemes are required to generate a preferably adult-like ventricular phenotype. Alternatively, tissue engineering approaches might be able to generate a mature and homogenous population with physiological properties resembling those of the adult heart.

In addition to arrhythmia risks, hPSC-CM could be useful for predicting other forms of drug-induced cardiotoxicity. For example, many chemotherapeutic agents are known to have adverse effects on heart function. One report showed that doxorubicin toxicity could be detected in hPSC-CM by assaying for release of cardiac troponin T [Andersson et al., 2010]. This study demonstrated the potential for using hPSC-CM to identify additional biomarkers useful for predicting other forms of drug-induced cardiotoxicity.

Finally, the availability of hPSC-CM allows research to establish novel assays and disease models for drug discovery. The spontaneously beating cells could enable screening of compounds to increase contractility. These cells could also be used to investigate mechanisms underlying cardiac hypertrophy and for screening drugs that inhibit this process [Foldes et al., 2011]. The ability to reprogram patient somatic cells further enables researchers to establish in vitro models of genetic diseases for drug screening. Two different groups have reported the reprogramming of skin fibroblasts from patients with congenital long-QT syndromes into iPSCs [Moretti et al., 2010; Itzhaki et al., 2011]. When the iPSCs carrying the genetic mutations were differentiated into cardiomyocytes, the diseased cardiomyocytes showed longer QT compared to wild type controls, reflecting the disease phenotype of the patients. In addition, the iPSC-CM carrying these mutations were more sensitive to proarrhythmic compounds. These studies are excellent examples of how hPSC can be used to generate relevant disease models for research and drug discovery.

CONCLUSION AND FUTURE OUTLOOK

Much progress has been made in the past decade in the field of stem cell biology, with the establishment of hESC lines and the discovery of iPS cells. Great improvements in cardiac differentiation and enrichment have been achieved with hPSC. Yet, much work remains to be done, especially in the area of translational research for clinical applications. Major hurdles to overcome include immature differentiation, lack of a robust scale-up platform, and the inability to direct differentiation into specific cardiomyocyte subtypes, particularly the ventricular myocytes. Although these represent significant challenges, further progress in understanding genetic and epigenetic control of cellular differentiation should ultimately enable efficient generation of cells with the desired phenotype.

The differentiation capacity of hPSC in culture should facilitate research to uncover novel genes and signaling pathways that are involved in early heart development. For instance, a recent study by transcriptome analysis of hESC derived cardiomyocytes reported identification of a large number of novel genes associated with cardiac development [Xu et al., 2009]. One of the candidate genes, RNA binding protein Rbm24, was further studied in zebra fish to elucidate its function in heart development. Loss-of-function study suggested that Rbm24 is an early factor of cardiogenic differentiation acting during assembly of sarcomeres and is required for cardiac contractility. The deficiency of Rbm24 could be one of the factors contributing to the sarcomere-based cardiomyopathy and thus points to a potential novel mechanism underlying certain forms of congenital cardiomyopathy [Poon et al., 2012]. This study demonstrates the power of studying cardiac differentiation from hPSC and validating the in vitro phenomenon in an in vivo model of heart development.

Given the high hurdles separating bench and bedside, the more attainable aim for hPSC-CM in improving human health is to serve as in vitro cell models for drug testing. Nevertheless, robust screening assays still require large scale production of hPSC-CM with consistent quality and defined characteristics similar to the in vivo phenotype. Focus should be on how to produce cells with adult ventricular phenotype in a consistent manner. Again, generation of cardiac tissues by engineering approaches might offer a more physiologically relevant model. Regardless of the format, adoption of the platform by pharmaceutical companies will only occur with extensive testing and validation.

Judging from the number of publications in the past few years, the rapid pace of hPSC-CM related research should continue in the near future. More attention will be given to human iPSC, which represents less of an ethical dilemma than hESC. Cardiomyocytes can be readily generated from human iPSC using similar protocols developed for hESC [Zhang et al., 2009; Mehta et al., 2011]. In particular, reprogramming of cells from patients with defined congenital cardiac disorders would allow study of disease phenotypes in vitro. Two models of congenital long-QT syndrome have already been established, and more models of cardiac disorders will surely be developed in the future. Availability of diseased cardiomyocytes would enable research into developing potential therapeutics or diagnostics to indicate risk of disease.

In conclusion, hPSC-derived cardiomyocytes are promising reagents that could have significant impact on cardiovascular medicine. Although technical bottlenecks exist, advances in stem cell research have been occurring at such a breathtaking speed that progress is inevitable. A multi-disciplinary approach that combines knowledge acquired from different fields, spanning developmental biology to tissue engineering, will likely be required to overcome existing hurdles.

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