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Prospects for Pluripotent Stem Cell-Derived Cardiomyocytes in Cardiac Cell Therapy and as Disease Models

Christian Freund and Christine L. Mummery*

Department of Anatomy & Embryology, Leiden University Medical Center, Postal Zone: S-1-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands

ABSTRACT

The derivation of embryonic stem cells (hESC) from human embryos a decade ago started a new era in perspectives for cell therapy as well as understanding human development and disease. More recently, reprogramming of somatic cells to an embryonic stem cell-like state (induced pluripotent stem cells, iPS) presented a new milestone in this area, making it possible to derive all cells types from any patients bearing specific genetic mutations. With the development of efficient differentiation protocols we are now able to use the derivatives of pluripotent stem cells to study mechanisms of disease and as human models for drug and toxicology testing. In addition derivatives of pluripotent stem cells are now close to be used in clinical practice although for the heart, specific additional challenges have been identified that preclude short-term application in cell therapy. Here we review techniques presently used to induce differentiation of pluripotent stem cells into cardiomyocytes and the potential these cells have as disease models and for therapy. J. Cell. Biochem. 107: 592–599, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: HUMAN EMBRYONIC STEM CELL; INDUCED PLURIPOTENT STEM CELL; CARDIOMYOCYTE; DISEASE MODEL; TRANSPLANTATION

During mammalian development the heart grows through proliferation of its component cell types, 60% of which are cardiac fibroblasts, 30% force-generating heart muscle cells (cardiomyocytes) and the remainder vascular endothelial and smooth muscle cells. After birth, proliferation of cardiomyocytes rapidly declines and the heart grows mainly by an increase of cardiomyocyte size (hypertrophy). In the adult heart, mature cardiomyocytes are highly organized, terminally differentiated cells which have lost their ability to divide [Ahuja et al., 2007]. Although potential progenitor populations in the heart have been identified (reviewed by Martin-Puig et al. [2008]), these seem to support mainly basal turnover of cardiomyocytes in the absence of injury. Where large numbers of cardiomyocytes are lost, for example through ischemic events like myocardial infarction, intrinsic cardiac regeneration is poor and mechanisms to restore heart function consist of hypertrophy of surviving cardiomyocytes and proliferation of cardiac fibroblasts. Ultimately, in a high number of cases the hypertrophied heart progresses to irreversible heart failure. Currently the transplantation of a donor heart is the only effective therapy for major cardiac cell loss but the shortage of donor organs has remained an intractable problem. In addition, any transplantation of an organ from an unrelated donor requires continuous administration of immunosuppressive drugs to prevent rejection. As a result, alternate ways to regenerate the heart by promoting the endogenous repair and remodeling processes or replacing lost cardiomyocytes with donor substitutes have been the subject of much recent research. With the first derivation of human embryonic stem cells (hESC) from pre-implantation embryos [Thomson et al., 1998], it seemed that a new potential source of cells for the treatment of many different degenerative diseases had emerged, including cardiomyocytes for cardiac repair. hESC are pluripotent, meaning that they can differentiate into cell lineages of the three germ layers (endoderm, mesoderm, and ectoderm) that will give rise to all organs of the body. In the past decade, directed differentiation of hESC into cardiomyocytes and other cell types as well as mechanisms controlling pluripotency have been areas of intensive investigation. The latter recently led to the identification of four pluripotency factors which are sufficient to reprogram somatic cells to an embryonic-like state (induced pluripotent stem cells or iPS cells) [Takahashi and Yamanaka, 2006]. Prior to this landmark study, the

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*Correspondence to: Christine Mummery, Department of Anatomy & Embryology, Leiden University Medical Center, Postal Zone: S-1-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands. E-mail: c.l.mummery@lumc.nl

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only way to reprogram somatic nuclei was through cloning or somatic cell nuclear transfer to enucleated oocytes. It has thus become possible to derive pluripotent cells from specific individuals including patients with diseases of interest without the requirement for donor oocytes. This has the potential not only to provide patient-matched lines for future therapy that would circumvent the need for immunosuppression but would also be a means of creating pluripotent stem cells bearing disease traits. Furthermore, since the direct reprogramming of somatic cells to pluripotency does not require the destruction of human embryos, the legal and ethical issues still associated with human embryonic stem cell derivation in many countries can be avoided. In the future, it is expected that cardiomyocytes and other derivatives of iPS cells may be useful as a basis for cell replacement therapies once the viral delivery methods for pluripotency genes in somatic cells have been replaced by non-viral alternatives. In addition, it is expected that in the short term these cells will provide excellent models to study mechanisms of disease and for drug and toxicology testing (Fig. 1).

Here we discuss the current state of cardiomyocyte differentiation from human pluripotent stem cells and their potential in therapy and as disease models.

HUMAN EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS

hESC are conventionally derived from the inner cell mass of day 5 human embryos at the blastocyst stage by plating on a monolayer of mitotically inactivated mouse embryonic fibroblasts (MEF), which serve as supporting feeder cells [Thomson et al., 1998]. Under these conditions, hESC self renew indefinitely whilst retaining the capacity to form in principle all somatic derivatives and germ cells. To induce differentiation in vitro hESC are usually removed from the feeders and cultured in suspension where they spontaneously form three-dimensional aggregates called embryoid bodies (EB) which contain derivatives of the three germ layers. Multiple variants of this protocol are now available in the literature, which support various degrees of lineage bias and enrichment for specific cell types. These are increasingly based on chemically defined culture media supplemented with hormones and growth factors, in sequences and concentrations often deduced from signaling pathways known to control differentiation in early embryonic development [Brand, 2003]. Pluripotency is more effectively demonstrated in vivo, however, by injection of hESC into immunocompromised mice. Here they form teratomas, benign tumors consisting again of derivatives of all three germ layers but more often as organized structures recognized morphologically; for example gut-like epithelium (endoderm), striated muscle (mesoderm) and neural tissue or rosettes (ectoderm). Pluripotency of hESC is controlled by a core set of transcription factors of which Oct3/4, nanog and Sox2 are known to be key players [Boyer et al., 2006]. During differentiation, these genes are downregulated. The understanding of mechanisms of pluripotency in hESC together with experiments of nuclear transfer laid the basis for direct reprogramming of terminally differentiated somatic cells, firstly in mouse [Takahashi and Yamanaka, 2006] and later in human (skin fibroblasts) by virus-mediated overexpression of the transcription factors Oct3/4, Sox2, Klf4 and c-Myc [Takahashi et al., 2007]. At the same time, another group also succeeded in direct reprogramming of adult human cells but using the transcription factors Oct3/4, Sox2 in combination with nanog and Lin28 [Yu et al., 2007]. It is thought that the exogenous transcription factors only initiate the reprogramming since their later expression declines and the endogenous pluripotency network is upregulated. This eventually maintains the emerging iPS cells in an undifferentiated state. The human iPS cell lines derived so far are similar to hESC in morphology, proliferation, gene expression, epigenetic status of pluripotency genes and differentiation potential both in vitro and in vivo [Takahashi et al., 2007; Yu et al., 2007]. Although still derived only at low efficiency (~0.01%), iPS cells have now been generated from a range of different somatic cells, including mouse liver and pancreatic cells [Aoi et al., 2008; Stadtfeld et al., 2008a] and human keratinocytes from hair and cells from bone marrow [Aasen et al., 2008; Park et al., 2008]. Depending on the expression levels of endogenous transcription factors, some cell types require fewer reprogramming factors. Adult mouse neuronal stem cells for example already express Sox and c-Myc and Klf4 and only need additional Oct3/4 to be reprogrammed [Kim et al., 2009]. Although the viruses used to generate first generation iPS cells represent a potent tool to deliver genes into a target cell they have the disadvantage of random integration into the host genome. This can lead to alterations in the expression of endogenous genes or even cause cancer-inducing mutations. In addition, incomplete downregulation of transgenes during differentiation may lead to iPS cell derivatives with an unstable or tumorigenic phenotype. Much current research is focused on reprogramming methods independent of integrating viral vectors which will be particularly important for any future therapeutic use. Transient expression of reprogramming factors with the help of non-integrative adenovirus or plasmid-mediated transfection [Stadtfeld et al., 2008b; Okita et al., 2008] have been described as well as the use of specific small molecules, which could replace two of the reprogramming factors [Shi et al., 2008].

CARDIOMYOCYTE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

During embryogenesis of multiple vertebrate species, endoderm is known to play an important role in the differentiation of cardiac precursors that are present in the adjacent mesoderm [Nascone and Mercola, 1995]. In this light, essentially two approaches have been developed to direct differentiation of hESC towards the cardiac lineage in vitro. One is based on co-culture with endoderm-like cells or cells secreting endoderm-like signals; the other is based on direct addition of the growth factors and hormones thought to be involved in heart development in vivo to hESC grown as EBs or in monolayer. We have shown previously that hESC effectively differentiate into cardiomyocytes with a human fetal ventricular phenotype when removed from MEFs and co-cultured with mouse visceral endoderm-like (END-2) cells [Mummery et al., 2003]. Omission of serum and insulin could further increase the cardiac yield [Passier et al., 2005; Freund et al., 2007]. Alternatively, growth as EBs in the

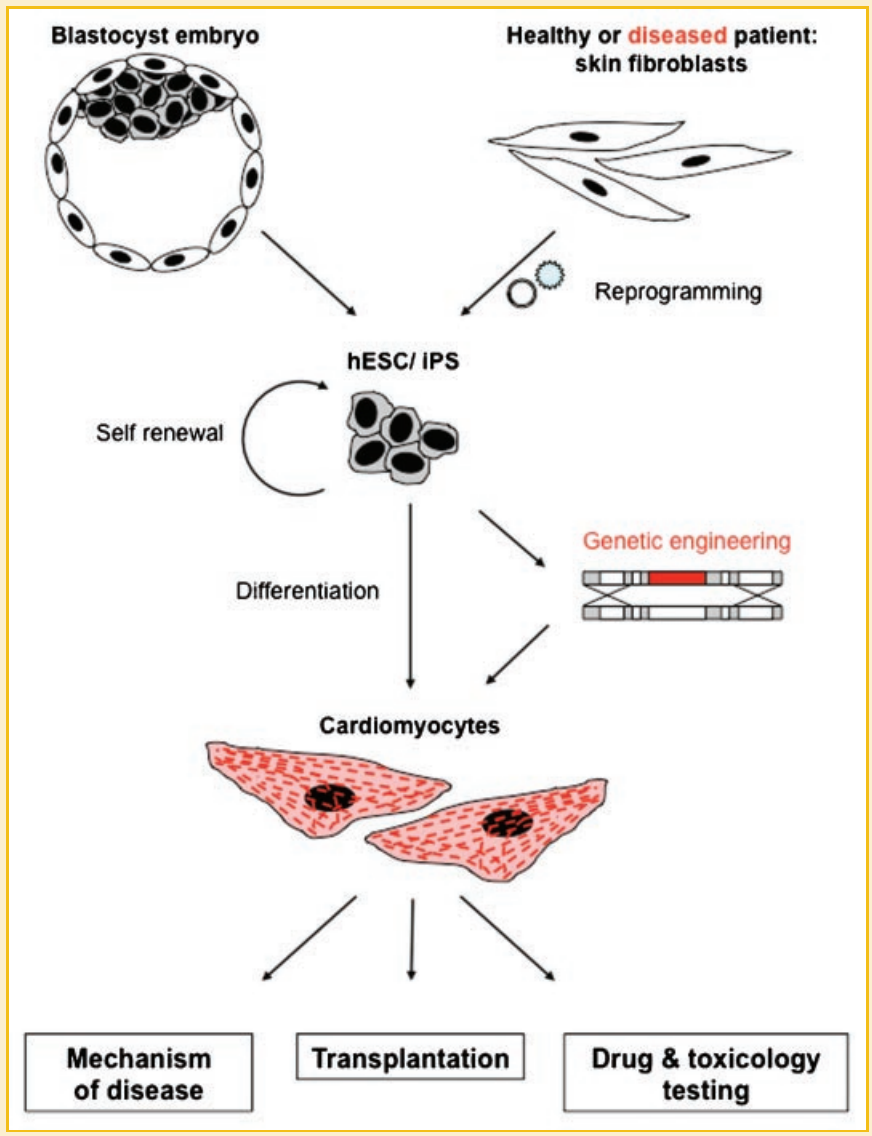

Fig. 1. Pluripotent stem cells as research models and in cell replacement therapy. Pluripotent stem cells are derived from human blastocysts (hESC) or by reprogramming of somatic cells (e.g., fibroblasts) using viral vectors or plasmids encoding selected transcription factors (iPS). In culture, pluripotent stem cells self-renew indefinitely and can be induced to differentiate into derivatives of the three germ layers, ectoderm, endoderm, and mesoderm. Cardiac progenitors that form cardiomyocytes, endothelial cells and vascular smooth muscle cells are mesoderm-derived. For studying the pathophysiology of genetic-based diseases, mutations can be introduced into hESC by homologous recombination (genetic engineering). Alternatively iPS can be generated directly from patients carrying the mutation. Derivatives of the mutated stem cells recapitulating the disease phenotype in vitro can be used for cell toxicity assays and drug discovery, particular if scaled production allows high throughput methods. hESC also provide a potential source of cardiomyocytes for cell replacement therapies, but would require immunosuppressive drugs. In this respect iPS-derivatives would be more suitable since they can be derived from the patient to be treated. In the case patient-specific iPS cells bearing monogenic diseases, these could be "cured" by replacing the mutated gene with a wild type functional sequence (genetic engineering) prior to the transplantation of the derivatives.

presence of serum causes contracting areas containing cardiomyocytes to start to appear within a few days [Kehat, 2001]. In EBs, it is thought that an outer layer of endoderm forms first and provides the inductive signals for cardiac differentiation. This ''spontaneous'' differentiation is now being replaced by addition of members of the transforming growth factor (TGF)-β superfamily (including bone morphogenetic proteins (BMPs) and activin as a surrogate to nodal) and of canonical Wnt signaling, which are essential for the specification of (cardiac) mesoderm [Yang et al., 2008]. It also appeared that defining EB formation using precise cell numbers and centrifugation (''spin EBs'') [Ng et al., 2005] combined with a defined set of growth factors [Ng et al., 2008] improved the efficiency of cardiomyocyte differentiation of hESC. Nonetheless, differences in the developmental potential of hESC lines exists, which are possibly due to their (epi)genetic heterogeneity [Osafune et al., 2008]. Replacement of (potentially infective) animal supplements both for growth and differentiation is not only important during development of future therapies based on hESC derivatives but is also

important for robust reproducibility of cardiomyocyte differentiation protocols without batch-to-batch variability associated with fetal calf serum. In addition, for both in vitro and future therapeutic use, it will also be essential to produce homogenous populations of each of the cardiovascular cells types and exclude residual undifferentiated cells which are potentially tumorigenic. Therefore much current research focuses on characterizing cardiac progenitor cell populations and on the identification of specific cell surface markers, which would allow their selection as living cells by fluorescent-activated cell sorting (FACS). Flk-1, a receptor for vascular endothelial cells growth factor (VEGF) has been identified as one such potential marker for cardiac progenitors which are mesoderm derivatives committed to the formation of cardiomyocytes, vascular endothelial cells (ECs) or vascular smooth muscle cells (VSMCs) [Yang et al., 2008]. Whilst there are multiple surface proteins with which to select live ECs (e.g., PE-CAM, VE-CAM, CD105, etc.) none are presently available for cardiomyocytes. Genetic methods, expressing fluorescent proteins under control of a cardiac promoter for example [Anderson et al., 2007] are at present the only option for selection.

For the use of stem cell derivatives in human therapy, it will also be particularly important to monitor cell karyotype to detect chromosomal abnormalities that could arise during prolonged cell culture [Spits et al., 2008]. Karyotypic changes have been repeatedly reported for hESC expanded in culture and might also be expected for human iPS cells; these could cause tumorigenicity independent of teratoma formation after transplantation of derivatives into patients.

CARDIOVASCULAR DERIVATIVES OF iPS CELLS

Recently, the first reports on the characterization of cardiomyocytes from iPS cells have been published. Mouse iPS cells could be differentiated into cardiomyocytes and other cardiovascular cells with a similar efficiency as mESC. The cardiomyocytes also expressed similar markers typical of cardiomyocytes [Narazaki et al., 2008]. Functional cardiomyocytes with nodal-, atrial-, or ventricular-like electrophysiological phenotypes have also been derived from human iPS cells, again using methods based on those effective for hESC [Zhang et al., 2009]. In both reports, down-regulation of transgenic reprogramming factors, including the oncogene c-Myc in certain mouse iPS derivatives, was blunted during differentiation.

The ability to differentiate iPS cells efficiently into a particular cell type may be influenced by the choice of the somatic cell type used for reprogramming. Data supporting this notion stems from experiments where a nucleus from a frog somite cell was transplanted into an enucleated oocyte [Ng and Gurdon, 2008]. The resulting frog embryo showed expression of the muscle-specific transcription factor MyoD, even in differentiating cells in which the marker is normally absent. In this case the gene expression pattern of a somatic cell was maintained after reprogramming the nucleus to an embryonic-like state. As a consequence, not only the efficiency of reprogramming of an individual somatic cell type and their accessibility as donated tissue but also the tissue origin of the cell used for reprogramming may require consideration for each particular application.

PLURIPOTENT STEM CELL-DERIVATIVES AS MODELS OF DISEASE

The derivation of the first mouse ESC line in 1981 [Evans and Kaufman, 1981; Martin, 1981] and their subsequent genetic manipulation initiated a new era towards a better understanding of mechanisms of disease. Numerous mouse strains carrying defined mutations in their genome have been generated and used for the analysis of gene function in vivo. In the context of the heart, even though the heart rate of small rodents is up to 10 times that of humans, useful models for human cardiac disease have been generated. They include hypertrophic disease, for example caused by mutations in the NFAT/calcineurin pathway [Bourajjaj et al., 2008] as well as disease associated with mutations in cardiac ion channels that can give rise to ''channelopathies.'' For example several mouse models with gain- and loss-of-function mutations of the *SCN5A* gene now exist [for a review see Charpentier et al., 2008]. The *SCN5A* gene encodes a subunit of a cardiac sodium ion channel; its malfunction is the underlying cause for several arrhythmic heart channelopathies in humans. Although the analysis of mutations in vivo has greatly contributed to the elucidation of pathophysiological mechanisms of SCN5A-related diseases, the models do not always recapitulate the phenotype seen in patients [Charpentier et al., 2008]. It remains unclear whether this discrepancy is related to species differences or to the nature of the mutation causing loss-of-function (point mutation vs. complete deletion). Secondly because the heart rates of humans and mice differ by orders of magnitude, the rapidly beating rodent heart may override the effect of arrhythmias which in a human heart could have severe consequences. The genetic background of mice can also influence the phenotype resulting from the mutations. For example 129P2 mice carrying a mutation in the *SCN5A* gene have a more severe phenotype than FVB/N mice with the same mutation [Remme et al., 2008]. Humans are by definition of mixed genetic background, resulting in phenotypical variations of genetic diseases. Indeed a single SCN5A mutation can result in multiple rhythm disturbances within the same family [Remme et al., 2008]. Therefore data from studies with transgenic mice on a single genetic background should be complemented by human models. Pluripotent stem cells, both genetically modified hESC or iPS cells derived from patients, are candidates on which to base human models.

Prior to human iPS cell isolation, hESC were among the most appropriate cell types on which to base models for cardiac disease although cardiac progenitors from adult human heart biopsies obtained during bypass surgery are alternatives [Smits et al., 2009]. However, in contrast to mouse ESC, gene targeting in hESC has proven more challenging. To date, only a few reports on successful targeting and genetic manipulation of hESC are available [for review see Giudice and Trounson, 2008]. In general, protocols for each hESC line have required individual optimization. Recently a generic method for gene delivery based on rapid adaption to defined, feeder-free culture conditions, has been reported which was shown to be

applicable to 12 different hESC lines; this was independent of whether cells were grown on human or mouse feeders, enzymatically or mechanically passaged and effective using transduction methods ranging from lenti-, adeno- and retro-viruses to electroporation and plasmid transfection [Braam et al., 2008]. In all cases (except electroporation), gene transduction efficiencies were above 70% and often 90%. This not only improves the efficiency and feasibility of generating stable hESC lines and targeting but also provides a platform for high throughput screening of expression of shRNA libraries for example for functional analysis. Generation of mutant hESC lines as disease models is pending in many laboratories.

With respect to iPS cell technology, only a few disease-specific lines have been reported to date [Park et al., 2008]. In general, it still needs to be demonstrated that the derivatives of these cells can indeed serve as in vitro models of the disease. Recently it has been shown that a human iPS cell model of spinal muscular atrophy (SMA) can recapitulate at least some aspects of this genetic disorder [Ebert et al., 2009]. The underlying cause of SMA is a mutation in the survival motor neuron 1 gene (SMN1), which results in the selective degeneration of a certain type of motor neurons. iPS cells carrying the mutation could initially produce similar number of motor neurons as wildtype iPS cells. However, mutated iPS-derived motor neurons were significantly fewer in number and of smaller size at later time points during cell culture [Ebert et al., 2009]. Potential caveats to the use of mutated iPS or genetically modified hESC as in vitro models for disease may be that many genetic diseases are only manifest in fully differentiated cells in later life (e.g., Parkinson's disease, ALS) and stem cell derivatives are generally immature. hESC-derived cardiomyocytes for example have sarcomeric organization resembling primary human fetal cardiomyocytes. They have low action potentials in patch clamp electrophysiology although in contrast to mouse ESC derived cardiomyocytes, the coupling of their ion channels to downstream signaling pathways seems relatively mature [Mummery et al., 2003]. For the future, it will be interesting to study mechanisms of disease in iPS cell lines from different patients carrying the same mutation with view to discovering the effect of genetic background in humans. The identification of the signaling pathways involved may aid development of specific therapeutic strategies. Furthermore disease-specific iPS cells represent promising targets as drug screening assays particularly if the production of derivative cardiomyocytes can be scaled up.

PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IN THERAPY

Despite the challenges of their basic biology, pluripotent stem cells remain interesting candidates for realizing cell replacement therapy since they are able to undergo directed differentiation to the three most important cellular components of the heart (cardiomyocytes, ECs, and VSMCs). However transplantation of suspensions of hESC-derived cardiomyocytes into animal models has demonstrated that many questions still need to be addressed before translation to clinical practice. Most studies to date have used a mixture of hESC-derivatives containing a significant proportion of cardiomyocytes but also other cell types [Caspi et al., 2007; Laflamme et al., 2007; van Laake et al., 2007]. Some studies have used hESC "primed" by growth factor treatment to be predisposed to form cardiac progenitors [Tomescot et al., 2007]. In general hESC derivatives have been injected directly into the myocardium of experimentally infarcted immunocompromised (SCID) rodent hearts. Follow up in most studies, particularly with respect to analysis of cardiac function, is relatively short, usually 4–8 weeks [Caspi et al., 2007; Laflamme et al., 2007]. In a study we carried out, follow up was over a longer period, in fact up to 6 months [van Laake et al., 2007, 2008]. For easy tracking of hESC derivatives after transplantation in vivo we used genetically marked, GFP-expressing cells. Differentiation to cardiomyocytes was induced by co-culture with END-2 and 1 million single cells containing 20–25% cardiomyocytes were injected into the ventricle of mice, that had undergone myocardial infarction. GFP-positive cells formed stable grafts within 3 weeks, which consisted almost exclusively of cardiomyocytes after 10 weeks. Cardiomyocytes matured and were found to form intercellular contacts, which are essential for a functional syncytium. In line with the other reports, functional improvement was observed in hearts with hESC-derived cardiomyocytes at 4 weeks. Surprisingly, despite graft survival even up beyond 6 months, functional improvement was not sustained at 12 weeks. The failure of long-term benefit could be due to the fact that transplanted cardiomyocytes-containing populations induced a transient paracrine effect (e.g., excretion of growth factors) that caused formation of neovasculature in the host and attenuated the immediate effects of ischemia, but did not contribute to contractile activity of the heart. The transplanted hESC-derived cardiomyocytes formed intercellular contacts with each other, but were usually separated from the surrounding host mouse myocardium by a thin layer of extracellular matrix [van Laake et al., 2007] so that functional coupling may not have taken place. In addition, it remains unclear whether human cardiomyocytes could keep pace with the much higher heart rate of rodents (80 beats per minute vs. 500 beats per minute). Larger animals with hearts more closely resembling the human heart in physiology and anatomy (e.g., from pigs or monkeys) would be a better model to address these questions. In particular, it will be important to determine whether introduction of an electrically active cell that because of its immature phenotype has intrinsic pacemaker activity, will cause arrhythmias.

So far transplantation experiments with iPS cell-derived cardiomyocytes have not been reported. A big advantage of the iPS technology will be the possibility to generate isogenic cardiomyocytes which are genetically equivalent to the cells of the transplant recipient (in contrast to allogenic transplants from an unrelated donor). Isogenic transplants do not require suppression of the immune system. By contrast allogenic transplants are rejected because their human leukocyte antigens (HLA) trigger the response of the recipient's immune system.

Transplantation of iPS cell-derived cardiomyocytes would also help to answer the question of whether these cells are indeed safe for cell therapy. In the experiments described above human cardiomyocytes were transplanted into the hearts of rodents. Although no teratomas were detected, these interspecies transplantations

(xeno-transplantation) may have camouflaged the actual tumorigenic potential of the transplanted cells in an immune neutral host. In support of this, Erdo et al. [2003] reported that mouse ESC differentiated into neurons after injection into rat brain but caused malignant tumors in mouse brain. Therefore it will be important to examine the tumorigenic potential of iPS cell-derived cardiomyocytes carefully, both at different developmental stages as well as after selection using methods outlined earlier. However, as a word of caution against premature optimism, it is important to note that individualized therapy of this nature is unlikely to be cost effective and the time taken to derive and characterize iPS cells from one patient (~four months) too long to treat anything but chronic ailments. In addition, like hESC lines, different iPS lines vary in their ability to differentiate into a certain cell type [Zhang et al., 2009]. Thus it may even be necessary to derive several iPS lines from the same patient and to test these for their differentiation potentials. To provide suitable cardiomyocytes for a patient in reasonable time, a bank of hESC lines which cover the HLA types of most individuals of a population and which are prescreened for their ability to differentiate could be a better option. This could be complemented by banks of iPS cells for rare genotypes for which no hESC lines were available, in the way banked cord blood from rare genetic backgrounds presently supplements bone marrow donations in the treatment of hematopoietic disease.

A recent alternative to generate patient-specific cells is conversion of one somatic cell type directly to another, a process called transdifferentiation. Theoretically fibroblasts which can be easily obtained from the patient's skin could be converted into cardiomyocytes without passing through the stage of pluripotency. A successful example for transdifferentiation is the conversion of mouse B lymphocytes to macrophages by the transcription factor CEBP [Xie et al., 2004]. More recently, exocrine pancreas cells were converted into endocrine β-cells in mice in vivo using three pancreatic transcription factors [Zhou et al., 2008]. Thus transdifferentiation could be used for regeneration in vivo—in contrast to the iPS technology which would represent an uncontrollable risk for the formation of teratomas. However it remains unclear if transdifferentiation is feasible between distantly related cell types (e.g., fibroblasts and cardiomyocytes) or restricted to more related cells.

Beside a potential role for iPS cell-derived cardiomyocytes for the replacement of lost cardiomyocytes through injury, iPS cells from patients with a *genetic* heart disease will help to develop new therapies based on genetic engineering. Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disorder affecting 1 in 500 individuals in the general population. Patients with HCM have an increased risk of arrhythmias, myocardial infarction and sudden cardiac death [Soor et al., 2009]. The genetic cause underlying the disease is often a mutation in one of 11 different sarcomeric genes (e.g., actin, β-myosin heavy chain). On a molecular basis, malfunction of proteins results in sarcomere and myofibrillar disarray, changes in Ca^{2+}-sensitivity and impaired contractile performance. In a first step of an HCM therapy the mutated gene would be replaced by the functional sequence by homologous recombination. After genetic engineering of iPS cells in vitro the repaired cardiomyocytes could be transplanted into the heart of the recipient (Fig. 1). Whilst gene therapies have been the subject of much potential clinical interest, random virus-mediated insertion of constructs rather than targeted integration has presented risks of oncogenic transformation. In addition, adult humans have already developed immunity against viruses from which viral vectors are developed; exposure to the vectors often causes a strong immune response in patients which blocks efficient gene delivery. In this respect the iPS cell technology represents a huge advance since these cells can be engineered by site-specific targeting ex vivo and tested prior to transplantation. An elegant example for a cure of a genetic defect using iPS cells was recently reported in a mouse model of sickle cell anemia [Hanna et al., 2007]. The cause of this hematopoietic disorder is a mutation in the hemoglobin gene leading to symptoms such as severe anemia due to the abnormal sickle-like morphology of the erythrocytes, splenic infarcts, etc. iPS cells were generated from tail tip fibroblasts from mice with the genetic defect. After replacement of the defective DNA sequence with the wildtype gene by homologous recombination, iPS cells were differentiated into hematopoietic progenitors in vitro. These were transplanted into diseased mice which had undergone irradiation to erase their own hematopoietic cells bearing the genetic defect. Reconstitution of the hematopoietic system with the repaired progenitors rescued the phenotype and "cured" the mouse [Hanna et al., 2007]. This work represents proof-of-principle for a therapy based on genetically engineered derivatives of iPS cells.

CONCLUSIONS

Despite 10 years of research on hESC, the dawn of research on human pluripotent stem cells is only just arriving. Politics and ethics have limited hESC research, but these objections do not apply to iPS cells. The 10 years of hESC research has however, taught us that the types of therapies that will be treatable by pluripotent stem cells requires a more conservative appraisal. The heart is a case in point where many unexpected challenges have arisen. Not only do we have to develop defined conditions for scaled production of pure populations of cardiovascular cells and ensure no rogue undifferentiated cells are co-transplanted, but we also have to ensure appropriate integration and alignment of the transplanted cardiomyocytes. This may ultimately require a tissue engineering approach [Zimmermann, 2009]. By contrast, with respect to developing human models for cardiac drug toxicity testing and as drug discovery platforms cardiomyocytes (and ECs and VSMCs) from hESC and human iPS cells are holding up to rigorous analysis and it seems likely that they may in the short-term complement existing in vitro systems for determining drug effects on the heart.

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