Review Article

Human Pluripotent Stem Cell-Derived Cardiomyocytes as Research and Therapeutic Tools

Ivana Acimovic, Aleksandra Vilotic, Martin Pesl, Alain Lacampagne, Petr Dvorak, Vladimir Rotrekl, and Albano C. Meli

1 Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5/A3, 62500 Brno, Czech Republic
2 ICRC, St. Anne’s University Hospital, 60200 Brno, Czech Republic
3 INSERM U1046, University of Montpellier I, University of Montpellier II, 34295 Montpellier, France

Correspondence should be addressed to Vladimir Rotrekl; vrotrekl@med.muni.cz and Albano C. Meli; albano.meli@inserm.fr

Received 6 December 2013; Accepted 4 February 2014; Published 2 April 2014

Academic Editor: Giancarlo Forte

Copyright © 2014 Ivana Acimovic et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Human pluripotent stem cells (hPSCs), namely, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), represent a powerful research tool in developmental biology, for drug screening, disease modelling, and potentially cell replacement therapy. Efficient differentiation protocols that would result in the cell type of our interest are needed for maximal exploitation of these cells. In the present work, we aim at focusing on the protocols for differentiation of hPSCs into functional cardiomyocytes in vitro as well as achievements in the heart disease modelling and drug testing on the patient-specific iPSC-derived cardiomyocytes (iPSC-CMs).

1. Introduction

Cardiovascular diseases are a leading cause of morbidity and mortality in developed countries, causing over 4 million deaths per year just in Europe [1]. They usually result in cardiomyocyte death [2]. Although there are indications that human adult heart has certain level of endogenous regeneration capacity, with different estimations of the rate of cardiomyocyte turnover between studies, adult human heart cannot effectively regenerate after injury [3–6]. Therefore, loss of cardiomyocytes causes permanent damage of heart that progressively decreases its functionality and could eventually lead to heart failure and death. Current treatments of cardiac disorders are mostly based on symptomatic treatment by medications and implantable cardiac devices. While heart transplantation constitutes the ultimate treatment for severe stages of heart failure, there are serious difficulties connected with organ transplantation such as limitations in organ supply and immunological incompatibility. Therefore, providing new tools for treatment of cardiovascular diseases, such as cardiac ischemia, myocardial infarction, and heart failure, is obviously needed. Theoretically, de novo cardiomyocytes for cell replacement therapy could potentially solve the problem of availability of human cardiac tissue.

Human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), with their ability of indefinite self-renewal and capability to differentiate into cell types derivatives of all three germ layers, represent a powerful research tool in developmental biology, for drug screening, disease modelling, and potentially cell replacement therapy. Efficient differentiation protocols that would result in the cell type of our interest are needed for maximal exploitation of these cells. In the present work, we aim at focusing on the protocols for differentiation of hPSCs into functional cardiomyocytes in vitro as well as achievements in the heart disease modelling and drug testing on the patient-specific iPSC-derived cardiomyocytes (iPSC-CMs).
oncogenic transcription factors such as c-Myc; viral vectors, and occurring random transgene insertions into the host’s genome during reprogramming raised concerns of hiPSCs tumorigenicity and safety of using hiPSCs for clinical applications.

Teratoma formation is one of the desired properties of PSCs demonstrating their ability to differentiate [13, 14]. Teratocarcinogenicity is a pathological property of PSCs when they do not differentiate in the in vivo environment. Possible occurrence of dangerous teratocarcinoma is the dark side of PSC potential use in the cell therapy [15, 16]. Teratocarcinogenicity is an intrinsic property of mouse ESCs due to their proliferative capacity and it is a consequence of epigenetic transformation of ESCs to embryonic carcinoma cells (ECCs) [13], while normal human ESCs do not readily form teratocarcinoma in immunodeficient mice and the transformation to human ECCs requires genomic changes (i.e., mutations) [17, 18]. Thus, unless hESCs are aneuploid, the residual undifferentiated cells are likely to form benign tumors only. Also the ability of iPSCs to create tumors seems to correspond to the level of genomic stability [19].

The hiPSCs created using viral vectors were shown to have elevated mutant frequencies and aberrant epigenome compared to hESCs or even differentiated cells [20], suggesting lower genome stability and thus higher risk of cancer development. Transplantation of progenitors or even terminally differentiated cells derived from pluripotent cells raises hopes for cell replacement therapy as PSC-derived differentiated cells similarly to mouse embryos after neurulation lose their ability to create teratocarcinoma and rather form benign teratomas [21]. But so far it is not technically possible to generate pure populations of terminally differentiated cells without traces of progenitors/stem cells. These data altogether suggest that finding reprogramming methods leading to lower mutant frequencies and higher genome stability might significantly contribute to the safety of iPSC products. Equally important is finding differentiation protocols leading to more defined and clearer populations of terminally differentiated cells intended for cell replacement therapy or development of robust transdifferentiation protocols eliminating the need and danger of PSCs.

To decrease tumorigenic potential different methods of generating hiPSCs were developed, including different combinations of reprogramming genes [22, 23] together with small molecules [24], which increased reprogramming efficiency, and use of different excisable [11, 25, 26] or nonintegrative vectors [27] for delivering reprogramming factors. A step forward to virus-free reprogramming methods was application of synthetic-modified mRNA [28] and recombinant proteins [29–31]. Therefore, application of improved reprogramming protocols for hiPSC generation, which will be safe for clinical use and production of patient-specific iPSC-derived cardiomyocytes (iPSC-CMs), would theoretically overcome immunological complications of transplanting organs and enable avoiding immunosuppressive treatment.

Differentiation of hPSCs to cardiomyocytes can be achieved in vitro by modulation of signalling pathways that are involved in cardiac development during embryogenesis. Potential applications of hPSC-CMs are numerous but the main goal is to get the highest output under the controlled culture conditions while major limits are low efficiency of current protocols and cardiac population heterogeneity (i.e., nodal, atrial, and ventricular cardiomyocytes). In the present discussion, we summarize the state-of-the-art methods for generating cardiomyocytes from hPSCs and their potentials as research and therapeutic tools.

2. How to Generate De Novo Cardiomyocytes from hPSCs

2.1. Cardiac Differentiation through Coculture with END-2 Cells. One of the first protocols for directed cardiomyogenesis of hESCs was developed by Mummery and colleagues and involved coculture of hESCs with mouse visceral-endoderm-like cells (END-2) [32]. Endoderm-secreted factors, such as bone morphogenetic proteins (BMPs), nodal/activin A, fibroblast growth factors (FGFs), and repressors of canonical Wnt/β-catenin pathway, have direct role in cardiac differentiation of hESCs. Overall efficiency of this protocol to generate hESC-CMs is quite low, resulting mostly in ventricular-like cardiomyocytes. Improvement of this protocol was achieved by switching from 20% fetal calf serum (FCS) in the medium to the serum-free conditions and by addition of L-ascorbic acid [33]. Similar protocol has been successful also to obtain hiPSC-CMs [34]. Decrease of FCS concentration led to increased percentage of beating areas in a dose-dependent manner and resulted in a 39-fold increase in total number of cardiomyocytes in the serum-free medium. Cardiac differentiation was further promoted in the insulin-free conditions. Phosphatidylinositol 3-kinase (PI3K) signalling inhibition increased the expression of mesendoderm markers [35], while insulin redirected differentiation in favour of neuroectoderm [36]. Enhancement of cardiac differentiation by ascorbic acid has also been shown in mouse PSCs (mPSCs) [37] through promotion of cardiac progenitor cell proliferation [38].

2.2. Embryoid Body-Based Cardiac Differentiation. Embryoid body-based method involved culturing of hPSCs as three-dimensional cell aggregates called embryoid bodies (EBs). Initially, after collagenase IV treatment small hPSC clumps were cultured in suspension to form EBs in the medium with 20% FBS. After 7–10 days EBs were plated on the gelatin-coated culture dishes, which gave 8.1% of spontaneously beating EBs [39]. The imperfection of the protocol with FBS is that interbatch differences of FBS can have significant impact on the efficiency of cardiac differentiation [40]. However, it seems that FBS enables cardiac differentiation in EB-mediated protocol in endoderm-dependent manner [41]. Addition of 5-aza-2′-deoxycytidine but not DMSO nor retinoic acid significantly enhanced cardiac differentiation of hESCs [42]. After induction of cardiac differentiation of EBs
in FBS-containing medium, EBs can be kept in the defined medium without serum [43].

Sometimes there is an intermediate step in the evolution of the protocols that mix two basic approaches and make it difficult to draw a clear line between methods. One of them includes differentiation of hESCs in the serum-free EBs [44]. It was shown that cardiogenic effect of EB-2 cells was independent of the direct interaction between hESCs and EB-2 cells [45]. In the same study insulin was found as an inhibitor of cardiomyogenesis in concentration-dependent manner predominantly during the early phases of differentiation, whereas prostaglandin I2 (PGI2) was discovered as enhancer. Cardiac differentiation of hESC-EBs in serum-free EB-2 conditioned medium yielded 10% CMs in the overall cell population with an increase to 20% by use of SB203580, specific p38 mitogen-activated protein kinase (MAPK) inhibitor, and resulted in approximately equal proportion of atrial- and ventricular-like cardiomyocytes [46].

Efforts that have been put to improve EB-based cardiac differentiation of hPSCs led to higher efficiency of the protocols through application of specific growth factors and chemically defined media and formation of uniform-sized EBs.

2.2.1. Specific Growth Factors and Small Molecules. Application of various combinations of specific growth factors in concentration- and time-dependent manner during cardiac differentiation of hPSCs in vitro can mimic signalling pathways responsible for cardiomyogenesis during embryonic development in vivo. Short-term BMP4 treatment promotes mesoderm induction [47], while long-term treatment leads to trophectoderm and extraembryonic endoderm differentiation [49]. Inconsistency in cardiac differentiation efficiency due to the interbatch differences of FBS used in the culture medium can be partially overcome with addition of BMP4 [40].

Canonical Wnt/β-catenin pathway has a biphasic role in human cardiogenesis. It should be activated during the early phase and inhibited during the late phase of cardiac differentiation [50]. Activation of Wnt signaling during the early phase of cardiac differentiation in hPSC-EBs by application of Wnt activators (Wnt3a, BIO, and CHIR99021) can be crucial for mesoderm induction [51, 52]. Early treatment with BMP4 followed by Wnt signalling inhibition, using inhibitor of Wnt response 1 (IWR1) or inhibitor of Wnt production 1 (IWP1), increases the efficiency of BMP4-directed cardiac differentiation of both hESCs and hiPSCs [53]. Some other small molecule inhibitors of Wnt pathway (i.e., Wnt3a, 53AH, and XAV939) also showed cardiogenic effect when applied after BMP4/activin A-mesoderm induction in hESC-EBs [54]. Use of stage-dependent combinations of BMP4, activin A, FGF2, Dickkopf 1 (DKK1), and VEGF in serum-free media and maintaining EBs under hypoxic conditions (5% O2, 5% CO2, and 90% N2) during the first 12 days of differentiation resulted in approximately 70% beating EBs [55]. After mesoderm induction, inhibition of TGFβ/activin/nodal and BMP4 signalling with small molecules SB431542 and dorsomorphin, respectively, can improve this system [56]. It was noticed that for each cell line it was necessary to optimize the protocol as different cell lines can differ in the levels of endogenous signalling. Our group also observed the interline variability in the cardiac differentiation efficiency as we applied the same growth factors (with substitution of DKK1 with IWR1) in FBS-free medium [57]. Seeking for more potent small molecule that could promote cardiac differentiation of hPSCs under defined cytokine- and xeno-free conditions, Minami et al. discovered KY0211, small molecule that acts as a Wnt inhibitor [52]. They also found as requirement for cardiac differentiation in the serum-free medium an addition of 0.4% human serum albumin or 1-2% bovine serum albumin. Recently, trichostatin A, histone deacetylase inhibitor, has been found as enhancer of EB-mediated cardiac differentiation [58].

2.2.2. Controlled EB Size. Studies on mESCs have shown that EB size and density were crucial for cardiac differentiation efficiency [59, 60]. The outcome of the EB-mediated differentiation of hESCs also depended on the size of the EBs [61]. Thus, some of the protocols included a step of forced aggregation of defined number of single hPSCs in the multwell plates (96-, 384-well) [62] that eventually finished in homogenous EB population. Stability of the aggregates was promoted by application of the Rho-associated protein kinase (ROCK) inhibitor, Y-27632 [62, 63]. Efficient EB formation was observed in U-, V-bottom well plates [64, 65], as well as AggreWell plates [57]. Stage-specific application of defined growth factors, polyvinyl alcohol, serum and insulin in the combination with V-96 plate aggregation system led to 94.7% of beating EBs and eliminated an interline variability in cardiac differentiation [66].

2.3. Monolayer-Based Cardiac Differentiation. High-density undifferentiated monolayer of hESCs on matrigel-coated culture dishes [67] or bone sialoprotein—peptide acyl surface (BSP-PAS) [68] can be differentiated into cardiomyocytes by combined application of activin A and BMP4 in Roswell Park Memorial Institute (RPMI) 1640 medium plus B27 supplement resulting in more than 30% cardiomyocytes [67]. In the same way as in the EB-mediated differentiation, addition of Nt3a in the early phase of activin A/BMP4-directed cardiac differentiation, as well as inhibition of Wnt/β-catenin signalling with DKK1 in the late stage, can enhance cardiac differentiation of hESCs [69]. Inhibition of Wnt pathway with IWR1 resulted in mostly atrial-like cardiomyocytes whereas inhibition with IWP4 gave both ventricular- and atrial-like cardiomyocytes based on expression of myosin light chain isoforms (MLC2v and MLC2a) [70]. The percentage of ventricular—versus atrial—like cardiomyocytes can be also modulated by alternation of retinoid signalling [71]. Further improvements involving matrix sandwich (overlay of monolayer-cultured hPSCs with matrigel), removal of insulin, and addition of FGF2 [72] resulted in up to 98% CMs, mainly ventricular-like [73]. In fully chemically defined medium (serum- and insulin-free) only by pretreatment with glycogen synthase kinase 3
afterdepolarizations (EAD and DAD) which are characteristic for CPVT patients [147]. Measurements in 3D beating clusters of CPVT-hiPSC-CMs showed that those clusters developed multiple Ca^{2+} transients when compared to wild-type clusters, indicating an arrhythmogenic phenotype in CPVT [148]. Arrhythmias could be exacerbated by isoproterenol (β-adrenergic receptor agonist) and prevented by KN-93, an antiarrhythmic drug that inhibits the calcium/calmodulin-dependent serine-threonine protein kinase II (CaMKII).

In all previously mentioned research CPVT-iPSC-CMs were generated from patients carrying mutations in RYR2 providing insights in pathophysiologic mechanisms and potential new drug treatment for this type of CPVT. Novak et al. were the first who reported modelling CPVT from the patient with mutation in CASQ2 [149].

8. Human Pluripotent Stem Cells and Cell Replacement Therapy

Human PSCs have the capacity to indefinite self-renewal and differentiation into all cell types in human body, including cardiomyocytes. Thus, theoretically hPSC could be unlimited source of human cardiac tissue which can be used in cell replacement therapies. Also, applying patient-specific iPSC-CMs would theoretically overcome existing immunological complications connected with organ transplantation.

Cell transplantation studies on infarcted animal hearts showed that hESC-CMs survive after transplantation, partially remuscularize scar tissue forming human myocardial graft and significantly improve mechanical and electrical function of the infarcted heart [67, 104, 169, 170]. Transplanted hPSC-CMs electrically couple and contract synchronously with host myocardium [169, 171] which indicates that improvement of contractile function of infarcted hearts after cell transplantation is due to creation of new force-generating units in host myocardium. Also, other indirect effects of transplanted cells such as production of paracrine factors may contribute to positive effects on cardiac function of infarcted hearts. These paracrine signals improve survival of host cardiomyocytes and reduce heart remodelling process after infarction [172].

Interestingly, Zwi-Dantsis et al. showed that iPSC-CMs from heart failure patients can integrate with pre-existing rat cardiac tissue and exhibit electrophysiological and pharmacological adaptation, bringing hope for cardiac regenerative medicine [26].

Since most transplantation studies were done on mice and rats, species with rapid heart rates, different cardiac physiology properties, and reaction to exercise and arrhythmogenic stimuli compared to humans, it is necessary to perform studies on large animal models with slower heart rate before clinical trials. Some of the research showed only transient beneficial effect in infarcted heart after cell transplantation [170]. Therefore, it would be of interest to carry out research on long-lasting effect of transplanted hPSC-CMs in host myocardium.

9. Summary and Outlooks

Methods to generate patient-specific iPSC-CMs have improved within the last years. Although most of the knowledge of the cardiomyogenesis using pluripotent stem cells have come from previous work achieved on ESCs, recent advances in this field make feasible a more efficient differentiation to human cardiomyocytes and a more defined resulting population of cardiac cells usable for disease modelling and drug testing in vitro. From the recent studies, it turned out that iPSC-derived CMs recapitulate the phenotype of patients with inherited cardiac syndromes and pathophysiology while the maturity and purity of those cells with the time stay much more unclear. From experimental and clinical investigations, it becomes clear that the development of protocols providing specific stimuli, combining electrical, mechanical or hormonal stimulation, is required to enhance the maturity and functionality of hiPSC-CMs in order to improve those tools for drug screening, disease modelling, and potentially for cell replacement therapy.

Conflict of Interests

The authors declare no competing financial interests.

Authors’ Contribution

Ivana Acimovic and Aleksandra Vilotic contributed equally to this work.

Acknowledgments

This work was supported by Grants of the Grant Agency of the Czech Republic (no. GA13-19910S), the Ministry of Education, Youth, and Sports of the Czech Republic (MSMT-179/2013-311, CZ.1.07/2.3.00/20.0011, and MSM0021622430), Project FNUSA-ICRC (no. CZ.1.05/1.1.00/02.0123), European Regional Development Fund, SoMoPro and South Moravian Region, and PHC Barrande (Duchenstem no. 28379TE). The research leading to these results obtained financial contribution from the European Community within the Seventh Framework Program (FP/2007–2013) under Grant Agreement no. 229603. Dr. Albano C. Meli was supported by the European Society of Cardiology (ESC), the French Muscular Dystrophy Association (AFM, Project 16073, MNM2 2012), and by the “Fondation de la Recherche Médicale” (FRM; SPF20130526710).

References


Notch inhibition allows oncogene independent generation of iPS cells

Justin K. Ichida1,2,9, Julia TCW1,2,3, Luis A. Williams1,2, Ava C. Carter1,2, Yingxiao Shi9, Marcelo T. Moura1,2, Michael Ziller1,4, Sean Singh1,2, Giovanni Amabile5, Christoph Bock1,4, Akihiro Umezawa6, Lee L. Rubin1, James E. Bradner7,8, Hidenori Akutsu6, Alexander Meissner1,4, and Kevin Eggan1,2,3

1 Harvard Stem Cell Institute, Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
2 Howard Hughes Medical Institute and Stanley Center for Psychiatric Research
3 Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA
4 Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02142, USA
5 Harvard Stem Cell Institute, Harvard Medical School, Boston, MA
6 Department of Reproductive Biology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya, Tokyo 157-8535, USA
7 Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA
8 Department of Medicine, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA
9 Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, 1425 San Pablo Street, Los Angeles, CA 90033, USA

# These authors contributed equally to this work.

Abstract

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:

Correspondence and requests for materials should be addressed to K.E. (keggan@scrbi.harvard.edu), A.M. (alexander_meissner@harvard.edu), or H.A. (akutsu-h@ncchd.go.go.jp).

Author Contributions


The authors declare no competing financial interests.

Accession numbers

Microarray data have been submitted to the GEO repository with accession number GSE35090.
The reprogramming of somatic cells to pluripotency using defined transcription factors holds great promise for biomedicine. However, human reprogramming remains inefficient and relies either on the use of the potentially dangerous oncogenes KLF4 and CMYC or the genetic inhibition of the tumor suppressor gene p53. We hypothesized that inhibition of signal transduction pathways that promote differentiation of the target somatic cells during development might relieve the requirement for non-core pluripotency factors during iPSC reprogramming. Here, we show that inhibition of Notch significantly improves the efficiency of iPSC generation from mouse and human keratinocytes by suppressing p21 in a p53-independent manner and thereby enriching for undifferentiated cells capable of long-term self-renewal. Pharmacological inhibition of Notch enabled routine production of human iPSCs without KLF4 and CMYC while leaving p53 activity intact. Thus, restricting the development of somatic cells by altering intercellular communication enables the production of safer human iPSCs.

Use of the potent oncogenes KLF4 and CMYC in the generation of induced pluripotent stem cells (iPSCs) limits their translational utility. Currently, elimination of these genes during human iPSC reprogramming requires suppression of p53 activity, which in turn results in the accumulation of genetic mutations in the resulting iPSCs. Therefore, there remains a real need for reprogramming approaches that enable iPSC generation without the use of KLF4 and CMYC while leaving p53 activity intact.

In part to address this need, several groups have undertaken chemical screens to identify small molecules that can improve reprogramming. Thus far, the majority of active compounds are thought to improve reprogramming by inhibiting chromatin-modifying enzymes or by reinforcing the transcriptional network associated with the pluripotent state. Consistent with their proposed mechanisms of action, these chemicals generally function in cellular intermediates that arise late in reprogramming, catalyzing their final conversion into iPSCs. It is currently unclear whether known chemicals are sufficient for generating iPSCs from adult human cells, which are consistently more difficult to reprogram than mouse embryonic fibroblasts.

Given the likely need for additional reprogramming chemicals and the knowledge that most known compounds act late in this process, we reasoned it would be valuable to identify small molecules that improve reprogramming by acting early, perhaps within the somatic cells themselves. We reasoned that one approach towards this goal would be to identify chemicals that could modulate signal transduction cascades in somatic cell populations to enrich for those cells with an enhanced capacity for reprogramming. We reasoned that if such compounds could be identified, they might expand the translational utility of chemical reprogramming.

It has been recognized that the extent of a target cell’s differentiation is an important determinant of the efficiency by which it can be reprogrammed. We therefore hypothesized that chemically driving somatic cells into a more potent “stem cell” state might improve their reprogramming. To test this hypothesis, we chose to ask whether known chemical inhibitors of the Notch signaling pathway could aid in reprogramming.

Nat Chem Biol. Author manuscript; available in PMC 2015 February 01.
Genes involved in embryonic heart development identified

Date: May 2, 2011
Source: Gladstone Institutes
Summary:
Scientists have identified networks of genes that play an important role in embryonic-heart development, advancing knowledge of how healthy hearts develop -- and offering clues about how to combat a common birth defect known as congenital heart disease.

Congenital heart disease affects nearly 1 out of every 100 babies born worldwide and is the most common cause of death from a birth defect. In the disease, cells in the embryo often fail to get the right instructions while the heart is being formed. Many genes and proteins must be deployed at just the right time and in the right amounts for healthy heart formation to occur. Disruption of the correct dosage of proteins can lead to congenital heart defects.

One way cells control the amount of protein made from genes is through a recently discovered molecule called a microRNA. MicroRNAs are tiny strands of genetic material that do not encode the information to make enzymes and proteins, as most RNAs do. Instead, microRNAs inhibit other RNA molecules from producing protein. In recent years, scientists have discovered that each microRNA inhibits protein production from hundreds of RNAs, regulating almost every process in every cell of the body by fine-tuning the dosage of key proteins.

"MicroRNAs provide an extra layer of regulation that helps ensure that the correct amount of protein is made from a particular gene at the right time," said Isabelle King, a Gladstone scientist and an assistant professor of pediatrics at the University of California, San Francisco. Dr. King is the lead author on the study, the results of which were published in the April 17 issue of Developmental Cell. "In the fetal heart, subtle changes in gene dosage and timing can yield heart defects in children."

While microRNAs are powerful, it has been challenging to identify the hundred or more genes that each microRNA regulates. To overcome this problem, the researchers devised a simple genetic test in fruit flies, a classic organism for genetic studies. By examining the impact of thousands of genetic mutations on the function of a muscle-specific microRNA, the scientists were able to better understand how the fly heart develops and found that the same genes were important in the mouse heart. These results may provide insight into what happens in humans because genes common to mouse and fly hearts are also typically critical for heart formation in humans.
"Flies and humans have a lot in common in terms of how their cells work, and so flies represent a powerful tool to explore human diseases," said Dr. King.

To examine genes that influence fetal-heart development, the Gladstone investigators created mutant flies with too much of a muscle-specific microRNA called miR-1. Flies and other animals without miR-1 are known to have heart defects. By selective breeding, the researchers found more than three regions in the flies' genome that made the effects of excess miR-1 even worse -- suggesting that genes in these regions work in concert with miR-1.

The identity of these genes will help researchers better understand why muscle and heart cells need miR-1, how birth defects develop in the fetal heart and where to target any potential therapies to prevent those defects. For example, one of the genes tells the cell which side should face up and which should face down, which is a critical event as heart cells come together to form an organ.

While the techniques used in this research revealed important information for heart research, a similarly structured experiment could also be used to reveal new knowledge about the function of microRNAs in other organs and tissues.

"The assay system we developed gives us a valuable tool to identify microRNA-responsive genes involved in other developing organs or disease states," said Deepak Srivastava, MD, director of the Gladstone Institute of Cardiovascular Disease and the study's senior author.

Gladstone scientists Joseph Shieh, Yu Huang and Chulan Kwon, and University of California, San Francisco scientist J. Liang, all contributed to this study. The National Institutes of Health, Gladstone, the California Institute for Regenerative Medicine, and the Younger Foundation all provided funding for this study.

Story Source:
The above post is reprinted from materials provided by Gladstone Institutes. Note: Content may be edited for style and length.

Journal Reference:
http://www.embryology.ch/anglais/pcardio/herzentwick01.html
16.1 Outer form and position of the heart

- Introduction
- First signs of cardiac development

Introduction

In the young embryo epiblast cells wander via the primitive streak between epi- and hypoblast and form the mesoderm layer out of which various structures arise. Cells, which are destined for cardiogenesis, take up a position that is cranial to the forming neural tube. Cardiogenesis takes place via a complex series of steps:

1. Determination of mesoderm- and neural crest cells for heart formation
2. Growth and differentiation processes to become cardiomyocytes
3. Migration and transformation processes in order to form the heart

First signs of cardiac development

The cardiogenic plate is formed by a collection of mesoderm cells in the most anterior part of the embryo. In the interactive diagram, please observe how the position of the pericardial cavity changes in relation to the cardiogenic plate due to the bending of the cranial end of the embryo.
Up to the early stage 9 (at roughly 25 - 27 days) this cardiac anlage is still located in the visceral part of the splanchnic mesoderm (splanchnopleura) above the umbilical vesicle. Out of this mainly develops the myocardium, which is responsible for the very early contractile ability of the embryonic heart. Through the rotation following the cranial folding of the embryo the pericardial cavity comes to lie ventrally from the cardiac anlage and, as things progress, will surround it.

The blood from the supplying vessels, the umbilical and omphalomesenteric veins, flows caudally over the inflow tract into the cardiac anlage and leaves it via the outflow tract and the aortic arches at the cranial end.

Fig. 1 - Embryo at stage 9 (roughly 25 - 27 day) after the rotation of the cardiac anlage

Fig. 1
Perspective side view of the embryo.
The formation of the cardiac tube out of vesicles is shown in an animation.
By means of various transplantation experiments on research animals, it has been shown that cell interactions play the main role in the differentiation of the mesoderm cells in the region of the subsequent cardiogenic plate. The anterior endoderm that lies ventral to the cardiogenic plate has a decisive influence by secreting inductive factors that over a short distance confer cardiogenic power to the mesoderm (5).

The cardiac tube itself consists of three layers: epicardium, myocardium and endocardium. The outermost layer and boundary of the pericardial cavity is the epicardium. The myocardial mantle follows as the next inner layer. Together they form the myoepicardium. The considerable distance from the myocardial mantle to the endocardial tube is filled with cardiac jelly and the cardiac lumen is coated with endocardial cells.
Fig. 2a
The cranial part of the embryo is shown in a medial sagittal section. The cardiac anlage is surrounded by the pericardial cavity. The heart consists of the myoepicardial mantle, the cardiac jelly and the endocardium.

Fig. 2b
In this transversal section the heart finds itself at the level of the rhomboencephalon, i.e., still very cranial. Note the position of the pericardium in relation to the heart, the many layers of the cardiac wall and the provisional existence of a dorsal suspension (mesocardium).
(see also the development of the pericardium)

The outer layer of the heart is formed by the epicardium that originally arises from the extracardial anlage, the proepicardial serosa cells. These grow over the myocardium and issue from a collection of cells in the septum transversum that lie ventrally to the liver bud near the sinus venosus. Presumably, the subepicardial mesenchymal cells also come from the epicardial layer (1). Besides the epicardial layer, it appears that the proepicardial serosal cells also form the endothelium and the smooth muscle cells of the coronary arteries. Moreover they play a modulating role in the development of the myocardium. (7)
One solution for the shortage of transplantable organs is creating artificial ones that last.

Already, researchers are developing bioartificial organs that can keep patients with serious organ failure alive and functioning for years. For now, the goal is to keep patients alive until they can receive a real organ, but one day, patients may be able to live for long periods of time with artificial hearts or kidneys. It is a reality!

Currently, SynCardia Systems, Inc. provides patients with a full-functioning artificial heart.

The manufactured heart replaces both failing ventricles and all four heart valves and eliminates the need for pacemakers and defibrillators. The longest a patient has been supported with the SynCardia Total Artificial Heart is nearly four years before receiving a successful transplant.

What Is Regenerative Medicine?

Imagine a treatment that frees a child from the daily insulin injections of diabetes, or helps a grandmother weakened by heart failure regain her independence. Imagine a treatment that helps a soldier disfigured by injuries regain his self-confidence. Imagine damaged organs regrowing and wounds healing without scars. These therapies may sound like science fiction, but many are available now thanks to regenerative medicine.

Regenerative medicine is a new way of treating injuries and diseases that uses specially-grown tissues and cells (including stem cells), laboratory-made compounds, and artificial organs. Combinations of these approaches can amplify our natural healing process in the places it’s needed most, or take over the function of a permanently damaged organ.
Review

Transforming the Promise of Pluripotent Stem Cell-Derived Cardiomyocytes to a Therapy: Challenges and Solutions for Clinical Trials

Andrew B.J. Prowse, PhD,a Nicholas E. Timmins, PhD,a Terrence M. Yau, MD, MSc, FRCSC,b,c,e,f,g Ren-Ke Li, MD, PhD,b,c,e,f,g,h,i Richard D. Weisel, MD, FRCSC,b,c,e,f,g,h Gordon Keller, PhD,f,h,j,k and Peter W. Zandstra, PhDа,g,i,l,m

а Centre for Commercialization of Regenerative Medicine, Toronto, Ontario, Canada
b Division of Cardiac Surgery, University of Toronto, Toronto, Ontario, Canada
c Peter Munk Cardiac Centre at the University Health Network, Toronto, Ontario, Canada
d Department of Surgery, University of Toronto, Waterloo, Ontario, Canada
e Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada
f University Health Network, Toronto, Ontario, Canada
g Heart and Stroke/Richard Lewar Centre of Excellence, Toronto, Ontario, Canada
h McEwen Centre for Regenerative Medicine, University Health Network, Toronto, Ontario, Canada
i Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada
j Princess Margaret Cancer Centre, Toronto, Ontario, Canada
k Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada
l The Donnelly Centre, University of Toronto, Toronto, Ontario, Canada
m Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada

ABSTRACT

Despite advances in coronary artery disease treatment and prevention, myocardial damage due to acute myocardial infarction (MI) remains a major cause of morbidity and mortality in the population. Cell-based clinical trials to treat MI have focused on cells derived from the bone marrow or those potentially possessing functional similarities such as skeletal myoblasts or cardiac progenitors isolated from heart biopsies. Any benefits provided by these cells in improving heart function, left ventricular ejection fraction, or extending life expectancy after MI have been credited mostly to paracrine effects. Functional restoration of cardiac function remains a major goal of cell therapy.

Since their derivation in 1998,1 human embryonic stem cells (hESCs) have been heralded for their potential to treat, or even cure, a range of previously intractable human diseases and disorders. In this article we focus on the potential of hESC- and induced pluripotent stem cell (iPSC)-derived cardiac cells in the prevention of heart failure after an extensive myocardial infarction (MI; Fig. 1). Collectively referred to as human pluripotent stem cells (hPSCs), with the exception of immunogenicity, we will not discuss the difference between hESCs and iPSCs, nor the merits of using one type over the other (neither technical nor ethical). Instead, both are discussed in common, as a pluripotent cell source with the capability to differentiate and give rise to functional human cardiomyocytes (CMs).

http://dx.doi.org/10.1016/j.cjca.2014.08.005
0828-282X/© 2014 Canadian Cardiovascular Society. Published by Elsevier Inc. All rights reserved.
damaged myocardium will require a functional cell type with similar phenotype and characteristics of the damaged tissue that can also integrate, survive, and electrically couple to the host. Human pluripotent stem cells (hPSCs) have the ability to differentiate into multiple cell types of the adult body. hPSC-derived cardiomyocytes represent a promising target population for cell-based therapies for MI because they are scalable and the product can be defined with a specific set of release criteria. The purpose of this article is to review the rationale for cell therapy in heart disease, discuss the properties of hPSC cardiomyocytes that define their usefulness for regenerative therapy, consider manufacturing issues and preclinical investigation, and finally examine the steps required to establish effective clinical implementation. Pluripotent stem cell-derived cardiomyocyte-based therapies have enormous potential to revolutionize the management of heart disease; expedient but careful development is needed to ensure that this potential is fully realized.

Underlying much of the excitement surrounding hPSC since their discovery is their potential for unlimited propagation and ability to differentiate and give rise to many cell types in the adult body (Fig. 1). This differentiation capacity however does not automatically facilitate their use in clinical therapy. Early hPSC cultures were grown on mouse feeder layers in media composed of nonhuman components, and maintenance culture and differentiation were more art than science. In the past 5 years there has been rapid advancement toward generating therapeutically relevant hPSC derivatives. Significant effort has been directed toward improved media composition (performance, cost, and regulatory compliance), enhanced differentiation methods (efficiency, specificity, and functionality), cell line derivation (efficiency, cost, and compliance), and most recently, 

[Figure 1. Derivation of human embryonic and induced pluripotent stem cells. Human embryonic stem cells (hESCs) are derived from a 5- to 6-day-old blastocyst after in vitro fertilization. The inner cell mass is transferred to a monolayer culture and expanded before differentiation to cells representative of the 3 adult germ layers. Induced pluripotent stem cells (iPSCs) are derived from patient-specific somatic cells that have been reprogrammed using pluripotent gene-inducing factors and exhibit many of the pluripotent properties of hESC, including differentiation to the 3 adult germ layers.]
Health & Wellness Resource Center

Stem cells in cardiac repair.

Robert J Henning, Future Cardiology, Jan 2011, vol 7, issue 1 p99 (19)

Author's Abstract:
Myocardial infarction is the leading cause of death among people in industrialized nations. Although the heart has some ability to regenerate after infarction, myocardial restoration is inadequate. Consequently, investigators are currently exploring the use of human embryonic stem cells (hESCs), skeletal myoblasts and adult bone marrow stem cells to limit infarct size. hESCs are pluripotent cells that can regenerate myocardium in infarcted hearts, attenuate heart remodeling and contribute to left ventricle (LV) systolic force development. Since hESCs can form heart teratomas, investigators are differentiating hESCs toward cardiac progenitor cells prior to transplantation into hearts. Large quantities of hESCs cardiac progenitor cells, however, must be generated, immune rejection must be prevented and grafts must survive over the long term to significantly improve myocardial performance. Transplanted autologous skeletal myoblasts can survive in infarcted myocardium in small numbers, proliferate, differentiate into skeletal myofibers and increase the LV ejection fraction. These cells, however, do not form electromechanical connections with host cardiomyocytes. Consequently, electrical re-entry can occur and cause cardiac arrhythmias. Autologous bone marrow mononuclear cells contain hematopoietic and mesenchymal stem cells. In several meta-analyses, patients with coronary disease who received autologous bone marrow cells by intracoronary injection show significant 3.7% (range: 1.9-5.4%) increases in LV ejection fraction, decreases in LV end-systolic volume of -4.8 ml (range: -1.4 to -8.2 ml) and reductions in infarct size of 5.5% (-1.9 to -9.1%), without experiencing arrhythmias. Bone marrow cells appear to release biologically active factors that limit myocardial damage. Unfortunately, bone marrow cells from patients with chronic diseases propagate poorly and can die prematurely. Substantial challenges must be addressed and resolved to advance the use of stem cells in cardiac repair including identifying the optimal stem cell(s) that permit transplantation without requirements for host immune suppression; timing of stem cell transplantation that maximizes chemoattraction of stem cells to infarcts; and determining the optimal technique for injecting stem cells for cardiac repair. Techniques must be developed to enhance survival and propagation of stem cells in the myocardium. These studies will require close cooperation and interaction of scientists and clinicians. Cell-based cardiac repair in the 21st century will offer new hope for millions of patients worldwide with myocardial infarctions who, otherwise, would suffer from the relentless progression of heart disease to heart failure and death.

Full Text: COPYRIGHT 2011 Future Medicine Ltd.

Author(s): Robert J Henning ¹
renewal, such as Sox2, Oct4 and Nanog\cite{16}. To date, retroviruses and lentiviruses have been used as vectors for the transmission of SOKM. However, viral integration into cells is not desirable, since viruses can integrate into host cell chromosomes, where they can cause mutagenesis, interfere with gene transcription and induce malignant transformation\cite{17}. For this reason, cell reprogramming strategies have been developed that rely on small molecules, small interfering RNAs or miRNAs, which can promote reprogramming by replacing some of the reprogramming factors, or increasing reprogramming efficiency and speed. The use of valproic acid has increased cell reprogramming efficiency and has permitted replacement of Klf4 and cMyc, which are oncogenes. As a consequence, full pluripotent stem cell clones can be developed with a combination of Oct4-Sox2-valproic acid\cite{18}. Nevertheless, iPSCs, which can differentiate into endodermal, mesodermal and ectodermal tissues, harbor the potential risk of producing teratomas in research animals and in patients.

In vitro, human iPSCs have been differentiated into cardiomyocytes with the expression of troponin-I, sarcomeric [alpha]-actinin, connexin-43 proteins, and calcium and potassium ion channels\cite{19,20}. These cardiomyocytes can increase their beating frequency in response to isoproterenol and phenylephrine, and decrease their beating frequency with carbamylcholine, verapamil and diltiazem\cite{19,20}. Nevertheless, the electrophysiological and mechanical properties of the iPSC-induced cardiomyocytes resemble primitive or immature myocytes, rather than adult myocytes. Moreover, the efficiency of differentiation of iPSC to cardiomyocytes appears to be highly variable and suggests the need for development of more specific differentiation-inducing reagents.

In vivo, murine fibroblasts transduced with human Sox2, Oct3/4, Klf4 and cMyc have engrafted into the infarcted myocardium of immunocompetent mice, and have restored contractile performance, as demonstrated by increased fractional shortening and ejection fraction, ventricular wall thickness and electric stability, in contrast to murine hearts treated with nontransduced fibroblasts\cite{21}.

Currently, there is a lack of scientific consensus regarding deriving, culturing and differentiating iPSCs. A single experiment can yield multiple iPSCs that are not entirely identical. Moreover, the gene expression of iPSCs suggests that they may be a subtype of ESCs, owing to differential promoter binding by reprogramming factors and epigenetic changes that occur when cells differentiate\cite{22}. Consequently, at the present time, large-scale cultivation of iPSCs and iPSC-induced cardiomyocytes is challenging. Nevertheless, with improvements in the methodologies for iPSC derivation, the efficient generation of safe, virus and transgene-free patient-specific iPSCs and iPSC-induced cardiomyocytes, should be achievable for the treatment of cardiac diseases.

Skeletal myoblasts

Skeletal muscular fibers harbor a reservoir of mononuclear, unipotent (tissue-committed) precursor cells, termed satellite cells, which normally lay in a quiescent state between the basement membrane and the sarcolemma of individual muscle fibers. Satellite cells are identified by the genetic markers PAX7 and PAX3\cite{23}. These cells are a heterogeneous cell population with different regenerative potentials. In uninjured muscle, the majority of the satellite cells are quiescent. Following muscle injury or disease, these cells rapidly mobilize, proliferate and differentiate into myoblasts that express Myf5 and MyoD, which are important in myogenic differentiation, and desmin, which is important in linking myofibrils. Ultimately, the myoblasts fuse to existing muscle fibers and also form new fibers. A 10-15-g skeletal muscle biopsy can
yield approximately 1 billion myoblasts when propagated in culture over 2-3 weeks \(^\text{[24]}\). These myoblasts can be used for autologous myoblast transplantation in patients with infarcted hearts.

Investigations in small and large animals with ischemic and nonischemic cardiomyopathies have established that myoblasts injected into damaged myocardium can survive in small numbers, proliferate and differentiate into skeletal multinucleated myotubes. Although these cells may align with cardiomyocytes, they do not express gap-junction proteins, such as connexion-43, and do not form connections with host cardiomyocytes. Lineage restriction of skeletal myoblasts prevents differentiation of myoblasts into cardiomyocytes and also prevents teratoma formation in the heart. Moreover, myoblasts injected into infarcted myocardium of research animals limits LV remodeling and increases the LV ejection fraction \(^\text{[24]}\). Although the precise mechanism of action of myoblasts in animal studies has not been determined, positive experimental results have facilitated the design and performance of clinical studies.

Several Phase I clinical studies in patients with myocardial infarction scars have demonstrated feasibility of directly implanting autologous skeletal myoblasts into the LV at the time of coronary artery bypass surgery (Table 1) \(^\text{[25-27]}\). Each of these studies has demonstrated with echocardiography that autologous myoblasts can increase LV regional wall motion and increase LV ejection fraction 7-18%. However, ventricular tachycardia has occurred in approximately 27% of the patients after myoblast transplantation into hearts. The absence of electrical integration of myoblasts with cardiomyocytes and electrical re-entry into myoblasts is thought to cause the ventricular arrhythmias in these patients.

The Phase I clinical studies \(^\text{[25-27]}\) made the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) study possible \(^\text{[28]}\). It was a randomized placebo-controlled double-blind trial to determine whether myoblasts were functionally effective and caused arrhythmias in patients with ischemic cardiomyopathies. A total of 97 patients with left ventricular ejection fractions between 15 and 35% received 400 million (33 patients) or 800 million (34 patients) myoblasts, or placebo (30 patients), which were injected at approximately 30 sites in the left ventricular myocardium. All patients also received coronary artery bypass grafts and automatic implantable cardioverter defibrillators (AICD).

At 6-month follow-up, the number of patients in whom LV segments improved contraction by at least 1 grade on echocardiography was not significantly different among the three groups: 48% in the placebo group, 35% in the myoblast low-dose group and 40% in the myoblast high-dose group \(^\text{[28]}\). Moreover, changes in the regional wall-motion score index and change in ejection fraction from baseline, were not significantly different among the groups. Nevertheless, patients who received 800 million myoblasts did have a significant decrease in LV end-diastolic and endsystolic volumes, and a decrease in LV remodeling.

Some of the factors that explain the disappointing results of the MAGIC study include a low rate of myoblast retention in the myocardium, a high rate of subsequent myoblast death and the inability of engrafted myoblasts to establish functional electromechanical connections with the patients’ cardiomyocytes \(^\text{[28]}\). In addition, multiple needle injections into the myocardium are associated with a high rate of myoblast leakage out of the myocardium, inhomogeneous distribution of cells, disruption of the extracellular matrix, and subsequent loss of signals modulating cell survival and differentiation \(^\text{[28]}\). Although myoblasts can be transduced to overexpress gap-junction proteins, such as connexion-43, there is no guarantee that the distribution of transduced myoblasts will be homogenous enough to allow synchronized contractions of the graft with the host cardiomyocytes. Nevertheless, studies of myoblasts,
DEVELOPMENT OF CARDIAC MUSCLE

Cardiac muscle develops from the lateral splanchnic mesoderm, which gives rise to the mesenchyme surrounding the developing heart tube (see Chapter 13, Figs. 13-1B and 13-7C to E). (Cardiac myoblasts differentiate from the primordial myocardium. Heart muscle is recognizable in the fourth week. It likely develops through expression of cardiac-specific genes. Studies suggest that PBX proteins interacting with the transcription factor HAND2 promote cardiac muscle differentiation. Immunohistochemical studies have revealed a spatial distribution of tissue-specific antigens (myosin heavy-chain isoforms) in the embryonic heart between the fourth and eighth weeks.

Cardiac muscle fibers arise by differentiation and growth of single cells, unlike striated skeletal muscle fibers, which develop by fusion of cells. Growth of cardiac muscle fibers results from the formation of new myofilaments. The myoblasts adhere to each other as in developing skeletal muscle, but the intervening cell membranes do not disintegrate. These areas of adhesion give rise to intercalated disks (intercellular locations of attachment of cardiac muscles). Late in the embryonic period, special bundles of muscle cells develop from original trabeculated myocardium that has fast-conducting gap junctions with relatively few myofibrils and relatively larger diameters than typical cardiac muscle fibers (Purkinje fibers) form the conducting system of the heart (see Chapter 13, Figs. 13-18E and 13-19C and D).

ANOMALIES OF MUSCLES

Absence of one or more skeletal muscles is more common than is generally recognized. Common examples are the sternocostal head of the pectoralis major, palmaris longus, of the pectoralis major is occasionally associated with absence of the mammary gland in the breast and/or hypoplasia of the nipple.
organs is made more complex than those for industrial applications"