Cellular Reprogramming & Induced Pluripotent Stem Cells

The Editors

The 2012 Nobel Prize in Physiology or Medicine was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka “for the discovery that mature cells can be reprogrammed to become pluripotent”. Sir John Gordon pioneered the field of somatic cell nuclear transfer, wherein the nucleus of a mature cell is transplanted into an enucleated egg, to produce a living organism (tadpole). The technique, which is commonly referred to as “cloning”, produced a paradigm shift in developmental biology and paved the way for genome reprogramming for reproductive gains. It led to subsequent cloning of a dozen or so species, with “Dolly the sheep” being the most famous cloned animal, cloned by Ian Wilmut and colleagues at the Roslin Institute in Scotland in July 1996. Although Dolly was euthanized in 2003 because of progressive lung and degenerative joint diseases, the success of the nuclear transfer technique demonstrated that the genome, even when isolated from adult cells, contains the information necessary to generate a living organism.

In parallel with these advances, pluripotent cells from mouse embryos, i.e., embryonic stem cells (ESCs) that possess the ability to differentiate into different lineages, were isolated and characterized. Culture conditions and differentiation regulators, such as the leukemia inhibitory factor and basic fibroblast growth factor, necessary to maintain pluripotency, were identified and characterized. Similarly, the transcriptional regulators of cell fate and lineage specification were identified, and it was shown that heterologous expression of so-called “master regulators”, such as MyoD and Antennapedia (ANTP), could lead to cellular transdifferentiation. Even though direct transdifferentiation of mature cells has not fully materialized yet, the discoveries advanced the notion that expression of certain “master” transcription factors could be harnessed to switch cell fate and differentiation. The convergence of these discoveries, namely somatic nuclear transfer, identification of “master” transcription factors, and isolation and characterization of ESCs, led Yamanaka and colleagues to hypothesize that a combination of transcription factors can reprogram somatic cells back to an embryonic state. Soon, factor-based reprogramming of somatic cells began. Yamanaka and colleagues were the first to generate stem cells, initially from mouse and subsequently from human fibroblasts, with properties similar to ESCs. This was accomplished by introducing four transcription factors, namely SOX2, KLF4, MYC, and OCT3/4, which are often referred to as the Yamanaka factors. This breakthrough as well as the contributions from others showing similarities between the iPSCs and the ESCs as well as germline transmission of iPSCs, had a watershed effect in biomedical research. Within 6 years after its initial description, a large number of patient-specific iPSC models were generated and characterized, including a dozen or so for cardiovascular diseases. The highly attractive nature of the iPSC models, wherein iPSCs generated from patients’ somatic cells are used as disease models in petri dish, has generated enormous interest in the potential use of iPSCs in deciphering the molecular basis of human diseases, identifying novel drug targets, and providing new therapeutic approaches.
These advances have not been without considerable challenges both in terms of efficiency, which typically is less than 1% of the transduced cells, and safety, because of the use of viral vectors and expression of oncoproteins. The gene transfer approaches using retroviral and lentiviral vectors have the potential for insertional mutagenesis and induction of an immune response. Consequently, various methods of induction that are free of vector DNA or use noninsertional vectors have been developed to generate iPSCs, including synthetic RNAs and proteins. Nevertheless, persisting vectors have been developed to generate iPSCs, including synthetic RNAs and proteins. 

Consequently, various methods of induction that are free of vector DNA or use noninsertional vectors have been developed to generate iPSCs, including synthetic RNAs and proteins.18,19 Nevertheless, persisting safety concerns have somewhat shifted the research focus from the direct therapeutic utility of iPSCs in humans to modeling of human diseases to gain mechanistic insights, identify therapeutic targets, and screen for drug toxicity.

Circulation Research has been the leading platform for disseminating state-of-the-art and novel discoveries pertaining to the use of iPSCs in the cardiovascular field through publication of original papers and Review articles. In 2013, the Editors of Circulation Research will be proud to present the thematic series on iPSCs edited by Nobel Laureate Shinya Yamanaka.

The following represents a selection of recently published Circulation Research articles on reprogramming, presented in their reverse order of publication. Articles highlighted in yellow represent the top 5 most read original research articles selected based on the number of Full Text/PDF downloads, adjusted to compensate for differences in the length of time since online publication.

The Editors

**Genome Editing of Human Embryonic Stem Cells and Induced Pluripotent Stem Cells with Zinc Finger Nuclease for Cellular Imaging; Wang et al**

**What Is Known?**
- Molecular imaging plays an important role in the characterization of stem cell behavior inside living organisms.
- Zinc finger nuclease (ZFN) technology bypasses the negative effects of current random genetic integration techniques.
- The AAVS1 locus is a safe harbor site in human genome and supports long-term transgene expression.

**What New Information Does This Study Contribute?**
- Use of ZFN to introduce the triple fusion reporter gene into the safe harbor AAVS1 locus for effective molecular imaging.
- Combines the latest genetic engineering techniques with state-of-the-art in vitro and in vivo imaging applications to create a platform with which to further investigate the translational potential of hESCs and iPSCs.

**Conclusions**
Our study demonstrates a novel application of ZFN technology to the targeted genetic engineering of human pluripotent stem cells (PSCs) and their progeny for molecular imaging in vitro and in vivo.

**Endothelial Cells Derived From Nuclear Reprogramming [Review]; Wong et al**

**Abstract**
The endothelium plays a pivotal role in vascular homeostasis, regulating the tone of the vascular wall, and its interaction with circulating blood elements. Alterations in endothelial functions facilitate the infiltration of inflammatory cells and permit vascular smooth muscle proliferation and platelet aggregation. Therefore, endothelial dysfunction is an early event in disease processes including atherosclerosis, and because of its critical role in vascular health, the endothelium is worthy of the intense focus it has received. However, there are limitations to studying human endothelial function in vivo, or human vascular segments ex vivo. Thus, methods for endothelial cell (EC) culture have been developed and refined. Recently, methods to derive ECs from pluripotent cells have extended the scientific range of human EC studies. Pluripotent stem cells may be generated, expanded, and then differentiated into ECs for in vitro studies. Constructs for molecular imaging can also be employed to facilitate tracking these cells in vivo. Furthermore, one can generate patient-specific ECs to study the effects of genetic or epigenetic alterations on endothelial behavior. Finally, there is the opportunity to apply these cells for vascular therapy. This review focuses on the generation of ECs from stem cells; their characterization by genetic, histological, and functional studies; and their translational applications.

**Induction of Cardiomyocyte-Like Cells in Infarct Hearts by Gene Transfer of Gata4, Mef2c, and Tbx5; Inagawa et al**

**What Is Known?**
- Gata4, Mef2c, and Tbx5 (GMT) can reprogram cardiac fibroblasts from intact hearts into cardiomyocyte-like cells in vitro.
- Cellular reprogramming is a potentially useful strategy for regenerating damaged organs.
- A polycistronic vector is a useful system to express multiple genes from the same promoter.

**What New Information Does This Article Contribute?**
- GMT can convert fibroblasts in infarcted hearts into cardiomyocyte-like cells in vitro and vivo.
- Retrovirus-infected cells are reduced in infarcted myocardium by the immune response.
- Gene transfer of a polycistronic vector encoding GMT enhances cardiac differentiation in infarcted hearts.

**Conclusions**
GMT gene transfer induced cardiomyocyte-like cells in infarcted hearts.
Extracellular Matrix Promotes Highly Efficient Cardiac Differentiation of Human Pluripotent Stem Cells: The Matrix Sandwich Method; Zhang et al

What Is Known?
- Human pluripotent stem cells (PSCs), including human embryonic stem cells and induced pluripotent cells, are a promising source of human cardiomyocytes that can be used for basic research such as disease modeling, drug development, and, potentially, in clinical applications.
- Current protocols to generate cardiomyocytes from human PSCs are primarily based on the sequential application of key growth factors, but these protocols have had variable success and often require optimization for different human PSC lines.
- Extracellular matrix signaling plays critical roles in development, but the efficacy of manipulating extracellular matrix has not been tested in protocols for generation of cardiomyocytes from human PSCs.

What New Information Does This Article Contribute?
- Sandwiching human PSC cultures between layers of Matrigel, a commercially available extracellular matrix preparation, promotes an epithelial-to-mesenchymal transition in cell phenotype and thus initiates the differentiation process needed to form cardiomyocytes.
- The matrix sandwich protocol combines dynamic application of Matrigel with sequential applications of growth factors (Activin A, basic fibroblast growth factor, bone morphogenic protein 4) and results in efficient generation of functional cardiomyocytes with high purity (up to 98%) and high yield (up to 11 cardiomyocytes per input PSC) from multiple human PSC lines.

Conclusions
Dynamic extracellular matrix application promoted epithelial-mesenchymal transition of human PSCs and complemented growth factor signaling to enable robust cardiac differentiation.

Microfluidic Single-Cell Analysis Shows That Porcine Induced Pluripotent Stem Cell–Derived Endothelial Cells Improve Myocardial Function by Paracrine Activation; Gu et al

What Is Known?
- Induced pluripotent stem cells (iPSCs) have been created from adult somatic cells of many small and large animals.
- More recently, iPSCs have been derived from swine, an animal that shares a cardiovascular anatomy and physiology similar to humans.

What New Information Does This Article Contribute?
- We successfully created iPSCs from porcine adipose tissue.
- For the first time, we also injected the iPSCs into the myocardium in a porcine model of myocardial infarction and tracked their localization to peri-infarct area by multimodality MRI and PET/CT imaging.
- In addition, we successfully differentiated porcine iPSCs into endothelial cells (iPSC-ECs) in vitro and demonstrated that intramyocardial injection of iPSC-ECs improved cardiac function in a murine model of myocardial infarction.

Conclusions
In summary, this is the first study to successfully differentiate piPSCs-ECs from piPSCs and demonstrate that transplantation of piPSC-ECs improved cardiac function after myocardial infarction via paracrine activation. Further development of these large animal iPSC models will yield significant insights into their therapeutic potential and accelerate the clinical translation of autologous iPSC-based therapy.

Differentiation of Human Embryonic Stem Cells and Induced Pluripotent Stem Cells to Cardiomyocytes: A Methods Overview [Review]; Mummery et al

Abstract
Since human embryonic stem cells were first differentiated to beating cardiomyocytes a decade ago, interest in their potential applications has increased exponentially. This has been further enhanced over recent years by the discovery of methods to induce pluripotency in somatic cells, including those derived from patients with hereditary cardiac diseases. Human pluripotent stem cells have been among the most challenging cell types to grow stably in culture, but advances in reagent development now mean that most laboratories can expand both embryonic and induced pluripotent stem cells robustly using commercially available products. However, differentiation protocols have lagged behind and in many cases only produce the cell types required with low efficiency. Cardiomyocyte differentiation techniques were also initially inefficient and not readily transferable across cell lines, but there are now a number of more robust protocols available. Here, we review the basic biology underlying the differentiation of pluripotent cells to cardiac lineages and describe current state-of-the-art protocols, as well as ongoing refinements. This should provide a useful entry for laboratories new to this area to start their research. Ultimately, efficient and reliable differentiation methodologies are essential to generate desired cardiac lineages to realize the full promise of human pluripotent stem cells for biomedical research, drug development, and clinical applications.
What New Information Does This Article Contribute?

- Drugs targeting repressive epigenetic marks induce myogenic plasticity in EPCs.
- Epigenetic reprogramming upregulates genome-wide transcription, including cardiomyocyte-specific gene expression in EPCs.
- Reprogrammed EPCs are therapeutically superior to untreated cells, resulting in improved left ventricular function in an acute myocardial infarction model.
- Secretion of proangiogenic factors is enhanced in drug-treated EPCs.
- Drug-treated EPCs from both mouse and humans show cardiomyocyte differentiation potential in vivo.

Conclusions
Taken together, our results suggest that epigenetically reprogrammed EPCs display a safe, more plastic phenotype and improve postinfarct cardiac repair by both neocardiomyogenesis and neovascularization.

What New Information Does This Article Contribute?

- The efficiency of direct cardiac reprogramming by GMT overexpression in cardiac fibroblasts (CF) and tail tip fibroblasts (TTF) is very low.
- While GMT overexpression upregulates a subset of cardiac genes and alters the electrophysiological phenotype in fibroblasts, this phenotype does not resemble those of a bona fide cardiomyocyte.

Conclusions
Significant challenges remain in our ability to convert fibroblasts into cardiomyocyte-like cells and a greater understanding of cardiovascular epigenetics is needed to increase the translational potential of this strategy.

Critical Factors for Cardiac Reprogramming [Editorial]; Srivastava & Ieda

Extract
Cellular reprogramming achieved by somatic nuclear transfer or cell fusion has long been recognized. The potency of specific transcription factors as cell fate determinants was first demonstrated by the discovery of MyoD, a master regulator for skeletal muscle differentiation, and by the subsequent identification of several genes as lineage-converting transcription factors in blood cells. These pioneering works led to the landmark study by the Yamanaka laboratory that demonstrated the generation of induced pluripotent stem cells from fibroblasts by transducing four stem cell-enriched transcription factors, Oct4, Sox2, Klf4, and c-Myc. Numerous subsequent improvements in techniques and additional factors have increased the efficiency and robustness of the technology, and such enhancements continue, as do analyses of the similarities and differences of induced pluripotent stem cells to embryonic stem cells. Increasingly efficient differentiation protocols now permit us to make significant quantities of many individual cell types from induced pluripotent stem cells.

Labor Pains of New Technology: Direct Cardiac Reprogramming [Editorial]; Yoshida & Yamanaka

Extract
Chronic heart failure primarily of ischemic origin still remains a major cause of morbidity and mortality. Because postnatal cardiomyocytes have little regenerative capacity, the current therapeutic approaches for heart failure are limited. Therefore, a new strategy to improve cardiac function must be established. The generation of cardiomyocytes from pluripotent stem cells is a promising source of cardiomyocytes for regeneration therapy because of the robust proliferation and capacity for differentiation of pluripotent stem cells. Pluripotent stem cells from the patients can be generated from their somatic cells by introducing reprogramming factors. This reprogramming technology simultaneously opened up a new avenue to generate not only pluripotent stem cells but also somatic cells. Thus far, successful direct reprogramming into neurons, pancreatic β-cells, hepatocyte-like cells, and cardiomyocytes has been reported.
Simultaneous Voltage and Calcium Mapping of Genetically Purified Human Induced Pluripotent Stem Cell–Derived Cardiomyocyte Monolayers; Lee et al

What Is Known?
- Human-induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) represent a new model for personalized study of heart disease.
- Transmembrane voltage, intracellular calcium homeostasis, and impulse propagation are three key parameters of interest in the study of heart disease and drug effects; however, a simple and scalable imaging platform for simultaneously measurement all three parameters in macroscopic human iPSC-CM tissue constructs is needed.

What New Information Does This Article Contribute?
- Large electrically coupled human cardiac monolayers can be produced using genetically purified human iPSC-CMs, permitting the study of the mechanisms of arrhythmia in human cells.
- The results demonstrate the potential of the system for simultaneous mapping of the three key parameters, transmembrane voltage, intracellular calcium concentration, and excitation propagation, for studying cardiac physiology and pathology and for high-throughput drug testing.

Conclusions
The multiparametric imaging system presented here offers a scalable enabling technology to measure simultaneously action potential and intracellular calcium wave amplitude and dynamics of cardiac monolayers. The advent of large-scale production of human iPSC-CMs makes it possible to now generate sufficient numbers of uniform cardiac monolayers that can be utilized for the study of arrhythmia mechanisms and offers advantages over commonly used rodent models.

MicroRNA-Mediated In Vitro and In Vivo Direct Reprogramming of Cardiomyocytes: Jayawardena et al

What Is Known?
- Cardiac injury is characterized by inadequate regeneration and excessive fibrosis.
- Cellular reprogramming could potentially be used for cardiac regenerative therapy.
- Cardiac fibroblasts represent therapeutic targets for in vivo reprogramming into cardiomyocytes.

What New Information Does This Article Contribute?
- Distinct miRNAs are capable of converting fibroblasts to cardiomyocyte-like cells in vitro.
- JAK inhibitor I treatment enhances miRNA-mediated reprogramming to cardiomyocytes.
- Cardiac fibroblasts could be reprogrammed to cardiomyocytes.

- In vivo reprogramming of fibroblasts in the heart using miRNAs may yield a more straightforward method for cardiac regeneration.

Conclusions
The findings from this study provide proof-of-concept that miRNAs have the capability of directly converting fibroblasts to a cardiomyocyte-like phenotype in vitro. Also of significance is that this is the first report of direct cardiac reprogramming in vivo. Our approach may have broad and important implications for therapeutic tissue regeneration in general.

Direct Reprogramming for Cardiac Regeneration: From Dream to Reality [Editorial]; Bruneau

Extract
Direct reprogramming of endogenous fibroblasts into functional cardiomyocytes to regenerate the heart after myocardial infarction (MI) has long been a dream. A few years ago, this would have been considered by most to be science fiction. Certainly, the most optimistic scientists would probably categorize this as “highly unlikely to ever work.” And yet, 2 papers, including one in this issue of Circulation Research, have achieved this, using two distinct approaches. These landmark studies will hopefully pave the way for effective approaches to restoring cardiac function after cardiac injury.

Islet1 Derivatives in the Heart Are of Both Neural Crest and Second Heart Field Origin; Engleka et al

What Is Known?
- Within the developing heart field, Islet1 is postulated as a selective marker of cardiac progenitor cells derived from the second heart field.
- The specificity of Islet1 as a marker for second heart field is critical to lineage tracing, gene inactivation, and differentiation analyses.
- Islet1 derivatives include cells populating the outflow tract, an area patterned and formed by derivatives of the cardiac neural crest and second heart field.

What New Information Does This Article Contribute?
- A reporter mouse RC::FrePe allows identification of cells undergoing dual Flpe- and Cre-mediated recombination and sensitively indicates intersection between lineages marked by separate Flpe and Cre drivers.
- Islet1 is not restricted to second heart derivatives in the heart but is also expressed by a subset of cardiac neural crest cells.
- The intersectional population revealed by Wnt1::Flpe;Islet1Cre++;Rc::FrePe resides in the cardiac outflow tract and includes smooth muscle cells of the tunica media of the aorta and pulmonary artery.
- Dual fate mapping using an alternative second heart field driver, a Mef2c enhancer regulating Cre, does not overlap with neural crest.
Conclusions

Isl1 is not restricted to second heart field progenitors in the developing heart but also labels cardiac neural crest. The intersection of Isl1 and Wnt1 lineages within the heart provides a caveat to using Isl1 as an exclusive second heart field cardiac progenitor marker and suggests that some Isl1-expressing progenitor cells derived from embryos, embryonic stem cultures, or induced pluripotent stem cultures may be of neural crest lineage.

MicroRNAs and Stem Cells: Control of Pluripotency, Reprogramming, and Lineage Commitment [Review]; Heinrich & Dimmeler

Abstract

Stem cells hold great promise for regenerative medicine and the treatment of cardiovascular diseases. The mechanisms regulating self-renewal, pluripotency, and differentiation are not fully understood. MicroRNAs (miRs) are small noncoding RNAs controlling gene expression, either by inducing mRNA degradation or by blocking mRNA translation. The expression of miRs was shown to regulate various aspects of stem cell functions, including the maintenance and induction of pluripotency for reprogramming. In addition, some miRs control cell fate decisions. This review summarizes the role of miRs in reprogramming and embryonic stem cell self-renewal, and specifically addresses the regulation of cardiovascular cell fate decisions by miRs.

Optical Imaging of Voltage and Calcium in Cardiac Cells & Tissues [Review]; Herron, Lee & Jalife

Abstract

Cardiac optical mapping has proven to be a powerful technology for studying cardiovascular function and disease. The development and scientific impact of this methodology are well-documented. Because of its relevance in cardiac research, this imaging technology advances at a rapid pace. Here, we review technological and scientific developments during the past several years and look toward the future. First, we explore key components of a modern optical mapping set-up, focusing on: (1) new camera technologies; (2) powerful light-emitting diodes (from ultraviolet to red) for illumination; (3) improved optical filter technology; (4) new synthetic and optogenetic fluorescent probes; (5) optical mapping with motion and contraction; (6) new multiparametric optical mapping techniques; and (7) photon scattering effects in thick tissue preparations. We then look at recent optical mapping studies in single cells, cardiomyocyte monolayers, atria, and whole hearts. Finally, we briefly look into the possible future roles of optical mapping in the development of regenerative cardiac research, cardiac cell therapies, and molecular genetic advances.

Induction of Vascular Progenitor Cells From Endothelial Cells Stimulates Coronary Collateral Growth; Yin et al

What Is Known?

• Somatic cells (eg, rat fibroblasts) can be reprogrammed into induced pluripotent stem cells (iPSCs).

• Stem cells (eg, iPSCs, mesenchymal stem cells [MSC], cardiac stem cells [CSC]) are being used for many types of regenerative therapies.

• Induced pluripotent stem cells have tumorigenic potential and can form teratomas.

• Repetitive ischemia (RI) can induce coronary collateral growth.

What New Information Does This Article Contribute?

• Because of epigenetic memory, reprogramming of vascular endothelial cells (ECs) can produce induced vascular progenitor cells (iVPCs).

• Induced vascular progenitor cells remain committed to a vascular differentiation program, becoming smooth muscle or endothelium, but not cardiomyocytes.

• Partially reprogrammed iVPCs have a much lower risk of tumorigenesis and teratoma formation.

• Induced vascular progenitor cells better augment coronary collateral growth than do native ECs, iPSCs, or MSCs in a rat RI model.

Conclusions

We conclude that iVPCs, generated by partially reprogramming ECs, are an ideal cell type for cell-based therapy designed to stimulate coronary collateral growth.

Reprogrammed Endothelial Cells: Cell Therapy for Coronary Collateral Growth? [Editorial]; Faber

Extract

Myocardial infarction, ischemic stroke, and atherosclerosis of arteries supplying the heart, brain, and lower extremities are leading causes of morbidity and mortality. The abundance of native preexisting collateral vessels in tissues, and their anatomic lumen enlargement induced by arterial obstruction (remodeling or arteriogenesis) are major determinants of the severity of ischemic tissue injury caused by these diseases. Unfortunately, findings in animal studies showing that both of these determinants vary significantly among individuals because of differences in genetic background and environmental factors (eg, cardiovascular risk factors) are beginning to find corroboration in humans. Although efforts to increase collateral growth in ischemia using small molecules, proteins, and gene therapy have shown some effectiveness in experimental animals, patient trials for therapeutic angiogenesis have been largely disappointing.

Empowering Adult Stem Cells for Myocardial Regeneration [Review]; Mohsin et al

Abstract

Treatment strategies for heart failure remain a high priority for ongoing research due to the profound unmet need in clinical disease coupled with lack of significant translational progress. The underlying issue is the same whether the cause is acute damage, chronic stress from disease, or aging: progressive loss of functional cardiomyocytes and diminished hemodynamic output. To stave off cardiomyocyte losses, a number...
of strategic approaches have been embraced in recent years involving both molecular and cellular approaches to augment myocardial structure and performance. Resultant excitement surrounding regenerative medicine in the heart has been tempered by realizations that reparative processes in the heart are insufficient to restore damaged myocardium to normal functional capacity and that cellular cardiomyoplasty is hampered by poor survival, proliferation, engraftment, and differentiation of the donated population. To overcome these limitations, a combination of molecular and cellular approaches must be adopted involving use of genetic engineering to enhance resistance to cell death and increase regenerative capacity. This review highlights biological properties of approached to potentiate stem cell–mediated regeneration to promote enhanced myocardial regeneration, persistence of donated cells, and long-lasting tissue repair. Optimizing cell delivery and harnessing the power of survival signaling cascades for ex vivo genetic modification of stem cells before reintroduction into the patient will be critical to enhance the efficacy of cellular cardiomyoplasty. Once this goal is achieved, then cell-based therapy has great promise for treatment of heart failure to combat the loss of cardiac structure and function associated with acute damage, chronic disease, or aging.

**Epigenetic Modifications of Stem Cells: A Paradigm for the Control of Cardiac Progenitor Cells** [Review]; Zhou et al

**Abstract**

Stem cells of all types are characterized by the ability to self-renew and to differentiate. Multiple lines of evidence suggest that both maintenance of stemness and lineage commitment, including determination of the cardiomyogenic lineage, are tightly controlled by epigenetic mechanisms such as DNA methylation, histone modifications, and ATP-dependent chromatin remodeling. Epigenetic mechanisms are intrinsically reversible, interdependent, and highly dynamic in regulation of chromatin structure and specific gene transcription programs, thereby contributing to stem cell homeostasis. Here, we review the current understanding of epigenetic mechanisms involved in regulation of stem cell self-renewal and differentiation and in the control of cardiac progenitor cell commitment during heart development. Further progress in this area will help to decipher the epigenetic landscape in stem and progenitor cells and facilitate manipulation of stem cells for regenerative applications.

**Imaging: Guiding the Clinical Translation of Cardiac Stem Cell Therapy** [Review]; Nguyen et al

**Abstract**

Stem cells have been touted as the holy grail of medical therapy, with promises to regenerate cardiac tissue, but it appears the jury is still out on this novel therapy. Using advanced imaging technology, scientists have discovered that these cells do not survive nor engraft long-term. In addition, only marginal benefit has been observed in large-animal studies and human trials. However, all is not lost. Further application of advanced imaging technology will help scientists unravel the mysteries of stem cell therapy and address the clinical hurdles facing its routine implementation. In this review, we will discuss how advanced imaging technology will help investigators better define the optimal delivery method, improve survival and engraftment, and evaluate efficacy and safety. Insights gained from this review may direct the development of future preclinical investigations and clinical trials.

**Cardiomyocytes Obtained From Induced Pluripotent Stem Cells With Long-QT Syndrome 3 Recapitulate Typical Disease-Specific Features In Vitro; Malan et al**

**What Is Known?**

- The pathophysiological consequences of ion channel mutations that cause long-QT syndrome (LQTS) cannot be analyzed directly in cardiomyocytes from patients.
- Induced pluripotent stem (iPS) cells can be generated from skin biopsy samples of patients and differentiated into cardiomyocytes.

**What New Information Does This Article Contribute?**

- Disease-specific iPS cells can be generated from murine fibroblasts that carry a human mutation of the Na+ channel that causes LQTS 3.
- Cardiomyocytes can be differentiated in the culture dish from LQTS 3–specific iPS cells and show the known biological features of the cardiac Na+ channel mutation.
- Action potential durations of LQTS 3 cardiomyocytes were found to be prolonged at slow heart rates, which is the pathognomonic feature of LQTS 3.

**Conclusions**

We demonstrate that disease-specific iPS cell–derived cardiomyocytes from an LQTS 3 mouse model with a human mutation recapitulate the typical pathophysiological phenotype in vitro. Thus, this method is a powerful tool to investigate disease mechanisms in vitro and to perform patient-specific drug screening.

**Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Long QT Syndrome: Is Personalized Medicine Ready for Prime Time?** [Editorial]; Priori

**Extract**

The study by Malan et al in this issue of the *Circulation Research* presents elegant data about induced pluripotent stem cell (iPSC)-derived myocytes from a mouse model of long QT syndrome (LQTS) type 3. The study is an important contribution that adds to a highly innovative field that is trying to define the role of iPSC technology in the understanding of inherited arrhythmias. In this accompanying editorial, I provide an overview of the previous studies in the field, comment on the contribution of Malan et al, and conclude by discussing some of the challenges in the field.
Small-Molecule Inhibitors of the Wnt Pathway Potently Promote Cardiomyocytes From Human Embryonic Stem Cell–Derived Mesoderm; Willems et al44

What Is Known?
- Human embryonic stem cells (hESCs) show great promise as a source for generating myocardial cells for use in cell transplantation therapies.
- hESCs form cardiac myocytes if they are treated appropriately; however, the yield is typically low, because the mechanisms that drive hESC toward a cardiac myocyte phenotype are poorly understood.

What New Information Does This Article Contribute?
- We developed an hESC-based high-throughput small-molecule screen assay using a cardiac myocyte–specific fluorescent reporter to identify molecules that drive hESCs to cardiac myocytes.
- Small-molecule inhibitors of the Wnt signaling pathways were identified as potent inducers of cardiac myocytes. They increased cardiac myocyte yield dramatically over recombinant protein inhibitors. Many other pathway modulators were inactive. Thus, Wnt inhibition is uniquely important for directed cardiogenesis.

Conclusions
Pharmacological inhibition of Wnt signaling is sufficient to drive human mesoderm cells to form cardiomyocytes; this could yield novel tools for the benefit of pharmaceutical and clinical applications.

Reprogramming of Skeletal Myoblasts for Induction of Pluripotency for Tumor-Free Cardiomyogenesis in the Infarcted Heart; Ahmed et al45

What Is known?
- Committed skeletal muscle progenitor cells (myoblasts) can be reprogrammed into pluripotent stem cells that are similar to embryonic stem cells.
- Skeletal myoblast–derived induced pluripotent stem cells (SiPS) are potential candidates for the regeneration of ischemic myocardium.
- SiPS transplanted into the ischemic myocardium have been reported to differentiate into cells of all 3 germ layers, leading to tumor formation in the heart.

What New Information Does This Article Contribute?
- Predifferentiation of SiPS into developing cardiomyocytes before transplantation is important for regeneration of infarcted myocardium with minimal risk of myocardial tumor formation.
- Cardiac progenitors derived from SiPS are an excellent source of cells for cardiac regeneration. Transplantation of these cells was found to reduce infarct size and improve heart function in a mouse model of acute myocardial infarction.
- In addition to known pluripotency-related miRs such as Let-7 family miRs and 290 cluster miRs, this study also reveals that other miRs—such as 125b, 16, 199, 214, 20a, and 200a/b, which are tumor-suppressive miRs—are expressed in differentiated cardiomyocytes. These miRs can be used as tumorigenic biomarkers of iPS cells–derived progenitors.

Conclusions
Successful reprogramming was achieved in SMs with ES cell-like microRNA profile. Given the tumorigenic nature of SiPS, their predifferentiation into cardiomyocytes would be important for tumor-free cardiogenesis in the heart.

Growth of Engineered Human Myocardium With Mechanical Loading and Vascular Coculture; Tulloch et al46

What Is known?
- Cardiomyocytes can be cultured in monolayer or 3-dimensional matrices of synthetic or natural origin; interactions with endothelial cells are necessary for normal myocardial development.
- Human cardiomyocytes can be generated from human embryonic stem cells or reprogrammed “induced pluripotent stem cells” derived from differentiated tissues.
- Rat neonatal cardiomyocytes cultured in 3-dimensional collagen matrix respond to mechanical strain with organization and hypertrophy.

What New Information Does This Article Contribute?
- In a 3-dimensional bioengineered cardiac tissue generated with a type I collagen scaffold, human cardiomyocyte proliferation and vascular structure formation is promoted in vitro by coculture with vascular and stromal cell types.
- Mechanical stress conditioning promotes human cardiomyocyte proliferation, intercellular organization, matrix/scaffold organization, and cellular hypertrophy.
- This bioengineered human cardiac muscle is spontaneously contractile, responds appropriately to stretch with increased force of contraction, and can engraft onto the heart, where it is perfused through anastomosis of engineered human vascular networks to the host coronary circulation.

Conclusions
Our results indicate that both mechanical load and vascular cell coculture control cardiomyocyte proliferation, and that mechanical load further controls the hypertrophy and architecture of engineered human myocardium. Such constructs may be useful for studying human cardiac development as well as for regenerative therapy.
The Beat Goes On: Human Heart Muscle From Pluripotent Stem Cells [Editorial]; Eschenhagen47

Extract
In 2003, C. Zandonella reviewed in Nature how much was achieved of the 1999 promise to grow a functioning heart in the dish in a decade and what the perspectives were at that time. Today, more than a decade after its public prediction, the heart in the dish is still an unfulfilled dream, but repairing injured hearts with engineered myocardial tissue patches is a viable and increasingly realistic perspective in regenerative cardiology.

Derivation of Human Induced Pluripotent Stem Cells for Cardiovascular Disease Modeling [Review]; Narsinh et al48

Abstract
The successful derivation of human induced pluripotent stem cells (hiPSCs) by dedifferentiation of somatic cells offers significant potential to overcome obstacles in the field of cardiovascular disease. hiPSC derivatives offer incredible potential for new disease models and regenerative medicine therapies. However, many questions remain regarding the optimal starting materials and methods to enable safe, efficient derivation of hiPSCs suitable for clinical applications. Initial reprogramming experiments were carried out using lentiviral or retroviral gene delivery methods. More recently, various nonviral methods that avoid permanent and random transgene insertion have emerged as alternatives. These include transient DNA transfection using plasmids or minicircles, protein transduction, or RNA transfection. In addition, several small molecules have been found to significantly augment hiPSC derivation efficiency, allowing the use of a fewer number of genes during pluripotency induction. We review these various methods for the derivation of hiPSCs, focusing on their ultimate clinical applicability, with an emphasis on their potential for use as cardiovascular therapies and disease-modeling platforms.

Developmental and Regenerative Biology of Multipotent Cardiovascular Progenitor Cells [Review]; Sturzu & Wu49

Abstract
Our limited ability to improve the survival of patients with heart failure is attributable, in part, to the inability of the mammalian heart to meaningfully regenerate itself. The recent identification of distinct families of multipotent cardiovascular progenitor cells from endogenous, as well as exogenous, sources, such as embryonic and induced pluripotent stem cells, has raised much hope that therapeutic manipulation of these cells may lead to regression of many forms of cardiovascular disease. Although the exact source and cell type remains to be clarified, our greater understanding of the scientific underpinning behind developmental cardiovascular progenitor cell biology has helped to clarify the origin and properties of diverse cells with putative cardiogenic potential. In this review, we highlight recent advances in the understanding of cardiovascular progenitor cell biology from embryogenesis to adulthood and their implications for therapeutic cardiac regeneration. We believe that a detailed understanding of cardiogenesis will inform future applications of cardiovascular progenitor cells in heart failure therapy and regenerative medicine.

References


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