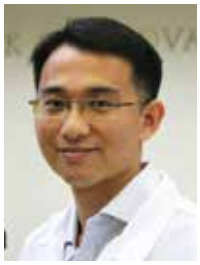
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INDUCED PLURIPOTENT STEM CELLS: HOW THEY WILL CHANGE THE PRACTICE OF CARDIOVASCULAR MEDICINE

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Abstract

Induced pluripotent stem cells (iPSCs) can be generated from adult somatic tissues by the forced expression of a few defined transcription factors, including Oct4, Sox2, Klf4, and c-Myc. iPSC technology holds tremendous promises for therapeutic cardiovascular regeneration because of the cells' unlimited capacity for proliferation and differentiation into all cell lineages. The iPSCs can be generated from somatic cells of patients with a genetic basis for their disease so as to understand the pathobiology of the disorder. This disease modeling can be adapted to high-throughput screens to discover new therapeutic molecules. Finally, the iPSC technology may enable personalized cell therapies, while avoiding the ethical concerns surrounding human embryonic stem cells. Intensive efforts are underway to develop reliable methods to guide stem cell differentiation into cardiovascular lineages in the treatment of peripheral artery disease and heart diseases. Studies of disease pathogenesis and drug discovery using iPSC technology shall advance the discovery of novel treatments for cardiovascular diseases.

Introduction

In 2012, the Nobel Prize in Medicine or Physiology was awarded jointly to Dr. Shinya Yamanaka and Sir John B. Gurdon "for the discovery that mature cells can be reprogrammed to become pluripotent." The work of these scientists has opened our eyes to the plasticity of cells and to the possibilities for true regeneration. As described below, the development of iPSCs will soon change the way we practice medicine.

Dr. Gurdon was the first to show that cell fate was fluid and that pluripotency could be restored in somatic cells. In 1962, he revealed that when the nucleus from a mature somatic cell (a frog intestinal cell) was placed into an enucleated egg cell, it could be reprogrammed. Specifically, the modified egg cell containing the nucleus from the frog intestinal cell developed into a normal tadpole. Thus, factors within the enucleated egg cell could act on the nucleus of the somatic cell, reprogramming its chromatin to express the genes required for pluripotency.

Four decades later, Dr. Yamanaka embarked upon a heroic project to determine which factors were responsible for pluripotency. He focused on transcriptional proteins that were known to be expressed by pluripotent stem cells in mice. Remarkably, he discovered that a small set of factors could reprogram mature cells to become pluripotent stem cells. Specifically, by overexpressing the genes encoding just four transcriptional proteins, he could induce mouse fibroblasts to become pluripotent stem cells. These iPSCs could generate any tissue in the mouse. In 2007, he showed that the same four genes, when overexpressed in human fibroblasts, could also generate iPSCs.

Dr. Yamanaka's generation of iPSCs galvanized the field of regenerative medicine and has led to intensive efforts to understand the mechanisms of nuclear reprogramming and to employ this technology to elucidate the pathobiology of disease, develop new therapies, and apply these cells for regenerative medicine. This review focuses on the use of iPSCs in cardiovascular medicine.

The Promise of iPSCs for Cardiovascular Medicine: a Comparison to Other Stem Cell Sources

A stem cell is defined by its capacity for both self renewal and directed differentiation. There are three broad categories of stem cells for application in regenerative medicine: iPSCs, embryonic stem cells (ESCs), and adult stem cells. The ESC is derived from the inner cell mass of the fetal blastula and is pluripotent, i.e., it is able to differentiate into any cell type of the adult body. ESCs can replicate via mitotic division while retaining their undifferentiated state (self-renewal) or differentiate into lineage-specific cells under the appropriate stimuli. ESCs can theoretically be used to create any tissue in the body for the purpose of regenerative medicine. Indeed, clinical trials are currently underway using these cells. However, a cell lineage or tissue created using ESCs will be immunologically distinct from the host, requiring immunosuppression. This concern may be partially addressed by creating banks of ESCs that would be matched for major histocompatibility antigens to recipients. However, ethical concerns have been voiced in using ESCs because they are derived from early human embryos.

By contrast, there are no such ethical concerns with the use of adult stem cells. These cells are multipotent rather than pluripotent; in other words, they partially lineage-committed, typically giving rise only to cells of a given germ layer. These multipotent cells are found in the bone marrow, the circulation, and within most tissues. Because they can be harvested from an

individual and expanded ex vivo, they do not need to overcome an immunologic barrier. Recent preclinical and clinical studies indicate that some common sources of stem cells—including hematopoietic stem cells, endothelial progenitor cells, cardiac stem cells, mesenchymal stem cells, or adipose stem cells—may reduce infarct size and improve cardiac contractile function in patients with myocardial infarction.[1-3] These beneficial effects are modest in humans and thought to be due largely to paracrine effects of secreted factors from the adult stem cells rather than the incorporation of the cells into the affected tissue.

In addition to the limited (if any) benefit of adult stem cell therapy, there are significant drawbacks compared to pluripotent stem cells such as ESCs or iPSCs. Adult stem cells are limited in their differentiation potential and replicative capacity; in other words, they can only give rise to a limited set of cells, and they have a limited number of population doublings before they senesce. Furthermore, in the case of autologous adult stem cells, the conditions that give rise to cardiovascular disease (e.g., hypercholesterolemia, aging, or tobacco exposure) are known to decrease their number and function. For this reason, allogeneic sources such as mesenchymal stromal cells derived from adipose or placental tissue are being employed for regenerative medicine. These allogeneic cells are easier to procure and amenable to tissue banking. Nevertheless, the limitations of adult stem/progenitor cells provide a rationale for deriving therapeutic cells from other sources.

A third type of stem cell that has remarkable potential for regenerative medicine is the iPSC. In 2006, Yamanaka and colleagues[4] reported that mouse fibroblasts could be reprogrammed into iPSCs by viral transduction of genes encoding four master regulators of pluripotency: octamer-binding transcription factor 3/4 (Oct 3/4) and SRY-related high-mobility-group (HMG) box protein-2 (Sox2), in combination with Krüppel-like factor 4 (Klf4) and c-Myc.[4] Successful reprogramming of adult human fibroblast cells into human iPSCs based on defined transcription factors has been reported independently by Yamanaka (Oct 3/4. Sox2, Klf4, c-Myc)[5] and James Thomson (Oct4, Sox2, Nanog, Lin28).[6] Human iPSCs are potentially better alternatives to human embryonic stem cells (hESCs) because they can be patient-specific and avoid the political and ethical dilemmas surrounding hESCs.[5, 6] Human iPSCs are already having a substantial impact on cardiovascular medicine, and their potential for regenerative cardiovascular therapies is promising.

How iPSCs are Changing Medicine: Their Use in Modeling Cardiovascular Diseases

Because somatic cells from any individual can now be induced into pluripotency, it is possible to make disease-specific cell lines from our patients. Thus, we can create "disease-in-a-dish" models with iPSC technology. Using iPSC-derived cardiovascular cells, investigators have already generated new insights into the molecular mechanisms of inherited cardiovascular diseases. Elegant studies have been reported using iPSC-derived cardiomyocytes from patients with long QT syndrome, [7, 8] LEOPARD syndrome,[9] and Timothy syndrome[10] in vitro. Exemplary of this approach is the work of the Dolmetsch group, which reprogrammed human skin fibroblasts from Timothy syndrome patients to generate human iPSCs and differentiated these cells into cardiomyocytes.

Electrophysiological recording and calcium imaging studies of the iPSC-derived cardiomyocytes revealed that the cells manifested irregular electrical activity and contraction, with abnormal calcium transients and prolonged action potentials. If the "disease-in-a-dish" model faithfully recapitulates the cardiovascular disease of the patient, then it may become a useful tool to determine if a drug has the potential to exacerbate the condition or to uncover new therapeutic avenues. In this regard, the Dolmetsch lab found that roscovitine restored the Ca^{2+} signaling and electrical properties of human-induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) from Timothy syndrome patients—a discovery that has led to a new therapeutic avenue for these patients and plans for a clinical trial. Thus, iPSCs provide new opportunities for drug screening and for studying the molecular and cellular mechanisms of cardiac pathophysiology in humans.

How iPSCs will Change Medicine Tomorrow: Their Use in Cardiovascular Regeneration

Following myocardial infarction, structural damage and functional impairment is often irreversible, and heart failure ensues.[11] The poor regenerative capacity of the human heart contributes to the difficulty in recovery from heart failure. Cardiomyocytes are terminally differentiated. Although there are resident cardiac stem cells in humans, their number and replicative capacity are limited. Heart transplantation is an option for severe heart failure but is limited by the availability of donors and the side effects of immunosuppressive agents.[12] The need for new approaches to rescue the failing heart provides a rationale for iPSC-derived therapeutic cells.

Numerous methods have been developed to enhance the efficiency of iPSC induction and to optimize their differentiation towards cardiac lineage. Experimentally, iPSCs have been shown to differentiate into each of the major cardiovascular components, including smooth muscle cells,[13] endothelial cells, vascular mural cells, and cardiomyocytes.[14, 15] Cardiovascular regeneration will require the effective generation of each of these cell types.

Our laboratory focuses on endothelial cells (ECs) as they are essential components in cardiovascular regeneration. Generally, approaches for differentiation of human or murine ESCs can also be applied in the differentiation of human iPSCs (Figure 1). Typically, we differentiate iPSCs to ECs using nonadhesive dishes to form embryoid body (EB) aggregates in endothelial growth media (with 5% fetal bovine serum, vascular endothelial growth factor 50 ng/mL).[16, 17] After 10 days, the EBs are added to gelatin-coated dishes. After 3 weeks of differentiation, the cells are dissociated and purified by fluorescence-activated cell sorting (FACS) using the EC markers VE-cadherin and CD31. Currently, our methodology yields 10% to 20% VE-Cad^{+}/$CD31^{+}$ cells that can be purified to between 75% and 90% with a second FACS. We have used noninvasive molecular imaging to document the survival of iPSCs-ECs injected into ischemic tissue in animal models of myocardial or limb ischemia. These cells incorporate into the microvasculature and improve tissue perfusion and organ function.[16, 17]

There remain substantial hurdles to overcome before iPSC-derived cardiovascular cells are ready for clinical trials. Currently, the methods to differentiate iPSCs into therapeutic cells are empirical, with combinations of growth factors, media, and matrices that have been optimized to favor the desired lineage. For cardiovascular regeneration, more robust selection markers and refined experimental protocols are required to reproducibly guide iPSCs to a cardiovascular lineage.[15, 18] Furthermore,

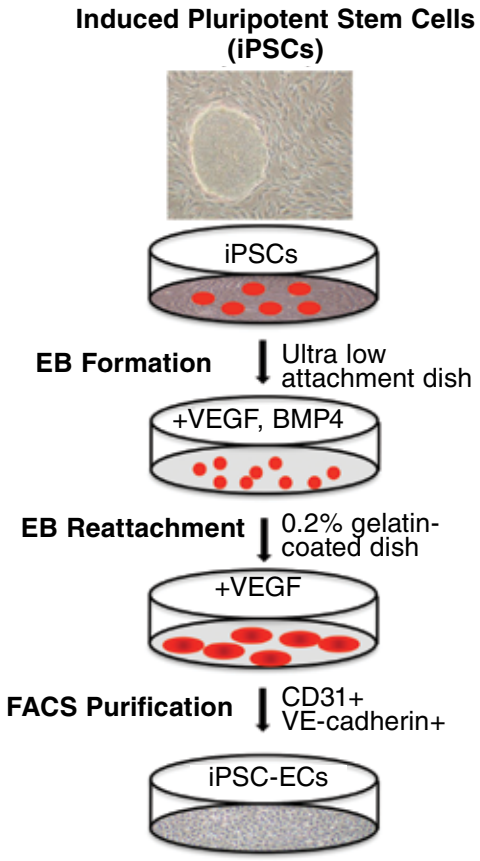

Figure 1. Differentiation of human induced pluripotent stem cells (iPSCs) into endothelial cells (ECs). Human iPSCs are grown on low adhesion dishes in the presence of vascular endothelial growth factor (VEGF) and bone morphogenetic protein-4 (BMP4) to induce the formation of embryoid bodies (EBs). Then EBs are cultured on 0.2 gelatin-coated dishes in differentiation media lacking BMP4. The pluripotent stem cell-derived ECs can be purified through fluorescence-activated cell sorting based on the expression of the EC markers CD31 and/or VE-cadherin.

effective negative selection against pluripotent cells is necessary to avoid teratoma formation by contaminating pluripotent stem cells.[19] There is also the concern that autologous iPSC-derived cells may acquire genetic or epigenetic alterations during the reprogramming or differentiation process and/or may recapitulate the vascular disease of the patient from which they were obtained.

However, great strides have been made in refining iPSC generation since Shinya Yamanaka first used a retroviral approach to overexpress the reprogramming factors. [4, 6, 20] Because this approach raised concerns regarding the integration of foreign DNA in the host genome, effective nonviral strategies for induction of pluripotency were developed. Our group has employed protein-based approaches to deliver reprogramming factors for generating iPSCs. In doing so, we have discovered the effect of innate immune activation in effective reprogramming, a finding that will lead to therapeutic ramifications.[20]

Conclusion

Induced pluripotent stem cells hold great promise for cardiovascular regeneration because of their unlimited capacity for proliferation and differentiation. iPSC technology already has enabled an exciting new approach for disease modeling and drug screening. Despite the great progress, the clinical use of iPSC technology is still in its infancy, and many technical hurdles remain. Ultimately, we and others intend to develop personalized cell therapies in the treatment of peripheral artery and heart diseases.[21, 22]

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Keywords: induced pluripotent stem cells, cardiovascular regeneration, disease modeling

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