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Review article

Induced pluripotent stem cells as a new strategy for cardiac regeneration and disease modeling☆

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ABSTRACT

The possibility to induce pluripotency in somatic cells or, even further, to induce cell transdifferentiation through the forced expression of reprogramming factors has offered new, attractive options for cardiovascular regenerative medicine. In fact, recent discoveries have demonstrated that induced pluripotent stem (iPS) cells can be differentiated into cardiomyocytes, suggesting that iPS cells have the potential to significantly advance future cardiac regenerative therapies. Herein, we provide an overview of the characteristics and differentiation potential associated with iPS cells. In addition, we discuss current methods for inducing their specification towards a cardiovascular phenotype as well as *in vivo* evidence supporting the therapeutic benefit of iPS-derived cardiac cells. Finally, we describe recent findings regarding the use of iPS-derived cells for modeling several genetic cardiac disorders, which have indicated that these pluripotent cells represent an ideal tool for drug testing and might contribute to the development of future personalized regenerative cell therapies.

Contents

1. Introduction

Cardiovascular disease is the leading cause of morbidity and mortality worldwide. It represents approximately 30% of all deaths, with nearly half resulting from myocardial infarction (MI) [1]. Fortunately, medical advances at the pharmacological, interventional, and surgical levels have significantly decreased the rate of mortality at the acute stage of the disease and have prolonged life expectancy. In spite of this remarkable progress, current treatment strategies have been unable to regenerate the diseased heart or provide a definitive cure. However, recent stem cell research has offered new hope that protective and regenerative therapies are possible (reviewed in [2]). Nevertheless, despite reports of successful *in vitro* differentiation of adult stem cells into cardiovascular

Abbreviations: BMPs, Bone morphogenetic proteins; CMs, Cardiomyocytes; CPCs, Cardiac progenitor cells; CPVT, Catecholamine polymorphic ventricular tachycardia; DMSO, Dimethylsulfoxide; ECs, Endothelial cells; END-2, Endoderm-like cell line; ESCs, Embryonic stem cells; FGF, Fibroblast growth factor; Flk-1, Fetal liver kinase-1; G-CSF, Granulocyte colony-stimulating factor; GSI, γ-Secretase inhibitor; GSK3, Glycogen synthase kinase-3; hESCs, Human embryonic stem cells; hiPS, Human induced pluripotent stem; iPS, Induced pluripotent stem; Isl1, Islet-1; JAK–STAT, Janus kinase–signal transducer and activator of transcription; LIF, Leukemia inhibitory factor; MI, Myocardial infarction; OSKM, Oct3/4, Klf-4, Sox-2, and c-Myc; SCF, Stem cell factor; SMCs, Smooth muscle cells; TGFβ, Transforming growth factor-β; VEGF, Vascular endothelial growth factor.

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lineages, only a few studies have demonstrated *in vivo* differentiation towards cardiomyocytes (CMs), particularly those derived from cardiac tissue [3,4]. It has been hypothesized that the principal mechanism of heart tissue regeneration involves replication and differentiation of cardiac progenitor cells (CPCs). The differentiation potential of CPCs to cardiac and vascular cells (*in vitro* and *in vivo*) has been demonstrated, along with their therapeutic potential following implantation into infarcted hearts of mice, which contributed to preservation of cardiac function and tissue viability [5–7]. However, despite these positive *in vivo* effects, CPCs were found to display limited potential for self-renewal and differentiation into CMs. Thus, their therapeutic benefits have been mainly attributed to release of complementary paracrine factors. Furthermore, the regenerative role of CPCs was recently challenged after pulse-chase studies demonstrated that pre-existing adult CMs were the dominant source of heart cell replacement during myocardial homeostasis and injury [8].

Although human embryonic stem cells (ESCs) have shown the greatest cardiac differentiation potential [9], their clinical use has been hampered by important limitations, including their potential tumorigenic and immunogenic properties as well as ethical issues related to their origin. For this reason, the discovery of so-called induced pluripotent stem (iPS) cells [10], which closely resemble ESCs and can be easily derived from adult cells, has provided an exciting alternative for bypassing these ethical and immunogenic concerns. In this review, we provide a detailed discussion of the origin, characteristics, and differentiation potential of iPS cells.

2. Discovery and derivation of iPS cells

The cell differentiation process was once believed to be irreversible. However, in 2006, the laboratory of Dr. Yamanaka reported the novel generation of embryonic stem-like cells from somatic cells (yielding iPS cells), a finding that resulted in a Nobel Prize in 2012 [10]. In order to produce iPS cells from adult mouse fibroblasts, cell de-differentiation was induced through retroviral transduction of several factors involved in pluripotency and self-renewal of ESCs. Initially, a total of 24 genes were selected and over-expressed in various combinations in order to identify those that might participate in cell reprogramming, which was ultimately found to depend on only four of the factors: Oct3/4, Klf-4, Sox-2, and c-Myc (OSKM). One year later, human iPS (hiPS) cells were also generated, using either the same combination of transcriptional regulators [11] or a different set of factors (Oct3/4, Sox-2, Nanog, and Lin-28) [12]. Following these discoveries, efforts to simplify the reprogramming process and to minimize the risk of chromosomal disruption revealed that a reduced set of reprogramming factors was sufficient to generate iPS cells (reviewed in [13]). Moreover, due to safety concerns surrounding spontaneous reactivation of viral transgenes or possible oncogene activation *via* lentiviral insertion, alternative iPS generation strategies have been developed and tested. These have involved the use of adenoviruses, RNA-based Sendai viruses, episomal vectors, DNA plasmids, excisable vectors, mRNAs, microRNAs, or even proteins (reviewed in [14]). Recently, the use of genetic factors, chemical inhibitors, and signaling molecules that can either replace core reprogramming factors or enhance reprogramming efficiency has also been investigated (reviewed in [15]).

Collectively, *in vitro* and *in vivo* studies have indicated that, like ESCs, iPS cells have the capacity to differentiate into cell types derived from any of the three germ layers. Indeed, this wide differentiation potential was confirmed through injection of iPS cells into either immunosuppressed mice to generate teratomas or into embryos to produce chimeras [16]. Also, the protocol developed for iPS cell generation has proved to be quite reproducible. Indeed, iPS cells with common pluripotent features have already been derived from many species, including humans, non-human primates, pigs, rats, and mice. However, like ESCs, these iPS cells can display differential phenotypes, morphologies, and/or culture requirements depending on the species of origin. For example, activation of the leukemia inhibitory factor–Janus kinase–signal transducer and activator of transcription (LIF–JAK–STAT) pathway is essential for self-renewal of ESCs and iPS cells derived from mice [17], with LIF [18] and bone morphogenetic proteins (BMPs) [19] necessary for cell culture and maintenance of pluripotency. In contrast, fibroblast growth factor-2 (FGF2) and Nodal/Activin signaling were found to be indispensable for human ESC and iPS cell maintenance [11,20]. Therefore, human iPS cell media is supplemented with FGF2 and/or Activin for efficient culturing of pluripotent stem cells [11,21]. In addition, several cell types have been successfully used to produce iPS cells (*e.g.*, adult β-pancreatic cells, neurons, keratinocytes, and hepatocytes; hematopoietic, neural, and adipose stem cells), demonstrating the striking plasticity of cells, which is independent of their origin and differentiation stage (reviewed in [13]).

3. Genetic and epigenetic profiles of iPS cells

Despite initial excitement regarding the ES-like features of iPS cells, deeper molecular analysis revealed differences between iPS cells and ESCs, mainly relating to aberrant gene expression [22]. In particular, incomplete silencing of somatic genes in reprogrammed cells, weak activation of ESC-specific pluripotency genes, and non-specific aberrations (distinct from either the cell of origin or ESCs) have been detected. Also, comparison of methylation marks in ESC and iPS cell genomes revealed significant variations. Similarly, consistent differences have been observed in miRNA expression patterns between human ESCs (hESCs) and hiPS cells [23]. Furthermore, two studies comparing global gene expression profiles in ESCs and iPS cells consistently identified the persistence of donor cell gene activation in iPS cells [24,25]. It has been suggested that this aberrant expression could lead to immune responses even after autologous transplantation. In fact, recent work from Zhao and collaborators demonstrated that ESCs derived from C57/B6 mice induced teratoma formation without any evidence of immune responses, whereas iPS cells derived from the same mouse strain failed to form teratomas due to rapid, T cell-dependent rejection. Moreover, global gene expression analyses of ESC- and iPS cell-derived teratomas revealed overexpression of teratoma-related genes in the iPS cells, which were responsible for the immune rejection [26]. However, these findings are now controversial after a recent study observed very limited immunogenicity of skin and bone marrow-derived iPS cells transplanted into mice. It was found that the immunogenicity of ten iPS cell clones was similar to that of seven different ESC clone-derived cells and that stable skin and bone marrow grafts derived from iPS cells formed without evidence of rejection [27].

4. Differentiation potential of iPS cells

Numerous protocols, most of them based on previous ESC techniques, have been used to differentiate iPS cells into diverse cell types *in vitro* (reviewed in [28]), demonstrating their pluripotency. Also, their *in vivo* differentiation potential has been confirmed in several animal models. For example, rat iPS cells were used to generate rat pancreas when injected into mouse blastocysts that were deficient in an essential gene required for pancreas development [29]. Additionally, when undifferentiated iPS cells were transplanted into an ischemic rodent heart, they efficiently differentiated into cardiac and vascular cells [30]. However, despite the proven pluripotency of iPS cells, differentiation patterns can be influenced by the origin and epigenetic characteristics of the derived iPS cells, thus leading to preferential differentiation into specific cell types. Although the molecular mechanisms for this phenomenon remain ill defined, it has been proposed that iPS cells can retain residual DNA methylation signatures characteristic of their somatic tissue of origin, which can favor their differentiation towards lineages related to the donor cell [31]. In an interesting study from Dr. Polo and colleagues, it was found that iPS cells generated from tail tip fibroblasts, splenic B cells, bone marrow-derived granulocytes, and skeletal muscle precursors exhibited gene expression