Application of iPS Cell Technology for Cardiac Disease Modeling and Repair

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Introduction
Since the advent of induced pluripotent stem (iPS) cell technology by Yamanaka and colleagues in 2006, demonstrating that mouse embryonic fibroblasts can be reprogrammed to an embryonic–like state by forced gene expression of Oct3/4, Sox2, c-Myc and Klf4 using retroviral vectors [1], several methods of generating iPS cells have been developed. In an important step forward, Yamanaka subsequently reported the generation of iPS cells from human somatic cells using the same factors, providing the stepping stone for human disease modeling in vitro and opening up the potential of cellular reprogramming for regenerative medicine [2]. The work was quickly validated by several groups, either using retroviral vectors with the original reprogramming factors on fetal, neonatal and adult human primary cells [3] or lentiviral vectors to demonstrate that Oct4, Sox2, Nanog and Lin28 are sufficient to reprogram human somatic cells [4]. Numerous protocols now exist for iPS cell generation and subsequent differentiation into diverse lineages, including cardiac cells. It will be important for the continued refinement and standardization of reprogramming techniques to focus on human therapeutic application, especially in the context of cardiovascular regenerative medicine.

iPS Cell Generation Techniques
While early work focused on generating iPS cells through genetic alterations and foreign transfer of DNA into target cells, several more recent methodologies were developed to remove potentially mutagenic molecules in their creation. Transient plasmid delivery through the use of non-integrating Epstein-Barr nuclear antigen-1 (EBNA1)-based episomal vector to reprogram human foreskin fibroblasts to pluripotency has been reported [5]. However, the oncogene SV40 large T was included in some combinations and the work was not demonstrated in adult cells, diminishing its clinical applicability [5]. Additionally, Yamanaka and colleagues recently reported the use of p53 suppression and non-transforming L-Myc to generate human iPS cells with episomal plasmid vectors at high efficiency [6]. However, transient p53 suppression may have unknown consequences on long-term cellular proliferation, survival and genomic stability. In developing a method that requires only a basic molecular biology background, minicircle vectors that are of eukaryotic origin were constructed to generate transgene free iPS cells from human adipose stem (hAS) cells [7]. Still, the reprogramming efficiency remained at a modest 0.005% using hAS cells and was ten-fold lower in neonatal fibroblasts [7]. A non-integrating Sendai virus-based method has also been reported as a safer way to establish iPS colonies; however, altering Sendai virus vectors can present as a technical hurdle for most applications [8]. Another method relied on the host-factor independent piggyBac transposon/transposese system to efficiently reprogram adult human fibroblasts to pluripotency and demonstrated the traceless removal of the reprogramming factors [9].

Working to address the pitfalls associated with viral integration methods, Rossi and colleagues utilized synthetic modified mRNA to repetitively transfect target cells with the reprogramming factors, giving rise to ES-like colonies by day 16 and at a 36-fold greater efficiency compared to retroviral methods [10]. Nonetheless, this relied on a technically complex protocol that required many rounds of treatment, which makes reproducibility more difficult. Protein based methods have successfully been used to generate stable iPS cells by fusing the original reprogramming factors to cell penetrating peptides, which eliminates the inherent risks of virus-based reprogramming [11]. Nevertheless, this method is marked by several shortcomings; namely, the significantly reduced reprogramming efficiency of 0.001% versus 0.01% with viral based methods, the doubled time of iPS colony formation of 8 weeks versus 4 weeks and a challenging and cumbersome protocol [11,12]. In moving towards the development of an efficient and robust system for generating transgene-free iPS cells from adult donors, a single excisable lentiviral cassette containing all four reprogramming factors flanked by loxP sites (hSTEMCCA-loxP) was created by Kotton and colleagues [13]. This accessible protocol achieved up to ~1% reprogramming efficiency in >100 lung-disease specific cell lines. Additionally, Cre/loxP technology makes excision of transgenes relatively simple. While representing a reliable platform for generating stable iPS cell lines, there remains a small theoretical risk of insertional mutagenesis by the inactive viral LTR left in the host genome after transgene excision [13]. The development of transgene-free iPS cells is an important advance in the rapidly growing field; however, methodologies will have to continue to be developed to ensure the consistent, efficient production of iPS cells with no genetic abnormalities.

Cardiac Differentiation of Human Pluripotent Stem Cells
Several exciting strategies have emerged over the past several years for the generation of cardiovascular progenitors from human embryonic stem (hES) cells and iPS cells, which facilitates our understanding of cardiac disease development and holds great promise.

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for therapeutic application. Spontaneous cardiac differentiation of human iPS cells from embryoid bodies has been reported and functional characterization of cardiomyocytes derived in this manner has revealed their cardiac-specific structural, molecular and functional properties, including electrophysiological and drug regulation hallmarks [14,15]. Other studies have implicated the role of many growth factors in the differentiation of hES cells into cardiomyocytes, including: basic fibroblast growth factor (bFGF), bone morphogenic protein 4 (BMP-4), vascular endothelial growth factor (VEGF) and activin A [16–19]. However, the efficiency of spontaneous differentiation of hES cells is low at less than 1% [20] and the use of multiple growth factors can be quite expensive. This motivated the findings that early treatment of hES and iPS cells with BMP-4, at specific concentrations [19], followed by late Wnt inhibition by small molecules can efficiently generate functional cardiomyocytes [18]. Therefore, it is highly important to optimize cardiac lineage development in an efficient, stage-specific and standardized manner.

Building upon these fundamental findings of the cardiogenic potential of iPS cells, their application for patient-specific cardiac disease modeling represents the next step. Particularly, iPS cells from patients with long-QT syndrome (LQTS) type 1 and type 2, a heritable disease characterized by prolonged ventricular repolarization leading to increased chance of death from cardiac arrest due to ventricular tachycardia, have been derived [21,22]. Cardiomyocytes derived from the iPS cells of LQTS type 1 patients showed molecular characteristics of the disease, including prolonged action potentials and diminished I\(_\text{K1}\) currents, owing to an R190Q-KCNQ1 mutation [21]. Disease modeling with iPS cells has also revealed that the phenotype of cardiomyocytes from LQTS type 1 patients is different from those of Timothy syndrome patients with regards to ventricular and atrial cardiomyocyte action potentials [23]. Overall, the use of diseased-patient iPS cells has set the stage for significant discoveries to be made regarding the molecular causes of disease and potential therapeutic treatments [21–23]. Similar insights have been made into LEOPARD syndrome and its associated phenotype, hypertrophic cardiomyopathy, using diseased-patient iPS cells [24].

The application of iPS cell technology for cardiovascular repair has also been tested in an in vivo model of acute myocardial infarction, which delivered iPS cells directly to the myocardium of nude mice to rescue cardiac performance [25]. Specifically, iPS intervention regenerated all three cardiac lineages, including endothelium, smooth muscle and myocyte, and improved ventricular wall thickness, contractile function, and electrical activity, while reducing fibrotic scar tissue [25,26].

**Challenges and Future Outlook for Clinical Application**

The first challenge will be the development of a reproducible, quantifiable and efficient system for generation of hES and iPS cell-derived cardiac cells. Another challenge will be the demonstration of new cardiac muscle formation in vivo using iPS-derived cells and the ability to heal scar tissue after myocardial infarction using this therapy. Further studies pertaining to the epigenetic memory of the tissue of origin that is present in iPS cells [27] will also be needed prior to any type of clinical application. Another important outlook will be the use of FDA approved small molecules to improve cardiomyocyte function, which will facilitate their function in patients. Additionally, direct reprogramming of cardiac fibroblasts into cardiomyocytes may have important therapeutic implications, but will need to be tested further [28]. Finally, the use of three-dimensional models and tissue engineering will be extremely important to recapitulate and mimic in vivo physiology in disease modeling and drug development.

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