Trends in Cardiovascular Medicine 24 (2014) 32–44

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Review article

Modeling heart disease in a dish: From somatic cells to disease-relevant cardiomyocytes

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ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form
7 June 2013
Accepted 10 June 2013
Available online 17 September 2013

ABSTRACT

A scientific milestone that has tremendously impacted the cardiac research field has been the discovery and establishment of human-induced pluripotent stem cells (hiPSC). Key to this discovery has been uncovering a viable path in generating human patient and disease-specific cardiac cells to dynamically model and study human cardiac diseases in an *in vitro* setting. Recent studies have demonstrated that hiPSC-derived cardiomyocytes can be used to model and recapitulate various known disease features in hearts of patient donors harboring genetic-based cardiac diseases. Experimental drugs have also been tested in this setting and shown to alleviate disease phenotypes in hiPSC-derived cardiomyocytes, further paving the way for therapeutic interventions for cardiac disease. Here, we review state-of-the-art methods to generate high-quality hiPSC and differentiate them towards cardiomyocytes as well as the full range of genetic-based cardiac diseases, which have been modeled using hiPSC. We also provide future perspectives on exploiting the potential of hiPSC to compliment existing studies and gain new insights into the mechanisms underlying cardiac disease.

Introduction

Despite important achievements over the last decade, heart disease remains the principal cause of death in developed societies. Intense research efforts have been directed at using human pluripotent stem cells (hiPSC) to invoke cardiac regeneration for heart repair and to model human cardiac development and diseases *in vitro* (Davis et al., 2011). hPSCs, which include both human-induced pluripotent stem cells (hiPSC) and embryonic stem cells (hESC), have the potential to differentiate into a variety of cell types, including cardiomyocytes. Seminal work using hESC demonstrated that by mimicking the cell-signaling environment during early stages of cardiogenesis, it is possible to differentiate hESC towards beating cardiomyocytes (Mummery et al., 2012). The contribution of hESC research to the cardiac field has been invaluable and their potential for addressing questions of a developmental nature is unmatched; however, their use for modeling human genetic-based diseases has certain limitations. hESC lines are typically isolated from the progeny of people who have no known genetic diseases, and as such genetic manipulation of the genome is required to introduce known mutations causative for specific diseases in order to emulate the genetic defect. In contrast, hiPSC offer unique advantages in that they are patient and disease specific, since they are generated through genetic reprogramming of an affected donor's cells (dermal skin fibroblasts (Takahashi et al., 2007), adipocytes (Sugii et al., 2010), nucleated blood

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1050-1738/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.tcm.2013.06.002

cells (Loh et al., 2009), and dental pulp (Yan et al., 2010)). In addition to carrying the genetic mutation of interest, these somatic cells can be isolated using relatively non-invasive procedures. Use of hiPSC also circumvents the ethical concerns associated with the use of cells from an embryonic origin. iPSC technology has been rapidly exploited by the biomedical research community as an invaluable tool to recapitulate human disease in a cell culture setting as a means to gain new understanding of underlying molecular mechanisms (Ebert et al., 2009; Israel et al., 2012; Marchetto et al., 2010; Soldner et al., 2009), and more recently the cardiac research field has begun to explore its potential (Davis et al., 2011; Hoekstra et al., 2012; Musunuru et al., 2010). To date, iPSC technology has been used to model several human genetic-based cardiac diseases and systemic diseases involving cardiac defects, which include LEOPARD Syndrome (Carvajal-Vergara et al., 2010), Long QT syndrome (Itzhaki et al., 2011; Lahti et al., 2012; Matsa et al., 2011; Moretti et al., 2010), Timothy Syndrome (Yazawa et al., 2011), Catecholaminergic Polymorphic Ventricular Tachycardia (Fatima et al., 2011; Itzhaki et al., 2012; Jung et al., 2012; Kujala et al., 2012), familial Dilated Cardiomyopathy (Sun et al., 2012), Arrhythmogenic Cardiomyopathy (Kim et al., 2013; Ma et al., 2012), as well as an overlapping syndrome of a cardiac Na^+ channel disease (Davis et al., 2012). Here, we discuss available methods to generate high-quality hiPSC and successfully differentiate them towards the cardiac lineage. We also review recent studies, which have modeled genetic-based cardiac diseases arising from structural, signaling, and electrophysiological abnormalities using iPSC technology, as well as provide perspectives on their contributions to the cardiac field.

iPSC generation

Classic hiPSC reprogramming methods as outlined by Yamanaka and colleagues consisted of retroviral-based gene delivery of oct4, sox2, klf4, and c-myc into human dermal fibroblasts, which triggered dramatic changes in cell morphology and behavior resembling hESC (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Validation of the pluripotent nature of hiPSC was demonstrated by their ability to express hallmark ESC-specific surface antigens and transcription factors typical of undifferentiated cells (Adewumi et al., 2007). In order to demonstrate the pluripotency of iPSC, several assays have been carried out, indicating their resemblance to hESC, which include their (i) ability to aggregate into spheroid cellular structures containing a mass of pluripotent cells resembling embryoid bodies (EB) (Takahashi et al., 2007), (ii) unguided, spontaneous differentiation of hiPSC in high serum into the three embryonic germ layers (endoderm, mesoderm, and ectoderm) (Takahashi et al., 2007; Takahashi and Yamanaka, 2006), (iii) subcutaneous implantation of hiPSC into mice (teratoma assays) resulted in tumors consisting of cell types and tissues originating from the three embryonic germ layers (Takahashi et al., 2007; Takahashi and Yamanaka, 2006), and (iv) karyotype and microarray analyses of hiPSC further demonstrated the chromosomal stability and genetic (transcript) resemblance of hiPSC to hESC, respectively (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Demethylation of pluripotency transcription factors (e.g., nanog) promoters in hiPSC also provided evidence of the epigenetic conversion of cells from a somatic to a pluripotent state (Takahashi et al., 2007). Modifications of the original method including the removal of c-myc (Li et al., 2010) and replacement of c-myc and klf4, with lin28 and nanog (Yu et al., 2007), also achieved similar results, with the possible advantage of reducing hiPSC's oncogenic potential. The production and validation of high-quality hiPSC is critical to disease modeling. Lesser quality cells (i.e., karyotypically abnormal or that are unable to differentiate properly) could produce artifactual phenotypes that are not related to the disease under study. Therefore, iPSC lines should be routinely taken through a panel of pluripotent-based assays to ensure their bona fide status. Additionally, standard approaches towards analyzing results from hiPSC have been to obtain results from at least three independent clones to rule out the possibility that the cell behavior observed was not due to random DNA insertion events. However, rising concerns towards their potential in vivo applications has prompted refinement of hiPSC generation strategies to allow for removal of transgenes after reprogramming, or to avoid integration altogether. These novel strategies could provide an appealing alternative to disease modeling and have been discussed in detail elsewhere (Li and Izpisua Belmonte, 2012). It could also shed light on whether reprogramming through newer methods (e.g., integration-free) is an important factor in producing cells that could qualitatively lead to better disease modeling, when compared to traditional methods.

Differentiation of iPSC towards the cardiac lineage

The most reproducible and efficient methods to differentiate hPSC to the cardiogenic path rely on defined and temporal manipulation of the Wnt (Wnt3a, Dickkopf-related protein (DKK)-1) and transforming growth factor (TGF)-β, (activin A, and bone morphogenetic protein (BMP)-4 and -2) signaling pathways (Kattman et al., 2011; Lian et al., 2012b; Paige et al., 2010; Zhang et al., 2012) (Fig. 1). These pathways are essential in the early steps of cardiac development (Davis et al., 2011) and must be utilized in a stage-specific manner to drive cardiac differentiation of hPSC (Mummery et al., 2012). The importance of these pathways is further highlighted as small molecules that inhibit specific downstream targets of these pathways can be used as an alternative to efficiently drive cardiac differentiation of hPSC (Hao et al., 2008; Kattman et al., 2011; Lian et al., 2012a; Minami et al., 2012; Willems et al., 2011) (Fig. 1). A number of cardiac differentiation protocols for hPSC are available, and their advantages and disadvantages have been discussed in greater detail elsewhere (Mummery et al., 2012). For simplicity, we broadly categorize these protocols into monolayer (Lian et al., 2012a; Paige et al., 2010; Zhang et al., 2012), embryoid body (Kattman et al., 2011; Kehat et al., 2001), and "co-culture" (Mummery et al., 2007) strategies, while providing details of efficiencies as well as chemically defined and undefined conditions to drive hPSC towards a cardiogenic path (Table 1). Key to the successful use of these approaches is the need to optimize