Advanced Drug Delivery Reviews 96 (2016) 3–17

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Human pluripotent stem cells: Prospects and challenges as a source of cardiomyocytes for in vitro modeling and cell-based cardiac repair☆

Matthew E. Hartman [a,b,c,d], Dao-Fu Dai [c,d,e], Michael A. Laflamme [c,d,e,*]

[a] Division of Cardiology, Department of Medicine, USA
[b] Department of Genome Sciences, USA
[c] Institute for Stem Cell and Regenerative Medicine, USA
[d] Center for Cardiovascular Biology, USA
[e] Department of Pathology, University of Washington, Seattle, WA, USA

ARTICLE INFO

Article history:
Received 5 March 2015
Accepted 7 May 2015
Available online 14 May 2015

Keywords:
Embryonic stem cells
Induced pluripotent stem cells
Cardiac regeneration
Cardiac repair
Heart failure
Cardiovascular disease
Drug discovery
Disease modeling

ABSTRACT

Human pluripotent stem cells (PSCs) represent an attractive source of cardiomyocytes with potential applications including disease modeling, drug discovery and safety screening, and novel cell-based cardiac therapies. Insights from embryology have contributed to the development of efficient, reliable methods capable of generating large quantities of human PSC-cardiomyocytes with cardiac purities ranging up to 90%. However, for human PSCs to meet their full potential, the field must identify methods to generate cardiomyocyte populations that are uniform in subtype (e.g. homogeneous ventricular cardiomyocytes) and have more mature structural and functional properties. For in vivo applications, cardiomyocyte production must be highly scalable and clinical grade, and we will need to overcome challenges including graft cell death, immune rejection, arrhythmogenesis, and tumorigenic potential. Here we discuss the types of human PSCs, commonly used methods to guide their differentiation into cardiomyocytes, the phenotype of the resultant cardiomyocytes, and the remaining obstacles to their successful translation.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Tissue engineering of the heart: from in vitro models to regenerative solutions".

* Corresponding author at: Center for Cardiovascular Biology, UW Medicine at South Lake Union, Box 358050, Brotman Building, Rm 442, 815 Mercer St. Seattle, WA 98109, USA. Tel.: +1 206 897 1518; fax: +1 206 897 1540.
E-mail address: laflamme@u.washington.edu (M.A. Laflamme).

http://dx.doi.org/10.1016/j.addr.2015.05.004
0169-409X/

1. Introduction

Human pluripotent stem cells (PSCs) have garnered a high degree of interest because of their potential use in applications ranging from basic research to novel cell-based therapies and tissue engineering. While the field has developed well-defined protocols for the expansion of human PSCs in the undifferentiated state as well as their subsequent differentiation into cardiomyocytes, we still need improved methods to derive specific cardiac subtypes (i.e. nodal versus ventricular cardiomyocytes) and to promote their maturation into a more adult-like phenotype. These improvements would most likely be applied first to in vitro applications for human PSC-derived cardiomyocytes, for example, drug discovery and cardiotoxicity screening as currently envisioned by the pharmaceutical industry and regulatory agencies [1–3]. Investigators are already using human PSC-derived cardiomyocytes as in vitro models of incompletely understood monogenic diseases, such as channelopathies and cardiomyopathies [4–9]. Moreover, if these cells are to advance to eventual clinical applications, a number of major hurdles must be overcome including graft cell death, immune rejection of the graft cells, and the risk of tumorigenesis. The heart is a particularly challenging target for human PSC-based therapies, at least if bona fide tissue regeneration ("remuscularization") is the goal, because the graft cardiomyocytes must undergo appropriate electromechanical integration and couple with host myocardium to contribute new force generating units without arrhythmias.

In this review, we will describe early progress in the field that led to the availability of human PSCs and human PSC-derived cardiomyocytes, the various approaches that have been pursued to generate populations of human PSC-derived cardiomyocytes at ever-improving purities and scales, our current understanding of the phenotype of these cardiomyocytes, and the challenges that must be overcome if human PSCs are to become a practical source of heart cells for in vitro modeling and in vivo cardiac regeneration.

2. Pluripotent stem cells: Definitions, origins, and early evidence for cardiac potential

In 1981, Martin reported the successful isolation and expansion of a PSC population from the inner cell mass of mouse blastocysts [10]. These cells were termed mouse embryonic stem cells (ESCs) to distinguish them from the previously described embryonal carcinoma cells, which also exhibit pluripotency (i.e. capacity to differentiate into cell types from all three embryonic germ layers). While mouse ESCs maintain their pluripotent phenotype when grown on a feeder layer of mouse embryonic fibroblasts (MEFs), Doetschman and colleagues found that, when removed from MEFs and placed into suspension culture with a high fraction of fetal bovine serum, mouse ESCs begin to differentiate and spontaneously form three-dimensional aggregates, termed embryoid bodies (EBs) [11]. These EBs contained differentiated cell types from all three embryonic germ layers, including a small fraction of spontaneously beating cardiomyocytes. When transplanted into syngeneic mice, these mouse ESCs formed teratomas that included cardiomyocytes among other cell types. In 1998, Thomson and colleagues reported the successful isolation of human ESCs from the inner cell mass of blastocysts that were left over from in vitro fertilization efforts [12]. As with their mouse ESC counterparts, human ESCs formed EBs when placed in suspension cultures in a high fraction of fetal bovine serum [13]. A subset of these EBs contracted rhythmically and expressed the cardiomyocyte marker α-cardiac actin. Kehat and colleagues more comprehensively phenotyped human ESC-derived cardiomyocytes obtained via the EB approach and showed that they expressed a variety of expected cardiac markers, including α- and β-myosin heavy chain, cardiac troponin I and T, myosin light chain (Mlc)-2a and 2v, atrial natriuretic factor, α-actinin, and the transcription factors Nkx2.5 and GATA4 [14]. Human ESC-derived cardiomyocytes also exhibited spontaneous electrical activity and intracellular calcium transients [14].

The National Institutes of Health Human Embryonic Stem Cell Registry currently lists 303 human ESC lines that are eligible for U.S. federal

Table 1
Comparison between two main pluripotent stem cell types as potential cardiomyocyte sources.

	ESCs	iPSCs
Derivation	Isolation from inner cell mass of blastocyst	Somatic cell reprogramming
Cardiac differentiation methods	Similar	Similar
Cardiac differentiation efficiency	Similar	Similar
Phenotype after cardiac differentiation	Similar	Similar
Relative advantages	Reprogramming not required (e.g. no use of viral vectors) Lack somatic epigenetic modifications Potentially more direct regulatory path for clinical use	No ethical issues with derivation Tissue specific derivation Particularly useful for modeling monogenic diseases Potentially useful for autologous cell therapies Possibly less immunogenic

ESCs: embryonic stem cells, iPSCs: induced pluripotent stem cells.