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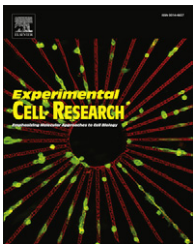

Cardiomyocytes derived from pluripotent stem cells: Progress and prospects from China

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ABSTRACT

Transplantation of embryonic stem cell- or induced pluripotent stem cell-derived cardiomyocytes (CMs) represents one promising approach for the treatment of myocardial infarction and failing hearts. Cardiac differentiation systems from these pluripotent stem cells (PSCs) can also be employed to better understand early developmental biology, drug discovery, toxicology testing, and disease modeling. A prerequisite to attain these goals is the ability to generate functional CMs in an efficient and reliable way. The lack of CM maturation must also be overcome, and appropriate methods for introducing PSC-CMs into heart while maintaining cell viability must be optimized. The past few years have seen major advances both in the differentiation, characterization and application of these cells to biological systems. Here we review recent progress, especially those performed in China, in basic stem cell biology involving studies of cardiogenesis and CMs through PSC differentiation, approaches for chamber-specific CM differentiation, maturation processes involving regulation of intracellular Ca^{2+} signals, and applications.

Contents

Introduction

Myocardial infarction (MI) and heart failure (HF) are the leading causes of cardiovascular morbidity and mortality in modern society. The incidence of these syndromes is rapidly increasing in the aging populations of Western and Chinese societies. In fact, over 10% of the very old will be afflicted with some form of heart diseases, and heart-related disease syndromes reach epidemic

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proportions in people > 80 years of age [1]. In the case of an MI, as many as one billion cardiomyocytes (CMs) are lost and the frequency of MIs dramatically increases with age. Because of the looming health care crisis in societies with rapidly aging populations, there is an urgent need to develop new therapeutic strategies aimed at myocardial regeneration through cell-based products or replacement of damaged tissues and scar tissue with new contractile cells. One possible source for these approaches is pluripotent stem cells (PSCs), which readily generate CMs *de novo* that can functionally couple with myocardial tissues.

PSCs, consisting principally of embryonic stem cells (ESCs) [2–5] and induced pluripotent stem cells (iPSCs) [6,7], retain the capacity for indefinite self-renewal and differentiation into cell and tissue derivatives from all three germ layers. These properties make PSCs valuable for studying early developmental biology, disease models, drug discovery, toxicology testing, and for regenerative medicine, including cardiac repair. Although beating CMs can be generated from PSCs, early attempts at differentiation were poorly reproducible, inefficient, and the yields relatively low. During the past decade, substantial progress has been made to understand the regulation of cardiac differentiation from PSCs and to develop strategies to efficiently and reliably direct PSC differentiation into cardiovascular lineages [8–12].

CM generation from *in vitro* differentiation of PSCs is now known to be a multistep process that involves initial epithelial to mesenchymal transitions, mesoderm specification and differentiation, cardiac specification and differentiation, and electrical maturation [13]. This well-organized process is tightly regulated by critical developmental signals, epigenetic controls, and extracellular microenvironments. Early cardiac differentiation of PSCs is regulated by BMP, TGFβ/activin/NODAL, WNT, and fibroblast growth factor (FGF), with highly specific temporal windows for effectiveness [13]. Factors including ascorbic acid also support CM differentiation. Differentiation approaches focusing on the induction of cardiac progenitor cells (CPCs) have also led to novel mechanistic insights [11,14,15].

The potential uses of human (h) PSC-derived cells are multifold [16]. These cells are clinically more relevant than animal cells. *In vitro* cultivated cells are amenable to detailed molecular, pharmacological, electrophysiological and functional analyses, which are invaluable for mechanistic insights into early cellular function. These cells can be employed to study diseases, including modeling of genetic-based cardiac diseases using iPSCs derived from patients [17], oxidative stress and ischemic preconditioning, and hypertrophy. Perhaps most importantly, PSC-derived CMs (PSC-CMs)represent a scalable cell source useful for cardiac myoplasty, regeneration and electrophysiological therapy. Despite these potential uses, a number of hurdles involving survival, immunogenicity, heterogeneity, maturation state, and electrophysiological safety must be resolved before these cells can be moved safely into clinic. While human (h) PSCs are therapeutically relevant, mouse (m) PSCs have often provided critical insights into the differentiation and function of these cells.

In this mini-review, we highlight studies in China that have expanded our understanding of PSC biology and differentiation to CMs. We will focus on selected topics of progress in cardiac differentiation, CPC induction, cardiac subtype specification, maturation of Ca^{2+} handling mechanisms as well as functional properties in differentiating PSC-CMs, and disease modeling.

PSC differentiation to CMs

Differentiation of PSCs occurs concomitant with reduced proliferation and augmented apoptosis [18]. In fact, approximately 30% of mESCs die by apoptosis within 3 days of the onset of differentiation [18,19]. This apoptotic response can be prevented by hsp27 accumulation and suppression of p38 mitogen-activated protein kinase (MAPK) [19]. Other reports suggest an important role of apoptosis in the regulation of cell lineage commitment and a possible role of Ca^{2+} signals in the regulation of apoptosis and specifically CM differentiation [18–20]. Using mESCs, Zhu et al. [21] showed that the induction of apoptosis through modulation of p53 by icariin contributes to CM differentiation. Icariin is thought to be the primary active component of *Epimedium* extracts found in traditional Chinese herbal medicine. Liang et al. [22] subsequently showed that apoptosis during early mESC differentiation is also regulated by Ca^{2+} signals released from type 3 inositol 1,4,5-trisphosphate receptors. These signals modulate CM differentiation through specific regulation of mesoendoderm lineage commitment. Suppression of apoptosis during initiation of rat (r) ESC differentiation was also found to be critical for establishment of stable *in vitro* differentiation protocol of rESCs to CMs [23]. rESC cultures require a combination of serum-free conditions and inhibition of MAPK and glycogen synthase kinase 3 or FGF. rESCs are capable of producing chimeras [4,5], but these cells proved extremely difficult to differentiate *in vitro*. By inhibiting apoptosis during early rESC differentiation with a Rho-associated kinase inhibitor Y-27632, we developed a stable *in vitro* differentiation system through which rESCs differentiate into three germ layers and generate functional CMs. These findings underscore the critical and generally overlooked role of apoptosis during ESC differentiation for cell lineage commitment or survival.

The findings that a co-culture system enhances cardiac differentiation highlight the unique role of the microenvironment and cell signaling during differentiation [24,25]. Recent studies from our group provide new evidence showing that enhanced collagen synthesis by ascorbic acid specifically promotes the proliferation of cardiac progenitor cells ($Nkx2.5^+$ CPCs) via the MEK-ERK1/2 pathway, and thus, leads to approximately 7.3-fold (miPSCs) and 30.2-fold (hiPSCs) increases in CMs [15]. Thus, it is an important way to generate CMs through intermediate stage during PSC differentiation. Ng et al. [26] demonstrated that apoA-I, the major protein component of high density lipoproteins, enhances cardiac differentiation of ESCs and iPSCs and promotes maturation of the calcium handling properties of ESC-CMs via the BMP4/SMAD signaling pathway. Over-expression of apoA-I resulted in a significantly higher percentage of beating embryoid bodies, an increased number of CMs, and expression of cardiac-restricted contractile protein RNAs. When recombinant apoA-I and BMP4 were added together, the percentage of beating embryoid bodies (EBs) was increased further, and functionally, CMs derived from the apoA-I-transduced cells exhibited improved calcium handling properties. Finally, thyroid hormone was also shown to promote cardiac differentiation of mESCs, and it enhanced electrophysiological maturation, as well as calcium homeostasis properties of ESC-CMs [27].

Electrophysiological studies demonstrate that PSCs from rat, mouse and human generate diverse types of CMs with traits