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Review article

Human embryonic stem cells for cardiomyogenesis

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Abstract

Myocardial cell replacement strategies are emerging as novel therapeutic paradigms for heart failure but are hampered by the paucity of sources for human cardiomyocytes. Human embryonic stem cells (hESC) are pluripotent stem cell lines derived from human blastocysts that can be propagated, in culture, in the undifferentiated state under special conditions and coaxed to differentiate into cell derivatives of all three germ layers, including cardiomyocytes. The current review describes the derivation and properties of the hESC lines and the different cardiomyocyte differentiation system established so far using these cells. Data regarding the structural, molecular, and functional properties of the hESC-derived cardiomyocytes is provided as well as description of the methods used to achieve cardiomyocyte enrichment and purification in this system. The possible applications of this unique differentiation system in several cardiovascular research and applied areas are discussed. Specific emphasis is put on the descriptions of the efforts performed to date to assess the feasibility of this emerging technology in the fields of cardiac cell replacement therapy and tissue engineering. Finally, the obstacles remaining on the road to clinical translation are described as well as the steps required to fully harness the potential of this new technology.

Keywords: Embryonic stem cells; Myogenesis; Tissue engineering; Cell therapy; Cardiomyocyte development

Contents

1. Introduction

The heart is one of the least regenerative organs and only a limited number of species (e.g. newts and zebrafish) are

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capable of significant myocardial renewal following injury. Consequentially, any major insult (due to ischemia, viral infection, or other pathologies) causing significant heart cell loss can result in the progression to irreversible heart failure. Congestive heart failure is a growing epidemic, and is already the most common cause of hospitalization in US citizens over 65 [1]. Moreover, due to the poor prognosis of patients with advanced heart failure and the shortage in donor organs for heart transplantation, a search for new therapeutic paradigms for heart failure has become imperative.

The recent advancements in stem cell biology, cell therapy, and tissue engineering have paved the way to the introduction of a new discipline in biomedicine, regenerative medicine. One of the goals of cardiovascular regenerative medicine is to develop cell replacement strategies to re-populate the scar tissue with new contractile cells, in an attempt to restore function in the failing heart. As reviewed recently [2–5], evidence from extensive animal experimentation suggest that transplantation of a wide array of different cell types can improve ventricular function following myocardial infarction.

Although originally proposed to regenerate the heart, it seems that several of these cell types, including skeletal myoblasts and hematopoietic and mesenchymal stem cells, probably do not transform to generate significant amounts of new myocardium [2–5]. This indicates that non-contractile mechanisms such as alteration of the infarct remodeling process, enhancement of angiogenesis, or augmentation of an endogenous repair mechanism probably underlie most of the benefit seen to date with cell transplantation [2–5]. This has led to the investigation of groups of cells that may have the promise for true re-muscularization of the infarcted heart. At present the two most intensely studied cell sources for this task are embryonic stem cells (which are the focus of this review) and resident cardiac progenitor cells [6].

2. Human embryonic stem cells

Embryonic stem cells (ESC) are pluripotent stem cell lines that have the potential to give rise to virtually every cell type in the body. Yet, unlike adult stem cells, these cells are not present in the adult organism. In fact, the cellular origin of the ESCs, the inner cell mass (ICM) cells, exist for only a short period in the developing mammalian embryo (Fig. 1). At this stage, the blastocyst is comprised of an outer layer of cells (the trophoectoderm) that will become supporting embryonal tissues and from the ICM cells that will give rise, through specialized progenitor cells, to all tissue types in the developing embryo. During the derivation of ESC, the trophectoderm layer is removed, the ICM cells are isolated, and plated on a mitotically inactivated mouse embryonic fibroblast (MEF) feeder layer where they form colonies, which are then selected, passaged, and expanded.

The derivation of mouse ESC (mESC) was first reported in 1981 [7,8] and during the next 25 years this technology has revolutionized developmental biology and the way we study gene function within the adult organism. It was not until 1998, however, that the derivation of similar human ESC (hESC) lines was first reported; initially by Thomson et al. [9] and later by Reubinoff et al. [10]. These human cell lines were derived from the ICM of human blastocysts, generated from IVF-produced embryos that were no longer designated for clinical use, and donated by individuals after informed consent.

Several lines of evidence confirmed the ESC-profile of the generated hESC lines. When cultured on a MEF feeder layer (or alternative conditions using human feeders [11], conditioned media, or cocktails of specific growth factors) the hESC could be propagated continuously in the undifferentiated state. The undifferentiated hESCs and their clonal derivatives [9,10,12] were shown to retain a normal diploid karyotype and to continue to display a high level of telomerase activity during long-term propagation in culture. Pluripotencey of the hESC was confirmed by injecting the undifferentiated cells into immunocompromised mice to produce teratomas containing differentiated derivatives of all three germ layers [9]. Pluripotency was also demonstrated *in-vitro*. When removed from the MEF feeder layer and cultivated in suspension, the hESC tend to spontaneously form three-dimensional differentiating cell clusters termed embryoid bodies (EBs), which contain cell derivatives of endoderm, mesoderm, and ectoderm origin [13].

The latter property has captured the imagination of both scientists, clinicians, and the lay public. If hESC can be coaxed to differentiate into specific cell lineages, then they may bring a unique value to several scientific fields such as developmental biology, functional genomics, patho-physiological studies, and drug screening and development. Furthermore, since many of the diseases that place the greatest burden on society (heart failure, neurodegenerative disorders, diabetes, etc.) result from cellular deficiency or dysfunction, having the ability to generate inexhaustible numbers of defined cell populations may allow the development of future cell replacement strategies for the treatment of these devastating disorders.

In this review we will focus on the cardiovascular applications of hESC research. Specific emphasis will be put on describing the strategies used to direct the differentiation of these cells into the cardiac lineage. This will be done in the context of insights gained throughout the years from several cardiac developmental models. We will next describe the structural and functional properties of the hESC-derived cardiomyocytes (hESC-CMs). Finally, current experimental evidence supporting the utility of these cells for cardiac repair will be described as well as the challenges remaining on the road to clinical utilization of these cells. The ethical and legal issues associated with hESC research will not be discussed in the current review but have received thoughtful coverage elsewhere [14–17].

3. Cardiomyocyte differentiation of ESC lines

The most common method used to initiate differentiation of mESC *in-vitro* requires their culturing in the absence of the self-renewal signals provided by MEF feeder layer or leukemia inhibitory factor (LIF), followed by cultivation in suspension to form EBs [18]. The EBs are then allowed to attach to a matrix