Chinese Journal of Physiology 51(4): 226-229, 2008

Review

Human ES Cell Derived Cardiomyocytes for Cell Replacement Therapy: A Current Update

Michael Xavier Doss, Agapios Sachinidis, and Jürgen Hescheler

Center of Physiology and Pathophysiology
Institute of Neurophysiology
University of Cologne
Robert Koch Str. 39
50931 Cologne, Germany

Abstract

Cardiovascular diseases are the leading cause of death globally. The pluripotency and indefinite proliferative capacity of embryonic stem (ES) cells make them a promising candidate for the cell replacement therapy where the damaged cells are replaced by the functional cells derived from stem cells *in vitro*. Emerging results with human ES cells for the myocardial repair are encouraging, but this approach is still in its infancy and is under extensive investigation. The daily upcoming experimental observations are reinforcing the solid hope that ES cells will be the potential source for use in cell replacement therapy. Although this demands serious considerations ethically and on practical applicability, the newly upcoming discoveries show that the adult human fibroblasts can be reprogrammed to embryonic stem cell like cells (called induced pluripotent stem cells) which can be used for cell replacement therapies. Remarkably, this obviates the need for the embryo destruction and overcomes related immunological problems which are the long time hurdles for the ES cell based cell replacement therapy. This review weighs the actual stand off in the human ES cells based cell replacement therapy for the treatment of cardiovascular degenerative diseases with a special emphasis on the hurdles and challenges to be resolved before the onset of clinical trials and the potential of the recently reported "induced pluripotent stem cells" for their use in cell replacement therapy.

Key Words: human embryonic stem cells, induced pluripotent stem cells, cell replacement therapy, cardiomyocytes, heart failure

Introduction

Cardiovascular diseases are the number one cause of death globally and are expected to remain the leading cause of death. Since adult cardiomyocytes have a very limited regenerative capacity, their loss permanently compromises myocardial contractile function. Heart failure is characterised by the loss of functional cardiomyocytes and thereby its inability to pump enough blood to maintain physiological functions. Heart transplantation is currently the last resort for end-stage heart failure but is hampered by a severe shortage of donor organs and immune rejection (13, 14). Cell replacement therapy is emerging as an innovative approach for the treatment of degenerative cardiac diseases, and embryonic stem cells (ESCs) appear to be an ideal source of cells for this paradigm. In particular, human embryonic stem cell (hESC)-derived cardiomyocytes theoretically fulfil most, if not all, of the properties of an ideal donor cell, but several critical obstacles need to be overcome (3, 7). This review enlists the progress made towards the embryonic stem (ES) cell derived cell replacement therapy to treat cardiovascular diseases and the obstacles to be resolved soon to improve the treatment of heart failure.

Corresponding author: Prof. Dr. Jürgen Hescheler, University of Cologne, Center of Physiology and Pathophysiology, Institute of Neurophysiology, Robert Koch Strasse. 39, Cologne, Germany. Tel: +49 221 478 6960, Fax: +49 221 478 3834, E-mail: J.Hescheler@uni-koeln.de
Received: February 12, 2008; Revised: May 26, 2008; Accepted: July 21, 2008.
 ISSN : 0304-4920. http://www.cps.org.tw

ES Cell-Derived Cardiomyocytes

Human ESCs, like mouse ESCs derived from the inner cell mass of blastocyst stage embryos, can be propagated continuously in the undifferentiated state when grown on top of a mouse embryonic fibroblast (MEF) feeder layer. When removed from these conditions and grown in suspension, they begin to generate three-dimensional differentiating cell aggregates, termed embryoid bodies (EBs), in which a variety of tissue types can be found. hESCs have also been shown to differentiate into the cardiac lineage (9, 18). Differentiation of human ESCs to the cardiac lineage creates a characteristic gene expression profile reminiscent of both murine ESC differentiation and the early stages of normal mouse heart development (1, 4, 6).

Advantages of ES Cell-Derived Cardiomyocytes Over Adult Stem Derived Cardiomyocytes

Four categories of stem cells have been examined for their ability to promote cardiac repair in animals. They are bone marrow derived/circulating progenitor cells and their subpopulations, skeletal myoblasts, resident cardiac stem cells and embryonic stem cells. The first three cell types are autologous or otherwise called adult stem cells. Partly for this reason, they have been the first to be used in clinical trials (19). These types of cells suffer from the major drawback that they are not an ideal renewable source of cardiomyocytes like the embryonic stem cells to meet the adequate number of cells required for implantation in the infarcted human heart to restore its function.

Functional / Physiological Integration of Human ES Cell-Derived Cardiomyocytes in the Host Tissue

hESC-derived cardiac clusters consist of a mixture of cell types (*e.g*. atrial, ventricular, nodal, and pacemaker cells) in various developmental stages (11, 12). Transplantation of *ex vivo*-differentiated human embryonic stem cell-derived cardiomyocytes (hESC-CMs) in the immunosupressed rat infarct model showed no formation of teratoma-like structures, the phenomenon exhibited by transplantation of undifferentiated hESCs. The grafted cardiomyocytes survived, proliferated, and integrated with host cardiac tissue (2, 12) .

Progress to Overcome Immune Rejection and Related Problems

It has been reported that human ESC derived cardiomyocytes do express MHC class I molecule although at low levels and expression increased upon differentiation *in vitro* (5). Hence, these cells will certainly evoke an immune response in the host upon transplantation. The recent breakthrough experiments show that adult human dermal fibroblasts and two other human dermal fibroblast populations (from synovial tissue and neonatal foreskin) from different human donors can be reprogrammed into induced pluripotent stem (iPS) cells by transducing them with retroviral vectors carrying human Oct4, Sox2, Klf4 and c-Myc and culturing them under human ES cell culture conditions (10, 15, 16, 17, 21). This raises the hope that someday, patient-specific iPS cells will overcome the problem of immune rejection of transplanted cardiomyocytes, and this will revolutionise the ESC-based cell replacement therapy (Fig. 1).

Quality and Quantity of the ES Cell-Derived Cardiomyocytes- A Major Concern

A major critical concern with respect to the clinical applicability is the purity and adequate quantity of human ESC-derived cardiomyocytes to improve the prognosis of heart failure. The routinely used differentiation protocols give rise to a mixture of different cell types apart from cardiomyocytes. Implantation of undifferentiated ES cells leads to formation of benign teratoma in the host (2). This demands a pure population of terminally differentiated cell phenotype. To harvest a single population of cardiomyocytes alone, several strategies have been developed for murine ESCs as follows. Magnetic bead tagged antibodies directed against the unique cell surface marker of the desired cell population can be directed and the desired population can be harvested by running through a magnetic column where the magnet tagged antibodies bound with the desired cell population will be retained, allowing other cell population to be washed out. Fluorescence-labelled antibodies raised against unique cell surface markers of the desired cell population can be lifted up by fluorescence-activated cell sorting (FACS). Some protocols employ selective culture conditions that promote the growth of one particular cell type at the expense of the other. ES cells may be genetically engineered to have selection markers or fluorescent markers under the control of a cardiomyocyte-specific promoter, for example Troponin T that is switched on during cardiomyogenesis. When the tissue/lineage specific promoter is activated during the particular lineage differentiation, the selection marker or the fluorescent marker will be expressed under the control of this promoter. Expression of selection markers (antibiotic resistance genes) makes the desired population resistant to that particular antibiotic when applied to kill the other cell population. These antibiotics block the endogenous translation or transcription machineries if cells do not express the particular enzyme/ protein

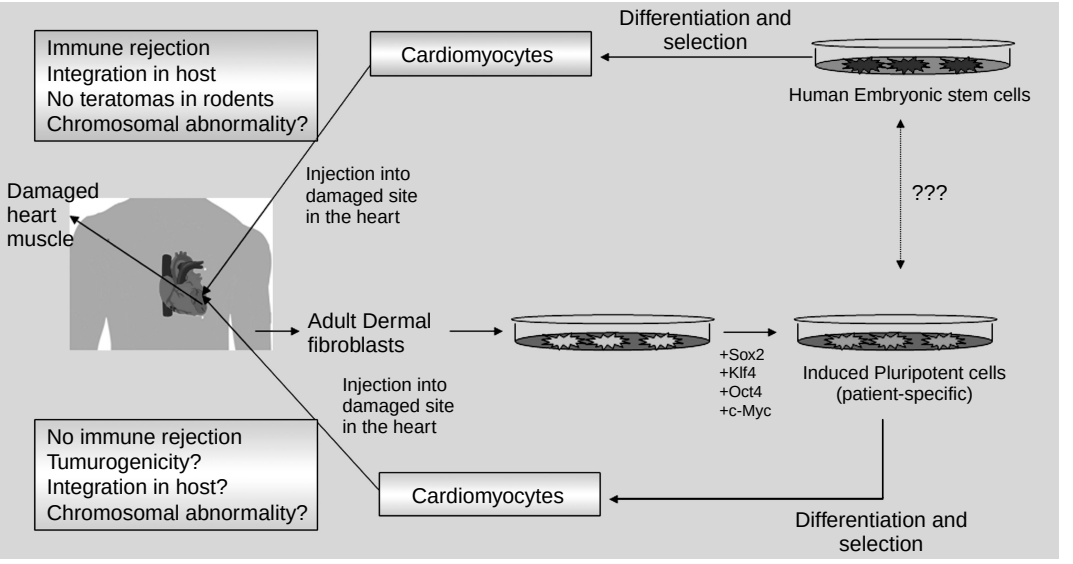

Fig. 1. Overview of challenges and hurdles to be overcome before the start of the clinical trials to treat patients with degenerative heart disease with cell replacement therapy with human ES / induced pluripotent stem cell derived cardiomyocytes.

which antagonizes the blocking activity of these antibiotics. Expression of the fluorescent marker allows the desired cell phenotype to be harvested by FACS. The drawback of these two approaches is that the genetically manipulated ES cell derivatives might be immunologically rejected since they express the foreign proteins – the selection and the fluorescent markers (3)

However, till now only a few suitable cell surface protein-antibody combinations have been identified for cardiomyocytes (19). Recently, only one report describes genetically marked human ESC-derived cardiomyocytes using lentiviral vectors (8). Even if available for experimental use, these genetically marked cardiomyocytes would be unlikely to be clinically acceptable due to the perceived risk associated with genetic manipulation. The only clinically suitable enrichment method described to date for human ES cell-derived cardiomyocytes used Percoll gradient purification, although other groups have found this difficult to reproduce (20).

Safety Issues

The major concern about the use of human ESC derived cardiomyocytes is the contamination by xenogens. The human ESCs need be cultivated on mouse embryonic fibroblasts and with sera obtained from animals like calf and bovine. This poses a major health threat to the human host due to the possible exposure to mouse retroviruses and other harmful substances. So, culture of human ESCs in serum free and feeder (xeno) free conditions is inevitable. Besides, the human ESCs during long time cultures *in vitro* develop chromosomal abnormalities and safety measures need to be formulated to avoid this situation and to abolish the chromosomally aberrant cells, if any, upon transplantation.

Conclusion and Future Perspectives

The uses of human embryos, however, face ethical controversies that hinder the applications of human ES cells. Now it seems to be possible that iPS cells, *i.e*. pluripotent, patient specific human embryonic stem cell-like cells can be obtained from adult fibroblasts without destroying any human embryo. Moreover, the cardiomyocytes obtained from this cell type are patient specific and hence immunological complications will be totally avoided. Still, this approach needs further extensive investigation before its clinical trials with respect to the reproducibility of the reprogramming approach with every individual patient, tumorigenicity, specific xeno- and serum-free culture conditions needed for the efficient self renewal and differentiation of the induced pluripotent stem cells into healthier and functional cardiomyocytes, their purity and the yield (Fig. 1). Once the above-mentioned obstacles have been removed, the ES cell-derived cell replacement therapy for the treatment of heart failure will be a new method for the patients in the near future.

Acknowledgment

We thank Dr. Johannes Winkler for his critical reading of this review and his valuable comments.

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