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The human embryonic stem cell-derived cardiomyocyte as a pharmacological model

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Abstract

Embryonic stem (ES) cells are specialised cells derived from the early embryo, which are capable of both sustained propagation in the undifferentiated state as well as subsequent differentiation into the majority of cell lineages. Human ES cells are being developed for clinical tissue repair, but a number of problems must be addressed before this becomes a reality. However, they also have potential for translational benefit through its use as a test system for screening pharmaceutical compounds. In the cardiac field, present model systems are not ideal for either screening or basic pharmacological/physiological studies. Cardiomyocytes produced from human ES differentiation have advantages for these purposes over the primary isolated cells or the small number of cell lines available. This review describes the methodology for obtaining cardiomyocytes from human embryonic stem cell-derived cardiomyocyte (hESCM), for increasing the proportion of cardiomyocytes in the preparation and for isolating single embryonic stem cell-derived cardiomyocyte (ESCM) from clusters. Their morphological, contractile and electrophysiological characteristics are compared to mature and immature primary cardiomyocytes. The advantages and disadvantages of the hESCM preparation for long term culture and genetic manipulation are described. Basic pharmacological studies on adrenoceptors and muscarinic receptors in hESCM have been performed, and have given stable and reproducible responses. Prolongation of repolarisation can be detected using hESCM cultured on multielectrode arrays (MEA). Human ESCM have a clear potential to improve model systems available for both basic scientific studies and pharmaceutical screening of cardiac target compounds.

Keywords: Embryonic stem cell; Cardiomyocyte; Screening; Differentiation

Abbreviations: βAR, β-adrenoceptor; EB, embryoid body; ES cell, embryonic stem cell; ESCM, embryonic stem cell-derived cardiomyocyte; FLPR, Fluorescence Lifetime Plate Reader; hESCM, human embryonic stem cell-derived cardiomyocyte; MEA, multi-electrode array; MHC, cardiac myosin heavy chain.

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1. Introduction

Embryonic stem (ES) cells are highly newsworthy at the moment, because of their obvious potential for tissue repair, coupled with their contentious origin. Their potential may have a tendency to be overstated, as the pro- and anti-camps become more polarized (Scolding, 2005) but it is nevertheless true that these cell lines can proliferate almost indefinitely in the undifferentiated state and then differentiate, when conditions are modified, into the majority of cell lineages. Advantages of these properties for generation of large quantities of tissue are evident. There are clearly problems to overcome before ES cell-derived tissue can be applied to human disease, in terms of immunogenicity; the production of teratomas; the xenogenic effects of culture using animal products and the ethical problems arising from the destruction of an embryo. Inroads are being made into each of these areas, including the ethical objections, by a concerted experimental effort from the scientific community. However, there are uses for ES-derived cells which are distinct from tissue repair, and which may be more immediately valuable. They are renewable and reproducible model system which can provide cells of almost any lineage for experimental purposes. Uses include investigation of development, basic physiological or pharmacological studies, and genetic manipulation. As well as representing a new tool for the basic researcher, ES cells and their derivatives have characteristics which make them well suited to high-throughput methods to screen for efficacy or safety in the pharmaceutical industry. In this review, we will consider the use of human embryonic stem cell-derived cardiomyocytes (hESCM) in relation to cardiac physiology and pharmacology, an area in which new models and new therapeutic paradigms are particularly needed.

1.1. The isolated cardiomyocyte as a tool for the cardiac researcher

The primary isolated cardiomyocyte has done sterling service as an experimental tool in cardiac research. Isolation methods for adult ventricular and atrial myocytes can be temperamental, but viable contracting cardiomyocytes have been routinely prepared from most experimental species including frog (Fischmeister & Shrier, 1989), mouse (Gong et al., 2000), rat (Powell et al., 1980), guinea-pig (Belevych et al., 2001), rabbit (Delbridge et al., 1996), ferret (Boyett et al., 1988), cat (Bailey et al., 1997), dog (Ravens et al., 1996), sheep (Kim et al., 2002) and pig (Louch et al., 2004). Importantly, they can also be obtained from human atrial (Harding et al., 1990) or ventricular (Brown et al., 1990; Beuckelmann et al., 1993; Houser et al., 2002) tissue, using myocardial samples as small as 40 mg (Davies et al., 1995a; Peeters et al., 1995). Their primary advantage is the exquisitely preserved function and morphology (Fig. 1**A**) (Slade et al., 1985), which has in turn encouraged the development of sophisticated measurement systems to take advantage of their properties. Contraction is stimulated by external electrical pacing, except in the specialised case of pacemaker myocytes isolated from nodal tissue, and can be maintained for some hours in a superfusion bath. Quantification of contraction is most usually done by tracking of the cell borders (Harding et al., 1988) or inter-sarcomere distance (Niederbichler et al., 2006) using optical methods, and is expressed as relative shortening as the unloaded cardiomyocyte contracts with each beat. Methods to measure force of contraction in loaded cardiomyocytes are more technically challenging, and commonly use carbon fibres to adhere to the cell, stretch it to the required sarcomere distance and sense the contractile force generated with each contraction

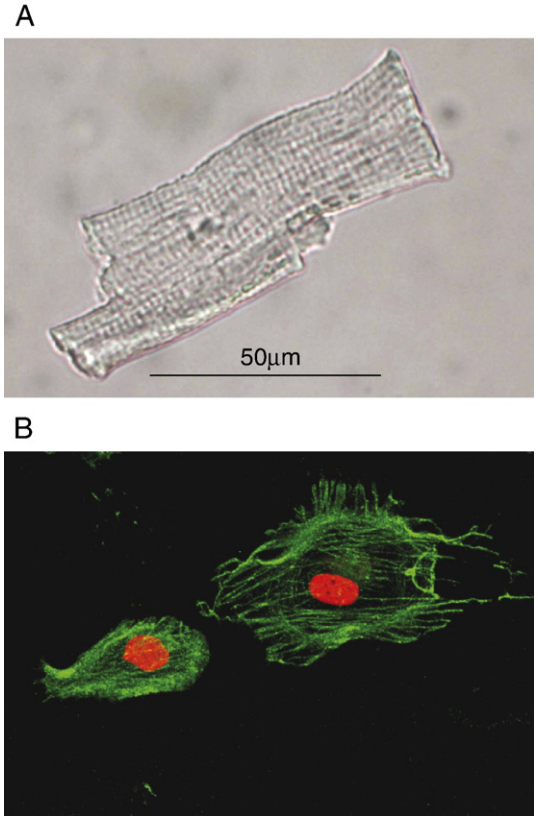

Fig. 1. (**A**) Adult ventricular cardiomyocyte from failing human heart. (**B**) Isolated hESCM stained with propridium iodide and antibodies for MHC, H7 line 18 days after induction of differentiation.