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Functional and developmental properties of human embryonic stem cells–derived cardiomyocytes ☆

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Abstract Cardiovascular diseases are the most frequent cause of death in the industrialized world, with the main contributor being myocardial infarction. Given the high morbidity and mortality rates associated with congestive heart failure, the shortage of donor hearts for transplantation, complications resulting from immunosuppression, and long-term failure of transplanted organs, regeneration of the diseased myocardium by cell transplantation is an attractive therapeutic modality. Because it is desired that the transplanted cells fully integrate within the diseased myocardium, contribute to its contractile performance, and respond appropriately to various physiological stimuli (eg, β-adrenergic stimulation), our major long-term goal is to investigate the developmental changes in functional properties and hormonal responsiveness of human embryonic stem cells–derived cardiomyocytes (hESC-CM). Furthermore, because one of the key obstacles in advancing cardiac cell therapy is the low differentiation rate of hESC into cardiomyocytes, which reduces the clinical efficacy of cell transplantation, our second major goal is to develop efficient protocols for directing the cardiomyogenic differentiation of hESC in vitro.

To accomplish the first goal, we investigated the functional properties of hESC-CM (<90 days old), respecting the contractile function and the underlying intracellular Ca^{2+} handling. In addition, we performed Western blot analysis of the key Ca^{2+}-handling proteins SERCA2, calsequestrin, phospholamban and the Na^+/Ca^{2+} exchanger. Our major findings were the following: (1) In contrast to the mature myocardium, hESC-CM exhibit negative force-frequency relationships and do not present postrest potentiation. (2) Ryanodine and thapsigargin do not affect the $[Ca^{2+}]_i$ transient and contraction, suggesting that, at this developmental stage, the contraction does not depend on sarcoplasmic reticulum Ca^{2+} release. (3) In agreement with the finding that a voltage-dependent Ca^{2+} current is present in hESC-CM and contributes to the mechanical function, verapamil completely blocks contraction. (4) Although hESC-CM express SERCA2 and Na^+/Ca^{2+} exchanger at levels comparable to those of the adult human myocardium, calsequestrin and phospholamban are not expressed. (4) In agreement with other reports, hESC-CM are responsive to β-adrenergic stimulation. These findings show that the mechanical function related to intracellular Ca^{2+} handling of hESC-CM differs from the adult myocardium, probably because of immature sarcoplasmic reticulum capacity.

Keywords: Human embryonic stem cells–derived cardiomyocytes; Excitation-contraction coupling; $[Ca^{2+}]_i$ transients and contractions; Sarcoplasmic reticulum Ca^{2+} release

Introduction

Cardiovascular diseases are the most frequent cause of death in the industrialized world. In the United States alone, congestive heart failure—the ineffective pumping of the heart caused by the loss or dysfunction of heart muscle cells, affects approximately 5 million patients, with 400 000

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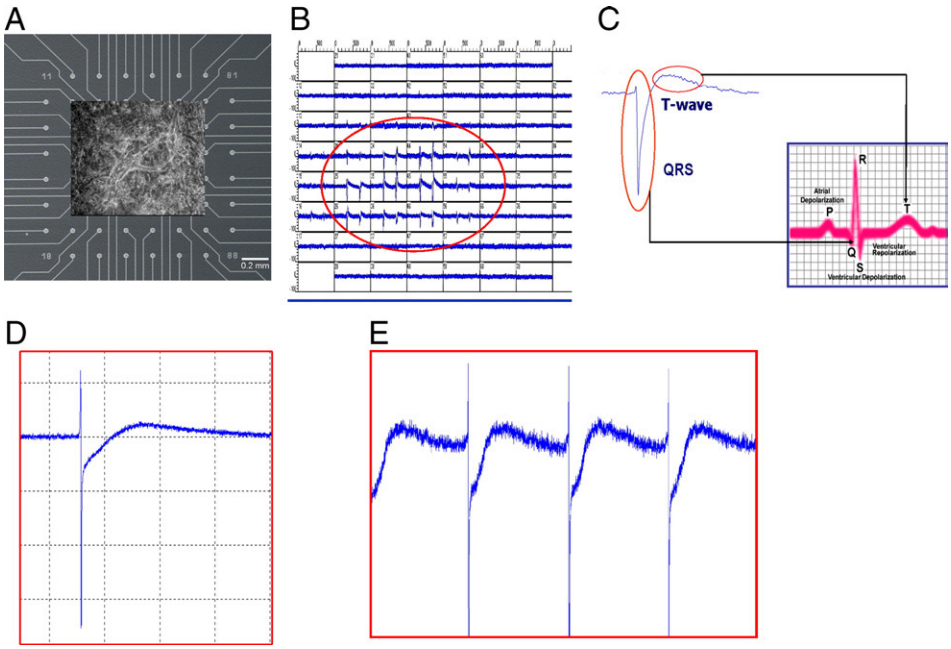

Fig. 1. Extracellular electrograms recorded by means of the MEA data acquisition system. A, The procedure for recording electrograms from hESC-CM: placing an embryoid body containing a spontaneously contracting area on top of the MEA. B, Electrograms recorded from a contracting embryoid body, which occupies the central area of the electrode array. C, A typical electrogram recorded from a 45-day-old embryoid body, with the corresponding pseudo electrocardiographic complexes. D and E, Electrograms recorded from cultured neonatal rat and mouse ventricular myocytes.

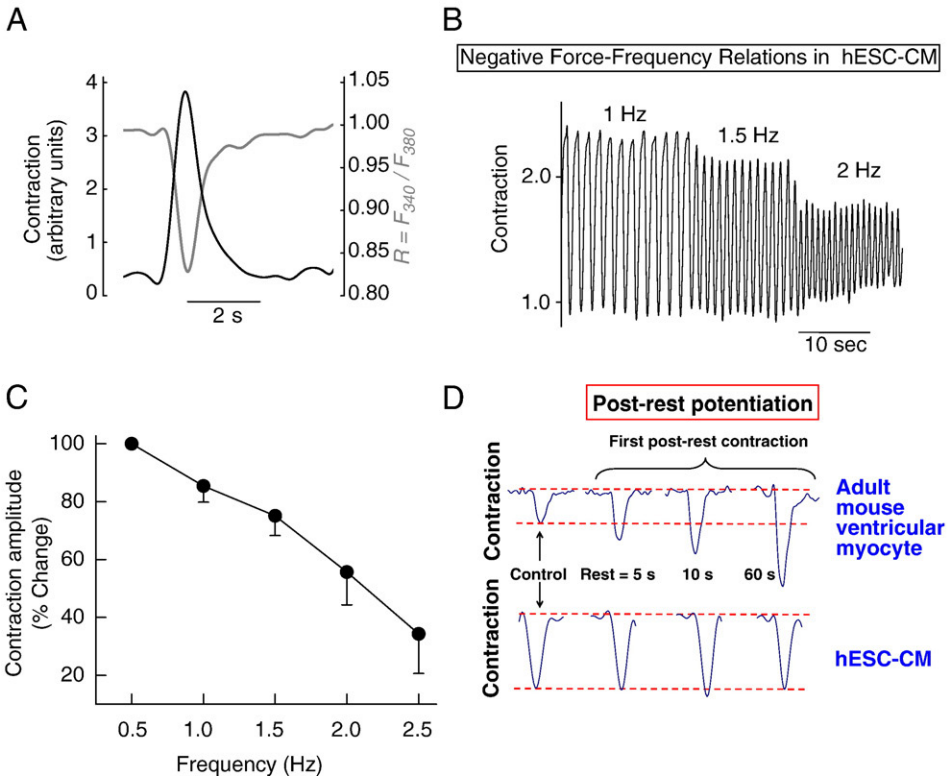

Fig. 2. $[Ca^{2+}]_i$ transients and contractile properties of hESC-CM. A, Simultaneously recorded traces of $[Ca^{2+}]_i$ transients and contractions of spontaneously contracting 47-day-old embryoid body. B, Representative recordings of contractions recorded from a 55-day-old contracting embryoid body stimulated at different rates. C, Force-frequency relations in 47- to 55-day-old embryoid bodies stimulated at different rates (n = 5 embryoid bodies). The results are expressed as percent change from the values at 0.5 Hz. D, Testing postrest potentiation in adult mouse ventricular myocytes and in hESC-CM. Representative contraction tracings recorded from an adult mouse ventricular myocyte and from a contracting embryoid body depicting the control contraction recorded at 1 Hz and the first postrest contraction after rest periods of 5, 10, and 60 seconds. Note the prominent postrest potentiation in the mouse ventricular myocyte and its absence in hESC-CM.