Electrophysiological Profiling of Cardiomyocytes in Embryonic Bodies Derived From Human Embryonic Stem Cells

Therapeutic Implications

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Human embryonic stem (hES) cells are derived from the inner mass cells of developing blastocysts and have the ability to generate cells from three embryonic germ layers. Since their initial culturing in 1981, ES cells have revolutionized the mouse genetics field1,2 by allowing the creation of mouse models of disease as well as the molecular study of the differentiation of pluripotent cells into various somatic cell types including cardiomyocytes and vascular smooth cells.3–5 Some key features of ES cells that have made them particularly useful in research include their ability for self-renewal, diploid karyotype stability, and continuous telomerase activity.6–9 Since human fetuses are not generally available for scientific study, hES cells represent an in vitro model for embryonic differentiation, with the ability to understand more about cell lineage commitment and the process of differentiation. Importantly, they also represent a potential source of cells for therapeutic uses such as the regeneration of functional myocardium and conducting tissue. As a consequence of these capabilities and their derivation from human embryos, hES cells have been a subject of numerous ethical and moral debates.10

In this issue of Circulation Research, He and coworkers characterize the electrophysiological and contractile properties of cardiomyocytes derived from hES cells.11 This work, together with previous studies by Gepstein’s group8,12 and other recent reports13,14 expands our understanding of cardiac differentiation of hES cells, building a foundation for future research and for new therapeutic strategies using hES cells. In agreement with other hES studies,8 He et al found that about 20% of the embryoid bodies (EBs) (three-dimensional cell aggregates of hES cells in culture) remained as spontaneous beating “cardiac-like” cell clusters after 45 days in culture. The number of cardiomyocytes within these EB outgrowths varied widely (between 2% to 70%). Others have previously reported that up to 70% of human EBs can be induced to beat spontaneously after 16 days in culture13 whereas approximately 90% of mouse EBs beat after differentiation.15 This observed variability may reflect differences in plating density, culture medium composition, or period of gestation.13 Another factor that may influence cardiomyocyte differentiation is the inductive interactions between anterior visceral endoderm-like cells and cardiac progenitor cells.14

He and colleagues used an array of tools to phenotype hES cell–derived cardiomyocytes. Immunostaining and ultrastructural analysis established that beating EBs displayed a sarcomeric pattern and Z-lines as reported previously by others8,13,14 Previous reports have also demonstrated that beating EBs express thin- (cardiac troponin I and T) and thick-filament (myosin heavy and light chains) proteins, creatine kinase-MB, cardiac transcription factors such as GATA-4, Nkx2.5, and MEF-2, and the cardiac-specific marker ANF.8,13,14 Collectively, these findings suggest that EBs contain cardiomyocytes. Consistent with this suggestion, in situ action potentials (APs) within a beating outgrowth recorded using microelectrode impalements (intracellular recordings) revealed typical cardiac-like depolarization patterns with AP heterogeneity resembling AP profiles observed in nodal, atrial, and ventricular cardiomyocytes. These observations build on recent findings showing similar electrical heterogeneity in hES cells cultured with visceral endoderm-like cells.14 Electrical heterogeneity was also observed as two distinct types of spontaneous electrical activity (ie, continuous and episodic) in EBs,11 which may depend on intercellular connxin-containing gap junction expression.12 The authors speculate that the episodic pattern could be due to conduction block related to tissue geometry, impaired cell-to-cell coupling, or reduced cellular excitability, although the role of altered intracellular Ca2+ cycling due to an immature Ca2+ regulatory system cannot be ruled out. The phenotyping and segregation of hES cell–derived cardiomyocytes into different subpopulations may allow highly specific cell therapies to be developed thereby minimizing possible side effects. For example, nodal cells may be used for pacemaker support in sick sinus syndrome and atrioventricular block, whereas the use of “pure” ventricular cells might allow selective therapy of myocardial diseases without the creation of a nidus of proarrhythogenic pacemaker/nodal cells. How undifferentiated hES cells transform into different types of cardiac myocytes will clearly be an important area of future research. Indeed, the ability of the vascular cytokine, endothelin, to transform avian embryonic heart muscle cells into impulse-conducting Purkinje fibers provides evidence that humoral factors can induce electrically distinct cell populations in the heart.16
Functional aspects of hES cell–derived cardiomyocytes were determined by He and colleagues using two different approaches. First, by increasing the EB outgrowth pacing rates, these authors observed AP shortening and absence of ventricular-like cardiomyocytes; this physiological response leads to systolic shortening at high heart rates thereby maintaining diastolic time for ventricular filling as shown in ventricular myocardiun of human embryos.15 Second, the impact of the specific Isk blocker, E-4031,16 on AP profile and triggered arrhythmias (early [EAD] and delayed [DAD] afterdepolarizations) was examined. Human cardiac APs represent complex interactions between depolarizing (such as Na+ and Ca2+ currents) and repolarizing currents (such as transient outward [Ito] and delayed rectifier [IKr and IK1] K+ currents).18,19 The application of E-4031 led to prolongation of atrial- and ventricular-like APs thereby providing pharmacological evidence that Isk contributes to repolarization in these myocytes. These data complement recent studies in hES cells showing expression of K, LQT1 (pore-forming subunit of Isk) and K, 4,3 (pore-forming subunit of Isk)14. Future studies will undoubtedly unravel the temporal and spatial expression patterns of pore-forming and accessory subunits contributing to cardiac ion channels in hES cell–derived cardiomyocytes, which are known to be developmentally regulated.10,20 In addition, although EBs are only 4 to 10 layers thick, it will be of great interest to determine whether regional “transmural” variations in AP profile occur within EBs, thus providing important new approaches for studying the regulatory factors governing the three-dimensional organization of AP profile and cardiac electrophysiology.

The application of E-4031 and the associated AP prolongation was also associated with EADs and DADs, suggesting that these myocytes have the capacity for arrhythmogenesis. Although this may be related to spontaneous Ca2+ release,19 it could also be at least partially related to myocyte injury after microelectrode impalement. In either case, relative high expression/activity of the Na+–Ca2+ exchanger observed in fetal/neonatal myocytes is likely to play an important role in the formation of these arrhythmias.21,22 Activation of G protein–coupled receptors, including β-adrenergic (β1- and β2-AR), α-adrenergic, and muscarinic (M2) receptors, is known to strongly influence contractile properties and beating rates of cardiomyocytes derived from hES.15,16 Consistent with this, He and coworkers showed that the acute application of the β-adrenergic agonist, isoproterenol, leads to a marked positive inotropic effect in hES cell–derived EB outgrowths.11 These effects could be related to activity of protein kinase A on L-type Ca2+ channel (α1c) and phospholamban, both of which are expressed in these preparations14 and are likely to elevate peak systolic intracellular Ca2+ concentration recorded previously in these preparations.8 In addition, the L-type Ca2+ channel blockers, diltiazem and verapamil, have profound negative chronotropic effects on hES cell–derived cardiomyocytes3,14 as expected from the observation that pacemaker activity is critically dependent on L-type Ca2+ channel current.23

Overall, this study by He et al,11 together with several other recent reports, provides a solid phenotypic analysis of the electrophysiological and contractile features of EB-derived cardiomyocytes and underscores the complex machinery that exists within these cells. The observation that fetal myocyte transplantation can lead to stable intracardiac grafts and improvement of cardiac function24 has stimulated interest in the use of ES cells, with their self-renewal and pluripotent characteristics, as a potential therapeutic tool for cardiovascular diseases. Because heart disease and failure are often characterized by a loss of functioning cardiomyocytes, which are terminally differentiated and show a very limited capacity for regeneration,35 transplantation of hES cells or cardiac grafts may be a means of reversing the functional changes observed in cardiac patients. In particular, the study by He et al suggests that it might be possible to introduce preselected nodal, atrial, or ventricular myocytes to appropriately affected regions, thus tailoring therapy for heart disease patients. While the idea has conjured much interest, many questions regarding the feasibility of cell-based therapy still exist. Questions concerning proarrhythmicogenic integration, functional coupling with host tissues in context of the extracellular matrix, the role of epigenetic factors (eg, hemodynamics), and rejection and vascularization of the graft (eg, concomitant use of endothelial precursor cells) will all need to be addressed.9,24,26 Undoubtedly, a complete understanding of the electrophysiology and excitation-contraction coupling in hES cell–derived cardiomyocytes will provide insights into human embryonic cardiomyocyte development, signaling pathways, and transcriptional events while fostering the potential therapeutic applications of hES cells.

References


**KEY WORDS:** embryonic stem cells *■* embryonic bodies *■* electrophysiology *■* cardiomyocytes *■* action potentials
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