Electrophysiological Importance of Embryonic Stem Cell-Derived Cardiac Progenitors

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Embryonic stem (ES) cells represent an important model system for gaining insight into the control of early development and provide a useful source of cells in regenerative medicine. The mammalian heart is composed of diverse cell types, including cardiac, smooth muscle and endothelial cells. ES cells differentiate into multipotent Is1 (islet LIM homeobox 1)-positive cardiovascular progenitors, which develop into Flk1 (fetal liver kinase-1)-positive vascular progenitors for endothelial cell lineage and Nkx2.5 (NK2 transcription factor related, locus 5)-positive bipotential cardiomyogenic progenitors for cardiac and smooth muscle cell lineages.\(^1\,2\)

There is good evidence that transplantation of ES cells and ES cell-derived cardiac progenitors can improve cardiac function through mechanisms involving generation of new cardiomyocytes and formation of new blood vessels.\(^3\) However, previous experimental studies have shown that several potential side effects are associated with ES cell-based transplantation strategies, such as teratoma formation and arrhythmogeneity.\(^4\,5\) Purification of ES cell-derived cardiomyocytes is required to minimize the risk of teratoma formation within the host heart. Arrhythmogeneity has been suggested to arise from the enhanced electrophysiological heterogeneity and spontaneous activity associated with transplanted cells.\(^5\)

The spontaneous beating of the PrP-positive cells is accelerated by isoproterenol, which is totally antagonized by further addition of carbamylcholine, suggesting that β-adrenergic and muscarinic receptors are functionally expressed and mutually interacted. Interestingly, spontaneous electrical activity is significantly inhibited by blockers of the Na\(^+\) channel (Nav1.5), voltage-gated L-type Ca\(^{2+}\) current (I\(_{Ca,L}\), Cav1.2 and Cav1.3) and inward rectifier K\(^+\) current (I\(_{K1}\), Kir2.1). However, the voltage-gated T-type Ca\(^{2+}\) current (I\(_{Ca,T}\), Cav3.1 and Cav3.2) and hyperpolarization-activated cation current (I\(_{H}\), HCN4) are barely detectable in PrP-positive cells. Despite the presence of spontaneous automaticity, PrP-positive cells were found to exhibit the functional expression pattern of ionic currents that is basically similar to that of mature ventricular myocytes under physiological conditions but is substantially different from that of pacemaker and conduction system myocytes (Table).\(^5\,9\)

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PrP, prion protein; SA, sinoatrial.

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positive cells is produced by RyR2-mediated Ca$^{2+}$ release and resultant activation of forward mode $I_{scx}$, which extrudes 1Na$^+$ in exchange for 3Na$^+$ influx and thereby causes a net depolarizing current.

Spontaneous activity of cardiac sinoatrial (SA) node pacemaker cells is initiated by the slow diastolic depolarization (phase 4 depolarization) phase.\textsuperscript{19} There are a number of evidences that the slow diastolic depolarization arises from the activation of inward currents such as $I_h$, $I_{Ca,T}$ and $I_{Ca,L}$, together with deactivation of outward currents through the rapidly and slowly activating delayed rectifier K$^+$ current ($I_{Ks}$ and $I_{Kr}$, respectively).\textsuperscript{8-11} The SA node automaticity is thus ascribable to a complex but coordinated interaction of multiple membrane currents, and this process is referred to as the “membrane clock” mechanism.\textsuperscript{10,12-14} On the other hand, a novel ionic mechanism has been proposed for the genesis of SA node automaticity, in which the slow diastolic depolarization is largely dependent on the inward (forward mode) $I_{scx}$ evoked by spontaneous and cyclical local Ca$^{2+}$ release from the sarcoplasmic reticulum, with membrane currents including $I_h$ having a minimum role, known as the “Ca$^{2+}$ clock” mechanism.\textsuperscript{12,13,15}

Based on the results of pharmacological experiments, the electrophysiological basis of the automaticity of PrP-positive cardiac progenitors can be typically ascribed to the Ca$^{2+}$ clock rather than the membrane clock mechanism.\textsuperscript{6} It is also important to note that because PrP-positive cells scarcely express HCN4 protein (which is a specific marker for the SA node and primarily underlies $I_h$), PrP-positive cells may have segregated from the lineage of SA node cells.\textsuperscript{26}

This article by Fujii et al highlights the promising possibility that PrP-based selection and enrichment of ES-cell derived cardiac progenitor cells can be used to treat damaged myocardium. In fact, transplantation of PrP-selected progenitors into the mouse kidney capsule can give rise to cardiomyocytes (as evidenced by the expression of cardiac troponin T), which survive for at least 3 weeks without tumor formation.\textsuperscript{2} Future study should examine whether PrP-selected ES cell-derived cardiac progenitors can stably engraft within the injured ventricle and differentiate into working cardiac ventricular myocytes (but not into pacemaker myocytes) without arrhythmogenicity. It is also important to investigate whether PrP can be used to identify cardiomyogenic progenitors derived from other pluripotent stem cells, including iPS (induced pluripotent stem) cells.

References