

Na⁺/Ca²⁺ exchanger is a determinant of excitation-contraction coupling in human embryonic stem cell-derived ventricular cardiomyocytes.

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Public Summary:

In adult cardiomyocytes (CMs), the Na⁺/Ca²⁺ exchanger (NCX) is a well-defined determinant of Ca²⁺ homeostasis. Developmentally, global NCX knockout in mice leads to abnormal myofibrillar organization, electrical defects, and early embryonic death. Little is known about the expression and function of NCX in human heart development. Self-renewable, pluripotent human embryonic stem cells (hESCs) can serve as an excellent experimental model. However, hESC-derived CMs are highly heterogeneous. A stably lentivirus-transduced hESC line (MLC2v-dsRed) was generated to express dsRed under the transcriptional control of the ventricular-restricted myosin light chain-2v (MLC2v) promoter. Electrophysiologically, dsRed⁺ cells differentiated from MLC2vdsRed hESCs displayed ventricular action potentials (AP), exclusively. Neither atrial nor pacemaker APs were observed. While I_{Ca-L}, I_f, and I_{Kr} were robustly expressed, I_{Ks} and I_{K1} were absent in dsRed⁺ ventricular hESCCMs. Upon differentiation (7+40 to +90 days), the basal [Ca²⁺]_i, Ca²⁺ transient amplitude, maximum upstroke, and decay velocities significantly increased (P < 0.05). The I_{Ca-L} antagonist nifedipine (1 μM) decreased the Ca²⁺ transient amplitude (to ~30%) and slowed the kinetics (by ~2-fold), but Ca²⁺ transients could still be elicited even after complete I_{Ca-L} blockade, suggesting the presence of additional Ca²⁺ influx(es). Indeed, Ni²⁺-sensitive INCX could be recorded in 7+40- and +90-day dsRed⁺ hESC-CMs, and its densities increased from -1.2 ± 0.6 pA/pF at -120 mV and 3.6 ± 1.0 pA/pF at 60 mV by 6- and 2-folds, respectively. With higher [Ca²⁺]_i, 7+90-day ventricular hESC-CMs spontaneously but irregularly fired transients upon a single stimulus under an external Na⁺-free condition; however, without extracellular Na⁺, nifedipine could completely inhibit Ca²⁺ transients. We conclude that INCX is functionally expressed in developing ventricular hESC-CMs and contributes to their excitation-contraction coupling.

Scientific Abstract:

In adult cardiomyocytes (CMs), the Na⁽⁺⁾/Ca⁽²⁺⁾ exchanger (NCX) is a well-defined determinant of Ca⁽²⁺⁾ homeostasis. Developmentally, global NCX knockout in mice leads to abnormal myofibrillar organization, electrical defects, and early embryonic death. Little is known about the expression and function of NCX in human heart development. Self-renewable, pluripotent human embryonic stem cells (hESCs) can serve as an excellent experimental model. However, hESC-derived CMs are highly heterogeneous. A stably lentivirus-transduced hESC line (MLC2v-dsRed) was generated to express dsRed under the transcriptional control of the ventricular-restricted myosin light chain-2v (MLC2v) promoter. Electrophysiologically, dsRed⁺ cells differentiated from MLC2vdsRed hESCs displayed ventricular action potentials (AP), exclusively. Neither atrial nor pacemaker APs were observed. While I_(Ca-L), I_(f), and I_(Kr) were robustly expressed, I_(Ks) and I_(K1) were absent in dsRed⁺ ventricular hESCCMs. Upon differentiation (7+40 to +90 days), the basal [Ca⁽²⁺⁾]_(i), Ca⁽²⁺⁾ transient amplitude, maximum upstroke, and decay velocities significantly increased (P < 0.05). The I_(Ca-L) antagonist nifedipine (1 microM) decreased the Ca⁽²⁺⁾ transient amplitude (to approximately 30%) and slowed the kinetics (by approximately 2-fold), but Ca⁽²⁺⁾ transients could still be elicited even after complete I_(Ca-L) blockade, suggesting the presence of additional Ca⁽²⁺⁾ influx(es). Indeed, Ni⁽²⁺⁾-sensitive INCX could be recorded in 7+40- and +90-day dsRed⁺ hESC-CMs, and its densities increased from -1.2 +/- 0.6 pA/pF at -120 mV and 3.6 +/- 1.0 pA/pF at 60 mV by 6- and 2-folds, respectively. With higher [Ca⁽²⁺⁾]_(i), 7+90-day ventricular hESC-CMs spontaneously but irregularly fired transients upon a single stimulus under an external Na⁽⁺⁾-free condition; however, without extracellular Na⁽⁺⁾, nifedipine could completely inhibit Ca⁽²⁺⁾ transients. We conclude that I_(NCX) is functionally expressed in developing ventricular hESC-CMs and contributes to their excitation-contraction coupling.

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