Non-cardiomyocytes influence the electrophysiological maturation of human embryonic stem cell-derived cardiomyocytes during differentiation.

Journal: Stem Cells Dev

Publication Year: 2010

Authors: Changsung Kim, Maryam Majdi, Peng Xia, Karen A Wei, Maria Talantova, Sean Spiering, Brandon Nelson, Mark Mercola, Huei-Sheng Vincent Chen

PubMed link: 20001453

Funding Grants: Chemical Genetic Approach to Production of hESC-derived Cardiomyocytes , Optimization in the Identification, Selection and Induction of Maturation of Subtypes of Cardiomyocytes derived from Human Embryonic Stem Cells, Development of Neuro-Coupled Human Embryonic Stem Cell-Derived Cardiac Pacemaker Cells.

Public Summary:
Cardiovascular diseases remain the major cause of death in the US. Human Stem and progenitor cell-derived cardiomyocytes (SPC-CMs) hold great promise for myocardial repairs. Recent progress in cellular reprogramming of various somatic cell types into induced pluripotent stem cells opened the door for developing patient-specific, cell-based therapies. However, most SPC-CMs displayed heterogeneous and immature electrophysiological (EP) phenotypes with uncontrollable automaticity. Implanting these electrically immature and inhomogeneous CMs to the hearts would likely be arrhythmogenic and deleterious. Furthermore, as CMs mature, they undergo changes in automaticity and electrical properties. We used human embryonic stem cell-derived CMs (hESC-CMs) as the model system to study the development and EP maturation of CMs in the embryoid body (EB) environment. Elucidating molecular pathways governing EP maturation of early hESC-CMs in EBs would enable engineered microenvironment to create functional pacemaker cells or electrophysiologically compatible hESC-CMs for cell replacement therapies. We have established antibiotic (Abx)-resistant hESC lines conferred by lentiviral vectors under the control of a cardiac-specific promoter. With simple Abx treatment, we easily isolated >95% pure hESC-CMs at various stages of differentiation from EBs. Using the Abx selection system, we found that hESC-CMs isolated at early stages of differentiation without further contacts with non-cardiomyocytes (non-CMs) depicted arrested electrical maturation. The intracellular Ca2+-mediated automaticity developed very early and contributed to dominant automaticity throughout hESC-CM differentiation regardless of the presence or absence of non-CMs. In contrast, sarcolemmal ion channels evolved later upon further differentiation within EBs and their maturation required the interaction with non-CMs. We further developed an add-back co-culture system to enable adding non-CMs back to early isolated hESC-CMs, which rescued the arrest of EP maturation. Therefore, our study showed for the first time that non-CMs exert significant influences on the electrical maturation of hESC-CMs during differentiation. Knowledge of our study opens the door for future identification of specific subtypes of non-CMs that influence chamber-specific differentiation of SPC-CMs. Result from our study will allow us to improve functional maturation of primitive hESC-CMs or to create functional pacemaker cells for a safe and effective cell transplantation.

Scientific Abstract:
Various types of cardiomyocytes undergo changes in automaticity and electrical properties during fetal heart development. Human embryonic stem cell-derived cardiomyocytes (hESC-CMs), like fetal cardiomyocytes, are electrophysiologically immature and exhibit automaticity. We used hESC-CMs to investigate developmental changes in mechanisms of automaticity and to determine whether electrophysiological maturation is driven by an intrinsic developmental clock and/or is regulated by interactions with non-cardiomyocytes in embryoid bodies (EBs). We isolated pure populations of hESC-CMs from EBs by lentivirus-engineered Puromycin resistance at various stages of differentiation. Using pharmacological agents, calcium (Ca(2+)) imaging, and intracellular recording techniques, we found that intracellular Ca(2+)-cycling mechanisms developed early and contributed to dominant automaticity throughout hESC-CM differentiation. Sarcolemmal ion channels evolved later upon further differentiation within EBs and played an increasing role in controlling automaticity and electrophysiological properties of hESC-CMs. In contrast to the development of intracellular Ca(2+)-handling proteins, ion channel development and electrophysiological maturation of hESC-CMs did not occur when hESC-CMs were isolated from EBs early and maintained in culture without further interaction with non-cardiomyocytes. Adding back non-cardiomyocytes to early-isolated hESC-CMs rescued the arrest of electrophysiological maturation, indicating that non-

cardiomyocytes in EBs drive electrophysiological maturation of early hESC-CMs. Non-cardiomyocytes in EBs contain most cell types present in the embryonic heart that are known to influence early cardiac development. Our study is the first to demonstrate that non-cardiomyocytes influence electrophysiological maturation of early hESC-CMs in cultures. Defining the nature of these extrinsic signals will aid in the directed maturation of immature hESC-CMs to mitigate arrhythmogenic risks of cell-based therapies.
