Abstract: P78

Electrophysiological characterization of induced pluripotent stem cell-derived cardiomyocytes from duchenne muscular dystrophy patients

Authors:
JM Pioner1, R Coppini2, L Santini2, C Palandri2, E Bennati3, M Regnier4, L Sacconi5, E Cerbai2, C Poggesi1, C Ferrantini1, 1University of Florence, Department of Experimental and Clinical Medicine - Florence - Italy, 2University of Florence, Department of NEUROFARBA - Florence - Italy, 3University of Florence, AOU Meyer Children's Hospital - Florence - Italy, 4University of Washington, Department of Bioengineering - Seattle - United States of America, 5University of Florence, European Laboratory for Non-Linear Spectroscopy - Sesto Fiorentino - Italy,

Topic(s):
Basic Science - Cardiac Biology and Physiology: Ion Channels, Electrophysiology

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S21

Funding Acknowledgements:
Telethon grant GGP16191

Background. Cardiomyopathy invariably affects teenage patients with Duchenne Muscular Dystrophy (DMD) and account for half of the mortality. However, the molecular sequalae due to loss of dystrophin is still poorly understood.

Purpose. We analyzed time-points of electrophysiological maturation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to provide insights into early-stage of disease mechanisms.

Methods. We combined a substrate nanopatterning approach with long-term cultures to improve maturation of hiPSC-CMs. We first studied time points of healthy hiPSC-CM maturation after 100 days in culture. To study the consequences of dystrophin loss, we used a CRISPR-Cas9 genome edited cell line targeting wild-type dystrophin locus in the control-hiPSC line (DMD-CM1). We then compared hiPSC-CMs derived from a DMD patient (DMD-CM2) with deletion of exon 50 on DMD gene causing total lack of dystrophin. We studied action potential (AP) and Ca2+ transients (Ca-T) of Control- and DMD-CMs at different pacing frequencies (0.5-1-2Hz and post rest potentiation) or with pharmacological compounds (i.e. Isoproterenol).

Results. Day 20 post-differentiated single hiPSC-CMs were plated onto nanogrooved surfaces and measured for 3 months at progressive time-points. Later stage control-CMs showed hyperpolarized resting membrane potential (RMP, -80 mV) and larger AP amplitude (110 mV) compared to the early-stage counterparts. Moreover, the AP duration (APD90) was prolonged with more resemblance to ventricular APs (600ms). Not least, later-stage control-CMs showed decreased spontaneous beating rate. In later-stages, Ca-T amplitude was almost 3-fold higher compared to early-stage CMs. Ca-T rise (time to peak, ms) became increasingly faster, while Ca-T decay (RT50) were prolonged. We evaluated the potentiation of Ca-T after a pacing pause (post rest potentiation), which was progressively increased, suggesting improved sarcoplasmic reticulum regulation of Ca2+-handling. Isoproterenol showed negligible positive inotropic and lusitropic effects and did not induce spontaneous Ca-T release in later-stage CMs, suggesting still immature β-adrenergic response.

We are investigating the electrophysiological consequences of dystrophin-null hiPSC-CM lines, which showed preserved rate of cell fractional shortening, but depressed post rest potentiation and prolonged cell relaxation (RT50) in later-stages. Both DMD-CM lines showed prolonged Ca-T decay. In addition, DMD-CM myofibril mechanics showed deficit of force, prolonged myofibril relaxation phase (slow tREL and fast kREL) and increased Ca2+-sensitivity of force development (pCa50).

Conclusion. (1) Modelling time points of DMD-hiPSC-CM maturation may predict developing disorders of DMD cardiomyopathy in vitro (2) Slower relaxation kinetics may be due to both impaired myofibril properties and Ca2+ handling abnormalities (3) Altered Ca2+ handling is a leading consequence of loss of dystrophin in hiPSC-CMs.
Conclusion. (1) Modelling time points of DMD-hiPSC-CM maturation may predict developing disorders of DMD cardiomyopathy in vitro (2) Slower relaxation kinetics may be due to both impaired myofibril properties and Ca2+ handling abnormalities (3) Altered Ca2+ handling is a leading consequence of loss of dystrophin in hiPSC-CMs.