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Molecular characterization and functional properties of induced pluripotent stem cells-derived cardiomyocytes from healthy and diseased individuals. Models for investigating inherited cardiac diseases

Introduction: Duchenne Muscular Dystrophy (DMD) caused by mutations in the DMD gene encoding the dystrophin protein, is an X-linked disease affecting boys and teenagers and rarely adult heterozygous females. DMD is characterized by progressive muscle degeneration and weakness, loss of ambulation and death by the late 20's or early 30's. Dilated cardiomyopathy (DCM) is a major cause of morbidity and mortality in DMD patients.

Hypothesis: Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) generated from the DMD patients exhibit intracellular [Ca2+]i handling and mechanical abnormalities. Our goal was to decipher the mechanical and molecular mechanisms underlying the abnormal [Ca2+]i handling and contraction in DMD patients.

Methods: Dystrophin-mutated iPSC-CMs were generated from male and female DMD patients. To test the hypothesis, [Ca2+]i transients and contractions were recorded from stimulated iPSC-CMs clusters using fura-2 fluorescence and video edge detector, in the absence and presence of the β-adrenergic agonist isoproterenol, which increases SR Ca2+ release through PKA-regulated Ryanodine (RyR2) channels. Specifically, we measured the inotropic response to 10-9-10-6 M of isoproterenol using the IonOptix calcium and contractility system. In addition, metabolic indices were evaluated using liquid chromatography followed by mass spectrometry and Seahorse XF analyser.

Results: Our experiments showed a concentration-dependent positive inotropic and lusitropic effects in healthy iPSC-CMs, on both [Ca2+]i transient and contraction parameters. In contrast, compared to healthy iPSC-CMs, the female and male DMD iPSC-CMs displayed a markedly depressed inotropic response to isoproterenol. To decipher the underlying mechanism, we determined SR Ca2+ release and capacity in DMD iPSC-CMs by means of a brief application of caffeine (10 mM) which serves as an opener of the RyR2 channel. In control iPSC-CMs, caffeine caused an abrupt increase in [Ca2+]i, followed by a gradual decline in [Ca2+]i level. In marked contrast to control iPSC-CMs, the male DMD iPSC-CMs exhibited a much shorter response to caffeine, while only 50% of the female DMD iPSC-CMs displayed abnormal [Ca2+]i handling in response to caffeine. The caffeine-induced Ca2+ signal area of DMD iPSC-CMs (male and 50% of female) was smaller than control. In addition, the caffeine-induced Ca2+ signal amplitude of DMD iPSC-CM (female) was significantly smaller than control. In addition, Seahorse XF analyser demonstrated decreased oxidative phosphorylation accompanied by a correlated increase in glycolysis in DMD iPSC-CMs. Accordingly, mass spectrometry analysis showed a dramatic fall in phosphocreatine levels in DMD iPSC-CMs.

Conclusion: DMD iPSC-CMs exhibit an attenuated β-adrenergic inotropic response, metabolic deficits and reduced energy stores.

Speaker Biography

Ofer Binah is Chair of Physiology, Biophysics and Systems Biology, at the Ruth & Bruce Rappaport Faculty of Medicine, Technion, Israel. He is a cardiac physiologist working for the past 33 years on research topics related to cellular electrophysiology, mechanics, signalling pathways and arrhythmias. In addition, he investigated the cellular mechanisms whereby cytotoxic T lymphocytes destroy cardiomyocytes in the course of heart transplant rejection and inflammatory heart diseases. Since 2001 he is investigating the functional properties of human embryonic stem cell-derived cardiomyocytes, and have published several papers in this area. Over 10 years ago he has begun investigating iPSC-derived cardiomyocytes generated from both dermal fibroblasts and keratinocytes, from healthy volunteers and from patients with inherited cardiac pathologies, including inherited arrhythmias and cardiomyopathies such as Duchenne Muscular Dystrophy.

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