Characterization of the PLN-R14Del mutation in hiPSC-derived cardiomyocytes

Maniezzi C.[1], Badone B.[1], Ronchi C.[1], Lodola F.[1], Eskandr M.[1], Florindi C.[1], Knaust A.E.[2], Eschenhagen T.[2], Hansen A.[2], and Zaza A.[1]
E-mail: claudia.maniezzi@unimib.it
[1] University of Milano-Bicocca, Milan, Italy
[2] University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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Abstract:
BACKGROUND. Phospholamban (PLN) is the natural inhibitor of the sarco/endoplasmic reticulum Ca2+ ATP-ase (SERCA2a). Heterozygous PLN-R14Del mutation is associated with an arrhythmogenic dilated cardiomyopathy (DCM), whose pathogenesis has been attributed to SERCA2a “super-inhibition”.

AIM. Test in human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM) harvested from a PLN-R14del carrier whether i) Ca2+ dynamics and protein localization were compatible with SERCA2a superinhibition and ii) functional abnormalities could be reverted by pharmacological SERCA2a activation with (PST3093).

METHODS. Ca^{2+} transients (CaT) were recorded at 36°C in hiPSC-CMs clusters during field stimulation. SERCA2a and PLN where immunolabeled in single hiPSC-CMs. Mutant preparations (MUT) were compared to isogenic wild-type ones (WT), obtained by mutation reversal.

RESULTS. WT and MUT differed for the following properties: 1) CaT time to peak (tpeak) and half-time of CaT decay were shorter in MUT; 2) several CaT profiles were identified in WT, “hyperdynamic” ones largely prevailed in MUT; 3) whereas tpeak rate-dependently declined in WT, it was shorter and rate-independent in MUT; 4) diastolic Ca2+ rate-dependently accumulated in WT, but not in MUT. When applied to WT, PST3093 turned all the above properties to resemble those of MUT; when applied to MUT, PST3093 had no effect. Preferential perinuclear SERCA2a-PLN localization was lost in MUT hiPSC-CMs. In conclusion, functional data converge to argue for PLN-R14del incompetence in inhibiting SERCA2a in the tested case, thus weakening the rationale for therapeutic SERCA2a activation. Mechanisms alternative to SERCA2a super-inhibition should be considered in the pathogenesis of DCM, including dysregulation of Ca^{2}+-dependent transcription.