Unraveling the pathophysiological role of the PLNR14del mutation in a novel heterozygous mouse model of dilated cardiomyopathy

Eskandr M.[1], Maniezzi C.[1], Florindi C.[1], Silljé H.H.W. [2], de Boer R.A.[2], Lodola F.[1], and Zaza A.[1]

E-mail: m.eskandr@campus.unimib.it

[1]Laboratory of Cardiac Cellular Physiology, Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy;

[2]Department of Experimental Cardiology, University of Groningen, University Medical Center Groningen, the Netherlands.

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Abstract:

The sarco/endoplasmic Ca^{2+} ATPase 2a (SERCA 2a) and its natural inhibitor phospholamban (PLN) play a key role in cardiac excitation-contraction (EC) coupling. Heterozygous PLNR14del mutation is associated with an arrhythmogenic dilated cardiomyopathy (DCM). Haghighi and colleagues showed that overexpression of PLNR14del mutation in a cell line (HEK-293) led to a substantial decrease in SERCA2a affinity for Ca^{2+}, which they defined as a “super inhibitory” effect of mutant PLN. However, this theory seems not to be consistently supported by the most recent experimental evidence. The aim of the project is to shed light on the still debated pathophysiological mechanisms of the DCM associated to heterozygous PLN-R14del mutation.

In this study a heterozygous transgenic mouse model that recapitulates the clinical human phenotype has been exploited. Ca^{2+} transients were recorded through an ion sensitive Fluorophore (Fluo4-AM) at 36°C during field stimulation in cardiomyocytes (CMs) isolated from 8-12 weeks old heterozygous PLN-R14del (Mut) and wild-type (WT) mice. To compare mutation effect with pharmacological SERCA modulation, CMs were perfused with a pure SERCA activator (PST3093). Furthermore, to test the involvement of metabolic defects, we investigated mitochondrial function (Seahorse, Mito Stress assay) and ROS (Operetta, DCFDA assay).

WT and MUT cells differed for the following properties:
(1) Ca^{2+} transient decay time was significantly shorter in Mut CMs compared to WT. When applied to WT, PST3093 decreased the Ca^{2+} transient decay time to approach the value found in Mut. The results indicate enhancement of SR Ca^{2+} uptake rate in PLN-R14del CMs. (2) Mitochondrial basal respiration, maximal respiration, spare respiratory capacity, and proton leak were decreased in Mut cells compared to WT. (3) ROS production was increased in in Mut CMs compared to WT.
Altogether, these results suggest that a DCM pathogenetic mechanisms other than defective SERCA function, which was actually improved in PLN-R14del cells. The observed changes in mitochondrial respiration suggest metabolic disfunction as an alternative pathogenesis for DCM. The link between PLN mutation and energy metabolism remains to be elucidated.