Basic Science – Cardiac Diseases, Fibrosis

Titin mechanical unloading disrupts sarcomere tensional homeostasis triggering fast, non-canonical myocardial fibrosis

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Background/Introduction: Stresses exerted during myocardial contraction and relaxation cycles are mainly braced by mechanical proteins located in the extracellular matrix (ECM) and cardiomyocytes (CMs). Titin (TTN) is a gigantic protein that scaffolds the sarcomere (i.e. the basic unit of contraction). Since most of the CM cytoskeleton is occupied by sarcomeres, titin is an essential structural hub for the maintenance of a homeostatic level of mechanical tension.

Purpose: It has been shown that mechanical disruption of the ECM affects the equilibrium forces between CMs and the ECM, leading to morphological changes in sarcomeres. However, potential alteration of tensional homeostasis resulting from defective mechanics of CM proteins remains unknown due to the lack of tools for in vivo studies. Therefore, the aim of this study is to explore the in vivo response of the myocardium to the abrupt cessation of the mechanical properties of titin.

Methods: For this purpose, we have experimentally challenged CMs to a tensional unloading by means of a titin cleavage model (TEVs-TTN) [1], both in vitro, using neonatal CM (NMCM), and in vivo. In this model, a cassette with a tobacco etch virus protease (TEVp) recognition sequence has been included in the I-band of titin. Transducing TEVp with adeno-associated virus (myoAAV), we were able to cleave titin in living CMs, ceasing only its mechanical properties. Samples were analyzed using an array of techniques such as histology, immunofluorescence and bulk and single-nucleus RNAseq.

Results: Our results show that while in NMCM we obtain a complete titin cleavage, in vivo we observe mosaic expression of TEVp and no more than 30% cleavage of the protein. In both cases, TEVp expression in TEVs-TTN does not affect cell viability. However, mild myocardial titin cleavage leads to a fibrotic response in less than six days, characterized by an increase in interstitial collagen and an expansion of cardiac fibroblasts population. Transcriptional and phospo-SMAD2/3 analysis results suggest that this fibrotic response occurs without activation of the canonical TGFβ pathway.

Conclusion: Taken together, our results suggest that the loss of titin mechanical-structural function in cardiomyocytes derived from the cleavage of a single peptide bond elicits a fast fibrotic compensatory response in the myocardium through intercellular communication with fibroblasts.