Platform: Cardiac Muscle Mechanics, Structure, and Regulation I

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Advanced Morpho-Functional Analysis on Ventricular and Atrial Tissue Reveals Cross-Bridge Kinetics Alterations and Sarcomere Energetic Impairment in Hcm Patients

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Mutations in cardiac myosin-binding protein-C (cMyBP-C), are the most common cause of Hypertrophic CardioMyopathy (HCM). The E258K-cMyBP-C is a penetrant missense mutation with poorly understood molecular mechanisms. Mechanics and kinetics of contraction as well the energy cost of tension generation were investigated using left ventricular (LV) and atrial tissue from E258K HCM patients and donor hearts. Kinetics of tension generation and relaxation were measured in LV and atrial myofibrils while ATPase and isometric active tension were simultaneously measured in permeabilized LV and atrial strips. The rate of tension generation following maximal Ca^{2+}-activation was faster in both LV and atrial E258K myofibrils compared to donors. The rate of isometric relaxation was also faster in E258K myofibrils, suggesting faster cross-bridge detachment and increased energy cost of tension generation. Direct measurements in skinned LV and atrial strips confirmed that tension cost was higher in E258K preparations compared to controls. To check whether cardiomyocyte disarray, typical of HCM hearts, may have contributed to artificially increase the tension cost measured in the HCM preparations, the strips used for mechanical investigations were clarified, immunostained and imaged at mesoscale level. An advanced tissue clearing method in combination with two-photon microscopy was employed to reconstruct the 3D image of the strips at sub-micrometer spatial resolution. A 3D cytoarchitecture analysis tool based on 3D Fourier Transform was developed and applied to determine cardiomyocyte orientation across and along the strips. Both global and local statistics of spatial disarray were derived and correlated to mechanical and energetic data. The results did not highlight structural differences between donor and HCM strips strengthening the conclusion that the E258K mutation primarily alters apparent cross-bridge kinetics and impairs sarcomere energetics. Acknowledgement: H2020EU SILICOFCM grant agreement 777204.

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Tropomyosin Cable Formation and Its Influence on the Structural Dynamics of Tropomyosin

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Head-to-tail association of coiled-coil tropomyosin dimers produce four-helix bundle ''overlapping domains'' connecting the dimers together. This polymerization process yields elongated super-helical cables that interact continuously with the surface of the actin filament. While considerable computationally generated information is available on the local and global interactions between isolated (unpolymerized) tropomyosin dimers and F-actin (Li et al., 2011), little is known about the influence of the overlapping domain on the structural-mechanics of tropomyosin and hence corresponding tropomyosin behavior on actin filaments. In an effort to elucidate localized overlapping domain - actin interaction and ensuing regulatory translocation across actin, we compared molecular dynamics simulations of models of single dimeric tropomyosin molecules (as in Li et al., 2010) with new models of tropomyosin cables. The simulations of tropomyosin cables were performed with the tropomyosin polymer constrained to a native super-helical configuration as if linked to a ghost F-actin filament at a radius of 39 Å, thereby maximizing native flexural and twisting motions without being dampened by interaction with F-actin. We have recently shown that torsional motions and sequence-dependent twisting of tropomyosin is initiated and propagated from aspartate-137 and surrounding amino acids and is critical for tropomyosin function (Lehman et al., 2018). We now have shown that the sequence-specific twisting pattern of tropomyosin is virtually unaffected by the additional presence of the overlapping domain, while the flexural motions of tropomyosin are dampened as expected. In addition, related MD shows that torsional (and flexural) mobility of the overlapping domain itself appears to be unaffected by the presence of the remainder of the molecule. We conclude that the material properties of tropomyosin are dominated by its dimeric coiled-coil region and that the main function of overlapping domain is to sponsor tropomyosin polymerization and cable formation.

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Protein Kinase C-Mediated Cardiac Troponin I S43/45 Phosphorylation Causes Contractile Dysfunction in Human Heart Failure and in Rodents

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Cardiac dysfunction is linked to increased protein kinase C (PKC) expression, but the role of downstream myofilament phosphorylation is debated. The current experiments evaluate the contribution of PKC-mediated cardiac troponin I (cTnI) S43/45 phosphorylation (p-S43/45) to contractile dysfunction. Western analysis showed increased cTnI p-S43 and PKC expression in failing human myocardium, and hearts from a rat pressure overload (PO) model. Myocyte contractile dysfunction was also present in failing (F) versus non-failing (NF) human myocytes, and in PO versus sham rat myocytes. Contractile function and cTnI p-S43 levels were partially restored in F human myocytes after ventricular assist device support, and gene transfer of dominant negative PKC-alpha. Based on these findings, transgenic mice were generated with a range of cardiac-specific phospho-mimetic cTnI S43/45D (SD) replacing endogenous cTnI, to directly test the contribution of p-S43/45 to cardiac dysfunction and remodeling. In young adults (4 wks), high (HE-), medium (ME-) and low (LE-) SD expression did not cause significant changes in cardiac morphology/remodeling compared to non-transgenic (ntg) littermates. By 8 wks, HE-SD mice developed increased heart weight/tibial length ratio which continued to increase at 12 wks compared to LE-, ME-SD and ntg mice. Echocardiography showed progressive increases in left ventricular systolic dimension (LVDs) and decreases in ejection fraction between 8 and 12 wks in ME- and HE-SD mice (8 wks: ntg EF% = 54.9$\pm$1.7, n=4, ME-SD = 38$\pm$1.3*, n=3; HE-SD = 27.1$\pm$ 5.9* n=3; 12 wks: ntg EF% = 52.8$\pm$4.6, n=3; ME-SD = 35.1+3.3*, n=5; HE-SD = 16.7$\pm$6.2*, n=3; *$p<0.05$ by ANOVA), and 95% of HE-SD mice died before 20 wks of age. Taken together, the results show that chronic phosphorylation of cTnI S43/45 is a significant contributor to progressive cardiac dysfunction and heart failure.

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Beta-Myosin Heavy Chain Post-Translational Modifications in Failing and Non-Failing Human Hearts

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Very little is known about post-translational modifications (PTM) of myosin heavy chain (MHC) in the human heart. Protein acetylation is a PTM, shown of instrumental importance as a modification of histone proteins. More recently it has been uncovered that the realm of lysine-acetylation extends beyond the nucleus. Protein phosphorylation is a well-known PTM found on several sarcomeric proteins in the heart and is crucial for fine-tuning myocardial contractility. To our knowledge, we were first to report alpha-MHC phosphorylation in murine hearts. Given the emergent importance of the broader lysine-acetylome and sarcomeric protein phosphorylation during heart failure, we examined PTMs in human heart beta-MHC. Here, using bottom-up proteomics, we identified six high-confidence lysine acetylation sites (three each in myosin-S1 and rod domains) and eight high-confidence phosphorylation sites (six in myosin-S1 and two in myosin rod regions). Furthermore, the acetylation and phosphorylation status of some of these sites changed in ischemic failing (IF) and non-ischemic failing (NIF) hearts compared to donor non-failing human hearts. Using label-free quantification, we found reduced acetylation of four lysine residues in IF and NIF human hearts. However, in IF human hearts differential acetylation was apparent, with two lysine residues in myosin-S1 exhibiting enhanced acetylation. Analysis of the phosphorylation sites is currently in progress. Additionally, we identified a region in the myosin-S1 ATP-binding pocket that exhibits both acetylated and phosphorylated residues, suggesting the importance of specific changes in the amino acid architecture in this region. It will be of interest to determine: (i) the functional role of these PTM sites; and (ii) whether altered acetylation of beta-MHC aberrantly effects human heart function and in turn contributes to