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ABSTRACT

In the diseased heart a multitude of cellular changes occur, either as a compensatory mechanism to deter the modifications brought on as a result of cardiac disease or a direct result of the pathophysiology. One such avenue for uncovering the molecular mechanisms underlying cardiac disease and their functional changes is to study post-translational modification (PTM) of proteins.

Initially, we sought to determine if the metabolic regulatory kinase AMPK phosphorylates cardiac troponin I (cTnI) at Ser-150 in vivo to alter cardiac contractile function directly at the level of the myofilament. Rabbit cardiac myofibrils separated by two-dimensional isoelectric focusing subjected to a Western blot with a cTnI phosphorylation-specific antibody demonstrate that cTnI is endogenously phosphorylated at Ser-150 in the heart. Treatment of myofibrils with the AMPK holoenzyme increased cTnI Ser-150 phosphorylation within the constraints of the muscle lattice. Compared with controls, cardiac fiber bundles exchanged with troponin containing cTnI pseudo-phosphorylated at Ser-150 demonstrate increased sensitivity of calcium-dependent force development, blunting of both PKA-dependent calcium desensitization, and PKA-dependent increases in length dependent activation.

We next wanted to investigate the effect of ischemic pH on Ser-150 and Ser-23/24 phosphorylation. We demonstrate phosphorylation of cTnI is simultaneously increased at Ser-150 and Ser-23/24 during in vivo myocardial ischemia. Myocardial ischemia is known to decrease intracellular pH directly resulting in depressed Ca^{2+} binding to Tn and impaired contraction. To determine the pathological relevance of these simultaneous TnI phosphorylations in ischemia we measured the individual effects of TnI Ser-150 (S150D), Ser-23/24 (S23/24D) or their combined (S23/24/150D) pseudo-phosphorylation on thin filament regulation at acidic pH similar to that in myocardial ischemia. Results demonstrate that while acidic pH decreased thin filament Ca^{2+} binding of all TnIs, TnI S150D attenuated this decrease such that it was similar to non-phosphorylated TnI at normal pH. The
dissociation of Ca\(^{2+}\) from troponin C (TnC) was unaltered by pH, such that TnI S150D remained slow, S23/24D remained accelerated and the combination of Ser-150 and Ser-23/24 pseudo-phosphorylation on the same TnI molecule retained accelerated dissociation.

Lastly, to investigate the remaining component of thin filament regulation, tropomyosin nitration or phosphorylation was investigated to determine the effect of PTM on structure and function. To investigate the kinetic regulatory role of αTm phosphorylation we expressed and purified native N-terminal acetylated Ser-283 wild-type, S283A phosphorylation null and S283D pseudo-phosphorylation Tm mutants in insect cells. Purified Tm's regulate thin filaments similar to that reported for muscle purified Tm. Steady-state Ca\(^{2+}\) binding to TnC in reconstituted thin filaments did not differ between the 3 Tm's, however dissociation of Ca\(^{2+}\) from filaments containing pseudo-phosphorylated Tm was slowed compared to wild-type Tm. Replacement of pseudo-phosphorylated Tm into myofibrils similarly prolonged the slow phase of relaxation and decreased the rate of the fast phase without altering activation kinetics. Additionally, we sought to investigate the effect of reactive nitrogen species to nitrate Tm Tyr residues, its structure-function impact and develop a mass spectrometry approach to identify Tm 3-nitrotyrosine (3-NT) PTM. Our data demonstrates the pathologically relevant reactive nitrogen species peroxynitrite modifies Tm Tyr residues to 3-NT with structural impact significant to modulate Tm function. We further developed and validated a novel and highly versatile target-driven MS/MS strategy to facilitate identification and quantification of Tm 3-NT without a priori knowledge of target residue modification.

In conclusion, the structural and functional modification of thin filament regulatory proteins described above provide an intricate glimpse into the mechanisms in which the heart can undergo to alter function both in normal physiology and in disease states. Gaining a better understanding of protein PTMs associated with cardiac disease will play a major role in the continued development of therapeutics to treat cardiovascular disease.


Benjamin Nixon, Presenter. 2012. AMP-activated Protein Kinase Phosphorylates Troponin I at Serine 150 to Alter Thin Filament Ca^{2+} Binding in a pH-Dependent Manner. Presented The Ohio State University Davis Heart and Lung Research Day Research Day.


RECENT PUBLICATIONS


AWARDS AND HONORS

2010 Finalist for Systems and Integrative Biology T32 Training Grant

2012 GRA Poster Presentation at the Davis Heart and Lung Research Institute Research Day 2nd place

2013 OSU Department of Physiology and Cell Biology Dr. Margaret T. Nishikawara Scholar Award

2013 Program of Muscle Health and Neuromuscular Disorders NIH/NINDS T32 Pre-doctoral Fellowship

FUTURE PLANS

Future endeavors will be centered on continued investigation into the mechanism underlying dysfunction in cardiovascular disease in the setting of academic research.