

digestion studies indicate that cTnI Delbp529AA is degraded at a faster rate than wild-type cTnI. These results suggest that the poor prognosis of patients carrying these RCM-linked mutants is due to protein dysfunction at multiple levels and suggest possible mechanisms of RCM pathology.

1851-Pos

Identification of Unknown Protein Kinase $\text{C}\alpha$ Phosphorylation Sites on Both Human Cardiac Troponin I and T

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Protein kinase C (PKC) isoforms have been shown to play an important role in the development of heart failure. Most research performed on PKC α has been done in rodents and direct evidence in human heart failure is limited. Our previous study showed a decrease in Ca^{2+} -sensitivity in failing tissue upon PKC α treatment of cardiomyocytes via phosphorylation of cardiac troponin I (cTnI), cardiac troponin T (cTnT) and myosin binding protein C (cMyBP-C). This study aims to determine the targets of PKC α on cTnI and cTnT. Western immunoblotting revealed that PKC α is less abundant but more active in failing compared to donor tissue. PKC α treatment of donor and failing tissue was able to phosphorylate Thr-143, which is a known PKC α site on cTnI, but endogenous phosphorylation levels were very low. LC-MS analysis of purified human recombinant cTn complex incubated with PKC α identified two novel phosphorylation sites, Ser-199 located on cTnI and Ser-189 on cTnT. Both sites are located in conserved regions on cTnI and cTnT. The PKA sites Ser23/24 on cTnI are phosphorylated by PKC α in purified human recombinant cTn complex, but there is no cross phosphorylation in donor and failing tissue. In conclusion, endogenous Thr-143 phosphorylation is low, which makes its involvement in heart failure unlikely. Exogenous PKC α phosphorylation of Thr-143 and Ser-199 on cTnI and Ser-189 on cTnT could possibly explain the decrease in Ca^{2+} -sensitivity observed and further research on the site-specific effects is warranted.

1852-Pos

Rescuing Myopathic Phenotype of Abnormal Cardiac Troponin T with a Single Amino Acid Substitution in Cardiac Troponin I

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Troponin T (TnT) and troponin I (TnI) are two subunits of the troponin complex. We previously found a single amino acid substitution in the TnT-binding helix of cardiac TnI (cTnI) in wild turkey hearts that expressed abnormally spliced myopathic cardiac TnT (cTnT) (Biesiadecki *et al.*, *JBC* 279:13825-32, 2004). To test the potential role of this cTnI modification in rescuing a cTnT abnormality, we developed transgenic mice expressing the cTnI variant (K118C) with or without a deletion of endogenous cTnI gene to mimic homozygote and heterozygote wild turkeys. Double and triple transgenic mice were created to combine the cTnI-K118C allele with an allele encoding the abnormally spliced cTnT (exon 7 deletion). Functional analysis in *ex vivo* working hearts found that cTnI-K118C had no destructive effect on cardiac muscle and baseline heart function but was able to rescue the decreases in cardiac function caused by cTnT exon 7 deletion. Further characterizations showed that cTnI-K118C significantly blunted the inotropic response of cardiac muscle to β -adrenergic stimulation whereas the PKA-dependent phosphorylation of cTnI was unchanged. These data indicate that TnI-TnT interaction is a critical link in Ca^{2+} signaling and β -adrenergic regulation of myocardial contraction, providing a novel target for the treatment of heart failure.

1853-Pos

H2-Helical Region of Cardiac Troponin T Contributes to Length-Dependent Regulation of Cardiac Contractile Activation

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An alpha-helical region of cardiac troponin T, cH2(T), is centrally positioned in the core domain of the troponin complex (Takeda *et al.*, *Nature* 2003 vol.424(6944):p35-41). cH2(T)-troponin I and cH2(T)-troponin C interactions have been illustrated through biochemical studies and visualized in the crystal structure of the core domain of human cardiac troponin, suggesting an impor-

tant regulatory role for cH2(T). However, little is known about how the cardiac-specific structure of cH2(T) relates to cardiac-specific contractile function. To better understand the functional significance of cH2(T), we created a chimeric rat cardiac troponin T (TnT) in which cH2(T) was replaced by the corresponding helical region of rat slow skeletal TnT and studied how replacement of native TnT with chimeric TnT affected contractile function of rat cardiac muscle. We measured isometric force, ATPase activity, and length-dependent contractile dynamics in detergent skinned papillary muscle bundles reconstituted with either wild-type or chimeric TnT, held at either sarcomere length (SL) 2.0 μm or 2.2 μm . Preliminary studies suggest that the SL dependence of Ca^{2+} -activated maximal force production and tension cost was depressed in bundles containing chimeric TnT. For example, bundles containing the chimeric TnT showed an 8.4% decrease in force production when SL was decreased from 2.2 μm to 2.0 μm , whereas bundles containing the wild-type TnT showed a 43.9% decrease in force production. In addition, length-dependent contractile dynamics were significantly altered in bundles containing the chimeric TnT. For example, the length-dependent rate constant of crossbridge recruitment was slower, and the rate constant of crossbridge detachment was faster in bundles containing the chimeric TnT. Thus, our data suggest that cH2(T) plays a role in cardiac-specific length-mediated myofilament activation, an important mechanism underlying the Frank-Starling relationship.

1854-Pos

Structural and Functional Characterization of the TNT1 Domain of Cardiac Troponin T

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Familial Hypertrophic Cardiomyopathy (FHC) is a primary cardiac muscle disorder and a common cause of sudden cardiac death among young people in the field. The majority of disease-causing mutations in the thin filament protein hcTnT are found within the TNT1 domain. This domain has not been crystallized and its structural details are poorly defined, limiting our ability to understand the mechanism of disease for these mutations. A highly charged region is found at the C-terminal end of TNT1 (158-RREEENRR-166) in which this highly alpha helical domain may unwind to create a flexible hinge that is necessary for normal function. We aim to determine the structural details and function of this region using SDSL-EPR and regulated in vitro motility (R-IVM) assays. The purpose of our R-IVM experiments is two-fold: to functionally analyze our spin labeled proteins and to gain insight into the function of TNT1 in the presence of cysteine substitutions. R-IVM data shows a progressive increase in the severity of the functional effects of cysteine substitution and spin labeling across the putative hinge region, suggesting that this region is dynamically important and may be making critical interactions with other components of the sarcomere. CW-EPR spectra of spin labeled hcTnT in Troponin ternary complexes show an increase in spin label mobility from residue 153 to 157 and 177, consistent with a decrease in alpha helical character across the putative hinge region. Preliminary doubly labeled CW-EPR experiments show that interspin distance between hcTnT residues 157 and 177 exceed 25Å. Interspin distance measurements using doubly labeled hcTnT will further elucidate the secondary and tertiary structure of this region. Additional spin label pairs are currently being investigated using both CW-EPR and DEER techniques to determine the structural details of this important region.

1855-Pos

Tropomyosin-Kappa Alters Cardiac Dynamics in a Mouse Heart Model

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Tropomyosin-kappa (TPM1 κ), a novel TM isoform, is exclusively expressed in the human heart. Alternative splicing of the α -TM gene generates TPM1 κ , in which the skeletal muscle exon 2b is replaced by the smooth muscle exon 2a. We previously reported that TPM1 κ expression was increased in the hearts of patients with chronic dilated cardiomyopathy (DCM). To understand how the TPM1 κ isoform affects cardiac dynamics, we generated transgenic (TG) mice expressing TPM1 κ in the myocardium. Most of the native TM (90%) is replaced by TPM1 κ . In situ cardiac dynamics were determined by echocardiographic analysis. Results demonstrated that the TG hearts exhibited a diastolic dysfunction associated with a dilation of the left ventricle compared with the non transgenic (NTG) controls. We also compared force-pCa relations in detergent extracted (skinned) fiber bundles isolated from NTG and TG-TPM1 κ hearts at sarcomere lengths (SL) 1.9 μm and 2.3 μm . Our data demonstrated a significant decrease in the Ca^{2+} sensitivity of the