Phosphorylation of cardiac myosin binding protein C abolishes myosin binding and preserves actin binding to accelerate relaxation kinetics in engineered cardiac tissue

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Cardiac myosin binding protein-C (cMyBP-C) is a sarcomeric protein that regulates contractility in response to increased circulatory demand. Phosphorylation of cMyBP-C during adrenergic stimulation increases cardiac contractility and reduced cMyBP-C phosphorylation in heart failure due to reduced adrenergic signaling contributes to decreased cardiac contractility. In addition, mutations in cMyBP-C linked to hypertrophic cardiomyopathy (HCM) may contribute to the development of the disease by altering phosphorylation. We tested the hypothesis that phosphorylation of cMyBP-C at Ser-273, Ser-282, and Ser-302 determines its ability to bind myosin and actin binding, thereby modulating cardiac function. The three phosphorylatable serines in cMyBP-C were replaced with phosphomimetic aspartate (DDD, n=11) and non-phosphorylatable alanines (AAA, n=7) and expressed in mouse engineered cardiac tissue (ECTs) lacking endogenous cMyBP-C to determine the primary contractile effects of phosphorylation in the absence of confounding remodeling events. AAA ECTs exhibited similar contractile kinetics to WT ECTs but ablated the kinetic response to adrenergic stimulation, manifesting the dephosphorylated state of WT ECTs. DDD ECTs had reduced time to 50% relaxation (0.026±0.01 sec vs. 0.037±0.01 sec in WT, $P<0.05$). Interestingly, DDD substituted cMyBP-C exhibited faster late relaxation kinetics compared to complete ablation of cMyBP-C (0.024±0.01 sec vs. 0.028±0.01 sec in KO, $P<0.05$). Isothermal titration calorimetry performed with purified cMyBP-C C0C3 domains containing the aspartate substitutions (n=3) abolished binding to myosin compared to the WT domains. C0C3 binding to actin assayed by co-sedimentation (n=3) showed identical binding affinity of WT and phosphomimetic cMyBP-C to actin filaments (K_d=13.52±0.72 uM vs. 13.67±0.44 uM in WT). Our data suggest that phosphorylation of cMyBP-C accelerates relaxation through the abolishment of myosin binding, without affecting its *in vitro* affinity for actin. These results also indicate that ECTs are a valuable pure expression model to characterize the functional and binding characteristics of each unique phosphorylation site of cMyBP-C.