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Should we treat heart failure with phosphatase inhibitors? Better to start at the end

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Heart failure (HF) is a syndrome characterized by the inability of the heart to pump sufficient blood to meet the metabolic demands of the body. Due to the fact that HF is the leading cause of death in Western society with limited medical treatment options, novel therapeutic agents for its treatment are desperately needed.

Heart Failure

There are many different causes of HF (chronic hypertension, ischemic heart disease, toxic drug treatment, mutations of sarcomeric or cytoskeletal proteins, etc.). Regardless of the pathological event, HF culminates with poor cardiac contraction and a progressive decline in pump function [1]. At the ventricular myocyte level, there is a dysregulation of sarcoplasmic reticulum (SR) Ca^{2+} handling. That is, there is a reduction in SR uptake due to decreased SR Ca^{2+} -ATPase (SERCA) expression and/or reduced phospholamban (PLB) Serine16 phosphorylation. This decrease in SR uptake lowers SR Ca^{2+} load, which in turn, results in diminished SR Ca^{2+} release and cardiac contraction [2]. Another cause of the decreased SR Ca^{2+} load is increased diastolic SR Ca^{2+} leak brought about via ryanodine receptor (RyR) hyperphosphorylation [3]. There is also an increased expression of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger resulting in a loss of Ca^{2+} from the cell. In addition to the intrinsic changes of the myocyte leading to the dysregulation of Ca^{2+} , there is a decline in the inotropic reserve with HF [1]. The β -adrenergic (AR) cascade is the dominant pathway in the heart responsible for positive inotropy [4]. Stimulation of this cascade via epinephrine and/or norepinephrine increases cyclic AMP levels to activate protein kinase A, which phosphorylates numerous proteins involved in the contraction of the heart (e.g., L-type Ca^{2+} channel, PLB, RyR, troponin I (TnI), myosin-binding protein C (MyBP-C), etc.). Unfortunately, HF results in a decrease in inotropic drive due to downregulation of β_1 -AR receptors and upregulation of GRK2. In addition to changes in function, HF also results in structural changes. For example in ischemic disease, there is a large increase in fibrosis (e.g., scar formation) at the site of myocyte loss. As HF progresses there is a dilation of the left ventricle. This dilation is a result of thinning and stretching of the free wall. Ultimately these events contribute to the decreased cardiac contraction and the poor pump function observed in HF.

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Disclosures

None

With the molecular mechanisms defined for the Ca^{2+} dysregulation and loss of inotropic reserve in HF, investigators have focused on correcting these alterations with the hopes of restoring myocyte contraction. For example, while increasing SERCA expression via adeno-associated virus was beneficial in animal models of heart failure, clinical trials have been disappointing. Another approach involved increasing cyclic AMP levels to restore the β -AR drive (i.e., phosphodiesterase inhibitors), however this approach actually increased death by inducing arrhythmias. A more recent approach to treat HF has been to alter aberrant HF induced alterations in signaling by targeting phosphatases.

Protein phosphatase 1

The function of numerous proteins in the heart is regulated via post-translational modifications through various signal transduction pathways (e.g., β -AR). The foremost post-translational modification in the heart is phosphorylation. A key aspect of signaling pathways is termination, which in the case of phosphorylation is dephosphorylation. Hence, a vast majority of these phosphorylations and their functional effects are reversible by removal of the phosphate through proteins termed phosphatases. Therefore, protein phosphorylation levels are determined by the balance of kinase *and* phosphatase activity. Since phosphorylation occurs on tyrosine, serine, or threonine residues, there are specific tyrosine phosphatases and serine/threonine phosphatases. The regulation of important excitation-contraction coupling proteins (RyR, PLB, TnI, etc.) occurs through the phosphorylation of serine/threonine residues. The two most important serine/threonine phosphatases in the myocyte are PP1 and PP2A. While significant work has been done on various kinases, much less is known about these phosphatases. Seminal work is now beginning to unravel the regulation and function of phosphatases in the heart [5, 6]. In terms of modulating various excitation-contraction coupling protein phosphorylation levels, and thus heart function, the PP1 enzyme is the most prominent [7]. Mammals have three PP1 genes that encode the catalytic subunit (PP1 α , PP1 β , and PP1 δ) [8]. While these three catalytic subunits are highly homologous in their catalytic domain, the N and C termini are very distinct. It is believed that this is important for substrate specificity and localization. Studies using pharmacological inhibitors and short hairpin RNA (shRNA) have suggested that PP1 (and specifically PP1 β) is a key protein responsible for SR Ca^{2+} handling via modulating PLB phosphorylation. Investigations using failing human heart tissue and experimental heart failure models have shown that PP1 expression and activity are increased [9, 10]. This increased phosphatase activity in turn results in decreased PLB phosphorylation and contractile dysfunction [11]. Hence, an attractive therapeutic approach would be to inhibit phosphatase activity. Indeed, recent work is now suggesting that inhibition of PP1 is a beneficial approach for the treatment of heart disease [12, 13]. Conversely, studies have demonstrated that inhibiting PP1 activity is actually detrimental [14, 15]. While, PP1 has a clear role in regulating heart function, its status in modulating specific protein phosphorylation levels and thus the direct effects on contractility is still unclear. The recent manuscript by Liu et al. [16] has addressed this uncertainty of PP1 regulating heart function by taking a genetic approach. The authors specifically focused on establishing the detrimental and potentially beneficial effects of directly decreasing PP1 activity on cardiac function.

PP1 knockout animals

In their manuscript titled “Cardiac-specific deletion of protein phosphatase 1 β promotes increased myofilament protein phosphorylation and contractile alterations” [16] Liu et al. created cardiac-specific PP1 isoform deleted mice via the Cre-lox system. Interestingly, unlike previous studies, knockdown of a catalytic subunit (α , β , or γ) did not result in any changes in myocyte Ca^{2+} handling nor PLB phosphorylation. While further studies must be done to resolve the discrepancy with previous results, we suggest that it may be due to compensatory adaptations in these mice. For example, the PP1 β knockout animal has significantly increased PP1 α expression with decreased I-1 expression. The altered cardiac function in the absence of altered Ca^{2+} transient and Ca^{2+} handling protein phosphorylation indicates a clear role of PP1 in the modulation of myofilament proteins. The authors identified the myosin regulatory light chain (RLC) and MyBP-C as specific myofilament targets for dephosphorylation via PP1 β . This is consistent with previous work demonstrating that RLC and MyBP-C are general PP1 targets, with the Liu et al. data identifying PP1 β as the primary modulator.

Unfortunately, it appears that inhibition of PP1 is not beneficial for the heart and may not be a suitable therapeutic approach. Knockdown of PP1 β (while improving cellular contraction) resulted in cardiac dysfunction with structural remodeling (e.g., dilation). The overall negative consequences of PP1 β knockdown are most likely due the altered phosphorylation of multiple targets. We therefore suggest a more direct approach to treat heart disease which is the need to “*start at the end*” by directly modulating the exact phosphorylation sites of specific proteins.

“Starting at the end”- myofilament protein phosphorylation

While knockdown of PP1 is clearly important to cardiac remodeling, our focus centers on PP1 regulation of cardiac contraction. The authors did a tremendous job identifying RLC and MyBP-C as targets for dephosphorylation by PP1 β . Both RLC and MyBP-C are phosphorylated to modulate myosin’s interaction with actin and thus cardiac contraction. The phosphorylation of RLC results in slowed relaxation [17]. The increase in RLC phosphorylation following PP1 β loss observed by Liu et al. is therefore a likely contributor to the resultant slowed relaxation. The functional effects of MyBP-C phosphorylation are more complicated since MyBP-C contains at least 4 phosphorylatable residues regulated by multiple kinases [18]. Generally, upon MyBP-C phosphorylation the rate of cardiac contraction and relaxation are increased [19]. However, there are varied effects of MyBP-C on function depending upon the degree and residue of phosphorylation (i.e., 1 site vs 4 sites phosphorylated) [20]. Thus, the Liu et al. data supports a role for PP1 β modulation of a specific MyBP-C residue. Importantly, increasing RLC or MyBP-C phosphorylation has been demonstrated to improve contractile deficits observed in cardiac disease [21, 22]. Therefore, RLC/MyBP-C phosphorylation (not PP1 β knockdown) may be key target protein post-translational modifications to alter cardiac function and used as a potential treatment for heart disease.

In addition to the observed altered phosphorylation changes in RLC and MyBP-C, it is entirely likely that PP1 β also directly modulates phosphorylation levels of other myofilament proteins, including tropomyosin, troponin T or TnI. Of specific interest, an elegant study demonstrated that the dephosphorylation of RLC resulted in altered TnI phosphorylation [23]. Thus, it appears that myofilament phosphorylation occurs via complex, interwoven signaling networks. The specific residue phosphorylation profile of myofilament proteins is both technically and quantitatively challenging to detect. These measurements often require in depth focused investigations to identify small but functionally relevant changes. Liu et al. clearly demonstrated RLC and MyBP-C are significant effectors of PP1 β functional effects. However, there are other myofilament phosphorylations that may play an equally important role in PP1 β modulation of cardiac function that were not examined. Therefore, these additional phosphorylations may be beneficial targets for the treatment of heart failure.

As an example, the phosphorylation of TnI Serine residues 23/24 (Ser23/24) is a key target to improve heart function in disease [24]. For decades, it is has been clear that the phosphorylation of TnI at Ser23/24 plays a significant role in cardiac adaptations with physiological and pathological insults. TnI Ser23/24 phosphorylation is maintained at a basal state in the normal heart, elevated with increased physiological demand through β -AR signaling and dramatically decreased in end stage heart failure. TnI, however, is also phosphorylated on at least 12 other residues in addition to Ser23/24 [25]. Many of these sites have also been demonstrated to play a significant role in cardiac disease. However, identifying and/or measuring these residue specific TnI phosphorylation events are difficult due the lack of commonly available TnI residue specific phospho-antibodies. Moreover, assessing changes in the phosphate occupancy of *other* (i.e., non-Ser23/24) TnI residue is challenging because of the high basal TnI phosphorylation background (i.e., upwards of 40% TnI Ser23/24 phosphorylation occupancy). For example, discovering a 5% increase in phosphorylation at one residue or a 5% increase at one with a concurrent 5% decrease at a second residue renders such detection technically challenging by global phospho-protein detection methods. To further exacerbate the situation, phosphorylation of other TnI residues can directly modulate the functional effects of Ser23/24. Remarkably, these effects occur without directly changing the TnI Ser23/24 phosphorylation levels [26, 27]. While Liu et al. thoroughly demonstrated PP1 β knockdown does not significantly alter TnI Ser23/24 phosphorylation; the global determination of TnI phosphorylation may have missed more subtle, but potentially significant, changes in other TnI residues. It will be interesting to investigate in further detail which specific myofilament residues PP1 β is able to modulate.

While the regulation and functional effects of the other TnI phosphorylation residues are not completely clear, recent studies are demonstrating these sites are indeed major nodes for the modulation of cardiac contraction. Work has shown that the phosphorylation of TnI Ser43/45 or Thr144 regulates contractility [28, 29]. Recent work is now finding novel TnI residues (i.e., Ser150 and Ser166) with altered phosphorylation in disease states [25, 27, 29]. Not only are new residues being identified, the myofilament modifications are evolving to demonstrate that there is an actual integration of these varied phosphorylations onto cardiac function allowing for an additional layer of contractile regulation via TnI. We have also just

characterized the first TnI tyrosine (residue 26) phosphorylation [30] identified in the human heart [25]. This phosphorylation represents the only tyrosine phosphorylation of any myofilament protein demonstrated to modulate cardiac contraction and therefore provides a novel signaling pathway to target towards the treatment of HF induced contractile dysfunction. Thus, the vast number of TnI phosphorylations, their diverse occupancy and their varied effects on multiple contractile and relaxation parameters provide the opportunity to develop specific phosphorylation target treatments for cardiac diseases.

The excellent study by Liu et al. [16] revealed protein targets of the specific catalytic subunits of PP1. However, with the wide range of protein targets and the diverse functions of PP1, a better approach may be to target precise phosphorylation sites of specific myofilament protein residues (i.e., start at the end).

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