Targeting Protein Phosphatase 1 in Heart Failure

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Over the past three decades the treatment of heart failure has shifted from a palliative care approach to a more successful focus on neurohormonal modulation through drugs that inhibit β-adrenergic, angiotensin, and aldosterone signaling. Despite these relatively recent advances, the clinical syndrome of heart failure remains progressive in nature. This observation begs the question as to a unifying aberrancy in cardiomyocyte phenotype that occurs in heart failure. More recently great attention has been paid to the alterations in calcium handling and has typically been associated with calcium-transients that are of a lower amplitude and slower decline than normal cardiomyocytes. This slower decline has been attributed to a decrease in the Ca$^{2+}$ uptake into the sarcoplasmic reticulum (SR); and particularly with the relative role of phospholamban (PLB) and the SR Ca$^{2+}$-ATPase (SERCA). Whereas previous work has successfully targeted PLB and SERCA in the investigational treatment of heart failure in mouse, rat, and human myocytes, more recent work has focused on the potential modulators of this system to elucidate the pathophysiologic changes that result in aberrant calcium handling.

In this issue, Pathak and del Monte et al show that targeted inhibition of protein phosphatase 1 (PP1) by increased activity of its inhibitor, inhibitor-1 in the heart, results in enhanced contractility and is protective against the development of cardiac hypertrophy and heart failure stimulated by hemodynamic load. In failing hearts, the downregulation of adrenergic receptor and cAMP-dependent protein kinase signaling leads to the inactivation of inhibitor-1 which, in turn, results in increased activity of PP1. This activation of PP1 leads to the dephosphorylation of phospholamban thus reducing calcium uptake by SERCA-2a. In an important series of experiments in transgenic mice and using gene transfer in rats, Kranias, Hajjar, and colleagues now show that the targeted inhibition of protein phosphatase 1 by overexpression of inhibitor-1 enhances cardiac contractility under normal hemodynamic conditions as well as in response to adrenergic stimulation with isoproterenol. Moreover, the authors show that targeted gene transfer of active inhibitor-1 in rats results in marked attenuation of the functional and morphometric changes associated with chronic pressure overload.

As a first step, the investigators determined the influence of the expression of a constitutively active, truncated analog of inhibitor-1 that was targeted to the cardiac myocytes. Both intact hearts and isolated myocytes expressing this activated inhibitor-1 exhibited enhanced contractility under basal conditions and augmented responsiveness to adrenergic stimulation. This enhanced contractility was associated with increased phosphorylation of phospholamban and the subsequent enhanced SERCA-2a activity.

When subjected to chronic pressure overload, mice expressing active inhibitor-1 exhibited less cardiac hypertrophy and interstitial fibrosis than wild-type mice. These beneficial effects were associated with a reduction in the activation of the MAP-kinase hypertrophic pathway, ERK1/2, but p38 activation was unchanged. Although phospholamban phosphorylation was reduced in the banded transgenic mice when compared with sham controls, this activation of the SERCA modulator remained higher than wild-type banded mice.

Because the clinical treatment of heart failure deals with preexisting conditions and is not generally directed at the prevention of disease, the authors turned to a model of chronic pressure overload–induced heart failure in the rat. In this series of experiments, adult rats subjected to aortic banding were transfected with a virus encoding the active inhibitor-1. Rats transfected with inhibitor-1 had a marked improvement in both systolic and diastolic left ventricular function. As with the studies in transgenic mice, this improvement in function was associated with an increase in SERCA-2a activity, albeit the total protein levels were reduced with heart failure.

This novel series of experiments further highlights the role of protein phosphatases in modulating not only cardiac function, but also a role in the response to stress. In recent years there has been a great deal of attention paid to intracellular mediators of hypertrophic signaling cascades, and data have been inferred to link some of these proteins and transcription factors to the early development of heart failure. To that end, many protein kinases and their substrates that mediate the expression of hypertrophy and progression to heart failure have been relatively well characterized. Much less relative attention has been paid to the protein phosphatases in this regard. The major Ser/Thr phosphatases such as type 1, type 2A, and type 2B (calcineurin) are a highly homologous family of proteins that play a critical role in the modulation of cardiac contractility, the expression of hypertrophy, and the progression to heart failure. In human and experimental heart failure, the type 1 phosphatase which is associated with the sarcoplasmic reticulum is activated through the dephosphorylation of its inhibitor protein, inhibitor-1. Molkentin and colleagues have recently shown that protein kinase C-alpha (PKC-α) plays a critical role in the regulation of cardiac contractility and the propensity of the...
PKC-/H9251-altered under conditions in which there is no change in cardiac hypertrophy where protein phosphatase 1 activity is divergent in the role of these proteins in the expression of heart failure (Figure).7–9 The findings of the present study and prior studies PKC-/H9251/ inhibit-1 are normal in size and histology but have expression and activity is increased, there is a divergence in the role of these proteins in the expression of cardiac hypertrophy where protein phosphatase 1 activity is altered under conditions in which there is no change in PKC-/H9251.9 Data from the present study showing marked prevention of cardiac hypertrophy under conditions of increased inhibitor-1 expression support this hypothesis and may suggest that the major influence on inhibitor-1 phosphorylation under conditions of hypertrophy is from PKA activation or another protein kinase. The present data support the hypothesis that although the stimuli may be different in hypertrophy and heart failure, the common pathway involves inhibitor-1 and the influence on protein phosphatase 1. Moreover, the observation that the hearts of transgenic mice overexpressing inhibitor-1 are normal in size and histology but have enhanced contractility suggests that under basal conditions, the role of inhibitor 1 and protein phosphatase 1 is relegated to another protein kinase. However, under stress from pressure overload, the role for this pathway also involves the expression of hypertrophy, remodeling, and the progression to heart failure. Unresolved, however, is the mechanism by which protein phosphatase 1 modulates hypertrophy signaling. It is unlikely that all of the observed effects are mediated solely by the effects of protein phosphatase 1 and its action at the level of its influence on phospholamban. Intriguingly, when Kranias and colleagues banded mice that were deficient in phospholamban, the greater contractility provided through enhanced SERCA-2a function did not impact the expression of cardiac hypertrophy nor the influence on the progression to heart failure. Furthermore, the genetic lack of phospholamban does not improve ventricular function in other models of heart failure such as overexpression of tropomodulin,13 Goq,14 mutant myosin binding protein C,15 or TNF overexpression,16 despite the fact that prolonged myocyte calcium transients are rescued. Although it is possible that the differences between the present article and previous reports rest in the differences between genetically altered mice and models used, it is unlikely that resolution of one aspect of heart failure (ie, calcium cycling) is sufficient to prevent or erase the heart failure phenotype. It is possible that enhanced inhibitor-1 expression/activity results in multiple benefits by providing some resolution of calcium transients through its effect on phospholamban, but also by other downstream effects on signaling pathways associated with the heart failure such as PKC17 and NCX18 or with cell survival through apoptosis regulators such as Bcl-2.19 The present publication opens the door for an intriguing study to determine these potential novel downstream targets of inhibitor 1 and/or protein phosphatase 1 that may work in conjunction with the better understood effect on calcium cycling.

The targeting of modulators of calcium handling proteins, and specifically the role of protein phosphatases in this modulation, have recently been thought of as therapeutic targets for the treatment of heart failure with the relatively recent observations that phospholamban and SERCA-2a play a central role in the disease. How should we integrate the
present data with the previously published body of evidence? It would seem that a disease that is as highly integrated as heart failure would not be one in which a single approach (ie, phospholamban ablation) would erase this clinical syndrome that is overwhelmingly complex. The present data support this hypothesis by demonstrating that working upstream of phospholamban may afford an additional benefit in the targeting of heart disease. As with the modulation of PKC-α, the prevention and treatment of heart failure by stimulation of inhibitor 1 may be more amenable to pharmacological targeting than the direct targeting of phospholamban or SERCA-2a.

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