Abstract: P304
Reprogramming of the protein phosphatase 1 interactome during heart failure progression

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Background: Heart failure (HF) is a complex disease with a rising prevalence despite advances in treatment. Protein phosphatase 1 (PP1) has long been implicated in HF pathogenesis but its exact role is both unclear and controversial. This is due to the fact that most prior studies measured only the PP1 catalytic subunit (PP1c) without investigating its diverse set of interactors, which confer localization and substrate specificity to the holoenzyme and constitute the PP1 interactome.

Purpose: To comprehensively define the PP1 interactome in cardiac tissue and test the hypothesis that this interactome becomes reprogrammed during HF progression at the level of specific PP1c interactors.

Methods: Mice were subjected to transverse aortic constriction and grouped based on ejection fraction (EF) into sham (n=24), hypertrophy (n=10), moderate HF (n=10; EF 30-40%), and severe HF (n=10; EF<30%). Cardiac lysates were subjected to affinity-purification using anti-PP1c antibodies followed by mass spectrometry, and subsequent western blot validation. To achieve cardiac knockdown of Ppp1r7, a key PP1c interactor, AAV9 vectors expressing shRNA targeting Ppp1r7 or scramble sequence were injected into aMHC-Cre mice. These mice were followed by echocardiography and ventricular myocytes were isolated for Ca imaging. PPP1R7 knockdown was also achieved in HeLa cells using siRNA and studied using western blots and mass spectrometry.

Results: 71 PP1c interactors and their binding to PP1c were quantified from mouse cardiac lysates, including numerous novel interactors. 9 interactors were strongly associated with HF progression including two known (Ppp1r7, Ppp1r18) and 7 novel (Des, Dbt, Prre1, Ccdc85c, Hnrpnm, Hsd17b8, Bckdha) interactors. Among all interactors Ppp1r7 had the highest binding to PP1c and its binding was significantly increased during HF progression (p<0.001). Cardiac-specific knockdown of Ppp1r7 in mice (n=9) led to a significant reduction in EF compared to controls (n=5) at 6 weeks (56±1.9% vs. 64±1.4%; p<0.05) and 8 weeks (50±1.6% vs. 65±2.5%; p<0.001) post AAV9 injection. Ventricular myocytes isolated from these mice showed increased Ca sparks frequency compared to controls (n=27 cells/4 mice vs. 20 cells/3 mice; 3.1±0.5 vs. 1.5±0.3; p<0.05). PPP1R7 knockdown in HeLa cells (n=4 vs. 4 plates; ~70% knockdown; p<0.0001) led to reprogramming of the PP1 interactome with a global increase in the binding of most other interactors to PP1c.

Conclusions: In this study we established the largest reproducible PP1 interactome to date, using mouse cardiac tissue and HeLa cells. We showed that this interactome is reprogrammed at the level of at least 9 PP1c interactors during HF progression, which may represent novel pathways for early therapeutic interventions. Of these we showed that Ppp1r7 plays a critical role in cardiac function and may serve as a compensatory sink that maintains homeostasis of the PP1 interactome during HF progression.
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