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EDITORIAL COMMENT

Unraveling the Mechanisms by Which Calpain Inhibition Prevents Heart Failure Development*

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Heart failure (HF) is a complex clinical syndrome defined as an inadequate cardiac performance to meet the metabolic demands of tissues in the body. HF affects 6 million people in the United States alone, and its prevalence is expected to grow by 40% over the next decade (1). The pathophysiology of HF is complex and may involve neurohormonal changes, altered energy metabolism, increased oxidative stress, and intracellular calcium (Ca^{2+}) overload. Adverse cardiac remodeling involves activation of proteases that play a role in the degradation of both intracellular and extracellular targets. The effects of these proteases that include matrix metalloproteinases, cathepsins, caspases, and calpains are synergistic and may represent targets to prevent the decline in cardiac function associated with HF (2).

Calpains are Ca^{2+}-activated proteolytic enzymes. More than 15 isoforms are encoded by distinct genes, and alternative splicing generates even more variants (3). The 2 main isoforms expressed in the heart are calpain-1 (μ-calpain) activated by μM Ca^{2+} concentrations and calpain-2 (m-calpain) activated by mM Ca^{2+} concentrations (4). Calpains may contribute to myocardial hypertrophy and inflammation, mainly through the activation of transcription factors such as nuclear factor-κB. They play an important role in the formation of interstitial fibrosis partly by activating transforming growth factor-β. Moreover, calpains have been implicated in apoptosis in part because they cause the breakdown of sarcolemma and sarcomeres (5).

Recent studies demonstrated that 1 of the protein targets cleaved by calpain is junctophilin-2 (JPH2), an intermembrane-linked protein that maintains the plasmalemma and sarcoplasmic reticulum at a fixed distance to ensure proper excitation-contraction (EC) coupling (6-9). JPH2 is essential for the maturation of transverse tubules (TTs) and development of efficient EC coupling in adult cardiac myocytes (10,11). Several studies have shown that HF precipitates a loss of the TT network, which in turn impairs cardiac contractility (12,13). Because reduced JPH2 levels have been observed in patients and animals with HF (13-17), loss of JPH2 has been implicated in TT remodeling in HF, although some have disputed a direct role for JPH2 (18).

Cleavage by calpains has been put forward as 1 of the main mechanisms underlying the loss of JPH2 levels in failing hearts (8,9,19). Because calpain activity is increased in myocardial tissue subjected to stress (i.e., ischemia, oxidative stress, HF) (20,21), it may not be surprising that Ca^{2+}-dependent proteolysis of JPH2 has been reported under pathological conditions (8,9,19). In mouse hearts, ischemia/reperfusion injury was shown to reduce JPH2 levels, a

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process that was reversible by endogenous calpain inhibitor calpastatin (19).

SEE PAGE 503

In this issue of *JACC: Basic to Translational Science*, Wang et al. (22) show that pharmacological inhibition of calpain activity protects against cardiac dysfunction in mouse models of HF. They showed increased calpain-1 protein and activity levels in heart samples from patients with ischemic or dilated cardiomyopathy. These studies confirm prior studies showing increased levels of both calpain-1 and calpain-2 in patients with New York Heart Association functional class III and IV HF (23). Because calpain-2 is activated at higher (pathological) Ca^{2+} concentrations, it would be interesting to assess in future studies whether calpain-2 expression and activity levels are also altered in human failing hearts. In addition, it is unknown whether other calpain isoforms are upregulated in the human heart under pathological conditions.

Next, Wang et al. (22) determined the effects of calpain inhibitor MDL-28170 in 3 established mouse models of HF, namely myocardial infarction as a result of left anterior descending coronary artery ligation, pressure overload by transverse aortic constriction (TAC), and isoproterenol infusion using an osmotic minipump. Administration of the inhibitor was started 3 days after the disease models were initiated; however, in prior studies, it was shown that calpain levels do not significantly increase in mice until 7 days post-MI. Similarly, in the present work, calpain activity did not increase until 2 weeks after TAC. Therefore, these studies assessed the effects of a prevention strategy as opposed to a more clinically relevant therapeutic intervention in which treatment would need to be initiated after the development of HF. The authors did conduct a secondary study in a small cohort of TAC mice in which MDL-28170 treatment was started 3 weeks after surgery; this revealed a reduced or delayed progression of HF. However, the compound induced only partial calpain inhibition, and the mechanism of reduce HF-related remodeling remains incompletely understood.

Although it was shown that the compound MDL-28170 normalized calpain levels at 5 weeks after myocardial infarction or TAC, or 2 weeks after isoproterenol, it remains unclear whether the protein and activity levels of calpain-1 and calpain-2 were altered in these HF mouse models, and in turn reversed by MDL-28170 treatment. The authors ascribed the therapeutic effects of calpain inhibition to inhibition of fibrosis and prevention of the loss of TT in failing hearts; however, it has been shown that calpain inhibition can also attenuate inflammation (24), apoptosis (20), and the disassembly of intercalated disks (20), among other things. Moreover, hypertrophic remodeling as a result of calcineurin activation has been attributed to enhanced calpain activity (23). It is unlikely therefore that the primary mechanism by which calpain inhibition improves cardiac function in animals with HF is the prevention of deleterious TT remodeling. Changes in TT remodeling could very well be the consequence of improved cardiac function.

The authors did perform additional experiments to address the question of whether JPH2 is a critical calpain substrate involved in HF development. Wang et al. (22) generated double transgenic mice overexpressing both calpain-1 and JPH2 by approximately 3-fold. The hypothesis tested by this experiment was whether overexpression of JPH2 could protect against the detrimental effects of calpain-1 hyperactivity. Despite an early protective effect, mortality rates and cardiac remodeling were not improved by JPH2 overexpression. Of interest, biochemical studies revealed that JPH2 levels declined in calpain-1/JPH2 double transgenic mice despite overexpression of JPH2, suggesting that calpain-1 activity levels in the transgenic mice were too high to be physiologically relevant. Regardless, these experiments did not prove the theory that loss of junctophilin levels is the mechanism by which increased calpain activity causes HF. Comparing calpain activity levels in calpain transgenic mice with those in the different models of HF would have provided useful information in this regard. If calpain activity levels were similar in all of these models, it would be important to rule out other pathways of JPH2 degradation (e.g., micro-ribonucleic acid-mediated decay) (25-27). In addition, it was quite surprising that the authors did not include JPH2 single transgenic mice in their studies, because this would have been an important control group for the double transgenic mice. Interestingly, prior studies have demonstrated that transgenic or adeno-associated virus-mediated overexpression of JPH2 slows down the progression of HF after TAC (28,29). In those studies, when calpain was not overexpressed, it was possible to prevent a reduction of JPH2 levels below those seen in sham control mice, providing further evidence that the calpain-1 transgenic mice are probably not suitable to model HF pathophysiology.

Cleavage of JPH2 by calpains yields several proteolytic peptides. Physiological Ca^{2+} concentrations can activate calpain-1, which in turn cleaves JPH2 into several peptides (8). Guo et al. (19) showed that calpain-1 cleaves JPH2 at multiple sites in both

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 3, NO. 4, 2018
AUGUST 2018:518–20

the N- and C-terminal regions. At present, it is unclear whether these peptides have any cellular functions. Overexpression of some of the N- and C-terminal JPH2 peptides in myocytes from JPH2-knockdown mice failed to normalize sarcoplasmic reticulum Ca^{2+} transients (19), unlike full-length JPH2 (7). Those studies suggest that the proteolytic JPH2 peptides might not be involved in regulating EC coupling in myocytes. Future studies are warranted to investigate whether these peptides have other roles in myocytes.

In conclusion, Wang et al. (22) showed increased calpain activity levels in failing human hearts. Using 3 different mouse models of HF, they demonstrated that pharmacological inhibition of calpain blunted the development of HF and preserved TT structure in ventricular myocytes. Overexpression of JPH2 delayed but did not prevent the development of end-stage HF and mortality in calpain-1 transgenic mice. However, these studies are inconclusive because JPH2 levels still dropped below those seen in control animals. Therefore, these preclinical studies suggest that calpain inhibition might represent a possible therapeutic strategy for HF treatment, although the underlying mechanisms remain poorly understood.

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REFERENCES

1. Writing Group Members, Mozaffarian D, Benjamin EJ, et al. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. Circulation 2016;133:e38–60.

2. Muller AL, Dhalla NS. Role of various proteases in cardiac remodeling and progression of heart failure. Heart Fail Rev 2012;17:395–409.

3. Huang Y, Wang KK. The calpain family and human disease. Trends Mol Med 2001;7:355–62.

4. Potz BA, Abid MR, Sellke FW. Role of calpain in pathogenesis of human disease processes. J Nat Sci 2016:2.

5. Letavernier E, Zafrani L, Perez J, Letavernier B, Haymann JP, Baud L. The role of calpains in myocardial remodelling and heart failure. Cardiovasc Res 2012;96:38–45.

6. Takeshima H, Komazaki S, Nishi M, Iino M, Kangawa K. Junctophilins: a novel family of junctional membrane complex proteins. Mol Cell 2000;6:11–22.

7. van Oort RJ, Garbino A, Wang W, et al. Disrupted junctional membrane complexes and hyperactive ryanodine receptors after acute junctophilin knockdown in mice. Circulation 2011;123:979–88.

8. Murphy RM, Dutka TL, Horvath D, Bell JR, Delbridge LM, Lamb GD. Ca2+-dependent proteolysis of junctophilin-1 and junctophilin-2 in skeletal and cardiac muscle. J Physiol 2013;591:719–29.

9. Wu CY, Chen B, Jiang YP, et al. Calpain-dependent cleavage of junctophilin-2 and T-tubule remodeling in a mouse model of reversible heart failure. J Am Heart Assoc 2014;3:e000527.

10. Reynolds JO, Chiang DY, Wang W, et al. Junctophilin-2 is necessary for T-tubule maturation during mouse heart development. Cardiovasc Res 2013;100:44–53.

11. Chen B, Guo A, Zhang C, et al. Critical roles of junctophilin-2 in T-tubule and excitation-contraction coupling maturation during postnatal development. Cardiovasc Res 2013;100:54–62.

12. Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. Proc Natl Acad Sci U S A 2006;103:4305–10.

13. Wei S, Guo A, Chen B, et al. T-tubule remodeling during transition from hypertrophy to heart failure. Circ Res 2010;107:520–31.

14. Minamisawa S, Oshikawa J, Takeshima H, et al. Junctophilin type 2 is associated with caveolin-3 and is down-regulated in the hypertrophic and dilated cardiomyopathies. Biochem Biophys Res Commun 2004;325:852–6.

15. Prosser BL, Ward CW, Lederer WJ. Subcellular Ca2+ signaling in the heart: the role of ryanodine receptor sensitivity. J Gen Physiol 2010;136:135–42.

16. Salnikov V, Lukyanenko YO, Lederer WJ, Lukyanenko V. Distribution of ryanodine receptors in rat ventricular myocytes. J Muscle Res Cell Motil 2009;30:161–70.

17. Landstrom AP, Weisleder N, Batalden KB, et al. Mutations in JPH2-encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. J Mol Cell Cardiol 2007;42:1026–35.

18. Guo Y, VanDusen NJ, Zhang L, et al. Analysis of cardiac myocyte maturation using CASAAV, a platform for rapid dissection of cardiac myocyte gene function in vivo. Circ Res 2017;120:1874–88.

19. Guo A, Hall D, Zhang C, et al. Molecular determinants of calpain-dependent cleavage of junctophilin-2 protein in cardiomyocytes. J Biol Chem 2015;290:17946–55.

20. Kudo-Sakamoto Y, Akazawa H, Ito K, et al. Calpain-dependent cleavage of N-cadherin is involved in the progression of post-myocardial infarction remodeling. J Biol Chem 2014;289:19408–19.

21. Takeshita D, Tanaka M, Mitsuyama S, et al. A new calpain inhibitor protects left ventricular dysfunction induced by mild ischemia-reperfusion in in situ rat hearts. J Physiol Sci 2013;63:113–23.

22. Wang Y, Chen B, Huang CK, et al. Targeting calpain for heart failure therapy: mplications from multiple murine models. J Am Coll Cardiol Basic Trans Science 2018;3:503–17.

23. Yang D, Ma S, Tan Y, et al. Increased expression of calpain and elevated activity of calcineurin in the myocardium of patients with congestive heart failure. Int J Mol Med 2010;26:159–64.

24. Poncelas M, Inserte J, Aluja D, Hernando V, Vilardosa U, Garcia-Dorado D. Delayed, oral pharmacological inhibition of calpains attenuates adverse post-infarction remodelling. Cardiovasc Res 2017;113:950–61.

25. Li RC, Tao J, Guo YB, et al. In vivo suppression of microRNA-24 prevents the transition toward decompensated hypertrophy in aortic-constricted mice. Circ Res 2013;112:601–5.

26. Xu M, Wu HD, Li RC, et al. Mir-24 regulates junctophilin-2 expression in cardiomyocytes. Circ Res 2012;111:837–41.

27. Zhang HB, Li RC, Xu M, et al. Ultrastructural uncoupling between T-tubules and sarcoplasmic reticulum in human heart failure. Cardiovasc Res 2013;98:269–76.

28. Guo A, Zhang X, Iyer VR, et al. Overexpression of junctophilin-2 does not enhance baseline function but attenuates heart failure development after cardiac stress. Proc Natl Acad Sci U S A 2014;111:12240–5.

29. Reynolds JO, Quick AP, Wang Q, et al. Junctophilin-2 gene therapy rescues heart failure by normalizing RyR2-mediated Ca(2+) release. Int J Cardiol 2016;225:371–80.

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