Myosin light chain dephosphorylation by ppp1r12c promotes atrial hypocontractility in atrial fibrillation

F.J. Gonzalez-Gonzalez1, S. Perike1, I. Abu-Taha2, F.W. Damen3, K. Lizama1, A. Aboonabi1, Y. Aguilar-Sanchez4, S.G. Ong1, D. Darbar1, C.J. Goergen3, B. Wolska1, D. Dobrev2, X.H.T. Wehrens4, M. Mccauley1

1University of Illinois at Chicago, Chicago, United States of America
2University of Duisburg-Essen - West-German Heart and Vascular Center, Essen, Germany
3Purdue University, West Lafayette, United States of America
4Baylor College of Medicine, Houston, United States of America

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Background: Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, increases thromboembolic stroke risk five-fold. Although atrial hypocontractility contributes to stroke risk in AF, the molecular mechanisms reducing myofilament contractile function remain unknown. We tested the hypothesis that increased expression of PPP1R12C, the PP1 regulatory subunit targeting atrial myosin light chain 2 (MLC2a), causes hypophosphorylation of MLC2a and results in atrial hypocontractility.

Methods: Right atrial appendage tissues were isolated from human AF patients versus sinus rhythm (SR) controls. Western blots, co-immunoprecipitation, and phosphorylation studies were performed to examine how the PP1c-PPP1R12C interaction causes MLC2a de-phosphorylation. In vitro studies of pharmacologic MRCK inhibitor (BDP5290) in atrial HL-1 cells were performed to evaluate PP1 holoenzyme activity on MLC2a. Cardiac-specific lentiviral PPP1R12C overexpression was performed in mice to evaluate atrial remodeling with atrial cell shortening assays, echocardiography, and AF inducibility with EP studies.

Results: In human patients with AF, PPP1R12C expression was increased two-fold versus SR controls (P=0.02, n=12,12 in each group) with >40% reduction in MLC2a phosphorylation (P=0.00001, n=12,12 in each group). PPP1R12C-PP1c binding and PPP1R12C-MLC2a binding were significantly increased in AF (P=0.03 and 0.007 respectively, n=8,8 in each group). In vitro studies utilizing drug BDP5290, which inhibits T560-PPP1R12C phosphorylation, demonstrated increased PPP1R12C binding with both PP1c and MLC2a, and dephosphorylation of MLC2a. Lenti-12C mice demonstrated a 150% increase in LA size versus controls (P=5.0x10-6, n=12,8,12), with reduced atrial strain and atrial ejection fraction. Pacing-induced AF in Lenti-12C mice was significantly higher than controls (P=0.02 and 0.04 respectively, n=6,6,5).

Conclusions: AF patients exhibit increased levels of PPP1R12C protein compared to controls. PPP1R12C overexpression in mice increases PP1c targeting to MLC2a and causes MLC2a dephosphorylation, which reduces atrial contractility and increases AF inducibility. These findings suggest that PP1 regulation of sarcomere function at MLC2a is a key regulator of atrial contractility in AF.
Figure Legend: (A) Western blot showing the expression of PPP1R12C in human ventricular tissue with stress-induction (SR) and chronic allopurinol (cAF). (B) Quantification of PPP1R12C protein abundance in each group. (C) Western blot and phosphorylation assays showing the phosphorylation of MLCK in cAF and SR tissues. (D) Quantification of bid change in phosphorylation of MLCK. (E) Co-immunoprecipitation assay showing PP1R12C and MLCK binding in human cAF and SR tissues. (F) Quantification of PP1R12C and MLCK binding. (G) Western blot showing increases in MLCK abundance in human hearts treated with leptin (Lenti-12C) versus controls. (H) Expression of PP1R12C in heart versus Lenti-12C mice, n = 5-7 in each group. (I) Quantification of MLCK protein expression in the three mice models, n = 3-5 in each group. (J) Representative tracing of contraction amplitude in the three groups. (K) Total collagen and total collagen in heart versus Lenti-12C mice, n = 5-7 in each group. (L) Western blot showing the expression of N-cadherin in heart versus Lenti-12C mice. (M) Western blot showing the expression of N-cadherin in heart versus Lenti-12C mice, n = 5-7 in each group. (N) Quantification of N-cadherin expression in the three mice models, n = 3-5 in each group. (O) Representative tracing of contraction amplitude in the three groups. (P)oky ejection fraction in heart versus Lenti-12C mice, n = 5-7 in each group. (Q) Quantification of N-cadherin expression in the three mice models, n = 3-5 in each group. (R) Representative tracing of contraction amplitude in the three groups. (S) Quantification of N-cadherin expression in the three mice models, n = 3-5 in each group. (T) Surface electrocardiogram in mice receiving transmyocardial laser perfusion, Lenti-12C mice are prone to perfusion-induced HP. (U) Quantification of HP among WT, Lenti-12C, and Lenti-42C mice.