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

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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 comparing the expression of PPP1R12C in human aortic tissues with aortic stenosis (SR) or chronic renal failure (cAF). (B) Quantification of PPP1R12C Protein abundance: n=12/group. (C) Western blot and immunoblot analysis showing the phosphorylation and abundance of MLCK in cAF and SR tissues. (D) Western blot showing a difference in the phosphorylation of MLCK in cAF and SR tissues. (E) Co-immunoprecipitation assay showing PPP1R12C-MLCK binding in human cAF and SR tissues. (F) Western blot showing increased PPP1R12C expression in human aortic tissues transduced with lentiviral PPP1R12C-shRNA constructs (Lenti-12C) versus controls (Lenti-shRNA). (G) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (H) Western blot showing increased PPP1R12C expression in aortic tissue of Lenti-12C mice. (I) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (J) Western blot showing increased PPP1R12C expression in aortic tissue of Lenti-12C mice. (K) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (L) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (M) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (N) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (O) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (P) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (Q) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (R) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (S) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (T) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice. (U) Representative Western blots and densitometry analysis of PPP1R12C expression in WT and Lenti-12C mice.