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Expression and regulation of the type 2A protein phosphatase-alpha4 signalling axis in cardiac health and hypertrophy

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Introduction: Cardiac physiology and hypertrophy are regulated by the phosphorylation status of many proteins, which is partly controlled by type 2A phosphatases and alpha4, a protein that is central to type 2A phosphatase biogenesis (1). The present study aimed to determine the expression and regulation of type 2A protein phosphatase catalytic subunit-alpha4 signalling axis in healthy and hypertrophied myocardium.

Method: Adult rat ventricular myocytes (ARVM) were processed for determination of mRNA (qRT-PCR) or protein (Western analysis) expression. Protein knockdown in cultured H9c2 cardiomyocytes was achieved using protein-specific small interfering RNA. Pressure overload-induced left ventricular hypertrophy (LVH) was achieved by 28 days of transaortic constriction in C57BL/6J mice. Data are presented as fold change (n=3-6) and statistical analysis was performed by Student's T-test or one-way ANOVA followed by a Dunnett's or Tukey's modified t-test for multiple comparison.

Results: qRT-PCR analysis (n=3) revealed that type 2A catalytic subunit (PP2AC, PP4C and PP6C) mRNA expression was significantly ($p<0.05$) different in H9c2 cardiomyocytes (PP2ACβ>PP2ACα>PP4C>PP6C) and ARVM (PP2ACα>PP2ACβ>PP6C>PP4C). Western analysis confirmed that all type 2A catalytic subunits were expressed in H9c2 cardiomyocytes, however, PP4C protein was absent in ARVM and only detectable following 26S proteasome inhibition with 1μM MG132 for 24 hours (n=3). Knockdown of alpha4 protein expression significantly attenuated expression of all type 2A catalytic subunits (n=4). LVH was associated with a significant increase in PP2AC (1.7-fold) and alpha4 (1.8-fold) protein expression (n=4-6). Although PP6C expression was unchanged, expression of PP6C regulatory subunits (i) Sit4-associated protein (SAP) 1 (2.2-fold) and (ii) ankyrin repeat domain 28 (1.9-fold) and 44 (1.5-fold) proteins were significantly elevated, whereas SAP2 expression was significantly reduced (2.8-fold) in LVH (n=4-6). Co-immunoprecipitation studies demonstrated that the cellular association between alpha4 and PP2AC or PP6C was either unchanged or significantly reduced in LVH, respectively (n=4). DNA damage assessed by histone H2A.X (ser139) phosphorylation (γH2A.X) was unaltered in LVH (n=4-6), however, exposure of H9c2 cardiomyocytes to 300μM H_2O_2 for 24 hours significantly increased levels of γH2A.X (3.4-fold), which was unaffected by knockdown of PP6C expression (2.6-fold) (n=5) and abolished by the short-term knockdown (4 days) of alpha4 expression (n=3). Mitochondrial function (MTT assay) and cellular viability (light microscopy) in H9c2 cardiomyocytes was significantly compromised by long-term (8 days) knockdown of alpha4 expression (n=4).

Conclusion: In conclusion, this study illustrates the significance and altered expression/activity of type 2A protein phosphatase-alpha4 complex in cardiac health and hypertrophy. (1) Kong *et al.*(2009). *Mol Cell* **36**:51-60.