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SIGNAL MODULATION IN CARDIAC FIBROBLASTS BY THE PLASMA MEMBRANE CALCIUM ATPASE 4 (PMCA4) CONTROLS CARDIAC HYPERTROPY

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Cardiac bypertrophy is an important pathological process leading to heart failure (HF). The identification of novel regulators of

myocardial hypertrophy is key in understanding the mechanisms of HF and in finding new treatment modalities. PMCA4 is a ubiquitously expressed Ca^{2+} pump that is involved in molecular signalling in the heart. Here we have investigated a novel role of PMCA4 in cardiac fibroblasts and the cross-talk signalling between PMCA4-deficient fibroblasts and cardiomyocytes in controlling myocardial hypertrophy.

To study the role of PMCA4 during hypertrophy we subjected $PMCA4^{-/-}$ mice to transverse aortic constriction (TAC) for 5 weeks. $PMCA4^{-/-}$ mice exhibited a significantly reduced hypertrophic response compared with wild type (WT) mice (Heart weight/tibia length ratio after TAC, $PMCA4^{-/-}$: 6.74+0.33 mg/mm vs WT: 8.34+0.88 mg/mm, $P<0.05$, $n>10$). This was accompanied by less fibrosis and lower expression of the hypertrophic marker BNP. However, cardiomyocyte specific knockout of PMCA4 did not produce any protective effect in response to TAC for 5 weeks. We reasoned that the phenotype might be due to PMCA4-mediated signalling in fibroblasts. Microarray analysis revealed a massive upregulation (~100 fold) of secreted frizzled-related protein 2 (sFRP2) in $PMCA4^{-/-}$ fibroblasts, which was confirmed by Western blot and real time PCR analyses. sFRP2 is a potent inhibitor of the pro-hypertrophic Wnt/β-catenin signalling pathway. To further test the anti-hypertrophic properties of $PMCA4^{-/-}$ fibroblasts, we co-cultured WT cardiomyocytes with either $PMCA4^{-/-}$ or WT fibroblasts. In response to phenylephrine stimulation cardiomyocytes cultured with $PMCA4^{-/-}$ fibroblasts displayed 88% less hypertrophy than those cultured with WT fibroblasts ($P<0.01$). Moreover, addition of anti-sFRP2 antibody to the culture medium abolished the anti-hypertrophic effect of $PMCA4^{-/-}$ fibroblasts, suggesting that the phenotype might be due to the paracrine effect of secreted sFRP2. Mechanistically, we found that $PMCA4^{-/-}$ fibroblasts showed a significant elevation in NFkB activity, an essential transcription factor regulating sFRP2 expression. Inhibition of NFkB activity using a specific inhibitor Bay11-7085 reduced the expression of sFRP2 in $PMCA4^{-/-}$ fibroblasts to the level comparable with WT expression.

In conclusion, our data provides new evidence that PMCA4-mediated signalling in cardiac fibroblasts plays a key role in controlling cardiac hypertrophy. Cardiac fibroblasts lacking PMCA4 produce higher levels of the protective protein sFRP2 which in turn inhibits the hypertrophic response in the neighbouring cardiomyocytes. Our study also demonstrates the importance of signalling crosstalk between cardiac fibroblasts and cardiomyocytes both *in vitro* and *in vivo*.