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REGULATION OF THE TNF-ALPHA SIGNALLING IN CARDIOMYOCYTES BY TUMOR SUPPRESSOR RAS-ASSOCIATION DOMAIN FAMILY PROTEIN 1A (RASSF1A)T Mohamed, M Zi, A Maqsood, S Prehar, L Neyses, D O'Ceandú *University of Manchester*

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Introduction Tumour necrosis factor- α (TNF- α) is a pro-inflammatory cytokine which plays key roles in the pathogenesis of heart failure. Cardiomyocytes express the TNF- α receptor (TNFR), however, the mechanism of TNF- α signal transmission in cardiomyocytes is not completely understood. Here we show a novel regulator of TNF- α signalling, the Ras-association domain family 1 isoform A (RASSF1A), which regulates cardiac contractility and intracellular calcium through interaction with TNFR1.

Methods and Results We used RASSF1A knockout (KO) mice and wild type (WT) controls and stimulated them with TNF- α (10 μ g/kg i.v.). In WT mice acute treatment with low dose of TNF- α increased cardiac contractility as indicated by the change in end systolic elastance (Ees) (baseline Ees (mmHg/ μ L), 3.3 ± 0.5 ; Ees after TNF- α stimulation, 5.7 ± 0.8 , $P < 0.05$) which is consistent with previously published data (Circulation 2004; 109:406-411). However, KO mice showed a blunted contractile response following acute TNF- α treatment (baseline Ees: 3.05 ± 0.4 vs Ees after TNF- α : 2.54 ± 0.3). Consistently, isolated cardiomyocytes from WT mice showed 40% increase in calcium transient amplitude in response to TNF- α (10 ng/ml) stimulation ($n=24$ cells, $p < 0.05$). However, KO cardiomyocytes showed no significant increase in calcium transient

amplitude following the same stimulation (n=24 cells). We also found that RASSF1A formed a molecular complex with TNFR1 in cardiomyocytes and this interaction was essential in the recruitment of TRADD and TRAF2, the major downstream effectors of TNF- α signalling. By mapping the interaction domain we found that the C-terminal region of RASSF1A was responsible for the formation of TNF- α receptor complex. Mechanistically, RASSF1A is essential in regulating calcium transients and contractility in cardiomyocytes downstream of TNF- α signalling through regulation of cytoplasmic phospholipase A2 (cPLA2) and phosphorylation of calcium handling molecules. Furthermore, using an adenoviral-mediated shRNA construct we found that cardiomyocytes lacking RASSF1A exhibited reduced activation of NF κ B, a downstream target of TNF- α .

Conclusion Our data indicates an essential role of RASSF1A in regulating TNF- α signalling in cardiomyocytes, with RASSF1A being key in the formation of the TNF receptor complex and in signal transmission to the downstream targets. Moreover, our present work contributes a novel effector pathway via RASSF1A which transmits the positive inotropic effect of TNF- α and should therefore be preserved or even stimulated in the treatment of heart failure. In addition, enhancement of RASSF1A function/expression might well benefit patients with heart failure.