Thrombospondin-1 drives cardiac remodelling in chronic kidney disease

Sohel M Julovi [1], Nikita Minhas[1], Kedar Ghimire[1], Jenifer Li[1] and Natasha M Rogers[1,2,3]

[1]Westmead Institute for Medical Research [2]Westmead Hospital, and [3]University of Sydney

AIMS:

To test the hypothesis that increased risk of cardiac remodelling in chronic kidney disease (CKD) is mediated by thrombospondin 1 (TSP1).

BACKGROUND:

Chronic kidney disease (CKD) is a global public health problem1 that shortens lifespan primarily by increasing the risk of cardiovascular disease[1]. Fifty percent of patients with CKD are more likely to die from cardiac events[2], underlying the so-called "cardiorenal syndrome".

Protein-bound uremic toxins including indoxyl sulfate (IS) represent important non-traditional risk factors in cardiorenal syndrome as they have demonstrable cytotoxic effects, and fail to be effectively cleared by dialysis[3].

Thrombospondin-1 (TSP-1) a well-characterised glycoprotein secreted by cells into the extracellular matrix (ECM), regulates cellular response to injury or repair and modulates cardiovascular homeostasis[4].

Recently, we have demonstrated that TSP1 is upregulated in acute[5] and chronic kidney injury[6] in animal models and elevated in CKD patient plasma.

Using a 5/6 nephrectomy model of CKD we now show that left ventricular hypertrophy (LVH) is a source of TSP1 and this protein is necessary to drive cardiac remodelling.

REFERENCES:

1. Chavers, BM, Solid, CA, Sinaiko, A et al. Diagnosis of cardiac disease in pediatric end-stage renal disease. Nephrol Dial Transplant, 26: 1640-1645, 2011.

2. Vanholder, R, Massy, Z, Argiles, A et al. European Uremic Toxin Work, G: Chronic kidney disease as cause of cardiovascular morbidity and mortality. Nephrol Dial Transplant, 20: 1048-1056, 2005.

3. Lekawanvijit, S: Cardiotoxicity of Uremic Toxins: A Driver of Cardiorenal Syndrome. Toxins (Basel), 10, 2018.

4. Rogers NM, Sharifi-Sanjani M, Csányi G et al.Thrombospondin-1 and CD47 regulation of cardiac, pulmonary and vascular responses in health and disease. Matrix Biol, 37:92-101, 2014.

5. Rogers, NM, Thomson, AW, Isenberg, JS. Activation of parenchymal CD47 promotes renal ischemia-reperfusion injury. J Am Soc Nephrol, 23: 1538-1550, 2012.

6. Julovi, S, Sanganeria, B, Minhas, N et al. Blocking thrombospondin-1 signaling via CD47 mitigates renal interstitial fibrosis. Lab Invest, 100(9):1184-1196, 2020.

METHODS:

1. **Culture of primary human cardiomyocytes**
2. **A 5/6 nephrectomy (5/6Nx) chronic kidney disease (CKD) model in Wild-type (WT) C57BL/6 mice and TSP1−/− mice**

ASSAYS AND ANALYSIS:

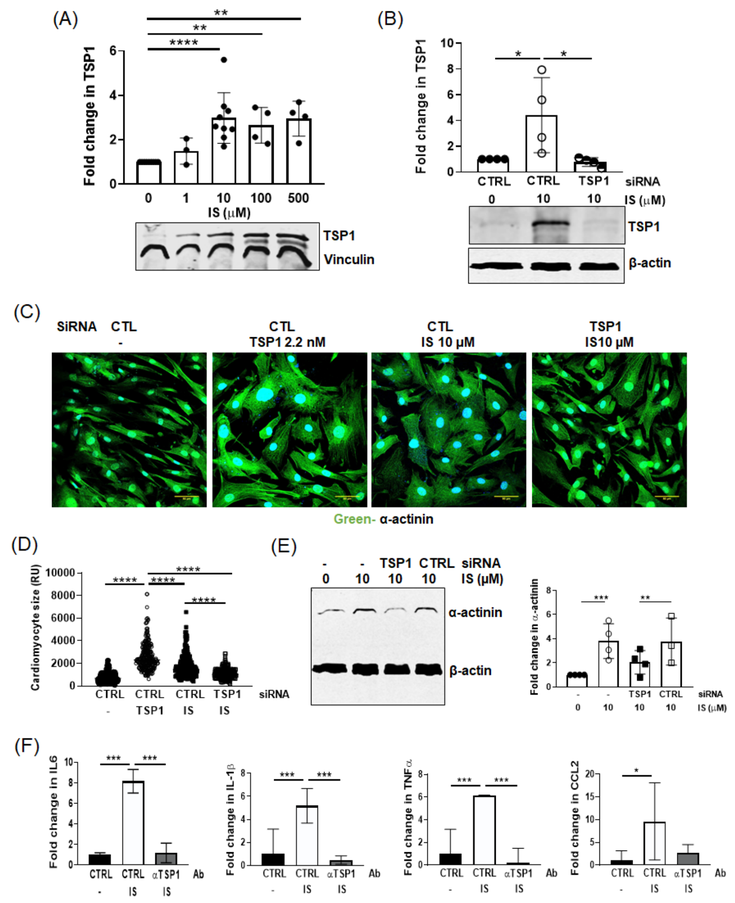
1. **Senescence and qPCR**
2. **Immunofluorescence (IF) microscopy**
3. **Immunoblotting and immunohistochemistry**
4. **Preclinical echocardiography**
5. **GraphPad Prism**

RESULTS:

KEY FINDINGS:

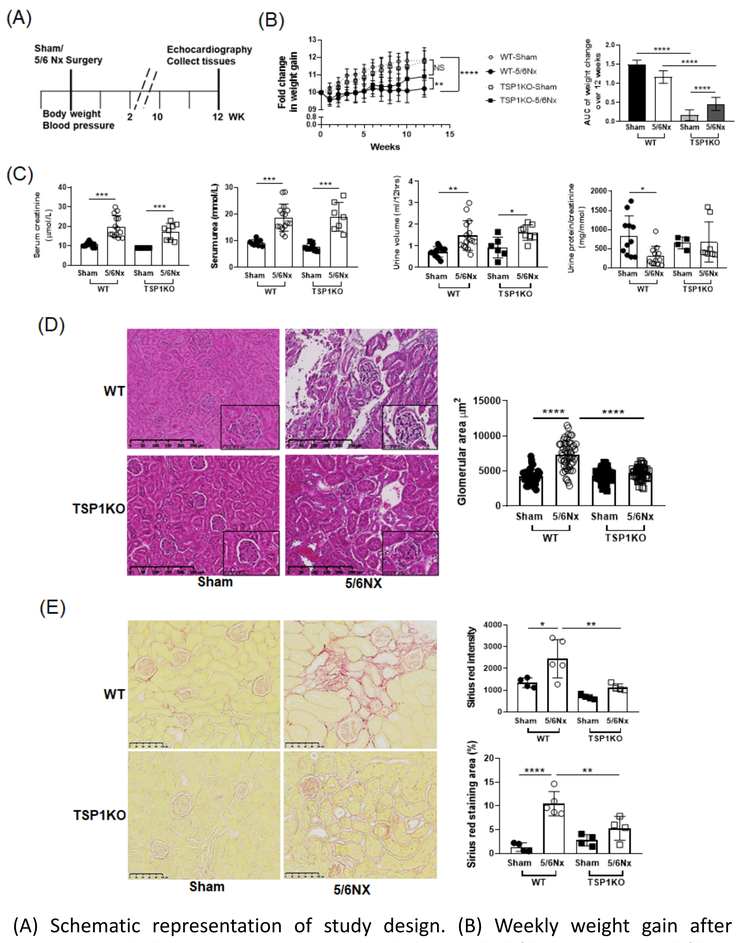
1. **TSP1 drives pro-hypertrophic effects of Indoxyl sulfate in cardiomyocytes**
2. **We have successfully developed preclinical cardio-renal disease model in TSP1 KO mice**
3. **LVH is a significant source of TSP1**
4. **TSP1$^{-/-}$ mice are protected from cardiac fibrosis and LVH in CKD**
5. **TSP1 is a marker of senescence-associated proinflammatory status in cardiac remodelling in CKD**

1. Activation of pro-hypertrophic genes by TSP-1 in cultured human primary cardiomyocytes



(A) Indoxyl sulfate (IS) enhanced expression of TSP1. (B) Knockdown of TSP1 inhibited IS induced TSP1 expression. (C) The cell size was determined by immunofluorescence staining using α-actinin antibody (green). (D) Significant increased in human cardiomyocyte surface area in response to TSP1 and IS. (E) TSP1 dependent IS induced activation of force-generating units (sarcomeres), α–actinin, essence of left ventricular hypertrophy. (F) Anti-TSP1 antibody suppressed IS-stimulated mRNA expression of pro-inflammatory cytokines. Statistical analysis displayed by mean ± SD, n=3 and >3, *p<0.05, **p<0.005, ***p<0.0005 and ****p<0.00005.

2. Induction of chronic kidney disease (CKD) in Wild-type (WT) C57BL/6 mice and TSP1KO mice



(A) Schematic representation of study design. (B) Weekly weight gain after surgery and AUC of weight change over 12 weeks. (C) 5/6 nephrectomy (5/6Nx) induced equivalent renal dysfunctions were observed in both genotypes, measured by serum creatinine, serum urea, urine volume and urine protein-creatinine ratio. (D) Histologic examination of the remnant kidney revealed tubular dilation and glomerular hypertrophy in WT 5/6Nx mice. (E) Sirius red staining revealed increased collagen deposition in WT 5/6Nx mice. Statistical analysis displayed by mean ± SD, n=3 and >3, * p<0.05, **p<0.005, ***p<0.0005 and ****p<0.00005.

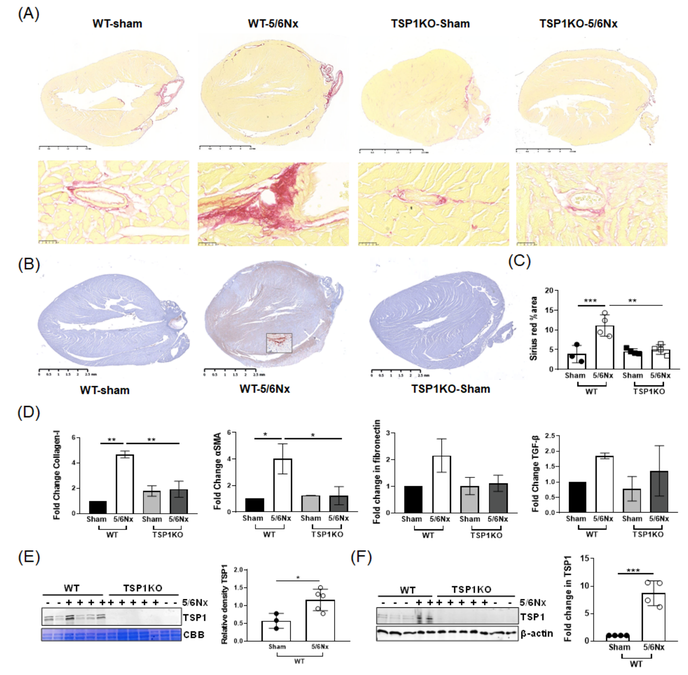
CONCLUSIONS:

This study underscore TSP-1 as a potential target of cardio-renal syndrome.

ACKNOWLEDGEMENT:

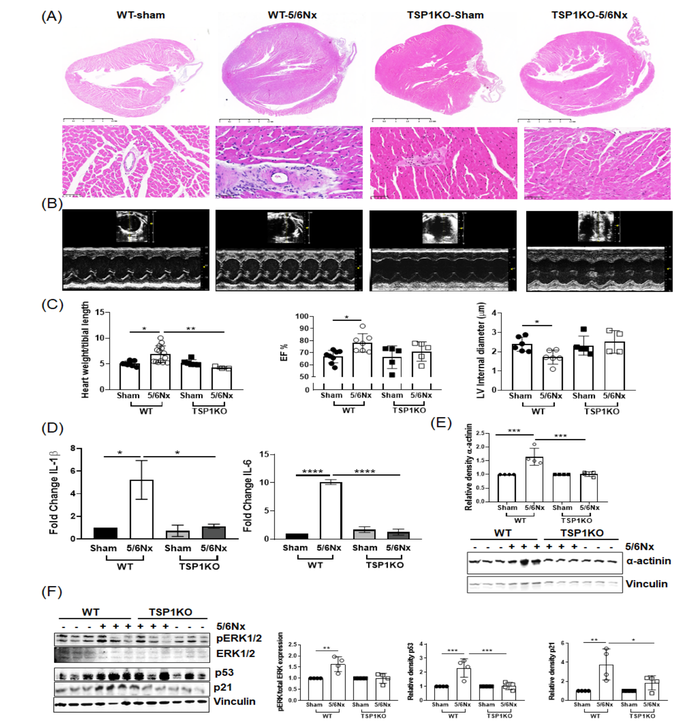
This work was supported by WMRF grant to Julovi SM, NHMRC and RACGP research grants to Rogers NM.

3. Association of TSP1 with cardiac fibrosis in CKD mice



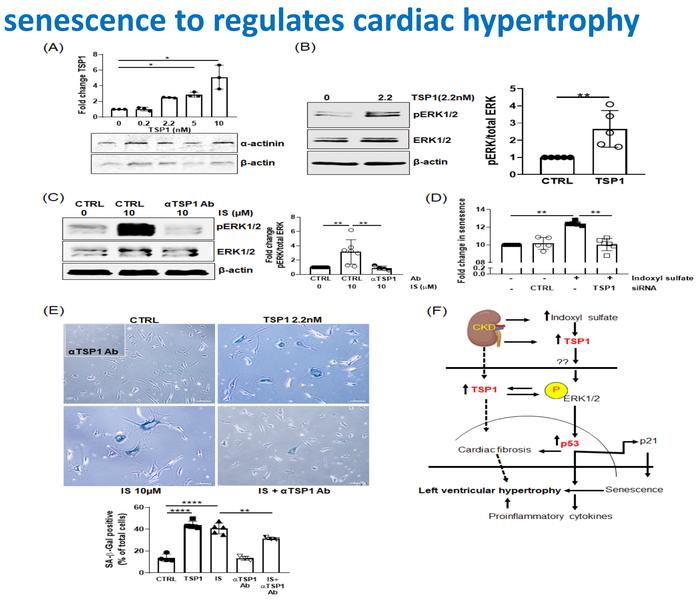
(A) Sirius red staining confirmed perivascular and interstitial fibrosis in WT 5/6Nx mice compared with TSP1KO 5/6Nx mice. (B) Immunohistochemical staining demonstrated upregulated interstitial and perivascular (inset) TSP1 expression in WT CKD heart, which was absent in TSP1KO mouse myocardium. (C) The fibrotic area (red area) was quantified by Image J. (D) Transcriptional upregulation of profibrotic markers collagen-1 and α-SMA were also significantly increased in WT 5/6Nx mice but not in TSP1KO 5/6Nx mice. Elevated TSP1 protein was confirmed in plasma (E) and heart homogenates (F). Statistical analysis displayed by mean ± SD, n=3 and >3, * p<0.05, **p<0.005, and ***p<0.0005.

4. Genetic disruption of TSP1 mitigates LVH in CKD mice



(A) Sagittal sections of the heart (hematoxylin-eosin staining) demonstrate increased size of cardiomyocytes and perivascular infiltration of inflammatory cells in WT 5/6Nx mice. (B and C) Ratio of heart weight to tibial length and m-mode echocardiography at week 12 confirmed histological evidence of left ventricular concentric hypertrophy with increased ejection fraction and decreased left ventricular internal diameter in diastole in WT 5/6Nx mice compared to TSP1KO 5/6Nx mice. (D) Analysis of mRNA expression of pro-inflammatory cytokines within the whole heart also demonstrated upregulated interleukin (IL)-β and IL-6. (E) Expression of α-actinin was increased only in WT 5/6Nx mice indicating greater sarcomeric content, essence of LVH. (F) Immunoblotting results from heart homogenates revealed significant over-expression of pERK1/2, p53, and p21 in WT 5/6Nx mice but not in TSP1KO 5/6Nx mice. Statistical analysis displayed by mean ± SD, n=3 and >3, * p<0.05, **p<0.005, and ***p<0.0005.

5. TSP1 modulates ERK-p53 pathways and senescence to regulates cardiac hypertrophy



(A) Exogenous TSP1 induced α-actinin expression in a dose-dependent manner in cardiomyocytes. (B) TSP1 increased p-ERK1/2 significantly in human cardiomyocytes. (C) Indoxyl sulfate (IS) induced p-ERK1/2 activity was dependent upon intact TSP1 signalling, as expression was downregulated in the presence of anti-TSP1 antibody. (D&E) SA-β-gal activity demonstrated that TSP1 and IS significantly increased SA-β-gal. The effect of IS on senescence was abrogated in the context of TSP1 siRNA. Senescent cardiomyocytes express β-galactosidase. (F) Schematic representation of involvement of TSP1 in cardiorenal syndrome. Statistical analysis displayed by mean ± SD, n=3 and >3, * p<0.05, **p<0.005, ***p<0.0005 and ****p<0.00005.