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REVOLUTIONISING MEDICINE - THE NEW FRONTIER

WWP2 Loss-of-Function in macrophages ameliorates pathological cardiac fibrosis

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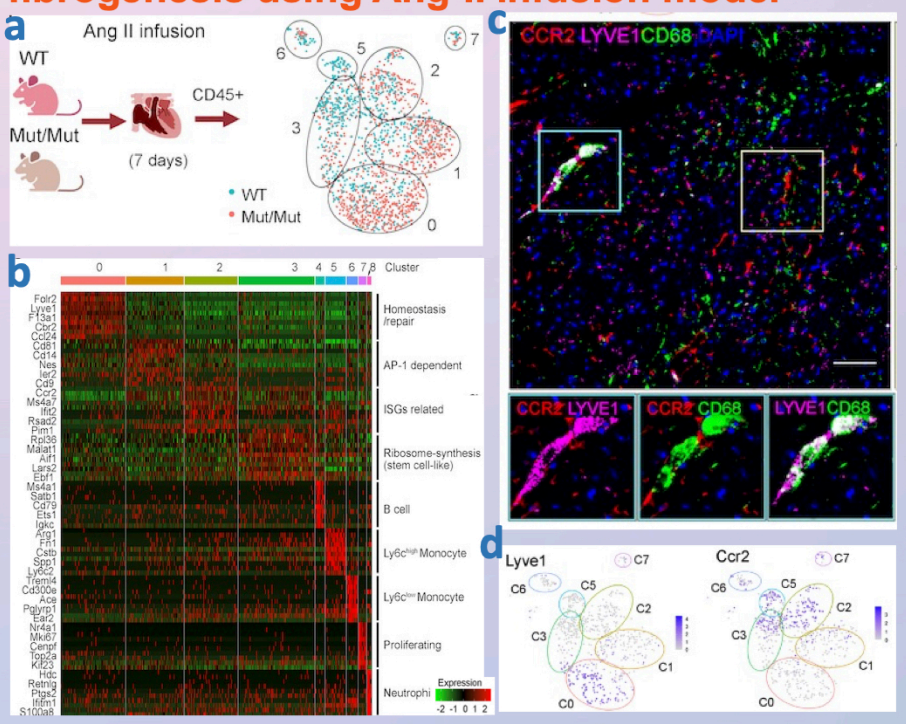
Background

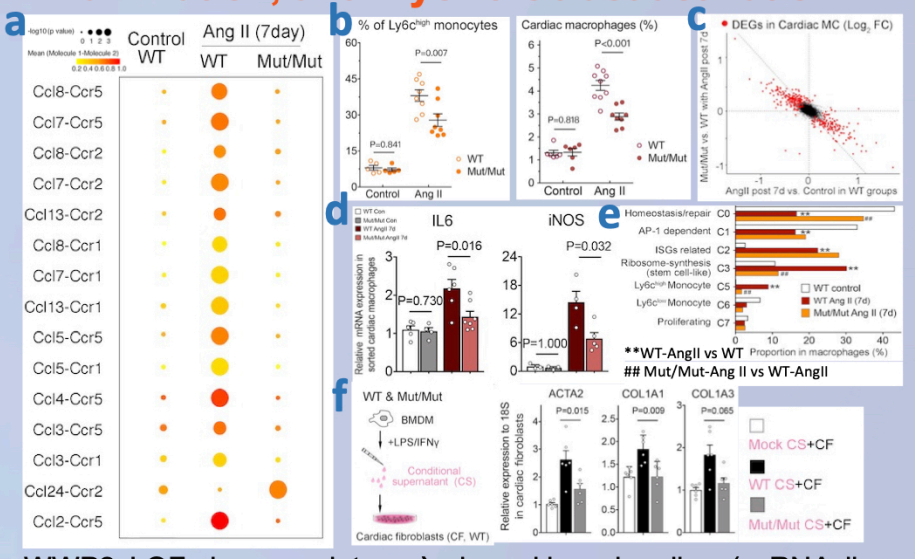
Cardiac fibrosis is the end-stage pathology of many cardiac conditions. We previously showed that the E3 ubiquitin ligase, WWP2, regulates a pro-fibrotic network conserved across different cardiac diseases[1]. However, the role of WWP2 in macrophages is unknown. Utilizing single-cell RNA sequencing of a murine model of hypertensive heart disease with or without WWP2-KO, we investigated the role of WWP2 in regulating macrophages during cardiac fibrosis. Transcriptomic analysis showed a downregulation of inflammation-related genes in the macrophages of WWP2-KO mice. Crucially, the therapeutic effect of WWP2 LOF was maintained in macrophage-specific-WWP2-KO mice. Lastly, IRF7 was identified as a mechanistic target for WWP2 in macrophages.

1) Profiling cardiac macrophages during fibrogenesis using Ang-II infusion model



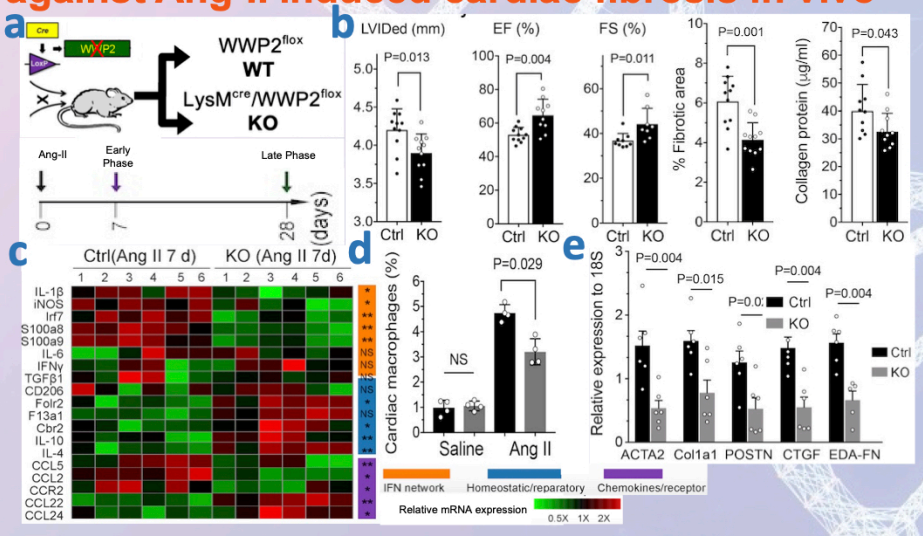
a) scRNA-sequencing of CD45⁺ macrophages from murine left ventricles (LV), **b)** Heatmap of top marker genes for macrophage clusters, **c)** Immunofluorescence of macrophage markers in murine LV, **d)** UMAP visualization of key marker expression

2) WWP2 LOF reduces chemokine signalling, inflammation, and myofibroblast activation



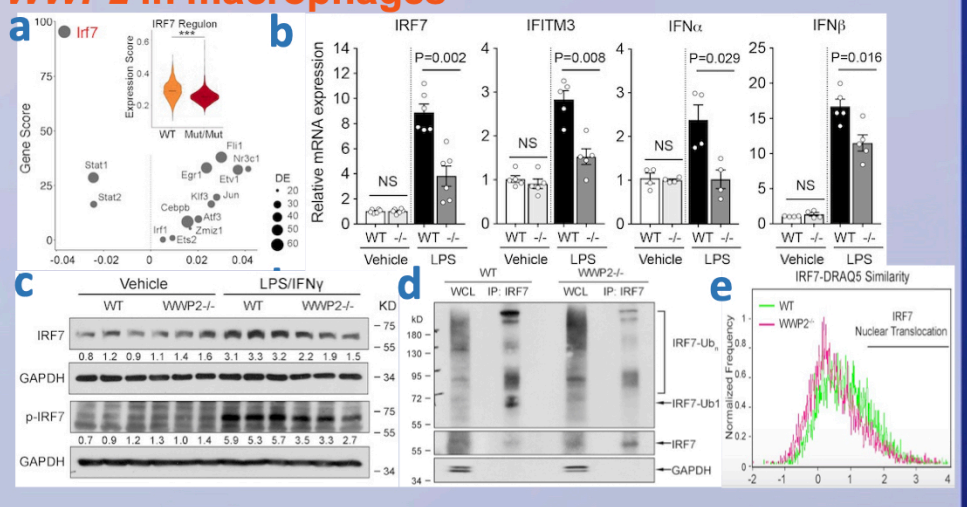
WWP2 LOF downregulates **a)** chemokine signaling (scRNA ligand-receptor analysis), **b)** % of Ly6c^{hi} and cardiac macrophages (flow cytometry, 7 day post Ang-II infusion), **c)** Ang-II induced signature (scRNA DE analysis), **d)** key inflammatory genes (qPCR), **e)** proportion of inflammatory Ly6c^{hi} monocytes (while upregulating proportion of homeostatic macrophages), **f)** myofibroblast activation

3) Macrophage-specific WWP2 LOF is protective against Ang-II induced cardiac fibrosis in vivo



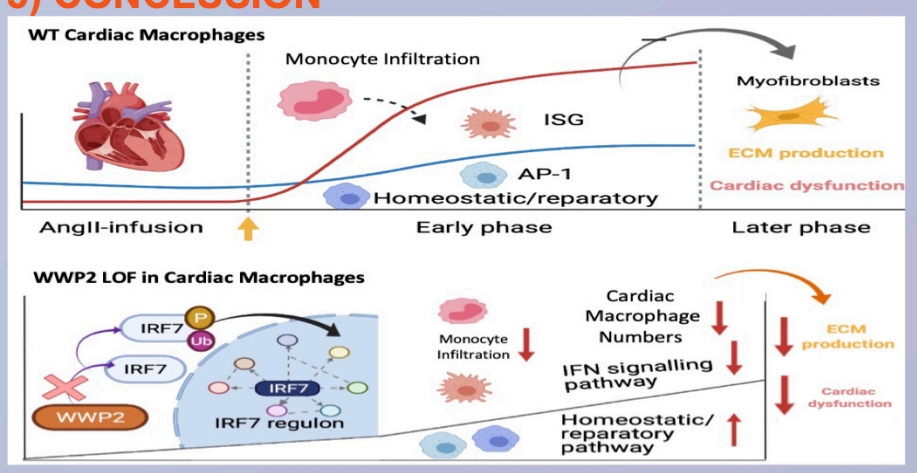
a) Cre/LoxP generation of LysM^{cre}/WWP2^{flox} mice and experimental schematic, **b)** Macrophage-specific WWP2 KO improves ejection fraction (EF), fractional shortening (FS), and reduces left ventricular internal dimension (LVID) end diastole, % fibrotic area, and collagen (28 days), **c)** Heatmap of qPCR of key inflammatory, homeostatic, and chemokine genes (7 days)**, d) %** Cardiac macrophages (7 days) **, e)** qPCR of key fibrogenic genes in murine LVs (28 days)

4) *IRF7* is a potential mechanistic target of *WWP2* in macrophages



a) Gene score vs differential expression (KOvsWT) of identified regulons, **b)** qPCR of IRF7 related genes, **c)** Western blot of IRF7/p-IRF7 in WT/WWP2$^{-/-}$ BMDMs +/- LPS/IFNɣ, **d)** Ubiquitination analysis of IRF7 in BMDMs, **e)** Imaging flow cytometry of non-nuclear/nuclear localization of WT/WWP2$^{-/-}$ BMDMS

5) CONCLUSION



IFN – Interferon AP-1 - Activator Protein-1 ISG – Interferon Stimulated Genes

References

1. Chen, H., Moreno-Moral, A., Pesce, F., Devapragash, N., Mancini, M., Heng, E.L., Rotival, M., Srivastava, P.K., Harmston, N., Shkura, K. and Rackham, O.J., 2019. WWP2 regulates pathological cardiac fibrosis by modulating SMAD2 signaling. *Nature communications*, *10*(1), pp.1-19.

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