FIBROSIS

Targeting ARNT to attenuate renal fibrosis

> modulation of the FKBP–YY1–ARNT–ALK3 signalling axis may be a promising target in chronic failure of multiple organ systems

Fibrosis is an important driver of end-stage organ failure and death in a variety of chronic diseases, including chronic kidney disease. However, approaches to attenuate the progression of organ fibrosis are limited. Several studies have demonstrated efficacy of the calcineurin inhibitor (CNI) FK506 (also known as tacrolimus) in protecting against acute experimental organ injury. Now, researchers show that low-dose FK506 exerts antifibrotic, renoprotective effects in a model of renal fibrosis via aryl hydrocarbon receptor nuclear translocator (ARNT)-mediated transcription of bone morphogenetic protein receptor type 1A (known herein as ALK3).

FK506 is well known for its immunosuppressive properties and is commonly used to lower the risk of rejection in organ transplant recipients. Although FK506 is nephrotoxic, studies suggest that low doses might induce renoprotective effects through calcineurin-independent mechanisms. Indeed, in an initial set of experiments, Tampe et al. found that administration of low-dose FK506 to mice undergoing unilateral ureteral obstruction (UUO) reduced chronic tubular injury and interstitial fibrosis. By contrast, the CNI ciclosporin, which unlike FK506 does not form a complex with FK506-binding proteins (FKBPs), did not attenuate histological renal damage induced by UUO in mice, suggesting that the renoprotective effects of FK506 are mediated by mechanisms independent of calcineurin inhibition.

Using gene set enrichment analysis of transcriptional expression data, the researchers found that FK506 induced the expression of genes involved in bone morphogenic protein (BMP) signalling responses, including ALK3, which has previously been shown to mediate the antifibrotic and pro-regenerative effects of BMP signalling. Pharmacological inhibition of BMP signalling blocked the renoprotective effects of FK506, supporting a role for BMP signalling in this process.

FK506 acts by forming a complex with members of the FKBP family of adaptor proteins. To identify the mechanisms by which FK506 induces ALK3 expression, Tampe et al. assessed the effects of depleting various FKBP family members on *Alk3* transcription in cultured mouse tubular epithelial cells (TECs). Of the FKBPs assessed, only deletion of FKBP12 induced *Alk3* transcription without the need for FK506 supplementation. Further experiments demonstrated that the effects of FKBP12 on *Alk3* transcription are mediated by direct interactions between FKBP12 and the transcriptional repressor protein YY1; the presence of FKBP12–YY1 complexes correlated with low *Alk3* transcription, whereas depletion of FKBP12 or YY1 or the addition of FK506 induced *Alk3* transcription. Moreover, TEC-specific deletion of *Yy1* induced *Alk3* transcription and protected mice from UUO-induced tubular injury and fibrosis.

The researchers then used array-based and computational prediction approaches to identify the transcription factor ARNT as a mediator of *Alk3* transcription downstream of FKBP12 and YY1. Small interfering RNA-mediated depletion of ARNT prevented FK506-induced transcription of *Alk3* in vitro. Conversely, disruption of the FKBP12–YY1 complex with low-dose FK506 increased expression of *Arnt* in vitro and in vivo, leading to activation of *Alk3*.

To assess the therapeutic potential of targeting the FKBP–YY1–ARNT signalling axis, the researchers used in vivo morpholinos (VMOs) targeting FKBP and YY1. Administration of these VMOs induced expression of intrarenal ARNT and protected mice from UUO-induced renal fibrosis. Similarly, a small-molecule inhibitor of FKBP12 induced intrarenal *Arnt* and *Alk3* transcription and protected mice from tubular injury and fibrosis. Moreover, this inhibitor also attenuated organ injury in models of cardiac and liver fibrosis, prompting the researchers to speculate that modulation of the FKBP–YY1–ARNT–ALK3 signalling axis may be a promising target in chronic failure of multiple organ systems.

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ORIGINAL ARTICLE Tampe, B. et al. Pharmacological induction of hypoxia-inducible transcription factor ARNT attenuates chronic kidney failure. *J Clin Invest.* https://doi.org/10.1172/JCI89632 (2018)