

WISP1 Neutralization Suppresses Fibrosis in Preclinical *in vivo* and *ex vivo* Models



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BACKGROUND

- The myofibroblast is the central pathogenic cell across all organ fibrosis. Myofibroblast activation is characterized by dysregulation of normal homeostatic function towards a state of persistent aberrant extracellular matrix deposition and remodeling that ultimately results in loss of organ function.
- To identify potential cellular mechanisms associated with reversion of activated hepatic stellate cells (HSC) back to a quiescent state, we performed a full-



genome microRNA screen coupled with high-content imaging of retinoid lipid droplets and identified miR-15a as a key regulator of HSC quiescence.

- Through transcriptomic analyses and experimental validation, we identified WISP1, a CCN family matricellular protein, as a key target of miR-15a and show that WISP1 is upregulated in myofibroblasts in human NASH samples in addition to other fibrotic diseases.
- We generated neutralizing antibodies to WISP1 and tested them in CCl₄ and bleomycin mouse models as well as in human *in vitro* and ex *vivo* preclinical models of fibrosis. In each model, anti-WISP1 neutralizing antibodies suppressed key markers of fibrosis suggesting WISP1 is a promising therapeutic target.

METHODS

• *High-Content Screening of the miRNA Library* HSCs were plated for 24h and transfected with miRIDIAN microRNA Mimic Library using DharmaFECT 1. Fresh media was added 24h after transfection. 72h later cells were washed, fixed, stained with BODIPY, and imaged. Retinoid individual confirmation
 existence of a human homologue



Anti-WISP1 neutralizing antibody suppresses fibrotic readouts in a CCl₄ mouse model of liver fibrosis and a bleomycin mouse model of lung fibrosis



droplets were quantified using CellProfiler.

• *Transcriptomic analysis of aHSC and qHSC* RNA from quiescent, activated, and induced-quiescent (miR-15a) HSCs was collected and RNA-Seq libraries were prepared using Illumina TruSeq. We performed differential gene expression and pathway analysis using gene set annotations from MSigDB.

HSC in vitro assays

Primary human hepatic stellate cells (ScienCell) were cultured for 48 hours in the presence of recombinant WISP1 or TGF β . Collagen levels were measured by ELISA (Takara Bio); α SMA (LS Bio); and, IL6 (MSD).

 Human precision cut liver slice (PCLS) model Human PCLS studies were conducted at Fibrofind (Newcastle, UK) as described in Paish et al (2019). PCLS were incubated for a 24hr rest period to recover from post-slice stress. Post-rest, PCLS were cultured in the presence or absence of TGF-β1 (3ng/ml) and PDGF-ββ (50ng/ml) in the presence or absence of novel antibodies or ALK5i as a positive control for 3 days (24h-96h). Endpoints included WISP1 ELISA (R&D Systems) and qRT-PCR for select genes. Data expressed in % Relative level of transcriptional difference.

Bleomycin and CCl₄ mouse models of fibrosis
 Mouse models of fibrosis were conducted at Aragen (Morgan Hill, CA) under
 IACUC approved protocols. Study designs were as shown in the Results.
 Histologic analysis was done at Dallas Tissue Research (Dallas, TX). IL6 and
 MCP-1 levels were determined by Luminex assays; Pro-collagen 1a1 by ELISA
 (Abcam); and, TIMP1 by ELISA (R&D Systems).

CONCLUSIONS

- miR-15a suppressed HSC activation
- WISP1 is a target of miR-15a
- WISP1 is up-regulated in human fibrotic disease
- WISP1 promotes fibrotic markers in vitro
- Anti-WISP1 suppresses fibrosis in vitro, in vivo and in ex vivo human explants

HSC reprogramming by WISP1 inhibition is an intriguing therapeutic approach for fibrotic disease

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