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

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BACKGROUND

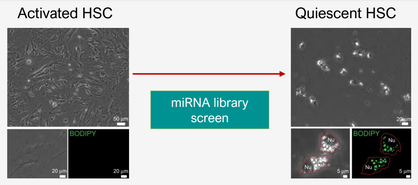
- The myofibroblast is the central pathogenic cell across all organ fibrosis. Myofibroblast activation is characterized by dysregulation of normal homeostatic function towards a state of persistent aberrant extracellular matrix deposition and remodeling that ultimately results in loss of organ function.
- 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 CCl_4 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 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 TGFβ. Collagen levels were measured by ELISA (Takara Bio); αSMA (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 CCl_4 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).

RESULTS

Full-genome microRNA genetic screen



Activated primary HSC:
Distinct morphology with loss of retinoid lipid droplets detected by BODIPY stain

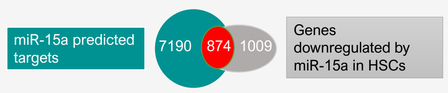
Quiescent HSC
High content screen for retinoid lipid droplets in quiescent cells by BODIPY stain

15 miRNA Hits in primary screen → miR-15a

- individual confirmation
- existence of a human homologue

WISP1 identified by transcriptomics

miRNA-15a from genetic screen
- Validated Hit
- Human orthologue

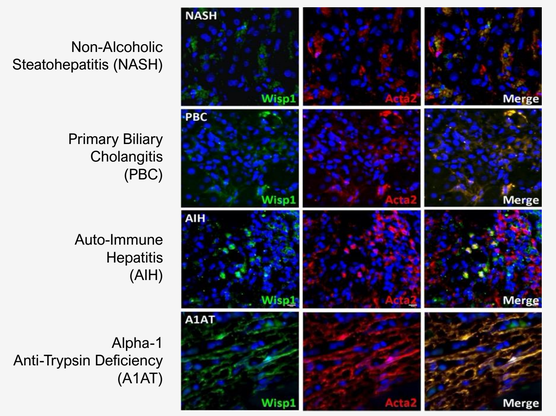


RNA-Seq (GSE 168617):
- Quiescent HSCs
- Activated HSCs
- Induced-quiescent HSCs (miR-15a)

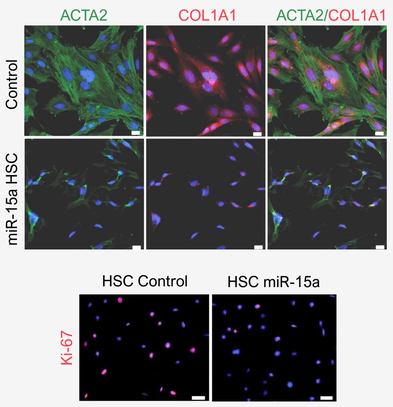
Pathway Analysis (21)

WISP1

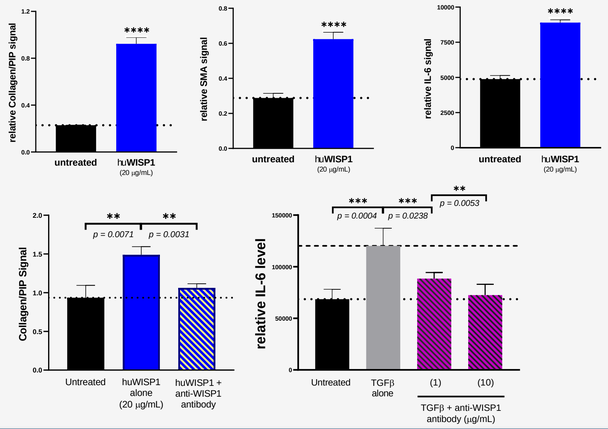
WISP1 is upregulated in human fibrotic diseases



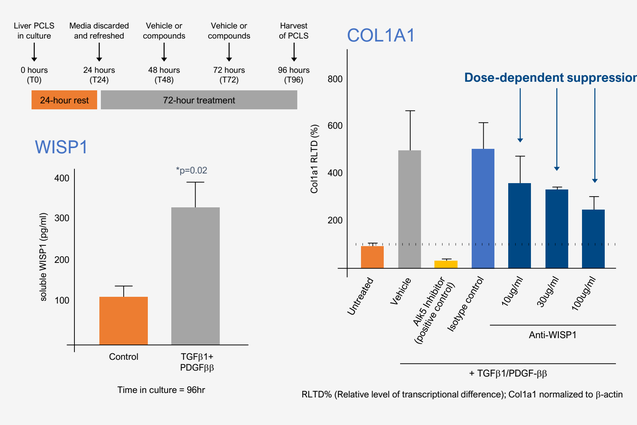
miR-15a suppresses fibrotic markers



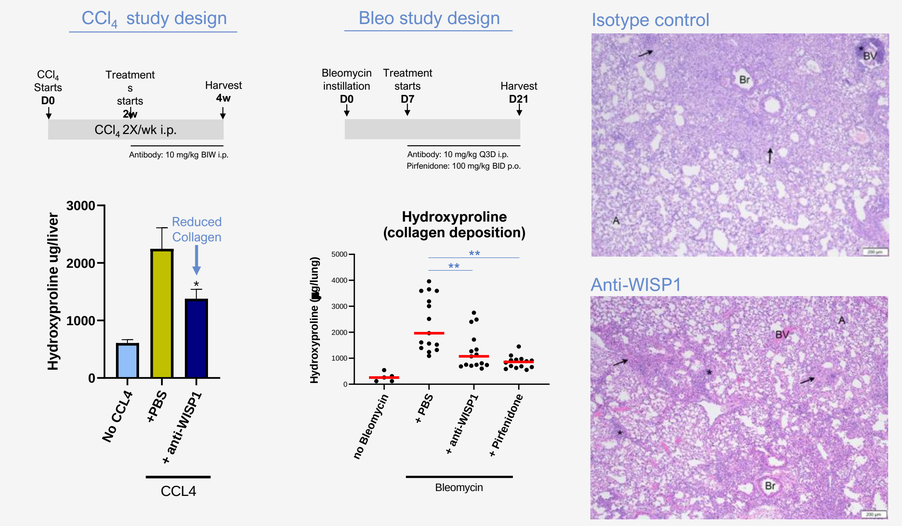
Anti-WISP1 reduces huWISP1 induced fibrotic markers in HSC

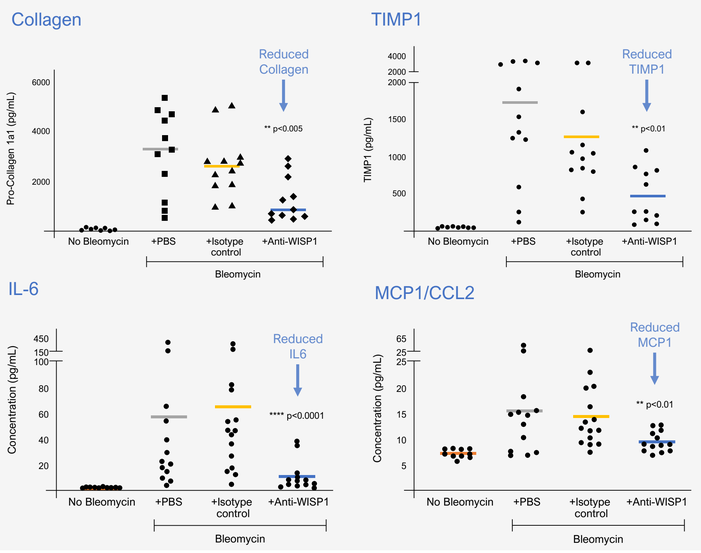


Human precision cut liver slice (PCLS) model of fibrosis



Anti-WISP1 neutralizing antibody suppresses fibrotic readouts in a CCl_4 mouse model of liver fibrosis and a bleomycin mouse model of lung fibrosis





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

ACKNOWLEDGMENTS

- Malavika Ghosh and Rashmi Munshi, Donovan Unks at (Aragen, Morgan Hill, CA)
- Lee Borthwick and Jelena Mann (Fibrofind, Newcastle, UK)
- Jessica Durrant, Kristin Panwell and Alex Van Engelenburg (Dallas Tissue Research, Dallas, TX)