Fibrosis Phenotypic Analysis of Collagen Stained Liver Histology Sections Discern Anti-Fibrotic Agents in DDC- Induced Cholangitis Mouse Model

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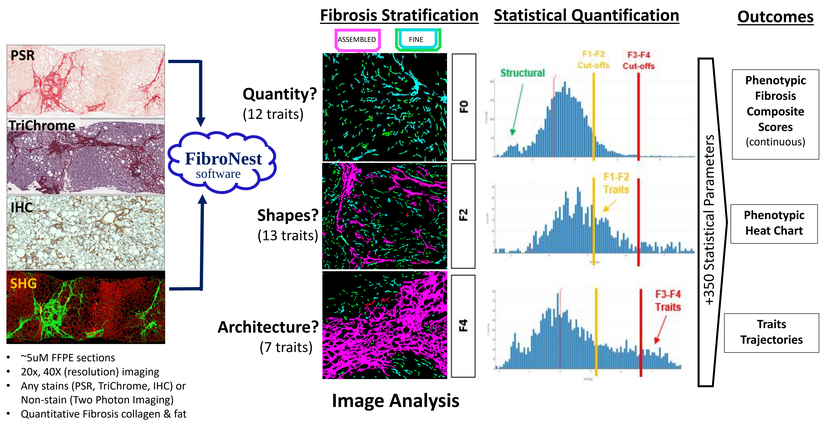
BACKGROUND

Primary sclerosing cholangitis (PSC) is a chronic liver disease caused by progressive inflammation, fibrosis, and strictures of hepatic bile ducts, often leading to biliary cirrhosis. Transforming growth factor beta (TGF-b) and its activation by alpha v beta 6 (avb6) integrin are key players in the pathogenesis and exacerbation of fibrosis. Here, we assess commercially available anti-fibrotic efficacy of SB525334 (TGF-b receptor I (ALK5) inhibitor) and 3G9 (anti-avb6) in a chemically induced cholangitis mouse model with a focus on the phenotypic quantification of fibrosis.

METHOD



- ~5uM FFPE sections
- 20x, 40X (resolution) imaging
- Any stains (PSR, TriChrome, IHC) or Non-stain (Two Photon Imaging)
- Quantitative Fibrosis collagen & fat

Cholangitis Mouse Fibrosis Model

Mice (8wks old, n=8-10/group) were fed with 0.1% DDC (3,5 diethoxycarbonyl- 1,4-dihydrocollidine)-diet for 20 days to induce biliary fibrosis and cholestasis.

A small molecule ALK5 inhibitor (SB525334, SB30) (30 mpk, PO, bid), and a blocking antibody against mouse avb6, 3G9 (10 mpk, IP injection, bid) were administered in DDC mice starting at diet initiation.

Liver histology sections stained with Picro-Sirius Red were imaged with Digital Pathology Imagers (light microscopy at 20X).

FibroNest©, a novel cloud-based image analysis platform, was used to quantify fibrosis the collagen content and structure, morphometric trait of each individual collagen fibers, and the fiber texture (relative arrangement / architecture of the fibers).

Each morphometric and texture trait is described by several quantitative fibrosis parameters (qFPs) to account for mean, variance, and progression. qFPs were combined to generate a Composite Fibrosis Score (CFS), a continuous phenotypic quantifier of fibrosis.

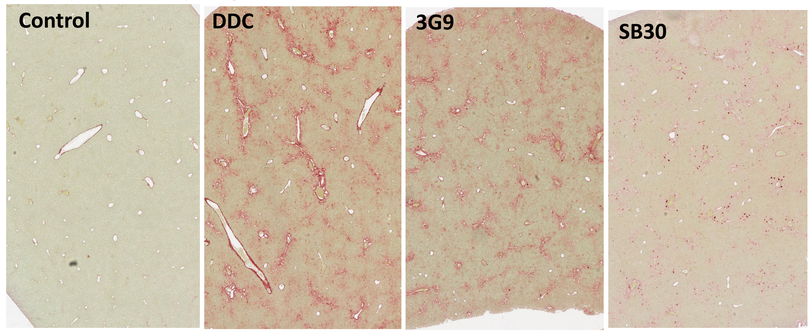
CONCLUSIONS

- SB30 reduced liver collagen fiber area and fiber network structures (74% and 10%, respectively, compared to DDC-Vehicle), while 3G9 decreased it to a lesser degree (19% and 18%, respectively). Both compounds reduces the Assembled and Fine Collagens.
- The qFPs, reported on heat charts, show highest values for DDC-Vehicle, mid values for SG9, and lowest values for SB30. SB30 is more effective than 3G9 in improving fibrosis area and structure index, qFPs, and Composite Fibrosis Scores (SB30 37% and 3G9 5% reduction compared to DDC- Vehicle).
- SB30 has higher anti-fibrotic effects compared to 3G9 in chronic DDC-induced PSC model for improving liver histopathology.
- FibroNest © is a reliable tool to evaluate fibrosis severity and progression in preclinical and clinical (previously shown) studies from digitized stained histological tissues. This will help assess and differentiate pharmacological agents.

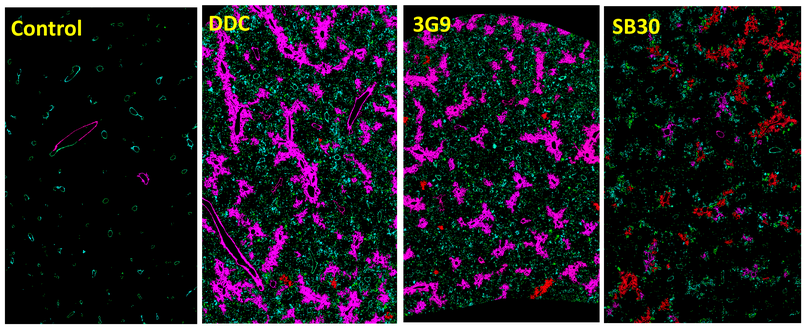
RESULTS

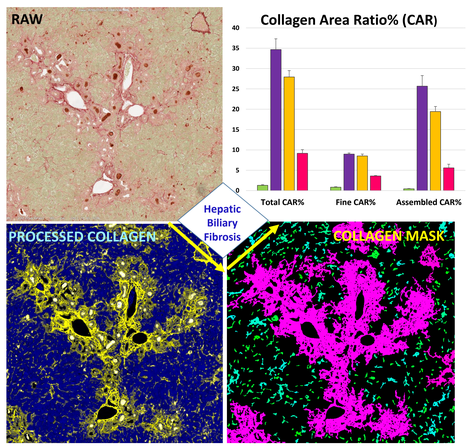
DDC- diet induced cholangitis hepatic fibrosis in mouse histological sections.

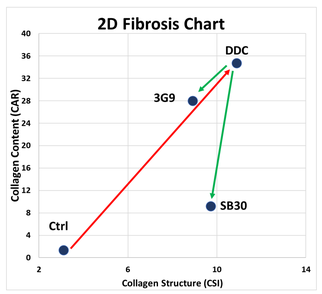
A. PSR (Picro Sirius Red) Collagen stains



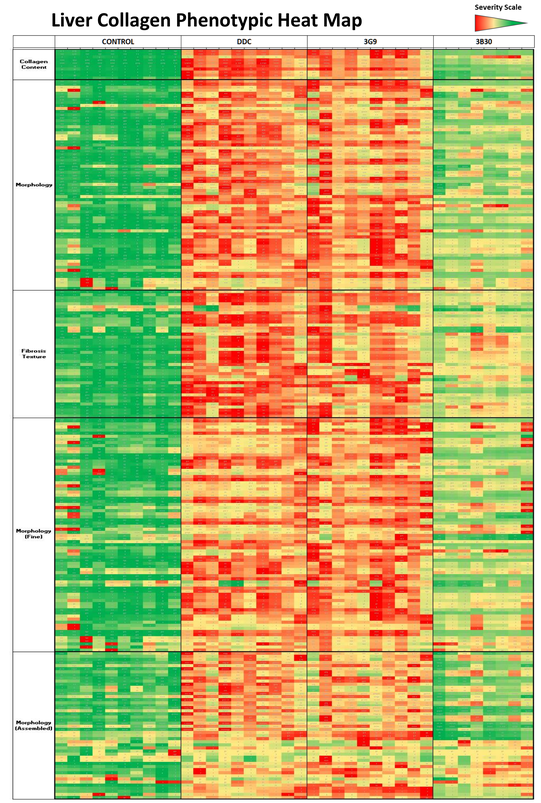
B. Cholangitis Fibrosis : Assembled Collagen and Fine Collagen



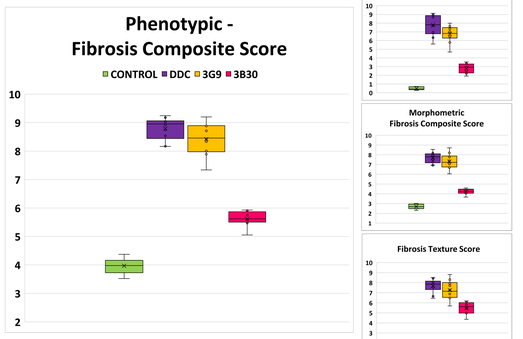




The coalesce of interstitial Fine collagen (blue/green) into Assembled collagen (magenta) or the reverse dissociation is a signal of fibrosis progression and regression.



Principal qFPs (quantifiable fibrosis parameters, row) are normalized and their progression is showed in color scale chart. Each column represents an animal. The values of the qFPs are combined to form the phenotypic Fibrosis Composite Scores (Ph-FCS).



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