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TLR-X (IOA-359) attenuates steatosis and fibrosis in a preclinical NASH model

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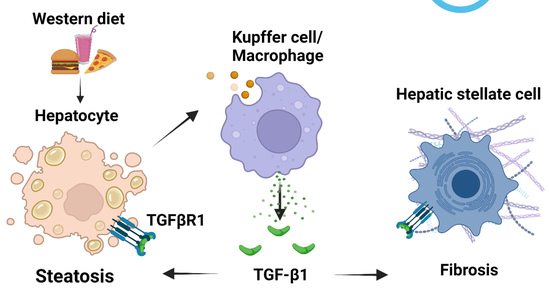
1 Introduction

Transforming growth factor-beta 1 (TGF-β1) is significantly upregulated in human NASH. The (canonical) TGF-β mediated Smad signaling pathway promotes NASH by inducing lipid accumulation in hepatocytes and contributing to hepatocyte death, and by activating hepatic stellate cells within the liver.

TGF-β receptor I (activin A receptor type II-like kinase, ALK5) is an attractive target for intervention in canonical TGF-β1 signaling due to its druggability, centrality, and specificity in the pathway. Silencing this pathway is expected to attenuate hepatic steatosis and fibrosis.



2 Aim

Investigate the novel, highly selective, and orally administrable ALK5 inhibitor TLR-X (IOA-359, provided by iOnctura) for the treatment of NASH to attenuate hepatic steatosis and fibrosis.

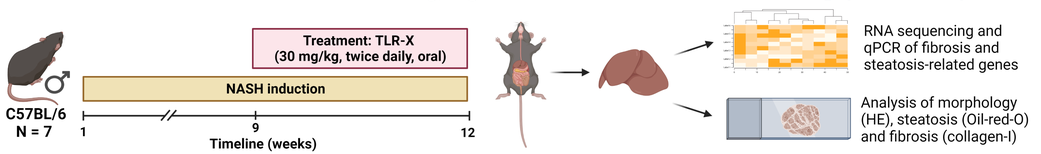
3 Method

Diet- and chemical-induced western diet fructose (WDF) NASH mouse model:

Western Diet – *ad libitum*
- 21.1% fat
- 34.0% sucrose
- 1.25% cholesterol

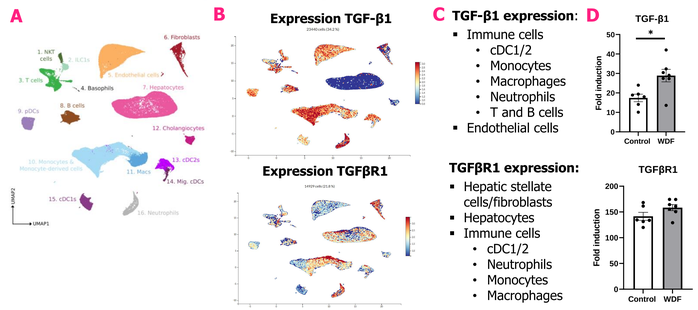
Fructose supplemented water – *ad libitum*
- 23.1 g/L fructose
- 18.9 g/L glucose

Carbon tetrachloride (CCl4) – once a week via i.p. administration
- 0.05 – 0.2 mL/kg (week 1-4)
- 0.2 mL/kg (week 5-12)



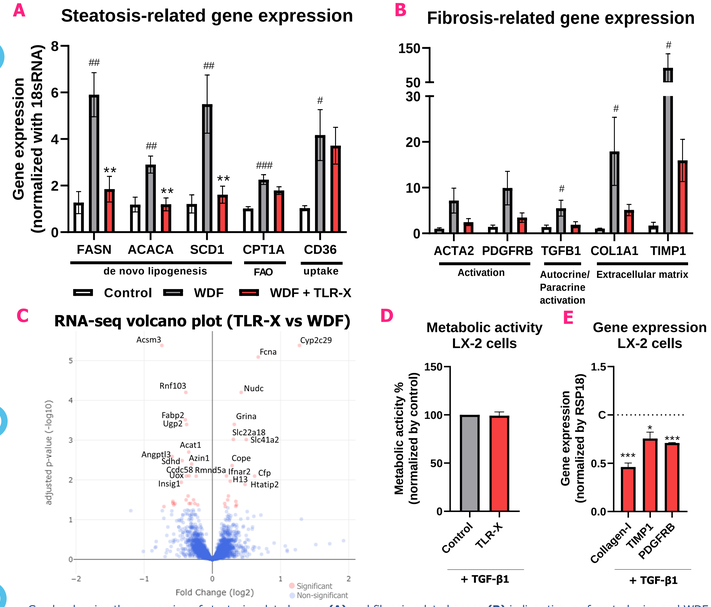
4 Results

TGF-β1 is upregulated in NAFLD/NASH



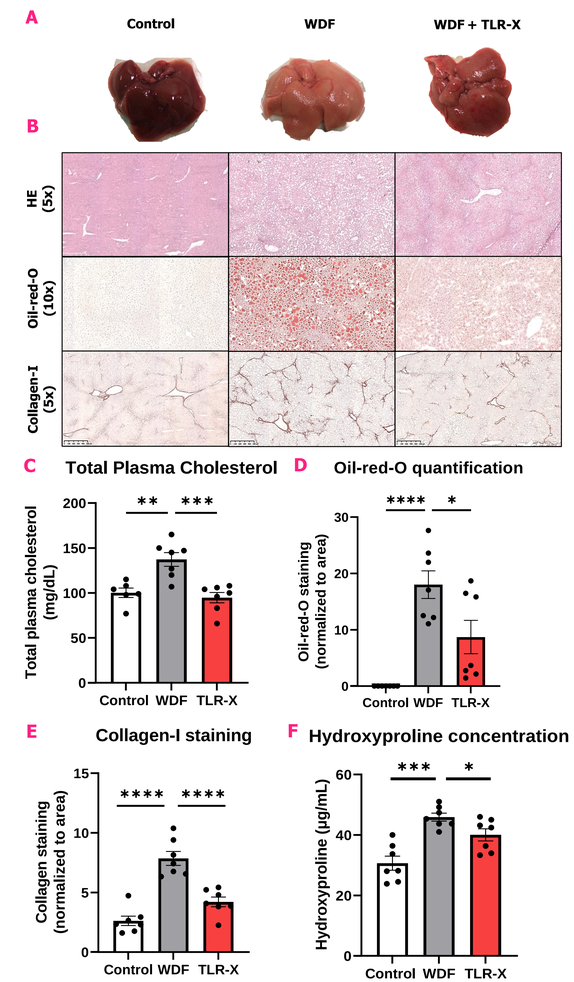
(A) Mouse NAFLD Atlas – All Liver Cells UMAPs clusters, provided by https://livercellatlas.org/. **(B)** Single cell expression of *Tgfb1* and *Tgfbr1* in Mouse NAFLD atlas visualized in all liver cell UMAPs clusters. **(C)** Clusters of cells that express *Tgfb1* or *Tgfbr1* in Mouse NAFLD. **(D)** RNA expression of *Tgfb1* and *Tgfbr1* retrieved from bulk RNA sequencing of liver tissue from WDF mouse model (n=6-7). Comparison between groups were performed by t-test. *p < 0.05.

TLR-X (IOA-359) significantly decreases steatosis and fibrosis-related gene expression in WDF NASH model



Graphs showing the expression of steatosis-related genes **(A)** and fibrosis-related genes **(B)** in liver tissue of control mice and WDF mice with and without TLR-X treatment. The results show significant downregulation of de novo lipogenesis related genes and downregulated trend in HSCs activation and extracellular matrix deposition. **(C)** Volcano plot showing the bulk RNA-sequencing data with significantly up- and down-regulated genes in TLR-X treated mice versus the diseased control (WDF). **(D)** Graphs showing no toxic effects of TLR-X in human HSCs (LX2) upon treatment, while **(E)** expression of major HSCs markers were significantly decreased upon treatment indicating a direct effect of TLR-X on (activated) HSCs. Multiple comparisons between different groups were performed by ANOVA with Bonferroni post-hoc test. *p < 0.05, **p < 0.01, ***p < 0.001.

TLR-X attenuates hepatic lipid accumulation and collagen-I deposition in WDF NASH model



(A) Representative macroscopic images of liver tissues. **(B)** Representative images of the liver sections stained with haematoxylin and eosin (H&E), Oil-red-O and collagen-I. **(C)** Treatment with TLR-X resulted in significant reduction in total plasma cholesterol levels. **(D)** Quantitative image analysis of Oil-red-O stained liver sections shows significant decrease in lipid accumulation after treatment. **(E)** Quantitative analysis of collagen-I IHC-stained liver sections, together with the **(F)** total hydroxyproline concentration (µg/mL) analysed in liver tissue indicate the reduction of fibrotic extracellular matrix upon treatment with TLR-X. Multiple comparisons between different groups were performed by ANOVA with Bonferroni post-hoc test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

5 Conclusions

Our results demonstrate that inhibition of the canonical TGFβ signaling using the novel ALK5 inhibitor TLR-X represents a potential therapeutic approach for the treatment of NASH, by attenuating steatosis and fibrosis, with possible clinical implications.

6 Acknowledgements

TLR Translational Liver Research

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