Endothelial-immune crosstalk contributes to vasculopathy in nonalcoholic fatty liver disease

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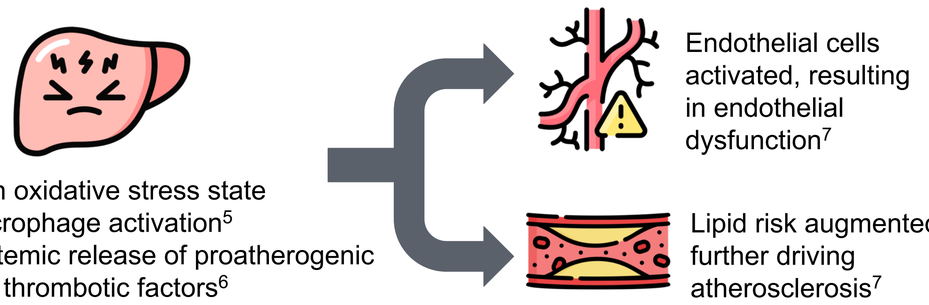
Introduction

Nonalcoholic fatty liver disease (NAFLD)

- Most common liver disease in developed countries[1]
- Covers a spectrum from simple steatosis to fibrosis[2]
- Top cause of mortality in patients with NAFLD is cardiovascular mortality from vasculopathy[3]

NAFLD and vasculopathy

NAFLD is associated with systemic vascular dysfunction: reduced flow-mediated vasodilatation, altered carotid artery intimal medial thickness, coronary calcification, and low coronary flow reserve[4].



- High oxidative stress state
- Macrophage activation[5]
- Systemic release of proatherogenic and thrombotic factors[6]

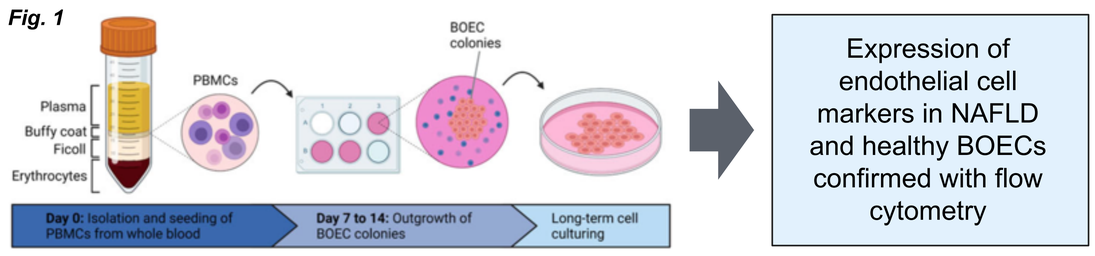
Despite current research, there remain knowledge gaps in NAFLD-related endothelial disease biology.

This study aims to discover the molecular basis of vasculopathy in NAFLD by investigating the effect of NAFLD on endothelial-immune crosstalk in the aspects of cell biology, chemokines and HLA typing.

Methods

Developing human blood outgrowth endothelial cells (BOECs) for disease modeling

Fig. 1



Analysing transcriptomic and protein differences between NAFLD and healthy BOECs

RNA sequencing and quantitative PCR

STRING
Protein-protein interactions from STRING database

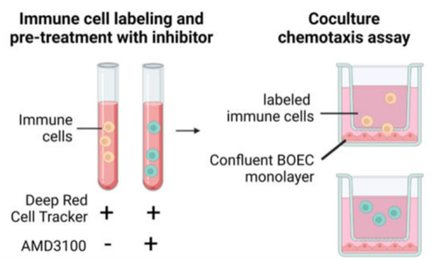
Enzyme linked immunosorbent assay (ELISA)

Immune profiling of NAFLD and healthy peripheral blood mononuclear cells (PBMCs)

DICE annotation[8] and chemokine ligand-receptor mapping[9] were used to identify the most predominant immune cell types and counter receptors to upregulated chemokines in NAFLD BOECs

Inhibition of CXCL12-CXCR4 axis

Fig. 2

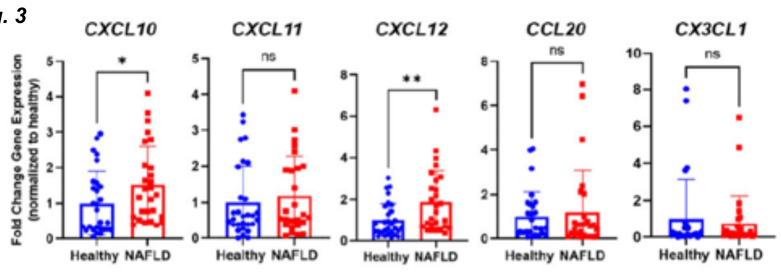


AMD3100 and anti-CXCL12 IgG were used to inhibit CXCR4 and CXCL12 respectively

Results

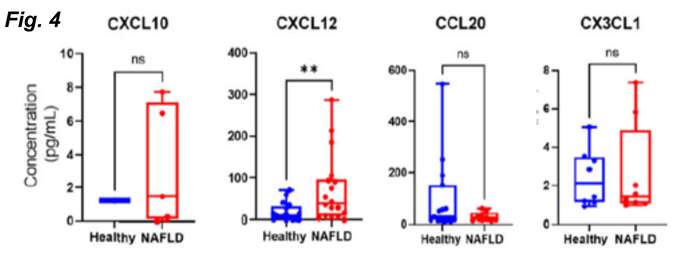
Enhanced chemokine hallmarks found in NAFLD BOECs

Fig. 3



CXCL10 and ***CXCL12*** genes were significantly upregulated in NAFLD BOECs, and were top hub genes involved in chemokine networks in these cells

Fig. 4



CXCL12 protein secretion was intensified in NAFLD BOECs-conditioned culture media, compared to that of healthy BOECs

T cells identified as key immune interactor, via CXCL12-CXCR4 axis

Fig. 5

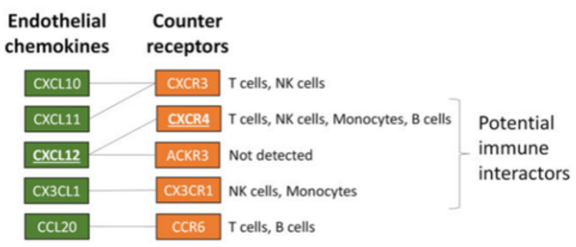
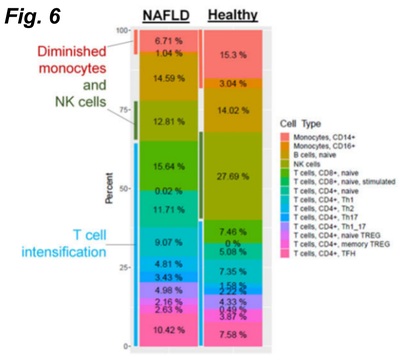


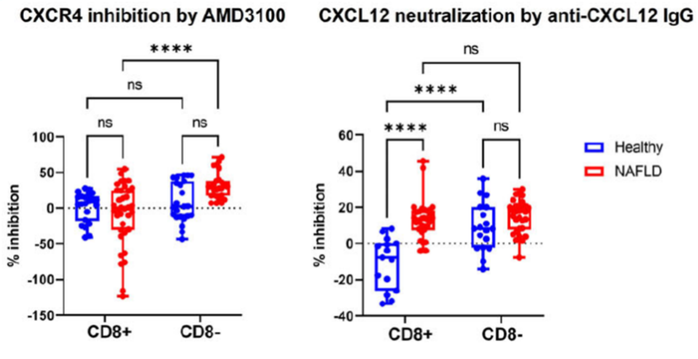
Fig. 6



- Chemokine ligand-receptor mapping showed **CXCR4** as counter receptor to CXCL12, with T cells, NK cells, monocytes, and B cells identified as potential immune interactors
- Percentage composition of identified cell types in NAFLD and healthy PBMCs single-cell analysis showed **T cell intensification**

CXCL12 neutralization with anti-CXCL12 IgG inhibited immune calls chemotaxis significantly

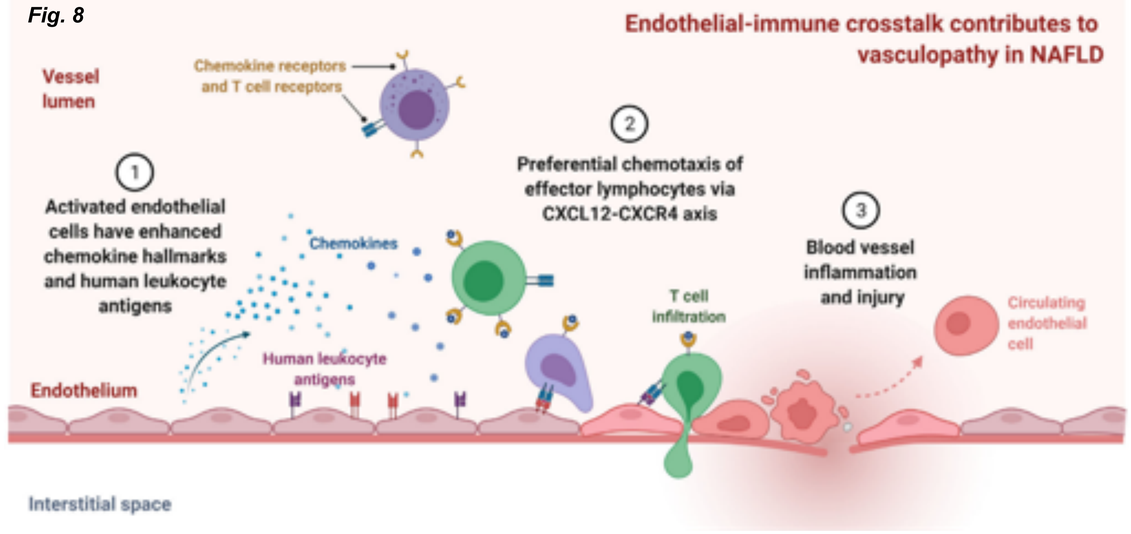
Fig. 7



- Chemotaxis assays showed that CXCR4 inhibition by AMD3100 was not significant
- Conversely, CXCL12 neutralization by **anti-CXCL12 IgG** resulted in significant inhibition

Discussion

Fig. 8



(1) Endothelial cells may be the source of enriched chemokine milieu in NAFLD, specifically **CXCL12 upregulation**

- CXCL12-CXCR4 axis is known to be involved in atherosclerosis[10]

(2) Such chemokine production could possibly play a role in **effector lymphocyte recruitment**, leading to **endothelial damage**

(3) **Increased numbers of circulating endothelial cells** in NAFLD patients suggest extensive damage to endothelium

- **CXCL12 neutralizing antibody** showed promising *in vitro* efficacy in reducing endothelial barrier permeability in NAFLD patient models

This project has advanced the knowledge about NAFLD, and these results have a considerable number of implications on future work.

Potential drug repurposing
Off-label use of existing small molecule drugs (e.g. NOX-A12) to treat the complications of NAFLD

Future pre-clinical testing
These findings suggest a residual inflammatory risk in NAFLD patients, hence future studies could look into ways to mitigate this risk

Does inhibition of the CXCL12-CXCR4 axis lead to decreased vascular permeability *in vivo*?

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