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MULTI-OMICS DEFINE DISTINCT FIBROGENIC REMODELING ENDOTYPES IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOHEPATITIS

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INTRODUCTION

- Fibrosis stage alone does not fully capture ongoing fibrogenic activity or the biological mechanisms driving fibrosis in metabolic dysfunction-associated steatohepatitis (MASH).
- Effective patient stratification may therefore require complementary assessment of fibrogenic activity using non-invasive tests (NITs), such as PRO-C3, AST [1], FAST score [1], LiverPRO [2] and biological drivers of fibrosis progression, captured through tissue-derived molecular and cellular features.
- Building on the “hot vs cold” fibrosis framework [3], we combined AI-derived macrophage quantification with PRO-C3 to define four fibrosis phenotypes—hot-progressive, hot-regressive, cold-progressive, and cold-regressive—and characterized their proteomic, metabolomic, and transcriptomic signatures.

AIM

Identify distinct MASH endotypes and evaluate candidate NITs for patient selection in early clinical trials.

CONTACT INFORMATION

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RESULTS

(A)

Protein	Biological function and potential relevance to fibrosis
SCLT1	Ciliogenesis/distal appendage protein; potential link to ciliary signaling and cellular remodeling
MTR	B12-dependent methionine synthase; regulates methionine and SAM metabolism
EN1	Homeodomain transcription factor; associated with fibroblast-to-myofibroblast differentiation and ECM production
ARHGAP11A	Rho GTPase-activating protein; regulates cytoskeletal dynamics, cell migration, and mesenchymal remodeling
FAM161A	Ciliary/centrosomal protein; potential link to altered ciliary and microtubule biology
LY96 (MD-2)	TLR4 co-receptor; mediates innate immune signaling implicated in macrophage–HSC profibrotic crosstalk
MZB1	ER chaperone enriched in antibody-secreting cells; marker of plasma-cell enrichment in inflammatory/fibrotic tissue

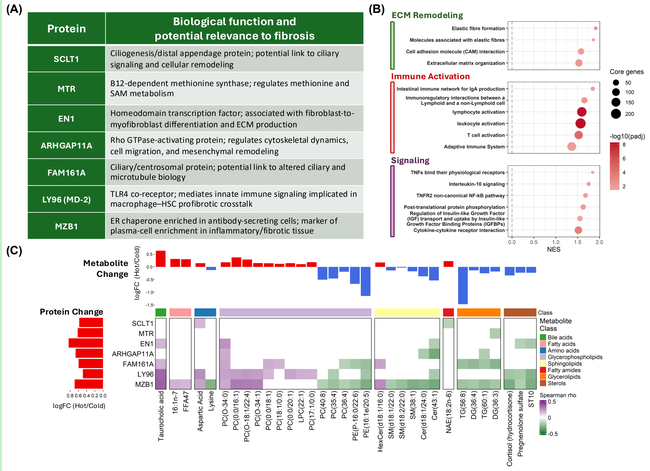


Figure 2. Proteomic and metabolomic characteristics of hot and cold fibrosis.

(A) Out of 174 proteins identified across five distinct models, the top 7 proteins listed in the table were found to be associated with inflammatory-enriched fibrosis phenotypes. (B) The majority of functions enriched in proteins differentiating hot from cold fibrosis involve ECM remodeling, immune activation, and cytokine signaling pathways, as determined by GSEA analysis using GO, KEGG, and Reactome databases (BH adjusted p < 0.05). (C) A heatmap illustrating the correlation among these 7 proteins and metabolites distinguishing hot and cold fibrosis—such as taurine acids, fatty acids, glycerophospholipids, ceramides, and sterols—revealed significant correlations. *The bar plot represents log fold-change (hot vs. cold). Correlations that were not significant were assigned zero (white). Candidate proteins and metabolites display consistent hot-cold patterns.*

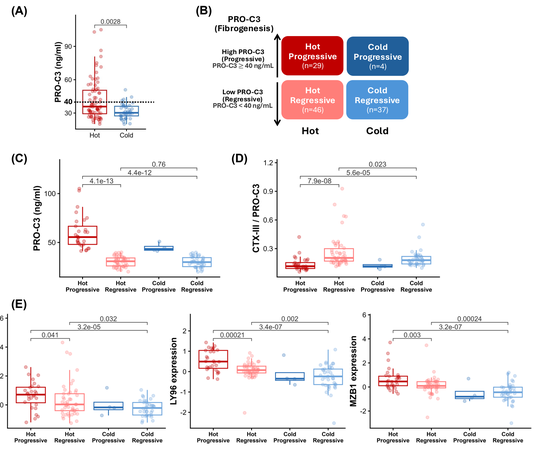


Figure 3. PRO-C3 further classifies hot and cold fibrosis endotypes into progressive and regressive phenotypes.

(A) The fibrogenesis marker PRO-C3 is markedly elevated in hot fibrosis. (B) Progressive phenotypes with elevated PRO-C3 levels (≥ 40 ng/mL) predominantly corresponded to hot fibrosis (88%, 29/33), while the majority of cold fibrosis cases exhibited low PRO-C3 levels (<40 ng/mL; 90%, 37/41). (C) PRO-C3 levels are significantly increased in progressive groups, and (D) the fibrolysis ratio, assessed by CTX-III / PRO-C3, is higher in regressive groups. (E) Expression profiles of the top seven proteins, exemplified by LY96, MZB1, and FAM161A, reveal distinct patterns that differentiate these four fibrosis endotypes. *All p-values indicated on the plots are from two-sided Wilcoxon tests.*

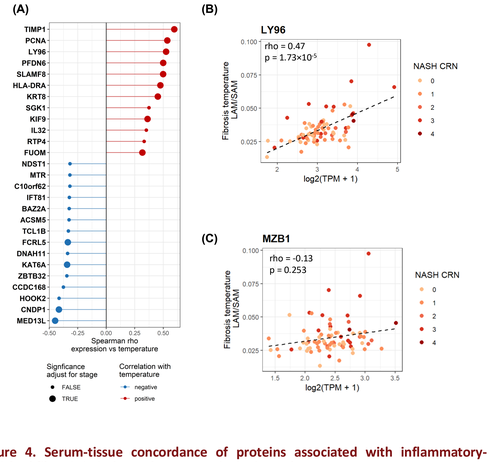


Figure 4. Serum-tissue concordance of proteins associated with inflammatory-enriched fibrosis in independent transcriptomic study.

(A) In an independent cohort with liver tissue transcriptomics, 27 (out of 174) proteins that differentiate hot and cold fibrosis demonstrated significant correlations between tissue RNA expression and macrophage-defined fibrosis temperature (lipid-associated/scar-associated macrophages). Of these, 13 proteins maintained significant correlations after adjusting for NASH CRN stage. (B-C) Tissue expression level of LY96 showed significant association with fibrosis temperature independent of NASH CRN stage, while MZB1 exhibited a correlation trend that did not reach statistical significance. *Stage-adjusted Spearman rho and p-values are indicated in the plot.*

METHOD

- Liver micro-architecture was quantified in 150 biopsy-confirmed MASH patients using LiverExplore (PathAI) and SHG/TPEF (HistoIndex) for qFibrosis assessment (qF0=24, qF1=22, qF2=33, qF3=40, qF4=22, NA=9); CRN fibrosis was centrally staged.
- Hot fibrosis (n = 75) and cold fibrosis (n = 41) were defined in patients with significant fibrosis (CRN F≥ 2): hot fibrosis had macrophage density ≥ 250 cells/mm² and cold fibrosis < 250 cells/mm² (Figure 1). Patients were further stratified into progressive (PRO-C3 ≥ 40 ng/ml) and regressive phenotypes (PRO-C3 < 40 ng/ml).
- Clinical variables and candidate NITs of fibrogenesis and fibrosis severity were evaluated to distinguish hot and cold fibrosis endotypes and compared with patients with no or mild fibrosis (CRN F0/F1).
- Serum proteomics and metabolomics were performed using OLINK Explore HT 5K and 508 metabolites across lipid and amino acid panels with Rubió Metabolomics.
- Differences between hot and cold fibrosis were assessed by univariate and multivariate regression, elastic net models (adjusted for age, BMI, and sex), and Wilcoxon rank-sum testing.
- Gene-protein concordance was assessed using tissue transcriptomic data from a published cohort[4], with correlations between hepatic gene expression and macrophage population defined fibrosis temperature[5] evaluated by NASH CRN stage-adjusted Spearman correlation.

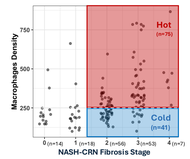


Figure 1. AI-based definition of hot and cold fibrosis endotypes.

CONCLUSIONS

- Hot and cold fibrosis represent biologically distinct MASH endotypes that are not fully captured by fibrosis stage alone.
- Hot fibrosis is characterized by greater immune activation, ECM remodeling and cytokine signaling, whereas cold fibrosis exhibits a more metabolically driven molecular profile.
- Combining macrophage-defined fibrosis temperature with PRO-C3 identifies four distinct phenotypes—hot-progressive, hot-regressive, cold-progressive and cold-regressive—with CTX-III / PRO-C3 providing additional evidence of differences in matrix turnover.
- LY96, MZB1 and FAM161A differentiated the four fibrosis phenotypes, while independent tissue transcriptomics provided orthogonal validation of the fibrosis-temperature-associated biology identified for LY96.

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