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Serum proteomics reveals association of CCL24 with key aspects of Primary Sclerosing Cholangitis

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Chemomab THERAPEUTICS

1 Introduction

Primary Sclerosing Cholangitis (PSC) is a chronic liver disease that is marked by the presence of a damaged peribiliary space. The pro-fibrotic chemokine CCL24 has been implicated in driving these self-reinforcing mechanisms in fibrotic-inflammatory diseases (1-2). CCL24 is overexpressed in livers of patients with primary sclerosis cholangitis (PSC), predominantly in areas of evident biliary injury. We previously showed that CCL24 blockade, using a monoclonal antibody, interferes with core pathways that induce PSC pathophysiology in pre-clinical models (3).

2 Aim

To demonstrate the role of CCL24 in PSC and its association with disease related pathways, we analyzed the serum proteome in healthy control and patients with PSC.

CCL24's association with key aspects of PSC was examined by:

- Correlation of serum CCL24 with inflammation, fibrosis and vascularization-related proteins.
- Exploring pathways enriched in patients with high CCL24 serum levels.
- Characterization of CCL24 dependent protein signature in hepatic stellate cell line, and assessment of this protein signature in healthy vs patients' sera.

3 Method

Sera from healthy controls (n = 30) and patients with PSC (n = 45) were analyzed using the Olink proximity extension assay (PEA) of 3072 proteins. We focused on extracellular proteins by extracting 991 intracellular proteins. Individuals' demographics, enhanced liver fibrosis (ELF) scores and alkaline phosphatase (ALP) levels were documented.

Differentially expressed proteins (DEPs; <0.05 by Welch two sample t-test) were submitted to pathway analysis using Ingenuity Pathway Analysis (IPA).

To evaluate the direct effect of CCL24 on hepatic cells, an LX2 hepatic stellate cell (HSC) line was stimulated with CCL24 and subsequently blocked by a neutralizing monoclonal antibody (CM-101). Conditioned media underwent proteomic profiling using a proteomic chip (L-507; RayBiotech).

5 Conclusions

CCL24 is associated with PSC-related pathways and severity.

A CCL24 related signature in hepatic stellate cells differentiates patients with PSC from healthy controls and by disease severity.

6 Acknowledgements

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7 References

1. Mor et al. Ann Rheum Dis 2019;78:1260–1268
2. Segal-Salto et al. JHEP Rep. 2020 Jan 2;2(1):100064
3. Greenman et al. JCI Insight. In-press

8 Contact information

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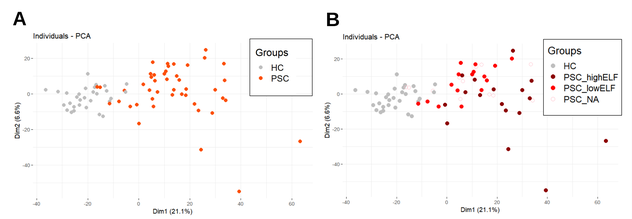
Results

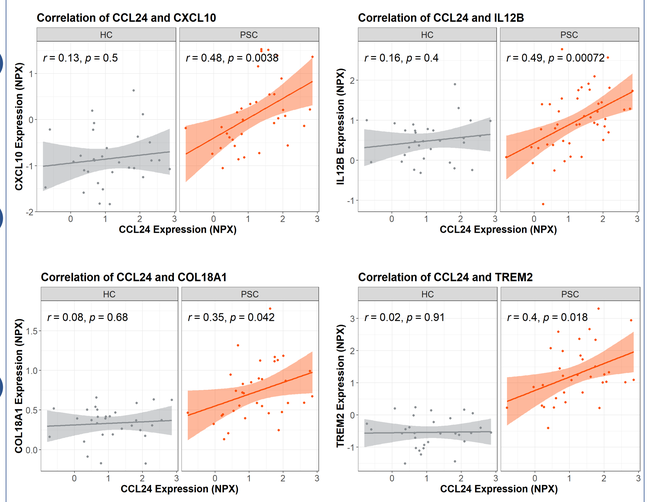
	N	Age [y], median (range)	Duration since diagnosis [y], median (range)	Male, n (%)	IBD any, n (%)	ALP [U/L], median (range)	ALT [U/L], median (range)	AST [U/L], median (range)	Bilirubin [mg/dL], median (range)	Fibroscan, n, median (range)	ELF, n, median (range)
Healthy controls	30	23.5 (18-38)	NA	30 (100)	0 (0)	74 (42-106)	16.5 (7-45)	19 (12-34)	11 (7-18)	NA	NA
PSC	45	45 (18-76)	4.9 (0-25.3)	26 (58)	30 (67)	246 (52-1064)	70 (10-796)	54 (15-919)	12 (3-41)	43, 10.1 (5.0-17.3)	33, 9.95 (7.85-12.84)

Serum Proteomics Separates Individuals by Disease and Its Severity



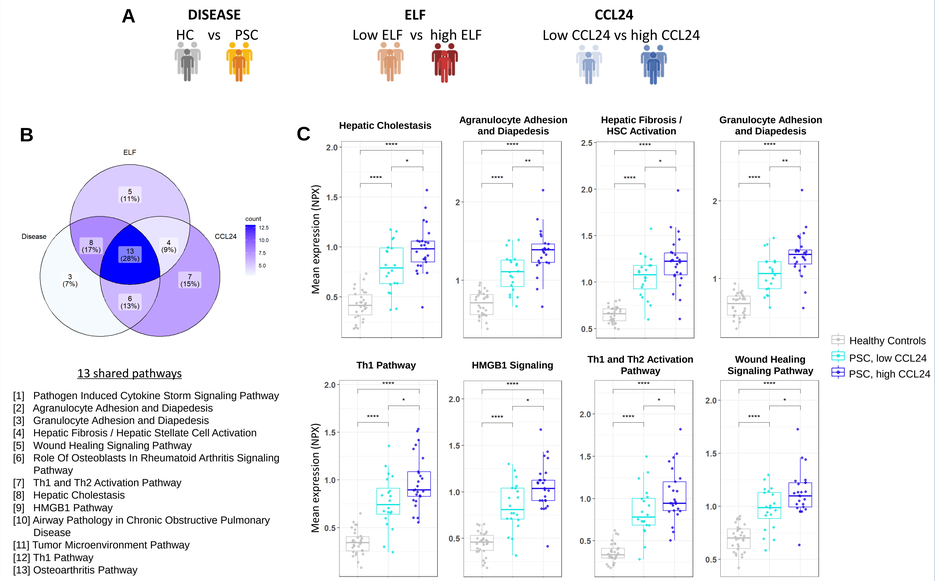
Principal component analysis (PCA) of 2081 analyzed extracellular proteins. (**A**) Scores of the first (Dim1) and second (Dim2) principal components in healthy controls (HC, grey dots) and PSC patients (orange dots). (**B**) Scores of the first and second principal components in in healthy controls (HC, grey dots), PSC patients with low ELF score (light red dots), PSC patients with high ELF score (dark red dots) and PSC patients with unknown ELF score (pink rings).

CCL24 Associates with Proteins of Inflammation, Fibrosis and Vascularization



Correlation of serum levels of CCL24 and protein markers of inflammation (CXCL10), fibrosis (COL18A1), vascularization (VEGFA) and myeloid activation (TREM2). Grey dots represent heathy controls (HC) and orange dots represent patients with active PSC disease (i.e., ALP > 1.5 upper limit of normal range).

Pathways Related to PSC and Its Severity Are Upregulated in Patients with High CCL24 Levels

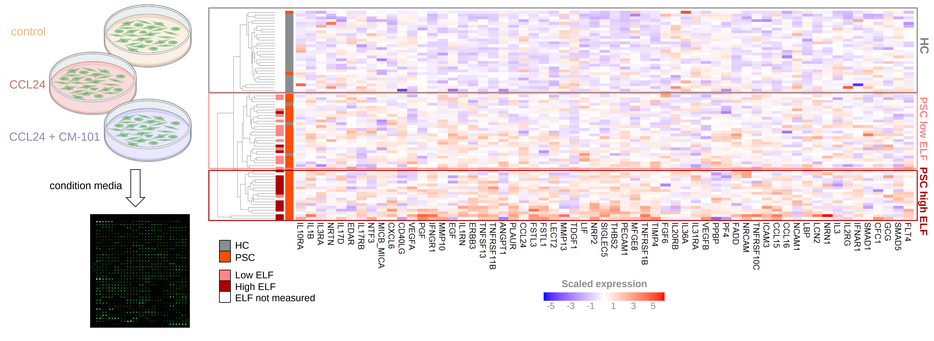


13 shared pathways

[1] Pathogen Induced Cytokine Storm Signaling Pathway
[2] Agranulocyte Adhesion and Diapedesis
[3] Granulocyte Adhesion and Diapedesis
[4] Hepatic Fibrosis / Hepatic Stellate Cell Activation
[5] Wound Healing Signaling Pathway
[6] Role Of Osteoblasts In Rheumatoid Arthritis Signaling Pathway
[7] Th1 and Th2 Activation Pathway
[8] Hepatic Cholestasis
[9] HMGB1 Pathway
[10] Airway Pathology in Chronic Obstructive Pulmonary Disease
[11] Tumor Microenvironment Pathway
[12] Th1 Pathway
[13] Osteoarthritis Pathway

(**A**) An overview of the analysis. Differentially expressed proteins (DEPs) were compared by disease (between health controls and patients with PSC), ELF (between patients with low ELF scores and patients with high ELF scores) or CCL24 (between patients with low serum CCL24 levels and patients with high serum CCL24 levels). (**B**) DEPs were submitted to pathway analysis using Ingenuity Pathway Analysis (IPA). Top 30 significant pathways were overlapped between the three comparisons (disease, ELF and CCL24). The Venn diagram is colored by the number of pathways. Shared pathways are presented below the Venn diagram, (**C**) Average expression of pathway-specific serum protein signatures in healthy controls versus PSC patients with low and high CCL24 serum. Boxes represent interquartile ranges with medians. HC, healthy controls.

An in-vitro Based CCL24 Dependent Protein Signature Differentiates by PSC and Its Severity



The LX2 hepatic stellate cell (HSC) line was stimulated with CCL24 and subsequently blocked by a neutralizing monoclonal antibody (CM-101). Conditioned media underwent proteomic profiling using a proteomic chip of 507 secreted proteins. A CCL24 dependent signature was generated by accounting proteins with a 2-fold change upon CCL24 treatment (relative to control), and that blocking CCL24 with CM-101 returned their expression to control levels. Differences in the serum proteomic profile of the CCL24 dependent was examined by an unsupervised heatmap of scaled expression values for each individual and identified protein. HC, healthy controls.