Identification of Novel Inflammatory Serum and Urinary Protein Biomarkers for PSC

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RATIONALE

Primary sclerosing cholangitis (PSC) is a rare disease with no FDA-approved therapies. The development of therapeutics for PSC has been hindered by the lack of robust biomarkers of progressive disease and treatment response. Here we combined two unique biomarker screening approaches, serum proteomics by proximity extension assay technology (Olink) and urine proteomics by unbiased LC-MS/MS technology (Biognosys) to identify novel candidate biomarkers for PSC.

METHODS

Serum Proteomics Analysis

Serum samples from 21 patients with PSC and 15 healthy, age-matched donors were analyzed on a 384 inflammatory protein panel using proximity extension assay technology by Olink. Normalized protein expression (NPX) levels were reported in a $\log_2$ scale. Proteins that failed Olink QC or were detectable in < 90% of the samples were excluded from analysis. The OlinkAnalyze R package was used to analyze the data running an unpaired t-test to compare values from PSC serum versus healthy serum. Significant differences are defined as $|\log2FC| > 0.5$ and FDR < 0.05. GO term analysis was run using EnrichR.

Serum Donor Demographics

Subject Group	n	Age (yr)	Gender
PSC (UCSF)	21	54 ± 13.6	9 M, 12 F
Healthy (BioIVT)	15	54.5 ± 8.4	8 M, 7 F

Urine Proteomics Analysis

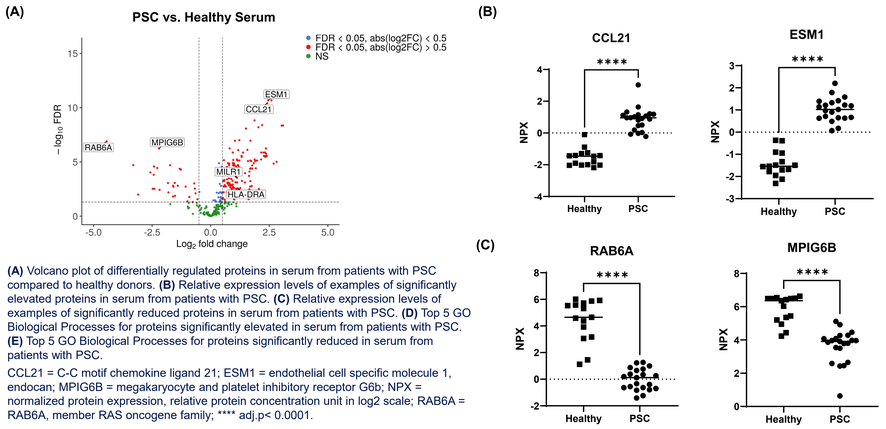
Urine samples from 12 patients with PSC (11 unique and 1 overlapping with serum analysis cohort) and 12 unique healthy, age-matched donors were analyzed by data-independent acquisition LC-MS/MS technology by Biognosys. Briefly, pooled urine proteins were digested, fractionated and analyzed via shotgun LC-MS/MS to generate sample-specific spectral libraries followed by individual patient sample analysis to quantify peptide abundance. Protein expression values were log2 transformed and median normalized using the DEqMS R package. Differentially regulated proteins were identified using the limma R package. Significant differences are defined as $|\log2FC| > 0.5$ and FDR < 0.1. GO term analysis was run using EnrichR.

Urine Donor Demographics

Subject Group	n	Age (yr)	Gender
PSC (UCSF)	12	58.5 ± 5.5	5 M, 7 F
Healthy (BioIVT)	12	53.9 ± 3.2	7 M, 5 F

RESULTS

SERUM PROTEOMICS



(A) Volcano plot of differentially regulated proteins in serum from patients with PSC compared to healthy donors. **(B)** Relative expression levels of examples of significantly elevated proteins in serum from patients with PSC. **(C)** Relative expression levels of examples of significantly reduced proteins in serum from patients with PSC. **(D)** Top 5 GO Biological Processes for proteins significantly elevated in serum from patients with PSC. **(E)** Top 5 GO Biological Processes for proteins significantly reduced in serum from patients with PSC.

CCL21 = C-C motif chemokine ligand 21; ESM1 = endothelial cell specific molecule 1, endocan; MPIG6B = megakaryocyte and platelet inhibitory receptor G6b; NPX = normalized protein expression, relative protein concentration unit in log2 scale; RAB6A = RAB6A, member RAS oncogene family; **** adj.p< 0.0001.

- 135 serum proteins were significantly elevated in PSC serum compared to healthy donors (FDR < 0.05, log2FC > 0.5)
  - Includes ESM1 (endocan) previously proposed as a biomarker of progression in liver cirrhosis[1]
- 31 serum proteins were significantly reduced in PSC serum compared to healthy donors (FDR < 0.05, log2FC < -0.5)
- Previously described PSC biomarkers were identified (e.g. CCL21, uPAR)
- Novel putative PSC biomarkers were also identified (e.g. ESM1, RAB6A, MPIG6B)

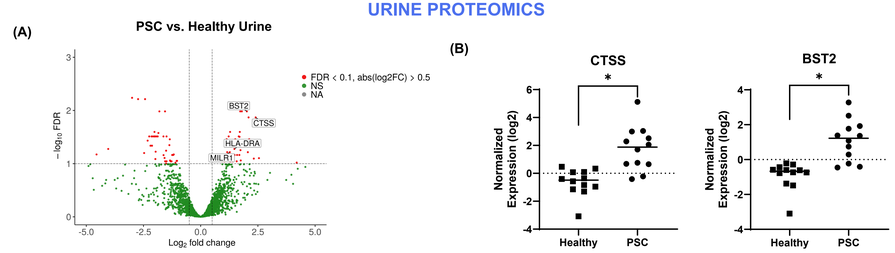
(D)

Top Upregulated GO Biological Processes	Adj. P-Value
Cytokine-Mediated Signaling Pathway (GO:0019221)	9.41E-13
Inflammatory Response (GO:0006954)	4.80E-07
Granulocyte Chemotaxis (GO:0071621)	6.45E-07
Chemokine-Mediated Signaling Pathway (GO:0070098)	1.41E-06
Cellular Response To Chemokine (GO:1990869)	1.50E-06

(E)

Top Downregulated GO Biological Processes	Adj. P-Value
Positive Regulation Of MAPK Cascade (GO:0043410)	2.07E-04
Cellular Response To Interleukin-1 (GO:0071347)	1.58E-03
Response To Interleukin-1 (GO:0070555)	1.58E-03
Inflammatory Response (GO:0006954)	3.91E-03
Cytokine-Mediated Signaling Pathway (GO:0019221)	4.70E-03

URINE PROTEOMICS



(A) Volcano plot of differentially regulated proteins in urine from patients with PSC compared to healthy donors. **(B)** Relative expression levels of examples of significantly elevated proteins in urine from patients with PSC. **(C)** Select GO Biological Processes for proteins significantly elevated in urine from patients with PSC. **(D)** Select GO Biological Processes for proteins significantly reduced in urine from patients with PSC.

BST2 = bone marrow stromal cell antigen 2, tetherin; CTSS = cathepsin S; * adj.p< 0.05.

- 2723 unique proteins detected in urine samples
- 89 urine proteins were significantly different between PSC and healthy urine (FDR < 0.1, $|\log2FC| > 0.5$)
  - Includes BST2 (tetherin) previously proposed as a biomarker of progression in primary biliary cholangitis[2]

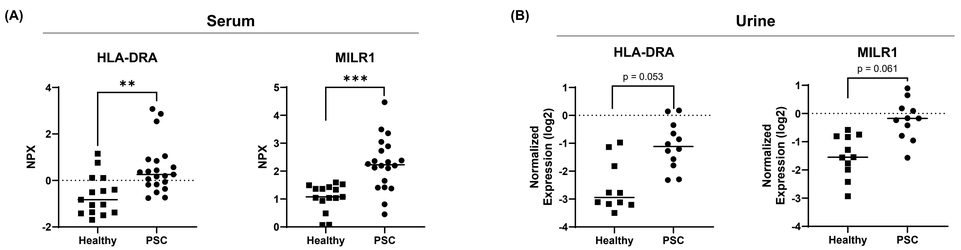
(C)

Upregulated GO Biological Processes	Adj. P-Value
Antigen Processing And Presentation Of Exogenous Peptide Antigen (GO:0002478)	1.48E-06
Response To Thyroid Hormone (GO:0097066)	2.50E-04
Ceramide Catabolic Process (GO:0046514)	4.29E-04
Regulation Of Immune Response (GO:0050776)	1.80E-03
Glycosaminoglycan Metabolic Process (GO:0030203)	1.82E-03

(D)

Downregulated GO Biological Processes	Adj. P-Value
Proteolysis (GO:0006508)	1.09E-05
Regulation of Fibrinolysis (GO:0051917)	9.91E-04
Retina Homeostasis (GO:0001895)	1.04E-02
Cholesterol Transport (GO:0030301)	1.75E-02
Detection of Bacterium (GO:0016045)	1.91E-02

OVERLAPPING PUTATIVE BIOMARKERS



(A) Relative protein expression of HLA-DRA and MILR1 in serum from healthy and PSC donors **(B)** Relative protein expression of HLA-DRA and MILR1 in urine from healthy and PSC donors

HLA-DRA = major histocompatibility complex, class II, DRA alpha; MILR1 = mast cell immunoglobulin like receptor 1, allergin-1; NPX = normalized protein expression, relative protein concentration unit in log2 scale; ** adj.p<0.01, *** adj.p< 0.001.

- Two putative biomarkers of PSC, HLA-DRA and MILR1, were found to be elevated in both the serum and the urine of patients with PSC

CONCLUSIONS

- **Two unique proteomic screening approaches were used to identify novel diagnostic biomarkers in patients with PSC**
- **HLA-DRA and MILR1 were identified as potential diagnostic biomarkers of PSC elevated in both serum and urine**
- **Previously proposed biomarkers of progression in other liver diseases were identified (e.g. ESM1 and BST2), suggesting a potential role in predicting progression in PSC**
- **Follow-up studies are required to validate the prognostic and/or therapeutic utility of these novel biomarker candidates in PSC**

References: 1. Toshikuni et al. Can J Gastroenterol Hepatol, 2015, 2. Hardie et al. eBioMed, 2016

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JS, RA, PK, and MD are employees and shareholders of Pliant Therapeutics. ST is a former employee of Pliant Therapeutics