The heparin-binding proteome in normal pancreas and murine experimental acute pancreatitis

Version 4

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ABSTRACT

Acute pancreatitis (AP) is acute inflammation of the pancreas, mainly caused by gallstones and alcohol, driven by changes in communication between cells. Heparin-binding proteins (HBPs) play a central role in cell communication. Therefore, we used heparin affinity proteomics to identify extracellular HBPs in pancreas and plasma of normal mice and in a caerulein mouse model of AP. Many new extracellular HBPs (360) were discovered in the pancreas, taking the total number of HBPs known to 786. Extracellular pancreas HBPs form highly interconnected protein-protein interaction networks in both normal pancreas (NP) and AP. Thus, HBPs represent an important set of extracellular proteins with significant regulatory potential in the pancreas. HBPs in NP are associated with biological functions such as molecular transport and cellular movement that underlie pancreatic homeostasis. However, in AP HBPs are associated with additional processes such as acute phase response signalling, complement activation and mitochondrial dysfunction. Plasma HBPs in AP included known AP biomarkers such as serum amyloid A, as well as emerging targets such as histone H2A. Pancreas HBPs are extracellular and so easily accessible and are potential drug targets in AP, whereas plasma HBPs represent potential biomarkers for AP. These need further investigation for potential applications in the management of AP.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

STEPS MATERIALS

NAME	CATALOG #	VENDOR
StrataCleanTM beads	400714	
RapiGest SF Surfactant	23221	
Iodoacetamide	AK-U470	P212121
DTT	D0632	Sigma Aldrich
Trypsin from porcine pancreas	T7409	Sigma-aldrich
Trifluoroacetic acid (TFA)		

MATERIALS TEXT

Blood obtained from 6-8 week old male adult CD1 mice (weight range, 24-30 g) with normal pancreas (NP) or experimental acute pancreatitis (AP), induced as described above, was collected in0.38% (w/v) sodium citrate (Sigma-Aldrich,Gillingham, UK) and centrifuged at 5,000 g at 4 ˚C for 15 min [27]. Supernatant plasma was extracted and frozen at -20 ˚C.

Heparin affinity chromatography

1 The frozen murine plasma was defrosted on ice and centrifuged at 16,100 g for 10 min, at 4 ˚C.

4 °C **16,100 g** 00:10:00

The resulting supernatant was diluted (1:8) in 75 mM NaCl, 6.85 mM Na_2HPO_4, 3.15 mM NaH_2PO_4, pH 7.2.

NOTE

NOTE

This method was effective in reducing the high abundance protein, albumin, down to levels that would not affect the MS analysis of plasma HBPs.

The diluted supernatant was centrifuged at 5,000 g for 5 min.

00:05:00 **5000 g**

and the resulting supernatant was then applied on a 1 mL Hi-Trap heparin column

EQUIPMENT

1 mL Hi-Trap heparin column
Affinity column
GE Healthecare Life Sciences 17040601

equilibrated in 75 mM NaCl, 6.85 mM Na_2HPO_4, 3.15 mM NaH_2PO_4, pH 7.2.

The heparin column was subsequently washed extensively with 50 mL of **modified PBS** (150 mM NaCl, 13.7 mM Na_2HPO_4, 6.3 mM NaH_2PO_4, pH7.2). The heparin-bound fraction was then eluted with 2 M NaCl in **modified PBS**.

NOTE

The receipt for PBS has been modified here. Please check carefullly.

[M]**2 Molarity (M) NaCl**

Protein adsorption using StrataClean for mass spectrometry

2 In order to eliminate the high concentration of electrolytes (2.15 M NaCl) in the eluate, so as not to influence MS analysis, StrataClean™ beads (Agilent Technologies, UK) were used to adsorb proteins.

StrataCleanTM beads
Catalog #: 400714

Briefly, 100 µg of the heparin-bound fraction was mixed with 30 µL StrataClean slurry, vortexed for 2 min. and centrifuged at 2,000 g for 2 min at 4 ˚C.

4 °C | 00:02:00

The supernatant was removed carefully and discarded.

The pellet was first washed with PBS and then further washed twice with H_2O.

Sample preparation for mass spectrometry

3 In the case of plasma samples, the StrataClean^{TM} beads were resuspended in 80 µL of 25 mM ammonium bicarbonate and 5 µL of 1 % (w/v) Rapigest (Waters, Manchester, UK) added and the samples shaken at 450 rpm for 10 min at 80 °C.

RapiGest SF Surfactant
Catalog #: 23221

80 °C for the denature by RapiGest | 00:10:00

Samples were reduced by the addition of 5 µL of 60 mM [M]60 Molarity (m) (DTT) and incubated at 60°C 60 °C for 10 min 00:10:00 and alkylated (addition of 5 µL of 180 mM iodoacetamide and incubation at room temperature for 30 min in the dark).

Iodoacetamide
by P212121
Catalog #: AK-U470

DTT
by Sigma Aldrich
Catalog #: D0632

Trypsin (Sigma, Poole, UK, proteomics grade) was reconstituted in 50 mM acetic acid to a concentration of 0.2 µg/µL and 5 µL (1 µg) 1 µg enzyme added to the sample followed by overnight incubation at 37 °C. 17:00:00 overnight | 37 °C

Trypsin from porcine pancreas
by Sigma-aldrich
Catalog #: T7409

NOTE
Samples were mixed on a rotating mixer

The following day the digestion was terminated and Rapigest removed by acidification with TFA (1 µL)

Trifluoroacetic acid (TFA)

and incubation at 37 °C for 45 min. 37 °C 00:45:00

Samples were centrifuged at 17,200 g for 30 min and the clarified supernatants transferred to tubes.

00:30:00 17,200 g centrifuge