Metabolite profiling applied to biomarker discovery in pancreatic cancer using high resolution LC-MS/MS

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Overview

- High resolution LC-MS/MS QTOF analysis was applied to pancreatic adenocarcinoma (PDAC) samples, a disease characterised by early metastasis and low survival rate.
- Previously published and new biomarkers were identified in this work; notably elevated bile acids and reduced LPEs, LPCs and PCs.
- Comparison to direct probe electrospray ionisation data provided added confidence in biomarker identification that may be considered in the future as part of a screening system for early detection of PDAC.

1. Introduction

In the present study, two metabolite profiling methodologies were used to detect metabolite changes between patient serum samples with pancreatic ductal adenocarcinoma (PDAC) and healthy controls; a standardised approach in LC-MS/MS based metabolite profiling and a direct analysis method (Direct Probe Ionisation Mass Spectrometry – DPiMS[1]). In this study high resolution LC-MS/MS was used to characterise the metabolic profiles of serum from patients with PDAC compared to healthy controls to reveal key metabolic differences in these phenotypes and confirm DPiMS measurements.

2. Materials and Methods

Serum samples included healthy controls (n=30) and PDAC (n=30). The research project was approved by the Pancreatic Cancer Research Fund Tissue Bank (PCRFTB) Tissue Access Committee.

- **Reverse phase LC Separation**.
  - Acquity C18 BEH (2.1x100mm 1.7µm); 50ºC, flow rate 0.4 mL/min
  - Binary gradient; water + 0.1% formic acid, and acetonitrile + 0.1% formic acid
  - Cycle time 35 minutes.
- **LC-MS/MS Mass Spectrometry Detection**. High resolution QTOF LC-MS/MS analysis (LCMS-9030, Shimadzu Corporation, Japan) using external mass calibration for positive and negative mode ESI. The untargeted metabolite profiling method used a TOF MS mass scan followed by a series of DIA-MS/MS mass scans.
  - TOF MS mass scan m/z 60-1000; 100 msecs
  - DIA-MS/MS mass scans m/z 40-1000; 33 msecs for each precursor isolation window; isolation width 35 Da; collision energy spread 5-55V; 27 mass scan events. Scan cycle time 0.99 second (28 mass scans in total).
- **DPiMS Mass Spectrometry Detection**. High resolution QTOF MS/MS analysis (DPiMS QT, Shimadzu Corporation, Japan); direct sample analysis (no LC).
  - TOF MS mass scan m/z 100-1500; 100 msecs
  - For DPiMS compound identification, DIA-MS/MS mass scans m/z 100-1500; collision energy spread 5-55V, precursor isolation window 1 Da, positive and negative
- **Data processing**. Several data processing tools are available on-line for feature detection, alignment and identification such as MS-DIAL, MZmine3, GNPS, XCMSonline plus. This study used MS-DIAL for peak processing and alignment, MetaboAnalyst for statistical analysis and LabSolutions Insight for metabolite identification.

2.1 Applying a generic reverse phase LC-MS/MS method

The method has been optimised for a broad range of metabolite classes

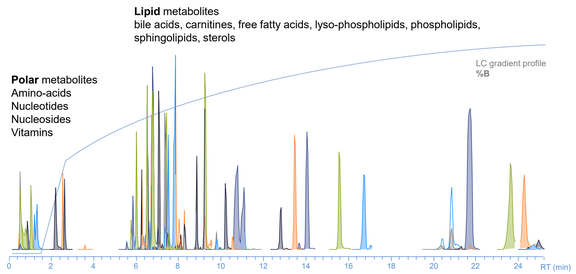

Figure 1. Separation of a broad range of metabolites using a single run reverse phase method with MS and DIA-MS/MS detection. In this study, the primary focus of the investigation was to profile the change in lipid distributions in PDAC samples compared to controls.

2.2 Data Processing Workflow for LC-MS/MS

One approach for processing untargeted metabolomics data;

- **MS-DIAL |** raw data file processing, feature detection, alignment, filters applied**.**
- **MetaboAnalyst 5.0 |** univariate statistical analysis, volcano plot.
- **Compound Identification |** LabSolutions Insight.

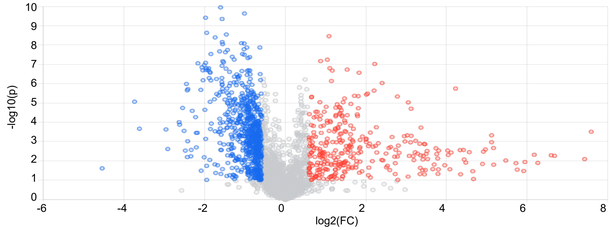

Figure 2. Volcano plot showing features extracted using HRMS LC-MS in positive and negative ion mode that were significantly increased or decreased in PDAC serum samples compared to healthy controls (fold change >1.5, p<0.1).

MS-DIAL generated an aligned data array that was subsequently filtered (metabolite feature was present in >50% of the QCs with QC RSD<20% and present in at least 80% of either the healthy or PDAC group).

2.3 Metabolite Identification using LC-MS/MS

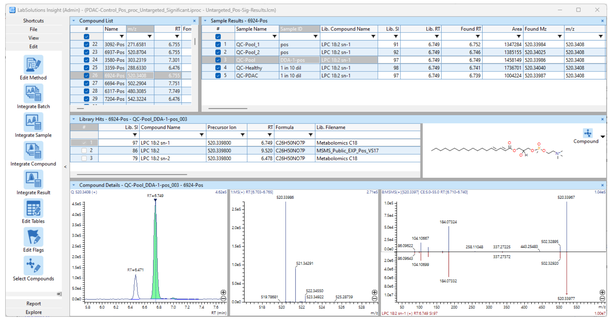

Figure 3. LabSolutions Insight software application was used for metabolite identification. DIA and DDA-MS/MS mass spectra were searched against an in-house development metabolomics library and the MS-DIAL MSP spectral kit (http://prime.psc.riken.jp/compms/msdial/main.html).

3. Results

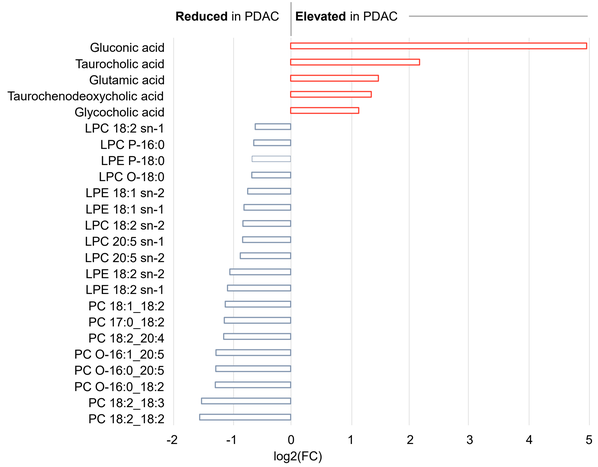

Figure 4. Bar chart highlighting the most significant changes (fold change >1.5) in PDAC samples relative to healthy controls that could be annotated at the MSMS level (Metabolomics Standards Initiative level 1 or 2).

3.1 HR LC-MS/MS comparison to PESI

Relative levels of biomarker candidates were compared between different acquisition methods; liquid chromatography electrospray ionisation (LC-ESI) and direct probe ionisation mass spectrometry (also known as probe electrospray ionisation (PESI)). Both high-resolution LC-MS/MS data and DPiMS showed the same direction of change.

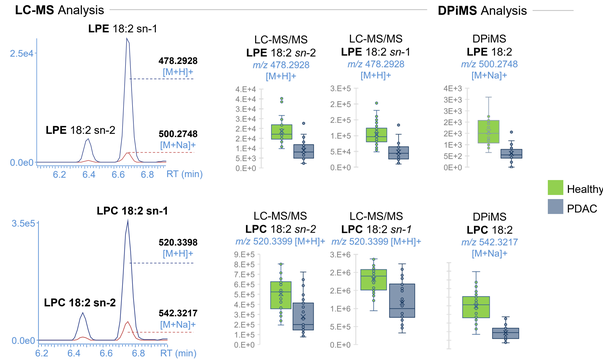

Figure 5. LC-MS/MS and DPiMS results for two lipids; LPE 18:2 and LPC 18:2. DPiMS shows the same direction of change as LC-MS/MS for both lipids, however, DPiMS cannot resolve the *sn*-1 and *sn*-2 isomers. As DPiMS is a direct analysis technique, adducts are often the dominant ion species (adducts are also present in LC-MS/MS, but the molecular ion is typically the dominant ion).

4. Conclusions

- High resolution LC-MS/MS detected a panel of potential biomarkers that may be used to differentiate between PDAC serum samples and healthy controls.
- Metabolites were identified to MSI level 1 or 2 using an in-house metabolomics library and the MS-DIAL MSP spectral kit (http://prime.psc.riken.jp/compms/msdial/main.html).
- Analysis by LC-MS/MS helped confirm candidate biomarker identification from DPiMS providing verification to a previous nominal mass study[2].

5. References

[1]Mandel et al. Application of Probe Electrospray Ionization Mass Spectrometry (PESI-MS) to Clinical Diagnosis: Solvent Effect on Lipid Analysis. J. Am. Soc. Mass Spectrom. 2012, 23, 11, 2043–2047

[2]Chung et al. Using probe electrospray ionization mass spectrometry and machine learning for detecting pancreatic cancer with high performance. Am J Transl Res. 2020; 12(1): 171–179.