Direct Probe Ionisation Mass Spectrometry applied to biomarker discovery in pancreatic cancer

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Overview

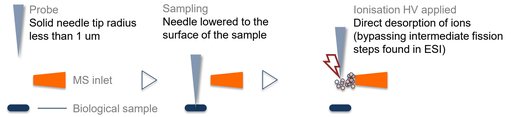
- Direct probe ionisation mass spectrometry (DPiMS) was applied to serum samples from pancreatic ductal adenocarcinoma (PDAC) patients and healthy controls using a high-resolution Q-TOF.
- Putative identification from iDIA data acquisition identified a number of potential biomarkers including LPC 18:2, LPE 18:2 and LPC 20:5.

1. Introduction

Several LC-MS/MS metabolomics-based studies have shown the potential use of metabolites as biomarkers for pancreatic ductal adenocarcinoma (PDAC) resulting in changes to amino acids, fatty acids and lipids. In this work a direct analysis method (DPiMS[1], requiring no LC separation) was applied to discriminate between PDAC serum samples from healthy controls using high resolution QTOF mass spectrometry.

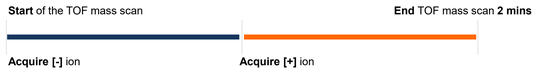
2. Materials and Methods

- **Serum samples.** Included healthy controls (n=30) and pancreatic ductal adenocarcinoma (PDAC) (n=30). The research project was approved by the Pancreatic Cancer Research Fund Tissue Bank (PCRFTB) Tissue Access Committee.
- **Sample preparation**. 50 µL of serum + 950 µL of 1:1 ethanol:water, centrifuged 16,000× g, 10 µL added to the sample plate.
- **Direct analysis using high resolution mass spectrometry**. Protein precipitated serum samples were analysed directly using a high resolution DPiMS QT (LCMS-9030 Q-TOF system, Shimadzu Corporation, Japan).
- **DPiMS.**
  - Probe needle sampling frequency ~3 Hz. Analysis time; 2 minutes

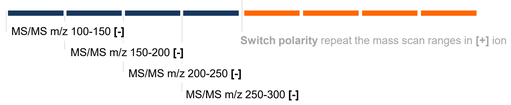


- **Mass spectrometry.** MS1 was used for biomarker discovery; iDIA for metabolite identification.

MS1 TOF Scan mass range m/z 100-1500; 2 min analysis, polarity switching



iDIA-MS/MS TOF Scans precursor ion isolation 1 Da; sequential mass range steps covering a mass scale of 200 Da for each polarity. Repeated across the mass scale m/z 100-1500



2.1 Data processing

- MS1 TOF data was acquired in both positive and negative ion modes in a single analysis for each sample. MS spectra for each sample and ion mode was extracted and filtered using the following criteria: MS bin width 5 mDa, group presence set to 80% (intensity >1000 counts).
- Ion features from each ionisation mode were statistically analysed (263 features negative mode; 468 features positive mode).
- Analysis was applied to the two groups using volcano plot (p<0.05 FDR corrected, fold change >2), lasso regression (λ = 0.1, selected via a 10-fold cross validation) and random forest analysis (number of trees = 1000, number of random variables used in each tree equal to the square root of the number of predictors all independently repeated over 10-fold cross validations).
- Feature importance was measured based on how much the Gini Index for a feature decreases at each split.
- 21 significant ion features were identified in positive mode and 30 in negative mode.

3. Results

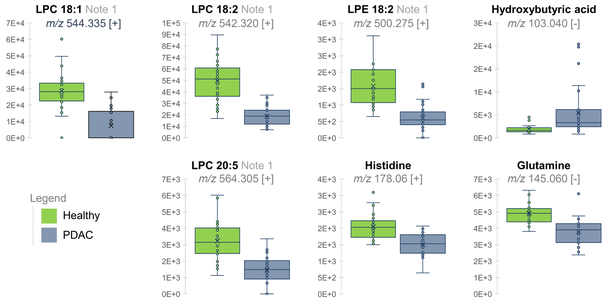


Figure 1. Box plots for a metabolic profiling analysis highlighting differences between PDAC patient serum samples and healthy controls using direct analysis DPiMS. Biomarker candidates include lipid classes (lysophosphatidylcholines such as LPC 18:1, LPC 18:2, LPC 20:5; lysophosphatidylethanolamines such as LPE 18:2) and amino acids (histidine, glutamine; hydroxybutyric acid is a partial-degradation product of branched-chain amino acids primarily valine).

Note 1. Data Processing Workflow and Metabolite Annotation.

- Statistical analysis initially identified mass to charge values of significance; for example, mass/charge 544.335 (within a 5 mDa bin).
- To annotate the mass/charge value m/z 544.335 with high reporting confidence, the bin was searched for an accurate mass of the precursor (MS1 m/z), iDIA-MS/MS spectra was used for library searching against in-house or other MS/MS data repositories.
- In the case of lyso PCs and PEs, isomers cannot be resolved by DPiMS and so the metabolite annotation considers both *sn*-1 and *sn*-2.

3.2 DPiMS Metabolite Identification

Two data acquisition methods were used in this study, MS1 TOF mass scan for biomarker discovery and narrow band iDIA for metabolite identification. The iDIA method used a precursor ion isolation window of 1 Da (mass scan time for each precursor was 20 msec, mass range m/z 100-1500, each method acquired data over a 200 Da mass range requiring a sequence of 7 methods). This approach was applied to pooled QC from healthy control serum samples.

DPiMS iDIA MS/MS data was compared to targeted LC-DDA-MS/MS spectra. In all cases there was a high similarity score between each MS/MS spectra enabling putative metabolite identification.

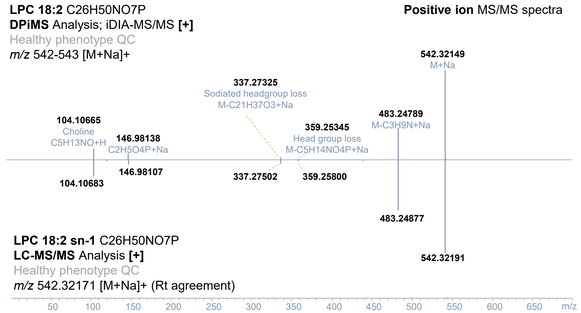


Figure 2. Metabolite annotation for LPC 18:2 in positive ion mode.

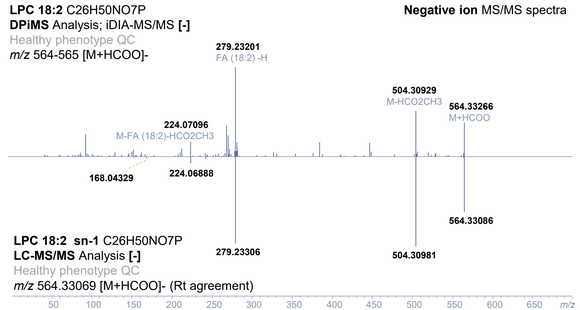


Figure 3. Metabolite annotation for LPC 18:2 in negative ion mode.

For negative ion mode, 0.1% formic acid was added resulting in the formation of the [M+HCOO]- adduct. DPiMS for both positive and negative ion MS/MS spectra were in agreement with LC-DDA-MS/MS spectra.

4. Discussion

Recent PDAC biomarker discovery studies by LC-MS/MS have identified panels of metabolites that can be considered as discriminative metabolites. Lyso PCs are reportedly down regulated in PDAC and it has been suggested this change may relate to carcinogenesis and progression of PDAC. PDAC is also associated with a fatty acid disorder resulting in a decrease in fatty acids determined by LC-MS/MS. Whilst LC-MS/MS based untargeted metabolomics has been applied to measure a high number of metabolite features in a biological sample, the cycle time is typically 20-30 mins. DPiMS is considerably shorter with an analysis time of 2 mins.

In this work high resolution LC-MS/MS and DPiMS was used in PDAC biomarker discovery studies, the key advantages of DPiMS include:

- **Rapid analysis cycle time**. Positive and negative MS1 data was acquired over a cycle time of 2 minutes in this study (the DPiMS has an oscillating sampling frequency of ~3 Hz which results in a stable ion current with an averaged mass spectrum).
- **Minimal sample preparation with minimal suppression effects.** DPiMS[2] can be used with minimal or no sample preparation, has a high tolerance to solutions of high salt concentrations, works with small sample volumes and results in sensitive detection.
- **Correspondence with LC-MS/MS for metabolite identification.** In this study DPiMS was primarily used to detect precursor ions in MS1 and statistical analysis tools were applied to differentiate between disease and healthy states (including linear tools such as lasso regression and machine learning techniques such as random forest analysis). Using narrow band iDIA with a precursor ion isolation width of 1 Da resulted in MS/MS spectra that were in close correspondence with MS/MS spectra acquired using LC-DDA-MS/MS (data acquired with the same collision energy spread of 5-55 V). Metabolites could therefore be annotated with high reporting confidence.

5. Conclusions

- High resolution direct probe ionisation mass spectrometry (DPiMS) was applied to distinguish metabolite profiles between PDAC patient serum samples and healthy controls.
- Using Volcano plots, lasso and random forest analysis significant ion features were identified in positive mode and negative ion modes.
- Putative biomarker candidates were putatively identified and included several lipid classes (lysophosphatidylcholines such as LPC 18:1, LPC 18:2, LPC 20:5; lysophosphatidylethanolamines such as LPE 18:2), amino acids (histidine, glutamine) and hydroxybutyric acid (a partial-degradation product of branched-chain amino acids).
- Statistically significant metabolite features were identified using a narrow band DIA-MS/MS method (iDIA) resulting in library searchable MS/MS spectra which agreed with LC-MS/MS spectra.
- The results suggest that DPiMS may be considered as an effective methodology for biomarker discovery without the need for LC separation.

6. 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.