An automated workflow for rapid alignment and identification of lipid biomarkers obtained from chip-based direct infusion nano-electrospray tandem mass spectrometry

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Introduction
Polar lipids (glycerophospholipids, saccharolipids) are routinely obtained from lung and plasma samples. After extraction, these lipids are isolated and identified either by direct nuclear magnetic resonance (NMR) analysis or by high-resolution mass spectrometry (HRMS) followed by tandem MS/MS analysis. A key step in this process is the alignment of mass spectra from infusion experiments. We present an automated workflow for rapid alignment and identification of lipids from infusion tandem mass spectra. Results from an environmental tobacco smoke study and plasma and lung tissue are shown.

Methods
To 30 µL plasma or 10 mg cryogenically ground lung samples 1.0 mL of chilled (isopropanol/acetonitrile, 3:2, v/v, -20°C) solvent was added. The solution was shaken for 5 minutes in a vortex and centrifuged 2.5 minutes at 13,000 rpm. After adding 10 µL of 100 mM ammonium acetate to 90 µL of the supernatant the lipid extracts were infused into a linear ion trap mass spectrometer coupled to an FT-ICR-MS using an Advion Nanomate chip-based infusion system. Mass spectra were acquired from 400-1000 m/z with positive ionization and 100,000 resolving power and 0V CID voltage. Data dependent MS2 scans were performed on the 40 highest peaks in the full scan spectrum and scanned in low-resolution mode with 30V CID voltage. The alignment data of the high-resolution and low-resolution mass spectra was performed using Genedata Expressionist Refiner MS. The statistical data evaluation was performed with Genedata Analyst using univariate and multivariate methods. Unique lipid species were identified by matching tandem mass spectra against a database of known fragment ions and validating results with LipidMaps tools and LipidMaps web services.

Results
Lipid extracts from heart, plasma and lungs from rats which were exposed to environmental tobacco smoke were analyzed. An alignment workflow for high-resolution and low-resolution mass spectra was built using Expressionist Refiner MS. It included spectrum grid alignment, spectrum averaging, intensity thresholding, peak detection, peak shaping and alignment export. The post-processing time for a sample setup of 70 infusion experiments took around 20 seconds. Additionally MS/MS spectra were extracted from data dependent scans. A matrix containing all samples and all aligned ions above a certain signal noise threshold was obtained as a result file. Identification of lipid species was performed by matching specific sn1 and sn2 fatty acid chain fragmentation from MS/MS data of the product ions. 50 lipid species were identified in plasma and 50 remained unknown. Different lipid patterns revealed alterations during exposure to tobacco smoke. This research project was supported by ROI ES019362 NIH/NIEHS.

Platform – nanoESI infusion with iontrap

Alignment of low-resolution and high-resolution mass spectral infusion data was implemented within Expressionist Refiner MS. The workflow performs peak detection, noise analysis, peak integration, isotope grouping and alignment of large datasets. The workflow runtime for 70 infusion samples is 30 seconds, allowing fast parameter optimizations. MS/MS data extraction is performed to obtain tandem mass spectra for assignment of phospholipid structure including head group and neutral loss analysis.

Parameter optimization and peak refinement

Depending on the mass spectral data acquisition mode, certain workflow parameters can be optimized to avoid peak detection errors or to adjust sensitivity if needed. The table shows a very high number of peaks after the initial step. The recursive consistency filter and the signal to noise filter ensure to retain peaks which have a very low abundance but good signal-to-noise (S/N) ratio.

Identified phospholipids from rat lung

Phospholipids were identified by accurate mass to obtain plausible phospholipid species and head group analysis from MS/MS scans. If the MS/MS spectra were clean to allow sn1 and fatty acid side chain analysis unique names were assigned (not shown here). A quality index was assigned to each lipid to specify high quality species. Data were validated with LipidMaps online and SDF tools, the Scionco LipidInspect and the LipidMaps MS-Prediction tools. A data table of 45,000 phospholipids was created for internal data matching of lipids.

Data dependent MS/MS for class analysis

The analysis of the different phospholipid classes including Glycerophosphocholines (GPCho), Sphingomyelines (SM), Glycerophosphoethanolamines (GPEtn), Glycerylphosphorylethanolamines (GPIns) and others can be performed by subtraction of specific neutral losses from the precursor ion in positive and negative mode. The precursor mass is then used to compute or lookup only species from a specific lipid class. In case of overlaps or uncertain spectra multiple species can occur.

Conclusion

• Lipid fingerprinting can be used for fast hypothesis generation
• Sn1 and sn2 fatty acid analysis from MS/MS
• Chip-based nanoESI infusion allows fast acquisition (one minute per sample) in high resolution mode
• Iontrap technology together with MS/MS scans used for phospholipid identification via head group and neutral loss analysis of fatty acids identified around 50 lipid components
• Expressionist Refiner MS workflow performs full data analysis in 20 seconds
• Expressionist Analyst platform performs multivariate and univariate statistics
• Perturbations in the phospholipid contents for rats exposed to environmental tobacco smoke (ETS) were observed