

Biochemical Mapping of Metabolic Alterations in Lungs of Rat Embryos

OLIVER FIEHN; Dinesh Kumar; Gert Wohlgemuth; Jesse Joad; Carol Hood; Kent Pinkerton;
Tobias Kind

UC Davis, Davis, CA

Keywords: Biomarker Validation; Carbohydrates; Lipid; Metabolic Profiling; Metabolism, Metabolites;

Novel Aspect: Using a lung biomarker study, an integrated workflow from chromatograms over compound identification to statistics and biochemical mapping is shown

Introduction

GC-TOF or LC-MS unbiased surveys of biological samples yield hundreds of resolved peaks per chromatogram. Statistical significant differences between semi-quantitative peak intensities can be routinely assigned to the classes of study designs. Metabolite peaks are then often called 'putative biomarkers' which must be validated in subsequent studies and confirmed to be specific for a diagnostic case. Consequently, the biomarker peaks must be unambiguously re-detectable over months in subsequent studies. This can best be performed by establishing standardized mass spectrometric metabolome databases. Secondly, valid biomarkers require a clear route to annotation of novel compounds to be implemented in routine clinical screens. Thirdly, interpretation of differential regulation of the identified metabolites should be guided by biochemical mapping to be of biomedical relevance.

Methods

PBS-perfused rat lungs were fresh frozen prior to homogenization and extraction. GC-TOF mass spectrometry (Leco Pegasus IV) was performed using Gerstel automatic liner exchange and cold injection conditions. GC-TOF spectra were filtered by the in-house BinBase mass spectral database. Metabolites were identified by a retention index/MS library of 713 authentic standards including PubChem and KEGG identifiers. Quantitative results were statistically evaluated, and significant differences were mapped to biochemical and chemical databases by open-access tools. Unknown metabolites were annotated using chemical ionization GC-TOF MS (Waters GC-T) and database queries according to accurate mass and accurate isotope data. Lipid fingerprints complemented the survey using nanoelectrospray (Advion) coupled to FT-ICR MS (ThermoFisher LTQFT). Data were aligned by the Expressionist software (Genedata).

Preliminary results

Timed pregnant rats were subjected to environmental tobacco smoke daily at 1 mg/m³ for 6 hours each day in controlled chambers from gestation day 5 to gestation day 20 (term is 21 days). After sample preparation, lung tissue GC-TOF chromatograms showed on average 852 deconvoluted peaks. However, many of these peaks were not consistently detectable in subsequent analysis of biological replicate samples. Consequently, this number of peaks was reduced to a much cleaner data set of 305 peaks using the in-house database BinBase by employing mass spectral metadata (peak purity, unique masses, s/n, apex masses). 155 of these metabolites were unambiguously identified by both retention index and mass spectral match criteria using a recently released mass spectral library and step-wise increase of similarity thresholds based on peak purity and peak abundance. Missing data were subsequently replaced from ion traces of unprocessed netCDF files, yielding a coherent result data sheet of 31,570 metabolic values. Multivariate statistics clearly proved that metabolic phenotypes in developing lungs were altered by cigarette smoke. 46 metabolites were differentially regulated in fetal lungs which were exposed perinatally to environmental tobacco smoke, indicating changes in carbohydrate and lipid metabolism. Only six statistically significant differences were annotated as unknown biomarkers. These peaks were subjected to identification by GC-TOF under chemical ionization and altered derivatization schemes. Additionally relative changes of polar membrane lipids were investigated by direct infusion nanoelectrospray mass spectrometry. Metabolic differences were eventually mapped onto biochemical pathways and plotted by chemical distances to enable straightforward interpretations of major metabolic disruptions.