Integrating MALDI imaging and ESI metabolomics for broadband identification and validation

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Introduction

Metabolomics is a huge field with many different technical approaches being used to gain insight about the metabolic processes in the samples of interest. Integrating these diverse techniques promises to enhance the amount of information. In this study we describe first results from an integrated spatial metabolomics workflow that combines MALDI imaging and ESI-based metabolomics. Our integrated approach offers a way of confidently identifying many ion images while providing a spatially correlated way of validating molecular changes with histologically relevant regions of interest.

Methods

Fresh frozen mouse brain specimen was sectioned sagittally to 10 µm thickness and mounted onto ITO slides. DHB/9AA matrix was deposited using an HTX TM sprayer. For direct infusion experiments 40 µm slices were cut and pieces of rat brain from different regions, weighed, homogenized and extracted to a constant w/v tissue/solvent. Extraction was performed by Chloroform:MeOH and Chloroform:H_2O extraction. Supernatant was solved in MeOH and diluted 1:10.

Images and direct infusion experiments were performed using a MRMS solariX 2xR 7T system. Direct infusion data were recorded with a mass resolution of 650.000 at m/z 400. Sample A (cortex region), sample B (cerebellum region) and sample C (hypothalamus region) were analyzed by direct infusion in positive and negative ion mode. Each sample was run in technical replicates five times. 64 single spectra were summed and analyzed in MetaboScape using Isotopic Fine Structure (IFS) and accurate mass. Data was differentially analyzed using the T-ReX 2D processing and two annotation workflows in MetaboScape. For imaging experiments SCiLS Lab Pro was used to find spatially relevant features. Regulated features detected in MetaboScape were loaded back to SCiLS Lab to visualize the local distribution. Additionally MALDI spectra from A, B and C were loaded directly into MetaboScape.

Results

Using the direct infusion data of region A, B and C, more than 3100 features could be assigned with a molecular formula. Of those 360 could be annotated by HMDB[1] and entries from the Lipid Maps database[2]. Statistical evaluation by t-test revealed significant differences in A (cortex), B (cerebellum) and C (hypothalamus) shown as volcano plots (Fig. 1B). The MALDI imaging data were visualized in SCiLS Lab and ROIs were drawn around visually distinct features (A, B and C) observed in the sagittal sections. The annotated differential features of the volcano plots were loaded into SCiLS Lab. Example features for an experiment in negative mode are shown in Fig. 1. In positive mode the results looked about the same, 3004 buckets could be annotated with a molecular formula and 233 by database search. MetaboScape also allows to merge datasets from positive and negative polarity to one bucket table.

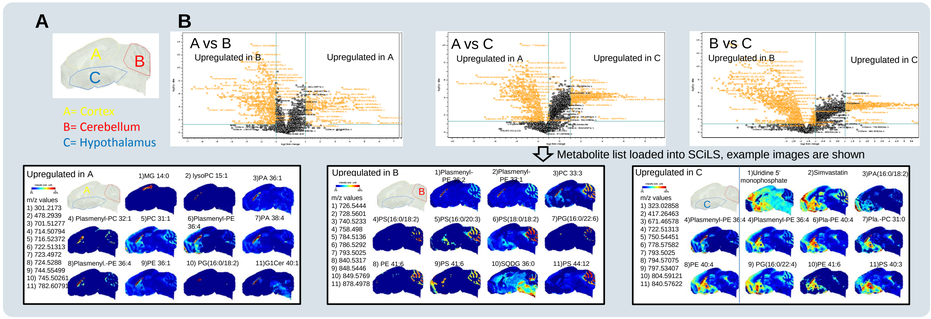

Fig. 1 In MetaboScape, a comparison of ESI direct infusion measurements of extracts of different brain regions A (cortex) B (cerebellum) and C (hypothalamus) was performed (A). By using the t-test differences between two groups were analyzed and shown via volcano plot (B). Example features for each region were extracted out of SCiLS and shown with the annotations and m/z values (D).

Starting from the imaging experiment a number of regulated ions were found by colocalization with the corresponding regions A, B or C. By acquiring MALDI data for the three specific regions MetaboScape was able to confidently assign molecular formulas. Examples are shown in Fig. 2.

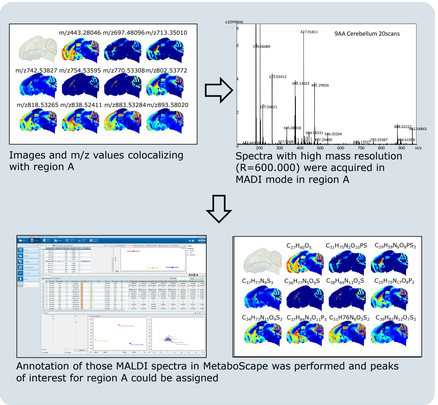

Fig. 2 Region A was used for a colocalization study in SCiLS Lab. Several features showed the specific region A pattern and are shown. MALDI spectra with a higher resolution were acquired from the region of interest. Those spectra were loaded into MetaboScape, and features were annotated.

References

(1) HMDB (http://www.hmdb.ca/)
(2) LipidMaps (http://www.lipidmaps.org/)

Summary

The combination of direct infusion Electrospray measurements of brain extracts and MALDI imaging gives additional insight into the localization of metabolites in specific tissue regions. Additionally MetaboScape can support MALDI imaging data by automatic annotation, recalibration and combination of negative and positive data in one bucket table. An easy upload of interesting features into SCiLS Lab allows direct usage of those data. Furthermore, single MALDI spectra can be uploaded to confirm unknown regulated compounds in MetaboScape.

Conclusions

- The combination of direct infusion MRMS experiments with MALDI imaging allowed the annotation of interesting features to distinct localizations by using MetaboScape and SCiLS Lab.
- Interesting peaks out of a MALDI imaging experiment can be annotated using MetaboScape allowing better interpretation of MALDI m/z values in imaging experiments.
- The combination of MetaboScape and SCiLS Lab offers a direct identification workflow including different statistical options such as PCA and t-test.

Metabolomics/Imaging