MSI analysis of changes in the lung as a result of inhaled drug dosing

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Introduction

Within drug development and pre-clinical trials a common, significant and poorly understood barrier to success is the development of lipidosis in tissues and cells as a result of administered treatments. A deeper understanding of lipid changes occurring as a result of drug dosing will allow better selection of compounds for preclinical trials, and thus decrease the number of animals required.

Methods

- Prior to sacrifice half of the animals underwent a bronchoalveolar lavage (BAL) lung wash procedure. Wash contained both phosphate buffered saline (PBS) and ethylenediaminetetraacetic acid (EDTA)
- Fresh frozen samples of mouse lung were sectioned at 14 µm
- MALDI samples coated with 2-Mercaptobenzothiazole (MBT) in 80 % MeOH by TM Sprayer (HTX)
- DESI sampling was performed using 95% MeOH with 0.001 mg/mL raffinose internal standard
- Imaging was carried out on a Synapt G2Si (Waters) at 45 µm pixel size for MALDI and 100 µm size for DESI

### BAL cell sampling – Effect on MSI data

**Effect on MALDI-MSI data**

- BAL shifts the primary adducts formed from [M+H]^+ to [M+Na]^+

**Effect on DESI-MSI data**

- BAL shifts the primary adducts formed from [M+H]^+ to [M+Na]^+
- Amiodarone and N-desyl amiodarone are not washed out of the tissue

### Detection of key molecules

- Amiodarone and three metabolites observed by DESI in positive mode
- Previous studies required an order of magnitude higher dosing to detect amiodarone
- Lipidosis biomarker BMP 22:6 observed by DESI in negative mode

### Repeatability of detection

- Detection of amiodarone is seen in multiple biological and technical replicates
- As are two of the primary metabolites (M11 and N-desyl amiodarone)

### Multivariate analysis of DESI data

- NMF of the positive mode data differentiates dosing and time
- Spectral factors show lipid profile differences between dosing
- Day 1 dosed factor is dominated by amiodarone signal
- Within analysis of changes in the lung as a result of inhaled drug dosing

Conclusions

- DESI MSI can be used to detect drugs and metabolites in tissues dosed via inhalation at low doses
- Lipid changes known to be associated with phospholipidosis can also be observed
- Other lipid profile changes can be detected using multivariate methods such as NMF
- Further molecular identification by MS/MS is required

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