LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY (LC-MS) BASED UNTARGETED LIPIDOMICS PLATFORM TO INVESTIGATE FERROPTOSIS IN MULTIPLE MYELOMA CELLS

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INTRO

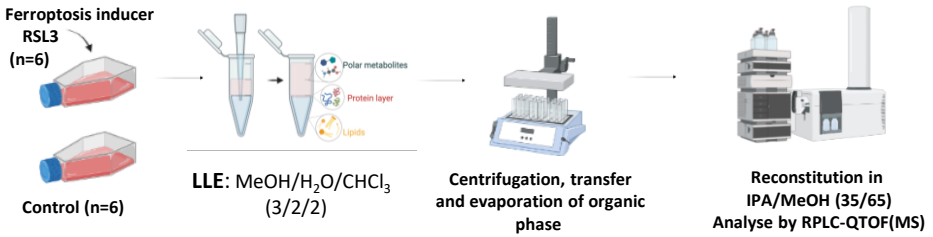
Ferroptosis is an iron-catalyzed mode of cell death characterized by lipid peroxidation, which is able to kill therapy resistant cancers. The development of analytical platforms that can reliably detect specific biomarkers for ferroptosis is crucial to understand its contribution in overcoming therapy resistance.

OBJECTIVE

Elucidation of endogenous lipid changes induced by ferroptosis in multiple myeloma (MM) cells

APPROACH

SAMPLE PREPARATION



DATA PROCESSING AND FILTERING

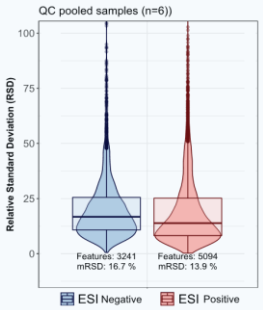
Preprocessing

- Raw data conversion .d to .mzML
- Peak picking & alignment: **MSDial 4.6**
- Deisotoping, duplicate removal and adduct flagging: **MS-FLO**
- Filtering: detection rate > 0.6, mRSD <30%

Treatment

- Within batch correction with
- QC pooled samples and
- Random forest Missing value imputation: **notameR package**
- Log transformation
- Pareto scaling for multivariate analysis: **MetaboAnalyst**

QA/QC



-**System suitability**: Evaluation of standard mixture before and after batch

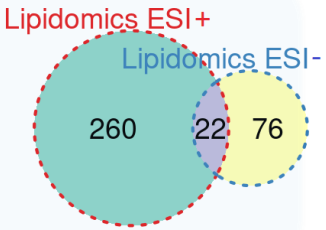
-**Conditioning QC**: Condition of the system and acquisition of MS/MS spectra

-**QC pooled samples spiked with ISs at regular intervals**: Repeatability

RESULTS AND DISCUSSIONS

UNTARGETED LIPIDOMICS DATASET

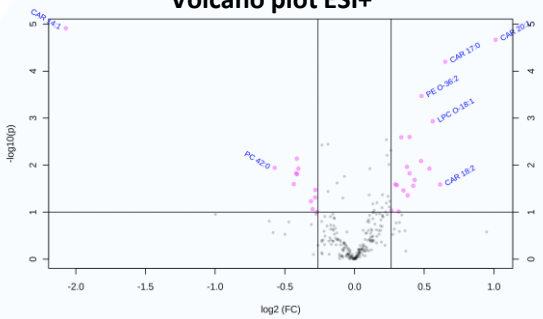
- Filtered dataset with >3000 features in ESI- and >5000 in ESI+
- **MS-DIAL MS similarity based lipidomics library** used for MS/MS matching: Score >70% + **Rule-based R script LipidMatch**
- Further **filtering** of matched lipids: Reversed-phase interactions (⇧RT with carbon chain, ⇩RT with N° of double bonds)
- **358 unique lipid species** were detected after data filtering
- Higher coverage in ESI+



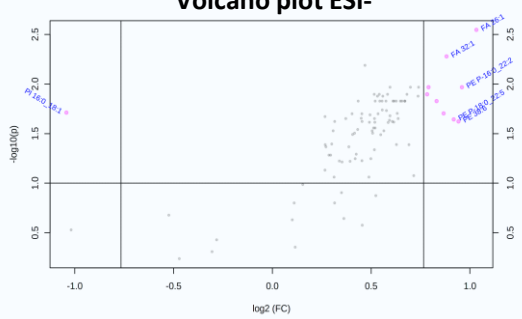
LIPID SIGNATURE OF FERROPTOSIS IN CANCEROUS MM CELLS

Treated vs Control

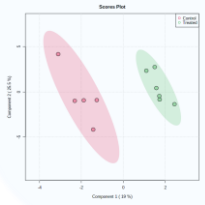
Volcano plot ESI+



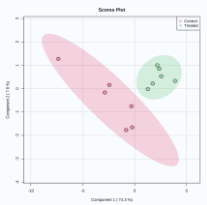
Volcano plot ESI-



PLDA ESI+



PLSDA ESI-



- Significant changes in **carnitine (CAR)** levels in ESI+: Higher VIP scores (>2). Long carbon chain species were dominant in the treated group.
- **Glycerophosphoethanolamines (PCs)** were down-regulated in ferroptosis induced MM cells.
- In ESI- mode, **Glycerophosphoethanolamines (PEs)** and **unsaturated fatty acids** were both up-regulated classes in the treated group (VIP scores > 1.2).

Further investigation: Validation experiment

- ➡ MM cells: require a higher number of replicates to reduce variability
- ➡ Targeted MS/MS acquisition for PE, PC, CAR and FA classes.
- ➡ Data mining: Oxidized glycerophospholipids: RT-MZ-MS/MS patterns