PROFILING THE LIPIDOME OF ADRENAL CANCER TISSUES USING FAST LC-MS METHODOLOGIES

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INTRODUCTION

Adrenocortical carcinoma (ACC) is a rare tumor with a poor prognosis and paucity of targeted therapies. Although 5% of the adult population have adrenal nodules, the incidence of ACC is rare at 1-2 cases per million in adults, mainly occurring at ages 40-50 years and in young children[1]. Surgical resection offers the only curative intervention, but many patients present with (inoperable) distant metastases, when prognosis is poor and 5-year survival is <50% [2]. Altered tissue and intracellular lipid composition has been described in other cancer types
where exploitation of lipid metabolism may pose a new therapeutic avenue[3,4]. Comprehensive lipid profiling for benign and ACC tissues was conducted utilizing fast chromatographic separation combined with high resolution mass spectrometry, identifying major dysregulated lipid classes and demonstrate highly confident identifications.

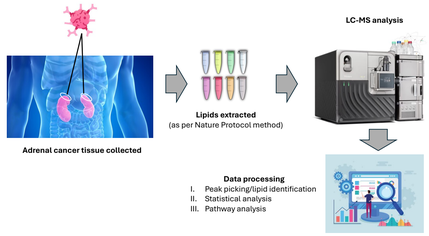
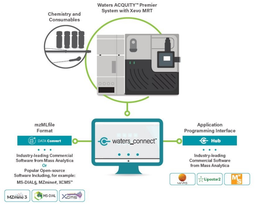


Figure 1. Experimental workflow. Adrenal cancer tissue was collected before being prepared for LC-MS analysis using the previously published Nature Protocols method.[5] Data were collected using MSE (DIA) and exported as generic mzML files using Data Convert. Peak picking and identifications were performed using LipoStar (Mass Analytica) prior to pathway analysis.



* HUPO Proteomics Standards Initiative, #https://mzu.to/#mzmlia, @http://prime.psc.riken.jp/compms/msdial/main.html

Figure 2. Seamless integration of raw data with 3rd party informatic packages, either directly interfaced through the application programming interface (API) or via generic mzML.

RESULTS

Following the data acquisition, mzML-based datasets (ESI+/-) were processed using LipoStar informatics for peak picking, normalization and feature identification. Multi-variate statistical analysis allowed for differential features to be established. Figure 3 highlights the features of significance and the criteria applied for selection. These features were subsequently searched against a variety of databases, including LipidMaps and an in-house database for comprehensive coverage. The distribution of lipid classes (figure 4) is particularly dominated with glycerophospholipids and glycerolipids. Example dysregulated lipids are provided as box-whisker plots with significant abundance changes between ACC and benign tissue (figure 5). Further interrogation of the data, involved pathway mapping to provide additional insight into the underlying biological mechanisms occurring during ACC development. Example pathways with high correlation are provided in figure 7.

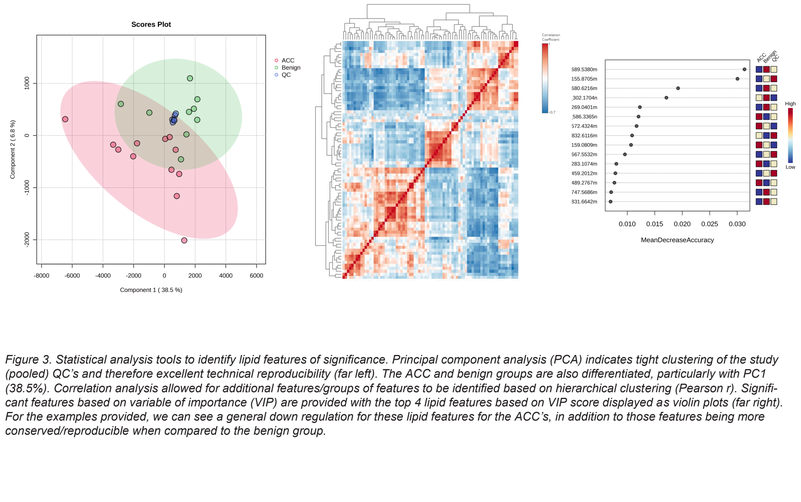


Figure 3. Statistical analysis tools to identify lipid features of significance. Principal component analysis (PCA) indicates tight clustering of the study (pooled) QC's and therefore excellent technical reproducibility (far left). The ACC and benign groups are also differentiated, particularly with PC1 (38.5%). Correlation analysis allowed for additional features/groups of features to be identified based on hierarchical clustering (Pearson r). Significant features based on variable of importance (VIP) are provided with the top 4 lipid features based on VIP score displayed as violin plots (far right). For the examples provided, we can see a general down regulation for these lipid features for the ACC's, in addition to those features being more conserved/reproducible when compared to the benign group.

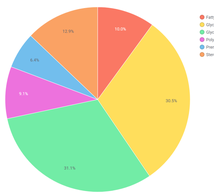


Figure 4. An overview of the lipid class distribution are provided in the pie chart (top left). Glycerolipids and glycerophospholipids are amongst the largest contributors to the overall lipidome analyzed by LC-MS.

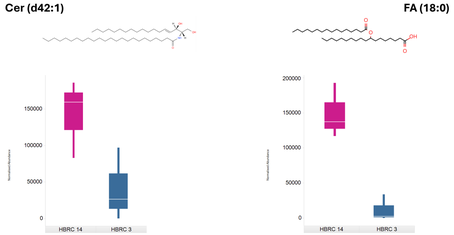
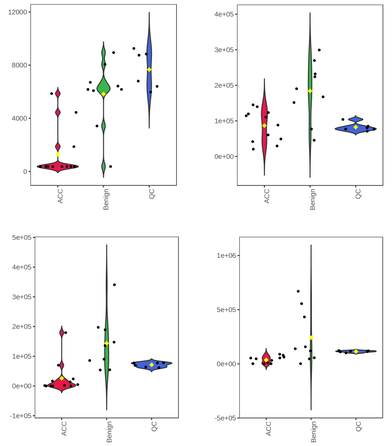


Figure 5. Box-whisker plots highlighting highly dysregulated lipids following identification for tissue originating from ACC versus benign tissues. In particular, these lipids were identified in ESI-, providing high scores/ fragmentation scores for comprehensive elucidation.



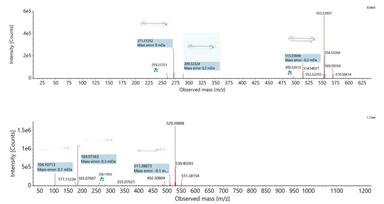
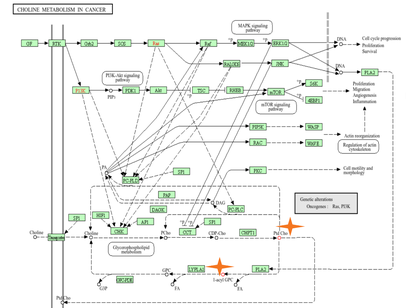
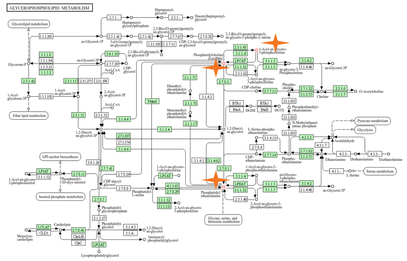


Figure 6. Fragmentation spectra relating to the spiked internal standards (EquiSPLASH). Ceramide (d7) and LPC (d7) components are provided as examples (upper and lower respectively). The spectra show the high level of fragmentation and associated mass accuracies, providing confident compound identification.





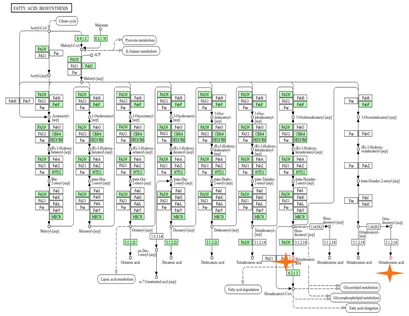


Figure 7. Pathway analysis based on the curated lipid identifications. A number of potential pathways were identified, with the three shown here representing the most significant pathways (based on p-value and Benjamini correction). Lipids mapped to these pathways are highlighted with orange stars. Choline metabolism (top left) has been well documented as being associated with cancer and tumour progression due to changes in signalling processes[5]. Additional pathways of interest include fatty acid biosynthesis and glycerophospholipid metabolism. Fatty acid biosynthesis is integral to cancer development with FFA's being implicated as a fuel source but also as secondary messengers and involved in the structure of the membrane matrix[6]. Similarly, glycerophospholipids are also involved in similar biological roles as FFA and are therefore also highly dysregulated within the ACC population[7].

CONCLUSION

- **LC-MS has been utilized to profile the lipidome and its associated changes with ACC, when compared with benign tumours.**
- **A comprehensive, seamless workflow has been applied with the Xevo MRT MS/waters_connect software to allow for data of high resolution and mass accuracy (sub 1 ppm) to be acquired for accurate and confident lipid identification.**
- **Integration with LipoStar allowed for high confident identifications and provided additional tools for data curation.**
- **A variety of lipid classes were identified, with significant regulation changes in glycophospholipids, ceramides and FFA's for ACC samples.**
- **Mapping these lipids to their biological roles/functions was conducted, highlighting a number of significant pathways (e.g., choline metabolism & fatty acid biosynthesis).**

References

1. Adrenal tumors in children and adolescents (vs. 8.0) - European Society for Paediatric Oncology
2. https://www.rogelcancercenter.org/adrenal-cancer/learn-about-adrenal-cancer/survival-rates#:~:text=Outcomes-,Adrenal%20Cancer%20Outcomes,the%20Stage%20of%20the%20tumor.
3. https://www.cancer.gov/types/adrenocortical/patient/adrenocortical-treatment-pdq
4. https://www.cancer.org/cancer/types/adrenal-cancer/treating.html
5. Glunde *et al.*, Choline metabolism in malignant transformation. *Nature Reviews Cancer*, **11**, 835-848 (2011).
6. Koundouros *et al.*, Reprogramming of fatty acid metabolism in cancer. *British Journal of Cancer*, **122**, 4-22 (2020).
7. Chen *et al.*, PLA2G4A and ACHE modulate lipid profiles via glycerophospholipid metabolism in platinum-resistant gastric cancer, *Journal of Translational Medicine*, **22**, 249 (2024).

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