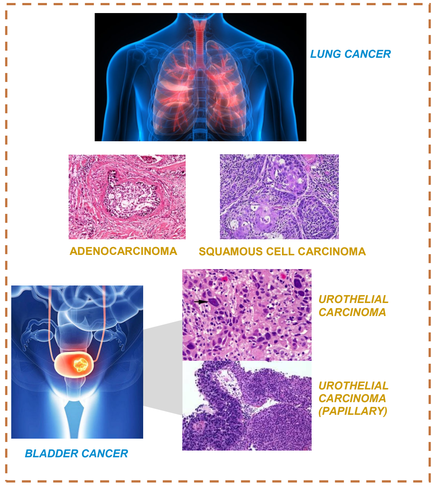
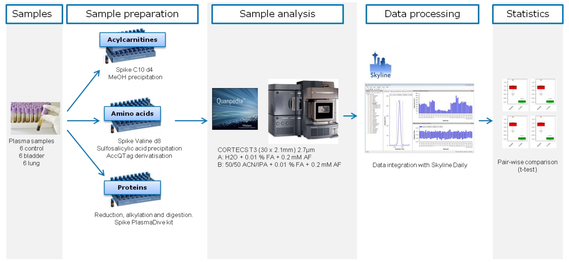
TARGETED MULTI-OMICS: RAPID PLASMA PROFILING OF A BLADDER AND LUNG CANCER HUMAN COHORT

Sarah Lennon[1], Billy J. Molloy[1], Lee A Gethings[1], Robert S. Plumb[2], Andrew Peck[2]
[1]Waters Corporation, Wilmslow, United Kingdom; [2]Waters Corporation, Milford, MA

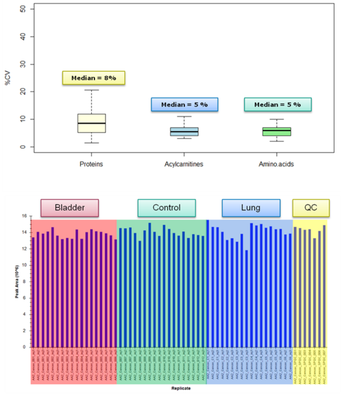
INTRODUCTION

Cancer is one of the most complex, life threatening diseases, existing in many forms which have unknown pathogenesis. A combination of genetic and lifestyle factors are known to contribute towards increasing the probability of encountering cancer. Lifestyle factors such as smoking are known to contribute towards both lung and bladder cancer, with lung cancer providing over 230,000 diagnosed cases in the United States annually. The exact mechanism as to how these carcinomas develop during various stages is still not well understood. Here, we describe a multi-omic approach to reveal molecular factors that may be involved in these biomolecular processes. Combining lipidomic, metabolomic and proteomic approaches have helped to identify multi-factorial disease associated components and their related pathways.





Sample preparation and data analysis workflow using MetaboQuan-R. Target assays included acylcarnitines, amino acids and proteins. The rapid speed of data acquisition allows for high throughput analysis, allowing a single sample to be analysed within 3 minutes.



MetaboQuan-R workflow demonstrates high analytical precision with low observed variance observed over the three assays. Peak area CV's of the pooled quality control samples yield median %CV's <10% in all cases, with the acylcarnitine and amino acid assays being at 5% for all 128 compounds identified (upper figure represented as a box-whisker plot).

A specific example is shown for the amino acid assay (lower bar chart) which represents the peak area of a spiked amino acid standard (valine d8) from all individual samples. This clearly shows the technical reproducibility of the assay with a median CV of 5%.

BILE ACIDS

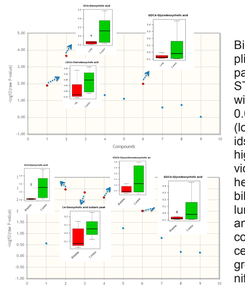


Figure 1

Bile acids are well documented as being implicated with cancer mechanisms and links to particular pathways such as the JAK2/STAT3 pathway. Figure 1 represents a t-test with a statistical ANOVA threshold applied at 0.05 for both lung (upper) and bladder (lower) cancer. Three representative bile acids adhering to the thresholding criteria are highlighted. An average profile over all individuals can also be demonstrated as a heatmap (Figure 2). Interestingly, the bulk of bile acids are shown to be down-regulated in lung cancer subjects, whilst only two (DCA and TCDCA) are shown to be elevated when compared with healthy controls. Bile acid receptors such as TGR5 responsible for cell growth and migration have been shown significant dysregulation for non-small cell lung cancer in particular.[2]

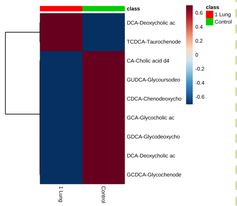
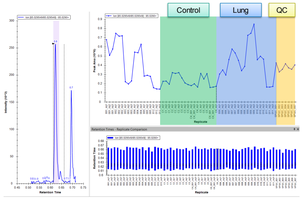


Figure 2

ACYLCARNITINES

Acylcarnitines play a pivotal role in cancer biology, with the carnitine system involved as a mediator and linking a variety of key pathways to provide the necessary energetics for cancerous cells.[3] The high throughput assay demonstrated here, shows an increased abundance of various acylcarnitines for lung cancer individuals. Figure 3 provides an example, with the C16:1 indicating elevated levels in the lung cancer cohort..

Figure 3



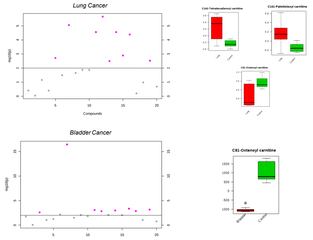


Figure 4

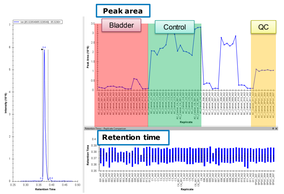
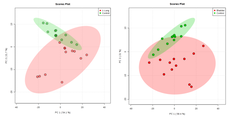


Figure 5

Following additional statistical curation, a total of 9 and 8 acylcarnitines for lung and bladder cancer respectively were identified as signficiant based on ANOVA/t-test with p-value thresholding of 0.01 FDR (Figure 4). Corresponding box-whisker plots for the most significant acylcarnitines, such as C14:2, C8:1 and C16:1 (lung cancer) are shown. All but the C8:1 show elevated expression levels for lung cancer but interestingly appears to be of significance for bladder cancer subjects when compared with healthy controls. Additionally, the data suggest that the variation in observed abundance is conserved within the healthy control population for the majority, however, the C8:1 is the only acylcarnitine to contradict this trend in the case of bladder cancer (Figure 5).

PROTEINS

Figure 6



In total 73 proteins were identified and quantified, those which showed statistical significance were further interrogated and molecular functions derived. The pie chart in Figure 9 represents the various molecular functions including metabolic processes (linking the bile acids and acylcarnitines) as well as immune system processes. Additional investigation of the data through pathway analysis ranks a variety of process networks in order of significance (Figure 10).

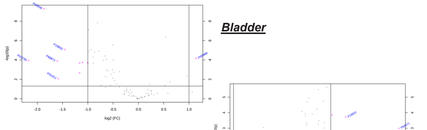


Figure 7

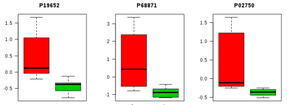


Figure 8

The targeted proteomics results show separation between healthy controls and lung cancer patients when using multivariate statistics. Principal component analysis (PCA) is shown in Figure 6. There is also separation within the lung cancer cohort and this can be attributed to those patients diagnosed with adenocarcinoma or squamous cell carcinoma. Additional statistical analysis based on P-value (0.1) and fold change (2.0) thresholding reveal 10 proteins of interest in both cases (Figure 7). Corresponding box-whisker plots (Figure 8) for some example proteins are provided from the lung cancer analysis, relating to Alpha-1-acid glycoprotein 2 (P19652), Hemoglobin subunit beta (P68871) and Leucine-rich alpha 2-glycoprotein (P02750). A similar trend is also observed for the bladder cancer cohort for these proteins, however with the exception of P00488 (Coagulation factor XII A chain) which is the only protein to show significant down regulation.

Figure 9

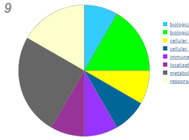
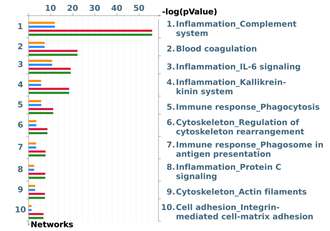


Figure 10



CONCLUSION

- **The MetaboQuan-R platform is shown to provide analysis on a rapid timescale (typically 3 mins) allowing high throughput sampling of plasma collected from a lung and bladder cancer cohort.**
- **Three assays covering acylcarnitines, bile acids and proteins have been demonstrated to show that significant variation is observed in both cancer cohorts.**
- **A total of nine (lung) and eight (bladder) statistically relevant acylcarnitines were identified and quantified over a linear range of 4.8 -625 ng/mL. Example acylcarnitines included C14:2, C8:1 and C16:1.**
- **Lung cancer subjects appeared to show a large number of bile acids to be down regulated, with only two significantly over expressed (DCA and TCDCA). The bile acids were typically quantified over a linear range of 156-2500 ng/mL.**
- **The protein assay identified 73 proteins, with the majority being over expressed for both cancer types. Detailed interrogation revealed the molecular functions implicated and associated process networks.**
- **This single platform approach is simple to implement, fast, reliable and robust. The results generated by MetaboQuan-R combine high sensitivity with sample high throughput.**

References

1. https://www.cancerresearchuk.org/about-cancer/lung-cancer/survival
2. Liu et al., *Cancer Lett.* **2018**; 412:194-207.
3. Melone et al., *Cell Death and Disease*. **2018**; 9:228.

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