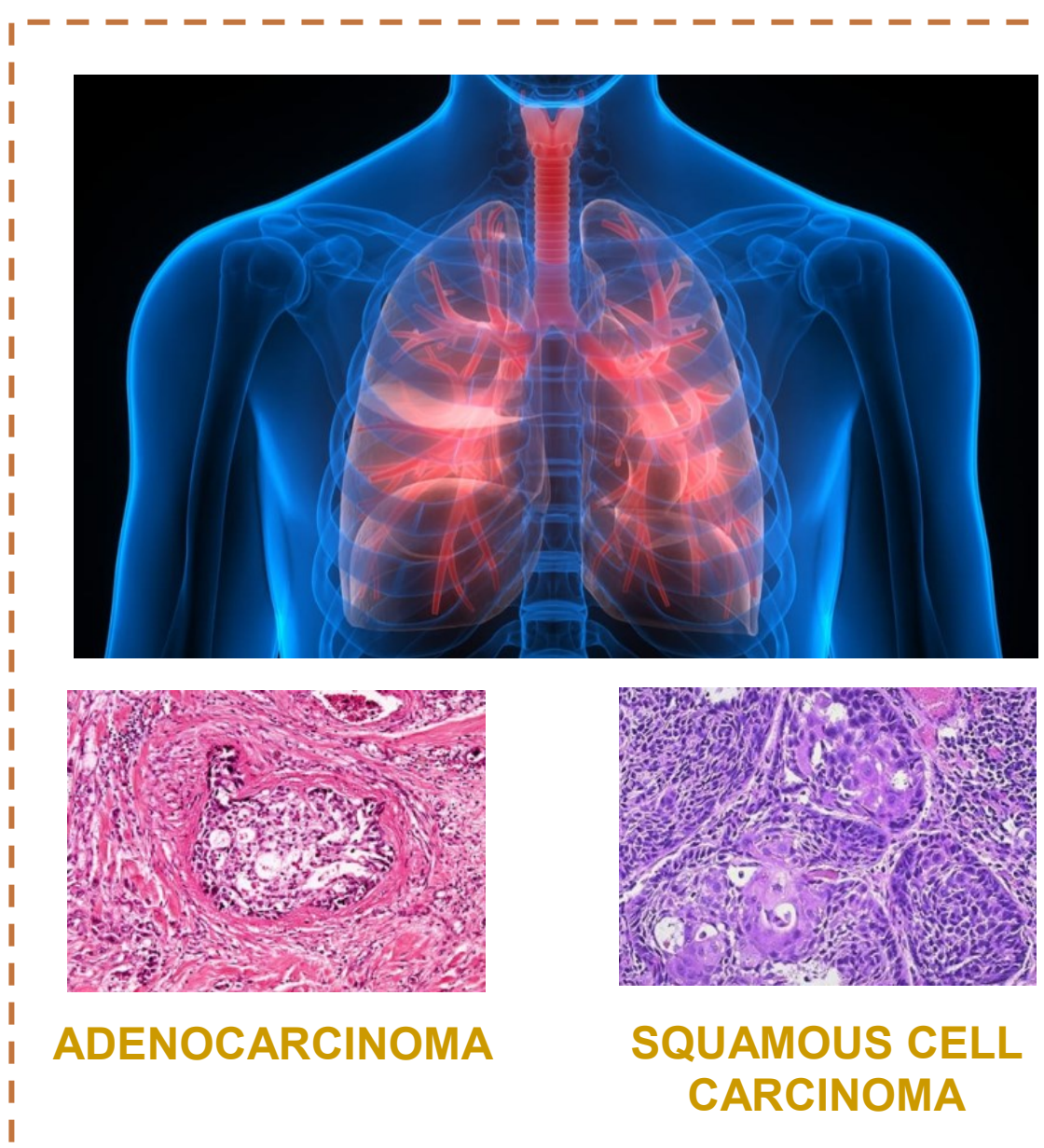# CHARACTERIZING LUNG CANCER USING A HIGH THROUGHPUT METABOLOMICS SCREEN

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INTRODUCTION

Lung cancer is one of the most common and serious forms of cancer, with over 44,000 individuals being diagnosed with the condition every year in the UK, with lifestyle factors such as smoking playing a major attributor to developing the disease. The 5 year survival rate is typically around 35% and 6% for grade 1 and 3 tumours respectively.[1] Therefore, having the ability to understand the underlying mechanisms of the disease is a crucial step in detecting the condition at early onset and therefore improving survival rates. Previous studies have shown a variety of molecules being implicated in a variety of key pathways which are associated with lung cancer ranging from small molecules to proteins. Typically, methodologies have to be created as 'bespoke' assays, which require significant optimisation, making multiplexing assays difficult. In this study, we introduce a methodology (MetaboQuan-R) which uses a single platform approach that is capable of measuring multiple assays on a rapid timescale that account for both small molecule and protein assays from the plasma of patients diagnosed with lung cancer.
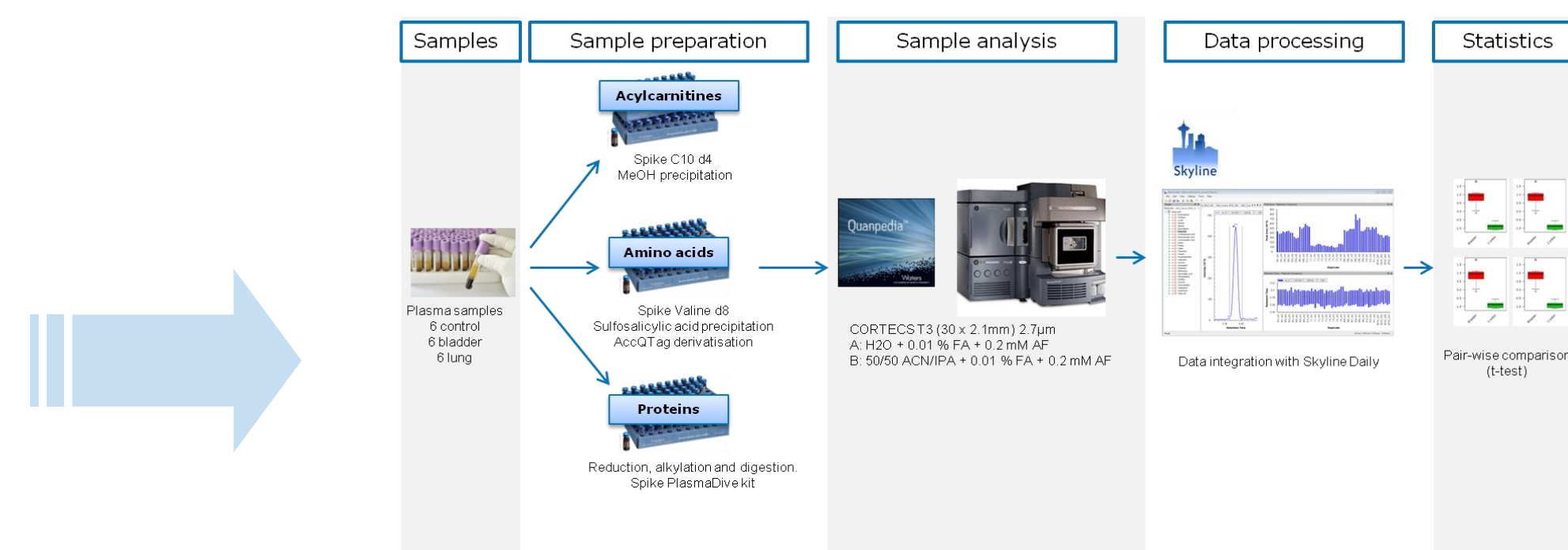

ADENOCARCINOMA   SQUAMOUS CELL CARCINOMA

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.

BILE ACIDS

Figure 1

Figure 2

Bile acids are well documented as being implicated with cancer mechanisms and links to particular pathways such as the JAK2/STAT3 pathway. 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 1 represents the T-test with a statistical ANOVA threshold applied at 0.05. 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.

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 lung cancer patients.
- Three assays covering acylcarnitines, bile acids and proteins have been demonstrated to show that significant variation is observed in a lung cancer cohort.
- A total of nine 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 lung cancer subjects. 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.

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

Following additional statistical curation, a total of 9 acylcarnitines are identified as significant based on an ANOVA/t-test with p-value thresholding of 0.01 FDR (figure 4). Corresponding box-whisker plots for C14:2, C8:1 and C16:1 are shown. All but the C8:1 show elevated expression levels for lung cancer. These box-whisker plots also suggest that the variation in observed abundance is conserved within the healthy control population.
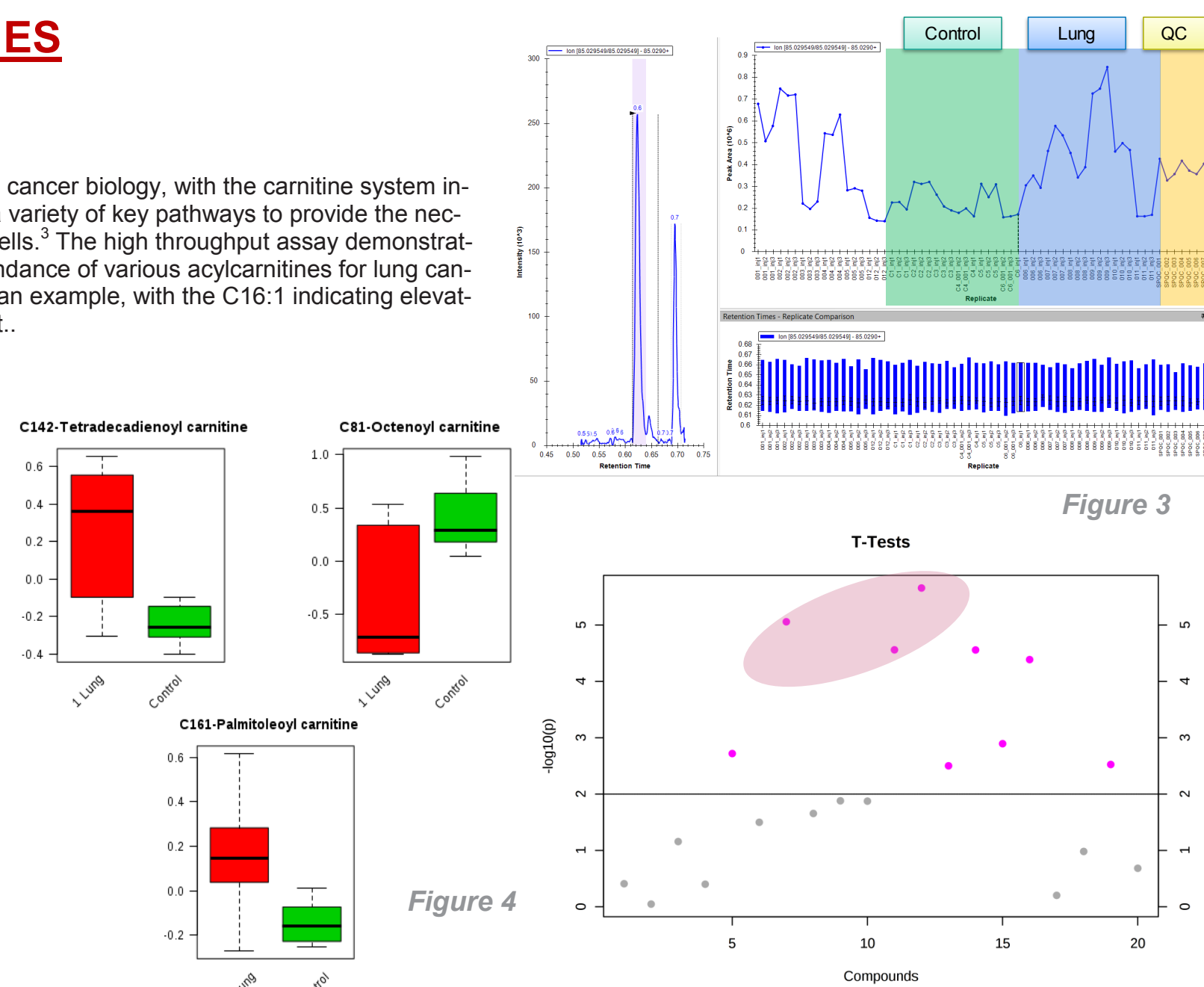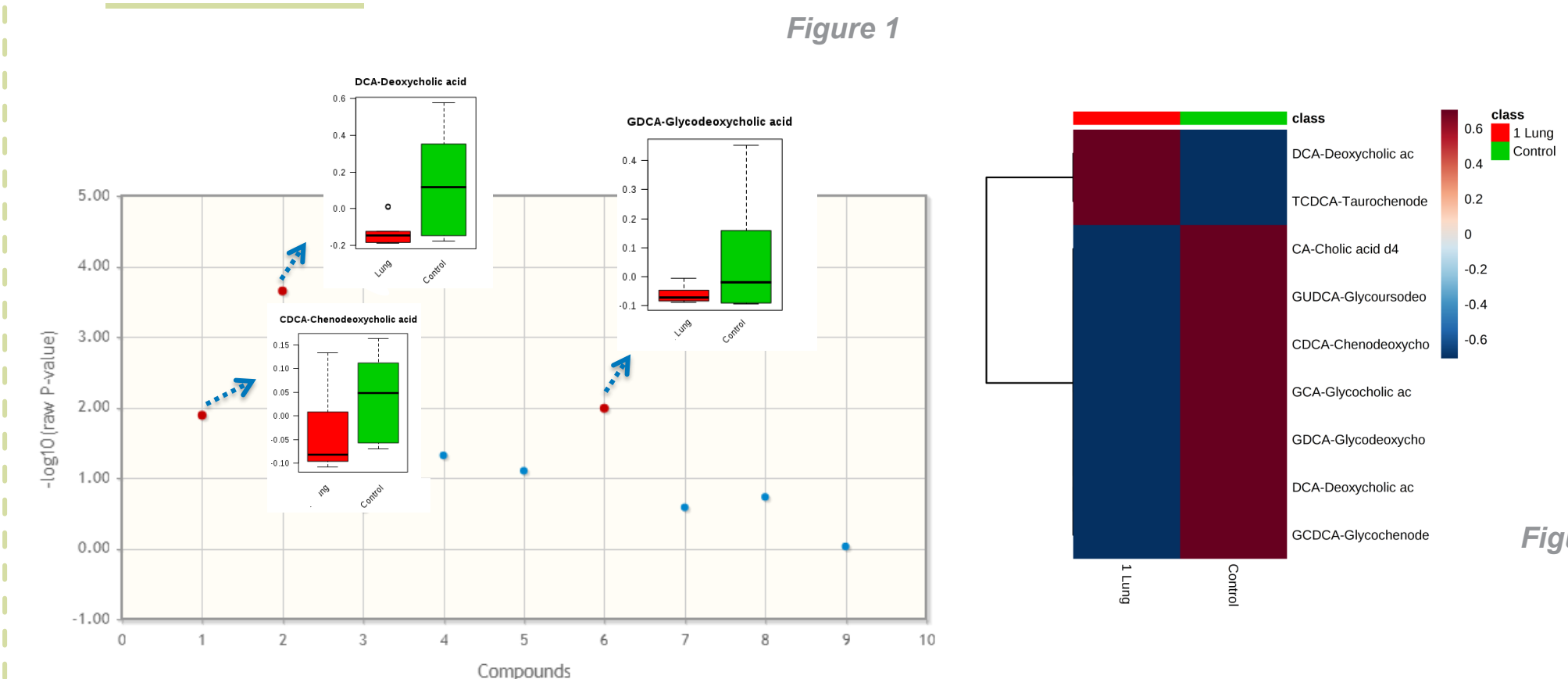

Figure 4

PROTEINS

Figure 5

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 7 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 8).
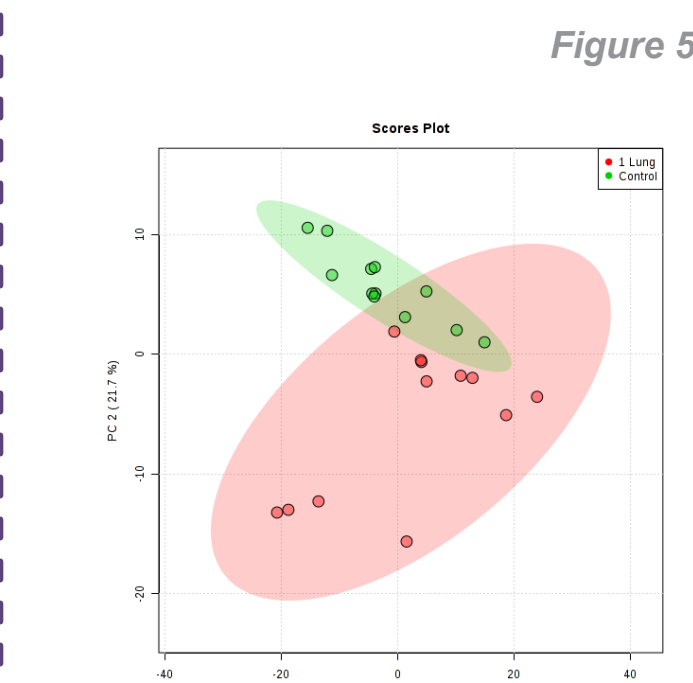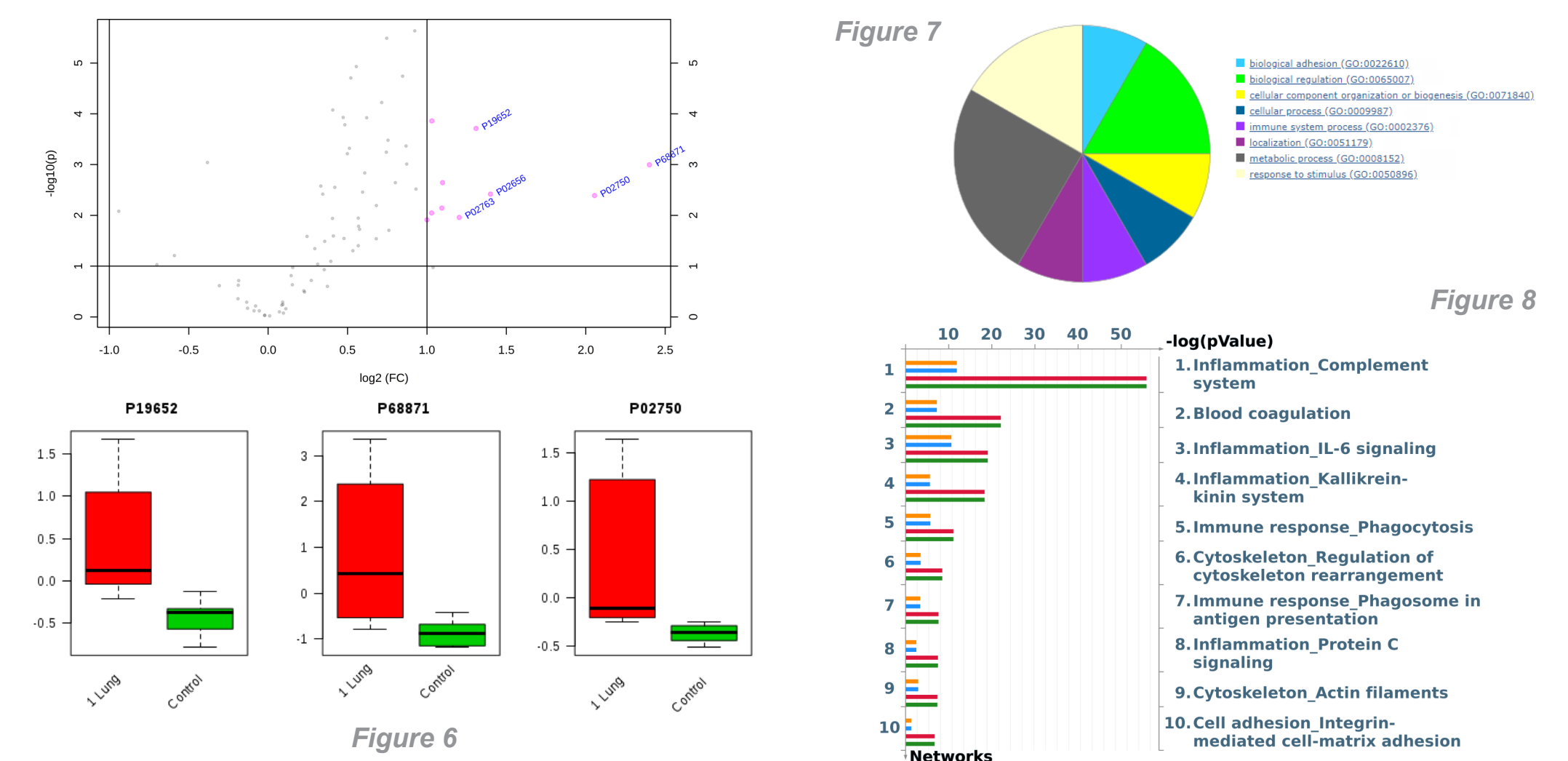

Figure 5

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 5. 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 (figure 6). Corresponding box-whisker plots for some example proteins are provided, relating to Alpha-1-acid glycoprotein 2 (P19652), Hemoglobin subunit beta (P68871) and Leucine-rich alpha 2-glycoprotein (P02750).

Figure 7

Figure 6

Figure 8

-log(pValue)
1. Inflammation_Complement system
2. Blood coagulation
3. Inflammation_IL-6 signaling
4. Inflammation_Kallikrein-kinin system
5. Immune response_Phagocytosis
6. Cytoskeleton_Regulation of cytoskeleton rearrangement
7. Immune response_Phagosome in antigen presentation
8. Inflammation_Protein C signaling
9. Cytoskeleton_Actin filaments
10. Cell adhesion_Integrin-mediated cell-matrix adhesion

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