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 (MetaQuan-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.

ACDCARNOINOS

SQUAMOUS CELL CARCINOMA

• The MetaQuan-R platform is shown to provide analysis on a rapid timescale typically in 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.
• Principal component analysis (PCA) and multivariate linear discriminant analysis (MLDA) shows separation for lung cancer subjects, whilst only two (DCA and TCDCA) have been shown significant dysregulation for non- lung cancer subjects.
• The protein assay identified 73 proteins, with the majority being over expressed in the lung cancer cohort. Additional statistical analysis based on an ANOVA/t test with a statistical ANOVA FDR (figure 4). Corresponding box whisker plots for some example proteins are provided, revealing 10 proteins of interest (figure 6). Corresponding box whisker plots for some example proteins are provided, revealing 10 proteins of interest (figure 6).

ACYLCAWNTINES

Bile acids are well documented as being implicated with cancer mechanisms such as the JAK2/STAT3 pathway. Bile acid receptors such as TGR5 responsible for cell growth and migration have been shown significant dysregulation in non-small-cell lung cancer (NSCLC) and lung adenocarcinoma (LUAD).2 Figure 1 represented the T test with a statistical ANOVA threshold applied at 0.05. Three representative bile acids showing statistical significance (Figure 1) are shown to be downregulated in lung cancer subjects, whilst only two (DCA and TCDCA) are shown to be elevated when compared with healthy controls.

PROTEINS

In total 73 proteins were identified and quantified, those which showed statistical significance were further interrogated and molecular function analyses derived. The pc plot in figure 7 represents the molecular functions including metabolic processes (glycerolipids, bile acids and acylcarnitines) as well as various system processes. Additional investigation of the data through pathway analysis reveals a variety of protein networks in order of significance (figure 8).

CONCLUSION

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 between the lung cancer patients and healthy controls. The proteomics data also show significant dysregulation for non-lung cancer subjects. Additional statistical analysis based on an ANOVA/t test and box change of 0.01 thresholding revealed a total of 9 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.

References
1.©2019 Waters Corporation
2. Lee et al., J Proteome Res 2011