Targeted UPLC-MS/MS Lipidomics for Biomarker Research of Prostate Cancer and Therapy Responses in Human Serum

Mass Spectrometry

Anthony Midey (anthony_midey@waters.com), Waters Corporation, **Nyasha Munjoma**, Waters Corp., **Chris Hughes**, Waters Corp., **Giorgis Isaac**, Waters Corp., **Lee Gethings**, Waters Corp., **Robert Plumb**, Waters Corp., **Ammara Muazzam**, University of Manchester, **Anthony Whetton**, University of Manchester, **Olivier Cexus**, University of Surrey, **Paul Townsend**, University of Surrey, **Nophar Geifman**, University of Surrey, **Hardev Pandhav**, University of Surrey, **Fowz Ahar**, Salford Royal NHS Foundation Trust

Hypothesis/objective:

Prostate cancer is commonly diagnosed using a blood-based protein biomarker method evaluating Prostate-specific antigen (PSA) levels. However, PSA by itself is not accurate especially as there is no reliable PSA range that is an explicit signifier for the presence. Thus, it remains a critical area of translational research area to develop robust, reproducible methods for distinguishing stages of prostate cancer and treatment responses. Several multi-omic and lipidomic studies have shown that specific lipids reflect prostate cancer signatures. Identifying key lipid markers for different stages of prostate cancer/therapies using UPLC-MS/MS offers a potentially more sensitive and selective method.

Methods:

Human serum from 7 different conditions were pooled to create phenotypic groups: controls of diagnosed prostate cancer patients, active surveillance (AS), Brachytherapy treated, hormone therapy only, healthy controls, combined radiotherapy and hormone therapy treated, and prostatectomy. Pre-mixed lipid standards (AVANTI) were spiked into plasma, generating a 10-point calibration curve at 3 QC levels in matrix. QC samples were prepared from pooling 7 groups (study reference) and NIST SRM 1950-Metabolites. These calibrants, QCs, and patient samples were extracted using a simple protein precipitation method. Lipids of the centrifuged supernatant were separated using a HILIC based LC-MS/MS on a ACQUITY Premier I-ClassTM UPLC coupled to a TQ-XSTM tandem quadrupole MS (Waters)

Results and Conclusions:

A targeted polarity-switching LC-MS/MS method developed using MRM transitions from the LipidQuanTM library (Waters), targeted 39 endogenous lipids previously found to be key differentiator of study cohorts: ceramide (Cer), lysophosphatidylinositol (LPI), lysophosphatidylethanolamine (LPE), phosphatidylinositol (PI), sphingomyelins (SM), and phosphatidylglycerol (PG) classes. The lipids were quantified with linear response of R2 from 0.985-0.996 and coefficients of variance <15%. Several key lipid species were shown to be at significantly different levels between study cohorts. For example, LPE lipids distinguished control prostate cancer patients from those treated with hormone therapy, the latter with double the concentration.