COMPARAITVE METABOLOMICS ANALYSIS OF PROSTATE CANCER CELLS WITH DIFFERENT ETHNIC AND TUMORIGENIC PHENOTYPES

Julius O. Nyalwidhe[1]; Tanya C. Burch[1], Johng S. Rhim[2], James Langridge[3]; Andy Baker[4], Giorgis Isaac[4]
[1]Leroy T. Canoles Jr. Cancer Research Center, Eastern Virginia Medical School, Norfolk VA; [2]Center for Prostate Disease Research (CPDR), Bethesda, MD; [3]Waters Corporation, Manchester, UK; [4]Waters Corporation, Milford, MA

OVERVIEW

A comprehensive high-throughput lipidomics and metabolomics analysis of prostate cancer cells with different tumorigenic phenotypes reveal potential markers for aggressive disease.

INTRODUCTION

- Prostate cancer is the most prevalent cancer amongst men and the second most common cause of cancer related-deaths in the USA.
- African American (AA) men have disproportionately high incidence and mortality rates of prostate cancer (PCa) when compared to Caucasian American (CA) men and other ethnic groups in the USA.
- Untargeted metabolite profiling may serve as surrogates for disease stratification and potentially as useful prognostic and diagnostic biomarkers.
- Metabolomics of prostate cancer is currently being studied to screen for biomarkers with high sensitivity and specificity. However, to date *no comparative* metabolomic analyses of disease stratified African and Caucasian Americans matched prostate clinical samples and cell lines has been done.
- Here for the first time we provide comprehensive metabolomics profiling data from CA and AA cell lines that include a novel set of African American cell lines which were only recently described.

METHODS

Sample Preparation

Prostate cancer cells with different ethnic backgrounds and tumorigenic phenotypes were homogenized and the lysates extracted using Bligh and Dyer method. The upper phase containing water soluble metabolites was collected for polar metabolite analysis and lower organic phase containing hydrophobic metabolites was collected for lipid analysis.

UPLC Conditions

The analysis was performed using an ACQUITY *i*-Class system coupled to a SYNAPT G2-*Si* mass spectrometer. For the lipid separation an ACQUITY UPLC CSH C_{18} (2.1 x 100 mm, 1.7μm) column was used with a flow rate of 400 μl/min [1]. For the polar metabolites BEH amide (2.1 x 150 mm, 1.7μm) was used in both basic and acidic conditions with a flow rate of 400 μl/min [2]. For detailed UPLC and MS conditions for the lipid and polar metabolites see references 1 and 2 respectively.

MS Conditions

MS System	Waters Synapt™ G2-*Si*
Mode of operation	TOF MS^E
Ionization	ESI +ve and –ve
Capillary voltage	Various (see ref. 1&2)
Cone voltage	30.0 V
Transfer CE	Ramp 20-40V (Function 2)
Source temp.	120.0
Desolvation temp.	500.0
Desolvation gas	800 L/hr (N_2)
Acquisition range	50-1200

Cell Line	Age	Race	Morphology	Clinical Stage	Tumor Grade	Gleason Score
RWPE-1	54	CA	Epithelial	Non-malignant	N/A	N/A
LNCAP	50	CA	Epithelial	Adenocarcinoma metastatic Lymph node tumor	N/A	N/A
RC77N-E	62	AA	Epithelial	Non-malignant	N/A[c]	N/A[c]
RC77T-E	62	AA	Epithelial	Primary Adenocarcinoma	Poorly differentiated	7
MDA PCa 2b	63	AA	Epithelial	Adenocarcinoma metastatic bone tumor	N/A	N/A

Table 1. Clinical features of the patients from whom prostate cell lines were derived.

RESULTS

A UPLC/MS^E data-independent method that provides molecular and structural information from every detectable component in a liquid chromatography separation was used to ensure maximizing data quality and coverage. Figure 1 and 2 show the chromatogram for the separation of various lipid classes and polar metabolites respectively in the pooled sample by ESI in both positive and negative ion mode. Both low and high energy data was acquired within the same run (MS^E) and the data from the two experiments were subsequently aligned for structural elucidation and identification.

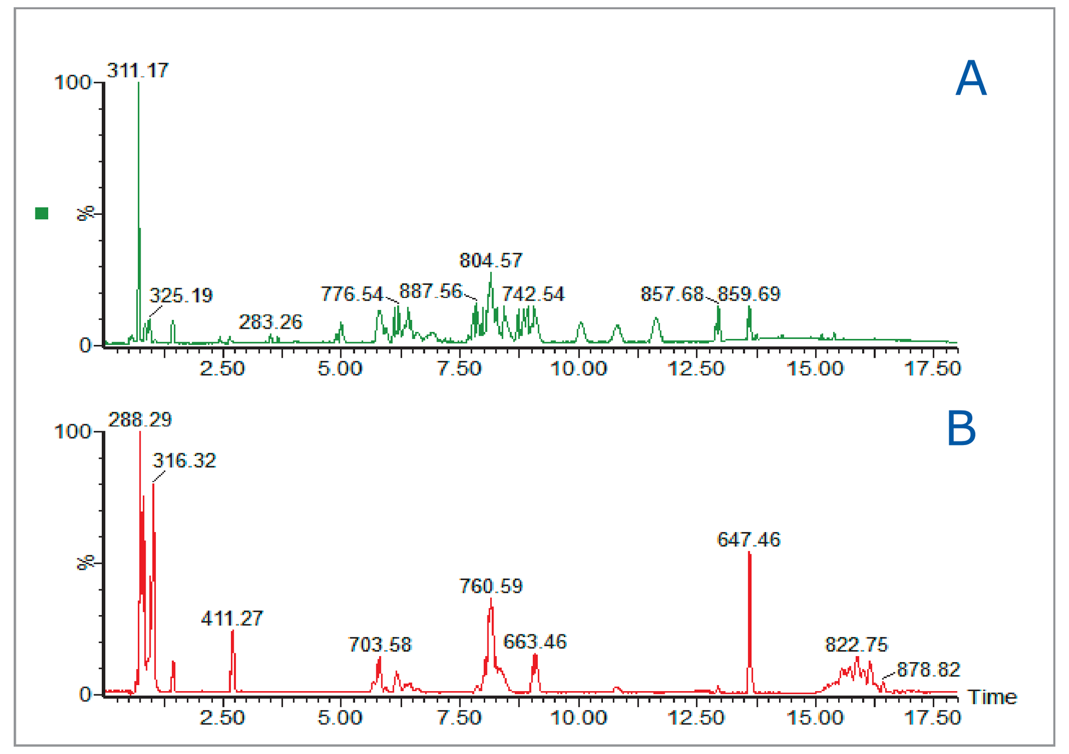


Figure 1. Analysis of lipids extracted from prostate cancer cell lines using UPLC/MS-MS. (a). Positive ion mode (b). Negative ion mode.

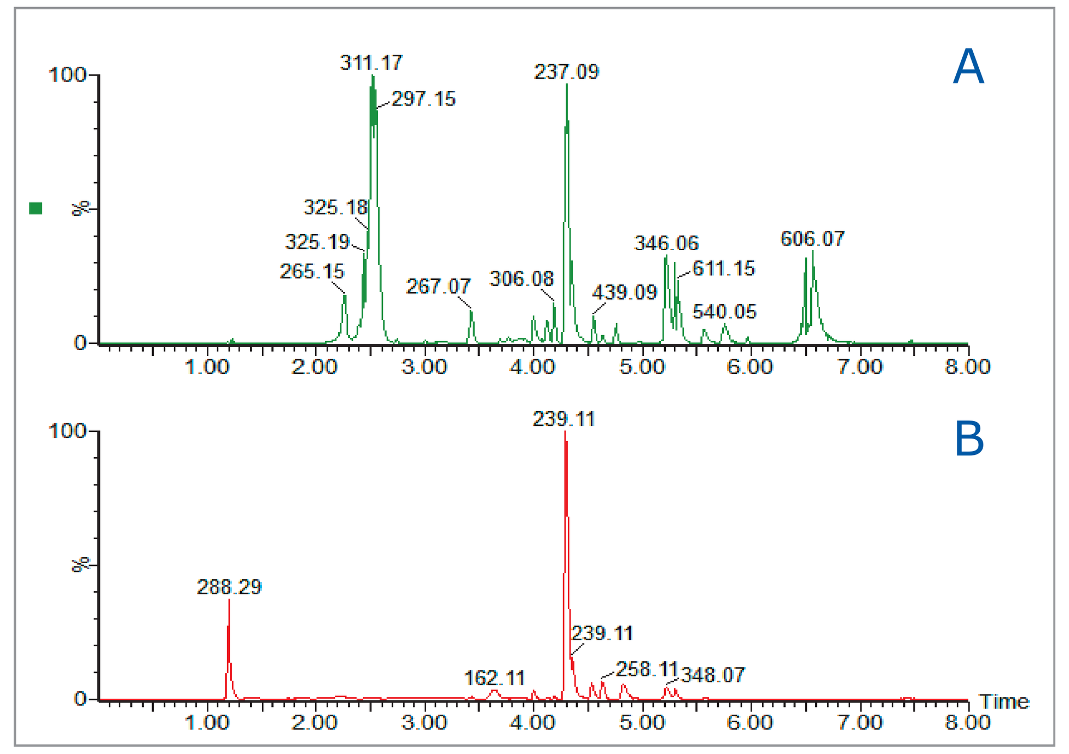
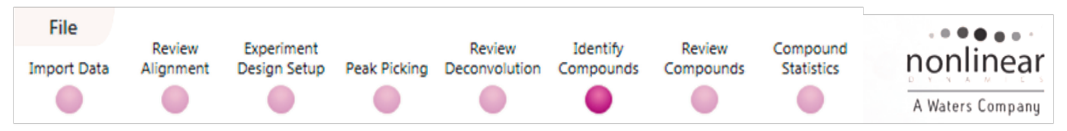


Figure 2. Analysis of polar metabolite extracted from prostate cancer cell lines using UPLC/MS-MS. (a). Positive ion mode (b). Negative ion mode.

Data Analysis



The data analysis is performed using novel data processing and statistical tool called Progenesis QI v1.0 (Nonlinear Dynamics, Newcastle, UK). With Progenesis QI, users can quickly perform differential analysis of results across different sample treatments thereby facilitating identification and quantitation of potential biomarkers. The Progenesis QI software adopts an intuitive step-by-step workflow to perform comparative high resolution UPLC-MS metabolomics and lipidomics data analysis.

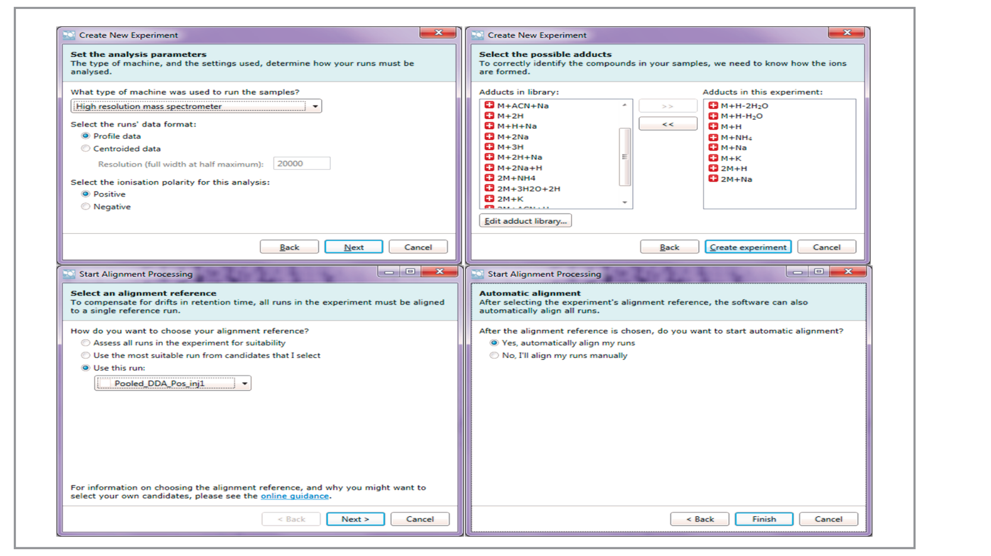


Figure 3. Data importing and retention time alignment. Progenesis QI supports multi vendor MS data.

The workflow starts with UPLC-MS raw data file importing. Details of data file format and a list of expected adducts are entered to facilitate the handling of data import followed by automatic retention time alignment (Figure 3). The imported data is presented as a low and high energy ion intensity map of m/z versus retention time enabling the visual check of the chromatography. Metabolomics experiments involve large amount of sample runs that may result in a shift in retention time. The LC/MS data was first aligned to correct any retention time drift between analytical runs. After retention time alignment, automatic peak detection, normalization, deconvolution, compound quantitation, identification and statistical analysis was performed. Alignment of the data runs allows a common pattern of compound ion detection to be performed across all the runs in the experiment. Adducts of the same compound are automatically grouped during deconvolution based on their retention time and mass difference. The deconvolution process uses the list of defined adducts provided when importing the raw data.

Identify Compounds

Confident compound identification was performed using the integral Metascope search engine and available databases using defined tolerances for precursor exact mass, retention time, fragment ion information.

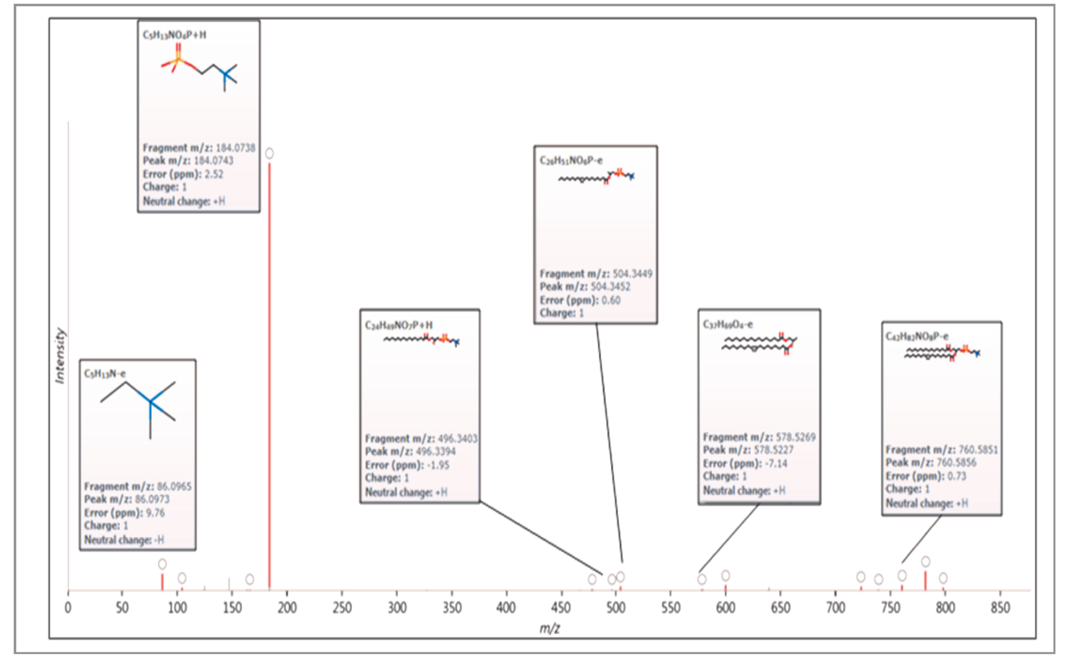


Figure 4. Fragmentation trace for compound 8.13_759.5775n in the positive ion mode with the identification of PC (16:0/18:1) (1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine). The measured spectra which are matched against the in silico fragment ions are highlighted in red. The circle on the top of the red spectra shows the structure, measured m/z, theoretical m/z and corresponding mass error in ppm.

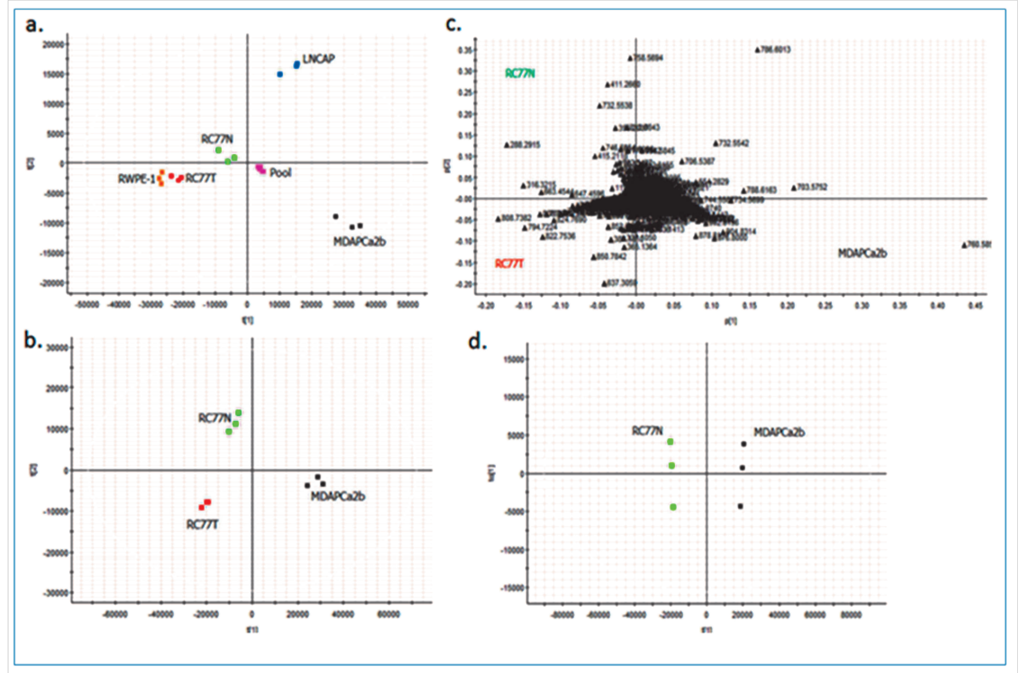


Figure 5. (a) PCA scores plot of the lipid profiles (+ve) of the five prostate cell lines, RWPE-1, LNCAP, RC77N-E, RC77T-E, MDAPCa2b and pooled sample. (b) PCA scores plot of the lipid profiles of the three African American cell lines RC77N-E, RC77T-E and MDAPCa2b. (c) Corresponding loadings plot from PCA analysis of the three African American cell lines. (d) Corresponding OPLS-DA analysis of the two African American cell lines RC77N-E and MDAPCa2b.

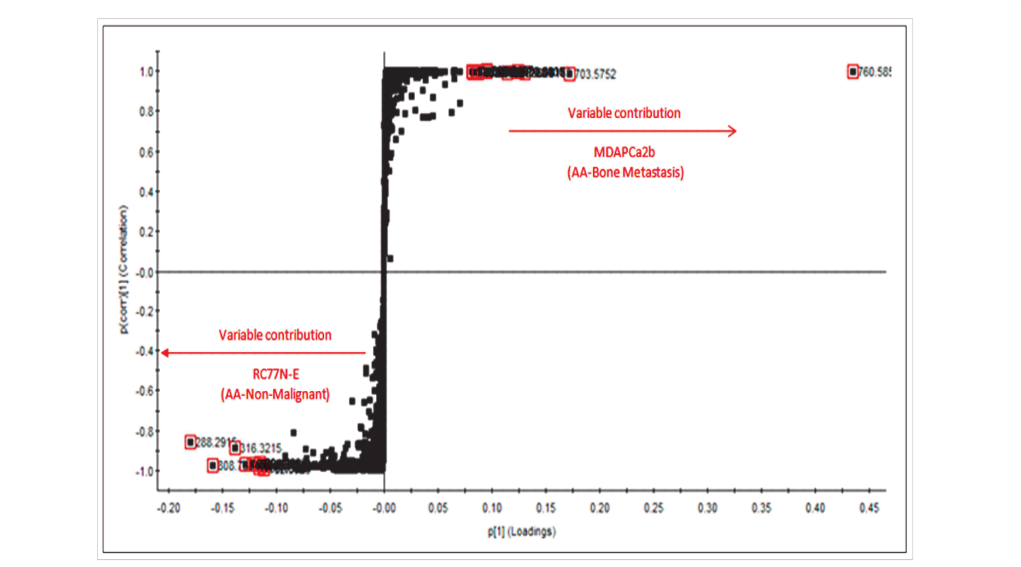


Figure 1. S-plot for the OPLS-DA data for the two African American cell lines RC77N-E and MDAPCa2b. The axis represents the reliability and magnitude of each EMRT.

Compound ID	LMID	Common Name	Maximum Fold increase	Highest Mean Cell Line	Lowest Mean Cell Line	P value
16.5_902.8157m/z			2.27	MDAPCa2b	RC77N-E	1.43E-06
16.15_858.7656	LMGP03010100	TG(16:0/18:1/18:1)	1.55	MDAPCa2b	RC77N-E	4.47E-06
6.31_782.5669m/z			18.0	MDAPCa2b	RC77N-E	4.86E-06
8.77_717.5328n			6.97	MDAPCa2b	RC77N-E	6.7E-06
6.42_807.5764n			12.7	MDAPCa2b	RC77N-E	7.21E-06
8.13_759.5775n	LMGP01010005	PC(16:0/18:1)	2.84	MDAPCa2b	RC77N-E	1.16E-11
16.40_912.8124n			3.32	MDAPCa2b	RC77N-E	1.99E-5
16.41_886.7921n	LMGL03010238	TG(16:0/18:0/20:2)	2.77	MDAPCa2b	RC77N-E	2.37 E-5
5.79_725.5567m/z			4.12	MDAPCa2b	RC77N-E	3.85E-5
8.14_1519.1517n			369	MDAPCa2b	RC77N-E	5.18E-5
6.45_731.5459n	LMGP01010566	PC(16:0/16:1)	4.77	MDAPCa2b	RC77N-E	9.13E-5
9.04_743.5509n			2.62	MDAPCa2b	RC77N-E	1.19E-4
16.43_860.7813n			1.57	MDAPCa2b	RC77N-E	1.2E-4
7.86_734.5698m/z	LMGP1010564	PC(16:0/16:0)	2.67	MDAPCa2b	RC77N-E	1.39E-4
10.84_787.6085n	LMGP1010764	PC(18:0/18:1)	2.11	MDAPCa2b	RC77N-E	1.79E-4
5.79_702.5656n	LMSP03010003	SM(d18:1/16:0)	2.24	MDAPCa2b	RC77N-E	2.02E-4

Table 2. Significantly upregulated lipid molecular species between the AA-bone metastatic MDAPCa2b cells and the AA-non-malignant RC77N-E cells.

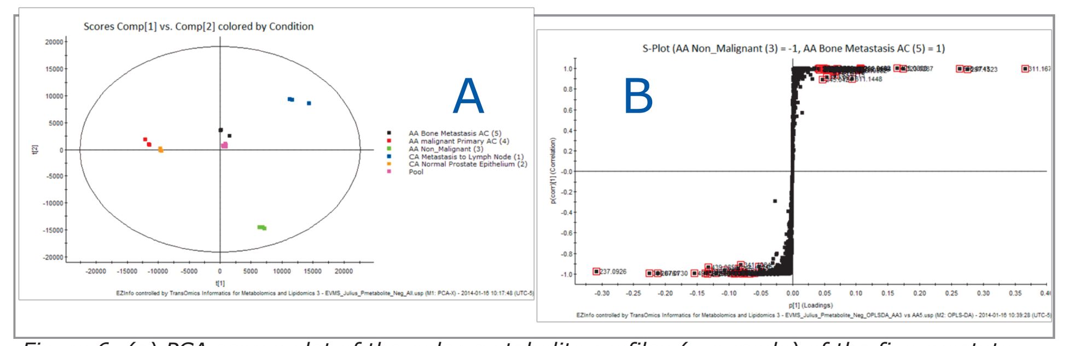


Figure 6. (a) PCA scores plot of the polar metabolite profiles (-ve mode) of the five prostate cell lines, RWPE-1, LNCAP, RC77N-E, RC77T-E, MDAPCa2b and pooled sample. (b) Corresponding OPLS-DA analysis of the two African American cell lines RC77N-E and MDAPCa2b.

Compound ID	HMDID	Common Name	Exact Monoisotopic Mass	Maximum Fold increase	Highest Mean Cell Line	Lowest Mean Cell Line	P value
3.43_347.0453m/z[2]	HMDB00195	Inosine	268.0808	8.12	MDAPCa2b	LNCAP	2.50E-3
4.19_307.0838n[1]	HMDB00125	Glutathione	307.0838	627	RC77N-E	MDAPCa2b	4.18E-2
5.22_346.0552m/z[1]	HMDB03540	3'-AMP	347.0631	14	RC77N-E	RC77T-E	1.08E-8
5.33_323.0287m/z[1]	HMDB11641	Uridine 2'-phosphate	324.0359	20.8	MDAPCa2b	RC77T-E	3.45E-8
5.57_362.0504m/z[1]	HMDB01397	Guanosine monophosphate	363.058	27.6	MDAPCa2b	RC77T-E	3.24E-9
6.50_606.0743m/z[1]	HMDB00304	acetylgalactosamine	607.0816	30.3	MDAPCa2b	RC77T-E	3.91E-9
2.29_276.185m/z[2]	HMDB13131	Hydroxyhexanoycarnitine	276.185	275	MDAPCa2b	LNCAP	1.60e-7
2.74_161.1055[2]	HMDB00062	L-Carnitine	161.1055	3.2	MDAPCa2b	LNCAP	1.10e-3
5.30_612.1524m/z[2]	HMDB03337	Oxidized glutathione	612.1524	1.97	MDAPCa2b	LNCAP	1.55E-5

Table 1. Significantly upregulated polar metabolites amongst the five cells lines in the negative[1] and positive[2] acquisition modes.

CONCLUSION

- **There is differential expression of specific lipid molecules including PC, TG, SM and metabolites with disease severity.**
- **Alterations in expression of specific lipids and metabolites may play an important role in the pathogenesis of prostate cancer and may be linked to metastasis and disease progression.**
- **Progenesis QI workflow provides a easy-to-use, scalable system for analysis of lipidomics and metabolomics data.**
- **Identification based on precursor exact mass, isotopic distribution, retention time, fragmentation and collision cross section (CCS) provides confidence in compound identification.**

References

1. Isaac G. et al. Lipid separation using UPLC with charged surface hybrid technology, Waters Apps Note, 2011, 720004107en.
2. Paglia G. et al. Development of a metabolomics assay for the analysis of polar metabolites using HILIC UPLC/QTof MS, Waters Apps Note, 2013, 720004612en.

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