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Fast Supercritical Fluid Chromatography Separation and Shotgun Lipidomics with High Resolution Mass Spectrometry for the Study of Breast Cancer Metastasis

Sheher Mohsin[1]; Sanjib Meitei[2], Peter Siegel[3]; Daina Avizonis[3]; Gaelle Bridon[1]

[1]Agilent Technologies Inc, Wood Dale, IL

[2]PREMIER Biosoft, Indore, Madhya Pradesh, India

[3]Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada

Introduction

Breast cancer cells can undergo metabolic reprograming to adapt to different metastatic sites. 4T1 murine breast cancer cells are a highly aggressive model of triple negative breast cancer that can spread to the lymph nodes, bone, lung, liver and brain. Their preferred metabolic program (glycolysis versus oxidative phosphorylation) was found to change depending on the tissue site that the breast cancer cells colonized. To further explore the metabolic adaptations that accompany organ-specific metastasis, parental 4T1 cells were subjected to an *in vivo* selection protocol to create subpopulations of cancer cells with increased selectivity for specific organs. These cells were shown to have distinct metabolic phenotypes(1,2). In order to explore the lipid adaptations that occur between the parental 4T1 cells and the liver-metastatic 2776 population, four replicate samples were generated from 8.87 million 4T1 cells per sample and 8.63 million 2776 cells per sample.

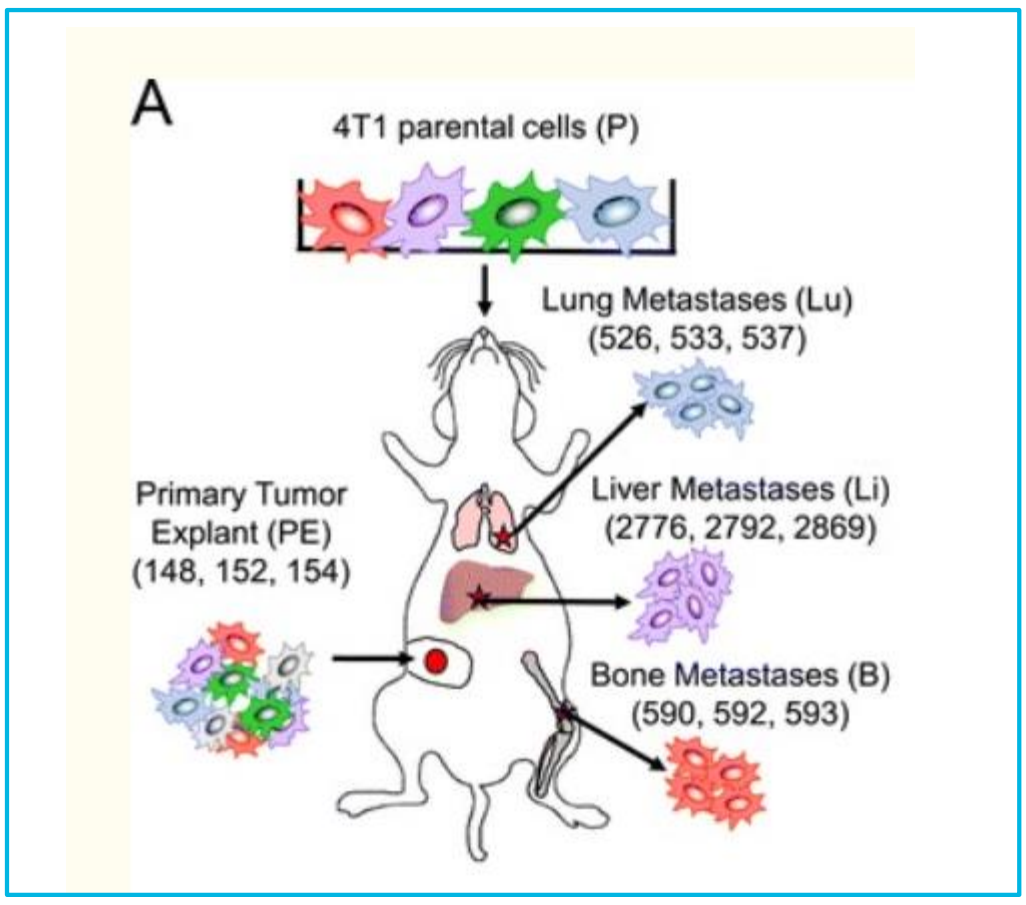

Figure 1: Depiction of 4T1 parental breast cancer cells and sub-populations that metastasize to different organs

Experimental

Four replicate samples were generated from 8.87 million 4T1 cells per sample and 8.63 million 2776 cells per sample. These samples were extracted using the Bligh-Dryer extraction protocol and dried under a nitrogen stream. Two different techniques were used to study differential lipidomics. SFC was used to separate the lipid classes followed by high resolution mass spectrometry. The SFC separation uses two columns to separate the whole range of lipids from polar to non-polar. The total analysis time was less than 14 minutes. Data independent acquisition with automated flow injection analyses (FIA) was also used for studying the lipidomic changes between these samples. Both sets of data were processed using SimLipid software and MetaboAnalyst software was used for statistical analysis.

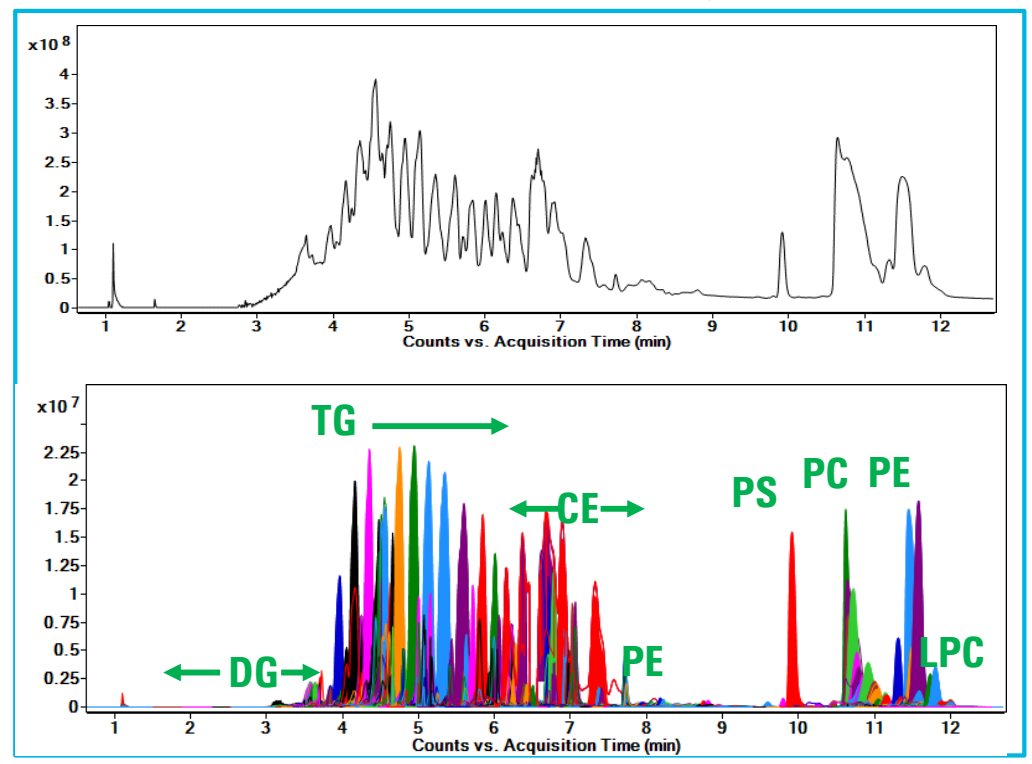

Figure 2: Lipids in liver metastasized 2776 cells separated by SFC

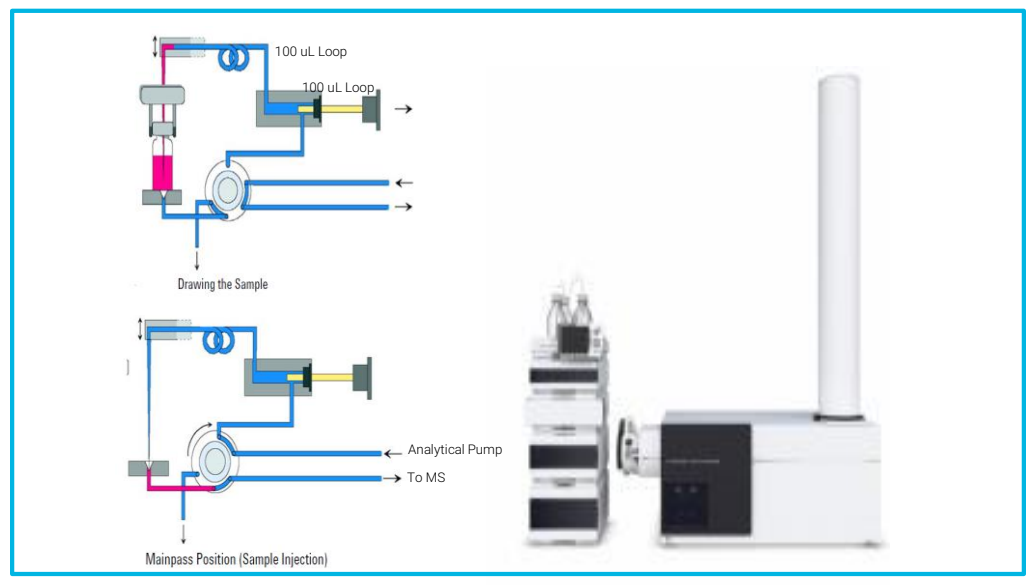

Figure 3. A 100 µL aliquot of sample is pushed at a rate of 12 µL/min by the HPLC pump for FIA. The Q-TOF is setup for MS/MS of 1 amu windows across the mass range for true data independent acquisition.

Results and Discussion

SimLipid Software was used to extract the lipid annotations from the samples. Total annotations irrespective of the score were 1,173 for the liver specific 2776 cells and 1,054 for the 4T1 murine breast cancer cells. The total lipids were next validated. Lipids from the sphingolipid, phospholipid, and sterol classes have their corresponding head group diagnostic ions observed in the MS/MS spectra e.g., peak at *m/z* 184.073 for phosphocholines, neutral loss of 141 from parent ion mass for PE lipids, etc. DG and TG were identified with at least 1/2 of the 2/3 fatty acid chains. After validation, 836 lipids between the replicates (all the 6 runs) were annotated. There were 654 annotations for the 2776 cells and 608 annotations for the 4T1 cells .

Comparing the two groups of samples using MetaboAnalyst indicated that the phosphatidylethanolamine, in general, was enhanced in the liver specific 2776 cells compared to the 4T1 cells. The other lipid groups that showed increased expression in the 2776 liver specific cells were the triacyl glycerides (TG) and phosphotidic acids (PA). Within the phosphatidylethanolamine group of lipids, the PE-O species were found to be at higher levels. This trend was observed for the samples separated with SFC as well as the shotgun FIA samples.

Summary Table

Group	SFC: 2776	SFC: 4T1	FIA: 2776	FIA: 4T1
TG	109	84	70	44
PC	23	23	119	121
DG	40	18	109	102
PC O-	18	13	68	68
SM	22	17	48	52
PE	14	22	60	36
PA	3	1	51	39
PS	22	18	24	24
PC P-	2	9	22	25
PE P-	16	10	13	11
Steryl_Esters	8	13	15	12
Cer	13	7	8	8
PE O-	7	2	15	12
LPC	5	4	4	6
Chol & Der	0	2	8	8
PG	0	0	12	6
PI	7	7	1	2
GlcCer	0	0	6	9
Oxidized PE	0	0	5	5
Oxidized PC	0	0	2	3
LPA	0	0	0	5
LPE	1	0	1	1
LPI	0	0	2	0
LPS	0	0	0	2
Cer-PE	0	0	1	0
Cer-PI	0	0	1	0
MG	0	0	1	0
LPG	0	0	0	1
Total	**310**	**250**	**666**	**602**

Table 1: Comparative lipid profiles between the 2776 vs 4T1 measured using SFC- and FIA-MS workflows

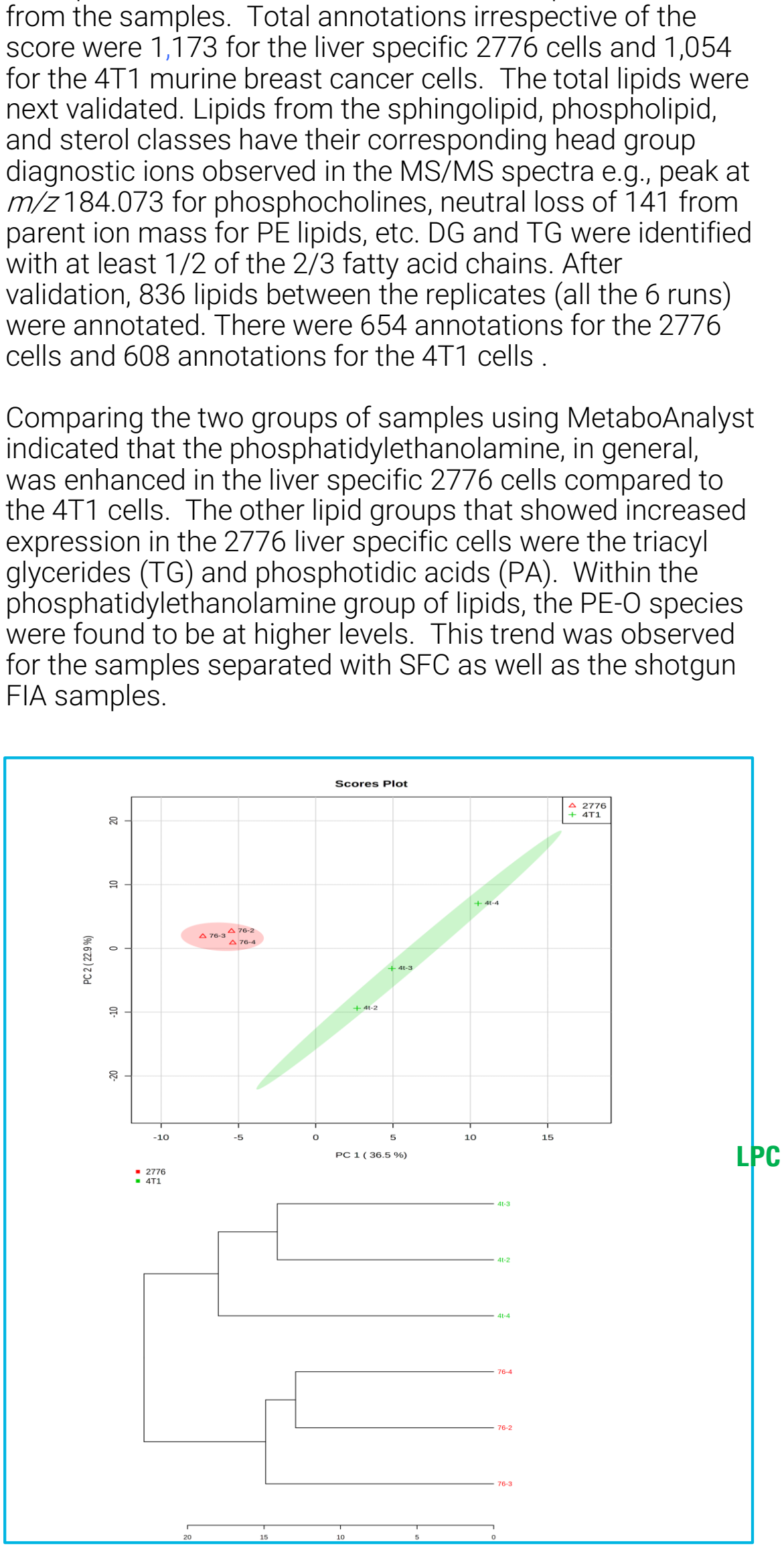

Figure 4. PCA plot and dendrograms showing good separation between the cell lines

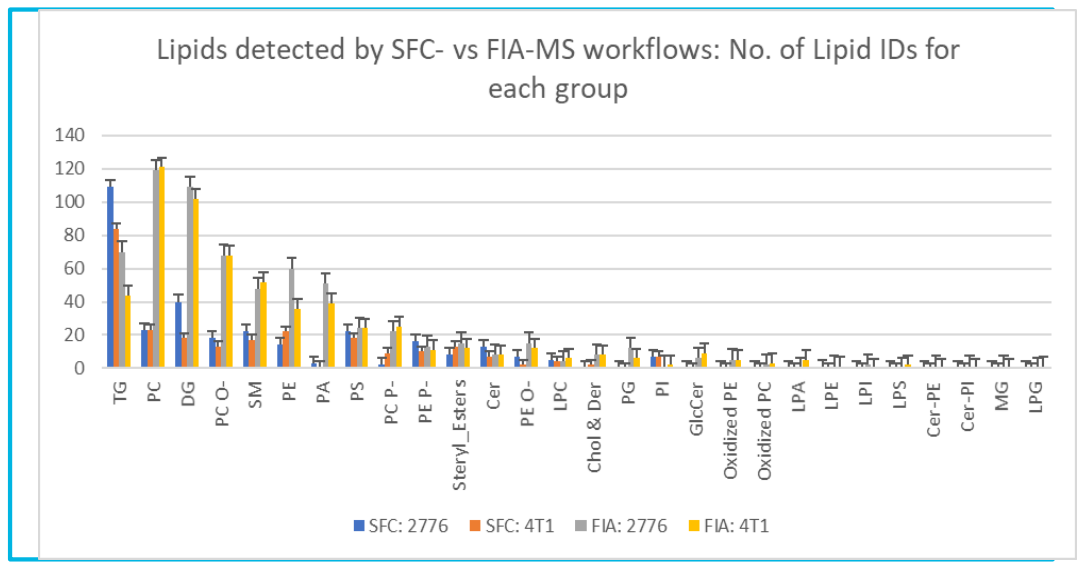

Figure 5. Bar graph showing lipids detected in each cell line with SFC and FIA

Results and Discussion

Figure 6. Heatmap showing the variation of ether PC between the two cell lines. MPP with the new Lipidomics workflow was used to generate the heatmap

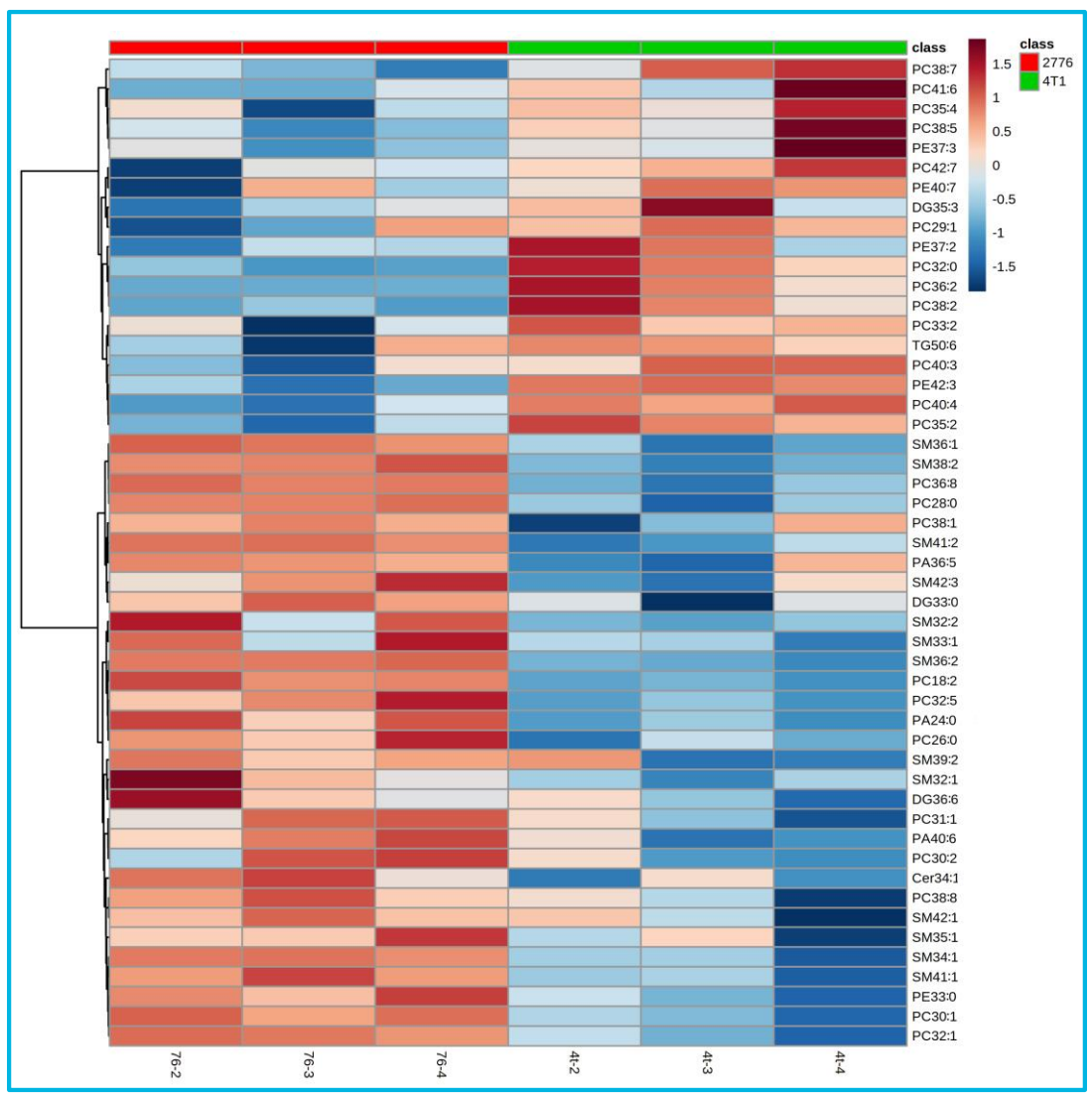

Figure 7. Heatmap showing the top-50 lipid species with highest variation; MetaboAnalyst software does not accept the special character ":" in the lipid names. Hence, we have manually inserted the character ":" into the lipid names displayed in the heatmap for better readability.

In this pilot study, we show an efficient means for lipid profiling using SFC-MS and FIA-MS. Both approaches revealed similar differential lipid patterns between 4T1 (parental) and 2776 (liver-metastatic) cell populations. Further investigation is required to ensure reproducibility and lipid identification validation through the use of authentic lipid standards when available. The detailed metabolic changes required for cancers (in this case triple negative breast cancer) to invade distal sights are poorly understood. We have shown previously that the cancer's oxidative phosphorylation capacity changes radically depending on the site of invasion (1,2). Thus the invading cancer cells somehow adapt to their new site's micro-environment. The pilot study presented here is a start in gaining an understanding of the changes in complex lipid biochemistry between highly aggressive and non-specific (4T1) compared to the highly specific liver-metastatic cells (2776). While we have much to decipher, it is interesting to point out that the 2776 cells show higher triglyceride content compared to the 4T1 parental cells. The major sites for triglyceride synthesis are in the liver and adipose tissues, which would indicate that metabolic adaptations may occur such that invading cancer cells mimic their new environment. Having reliable profiling techniques as described here will allow us to interrogate the changes in lipid biochemistry that occur in cancer cells allowing malignancies to occur.

Conclusions

We show two different automated techniques to study differential lipidomics: SFC with two columns to separate the lipid classes followed by high resolution mass spectrometry and data independent acquisition with automated flow injection analyses (FIA). Both techniques reveal similar differential pattern between 4T1 (parental) and 2776 (liver-metastatic) cell populations.

References

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