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Global profiling studies in tumor bearing mouse models using high mass accuracy MSn analysis

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Overview

Global metabolite profiling provides an insight into the phenotype of biological systems and the patho-physiological process related to disease.

In this paper, we describe the application of a LCMS-IT-TOF to metabolite profiling in several colorectal and non small cell lung cancer cell tumors.

Ion signals with a peak area variance of less than 30% were submitted for subsequent PCA and detection of metabolite changes (ion signal stability was assessed by a pooled QC sample analyzed throughout the batch).

Endogenous metabolites were identified using high accuracy MS data and verified using external search engines (http://www.lipidmaps.org; http://www.hmdb.ca; http://www.genome.jp/kegg/).

Changes in the levels of amino acids and lipids accounted for the different phenotyping of the cell lines.

Introduction

A major factor affecting pharmaceutical drug discovery is the ability to predict the human efficacy/safety of new chemical entities (NCEs) from preclinical discovery and development data. This is far from being a new problem but it remains a critical challenge in the drug discovery process. Determining which animal species/model adequately predicts human metabolism, efficacy and side effects remains a complex concept. In this study, mass spectrometry-based metabolite profiling was used to identify changes in endogenous metabolite levels in several mouse cancer models including colorectal and non small cell lung cancer cell tumors using high accuracy MSn analysis.

Methods

Several human colon cancer cell lines (HCT116, SW-620, COLO205 and HT-29) together with NSCLC line (CALU6) were implanted into mice at 8-10 weeks. Samples were harvested at 33 and 35 days for colorectal tumors HCT116 and SW620; 7-14 days for colorectal HT-29; 7,14,21 and 28 days for NSCLC tumor. 100mg of manually disaggregated tumor tissue was mixed with 1mL (50% acetonitrile in water) and sonicated for 5 min followed by 10 min centrifugation (17,900 rcf). Keeping the precipitated pellets, the supernatants were separated out and put in autosampler vials as the aqueous extract. To extract the non-polar analytes the pellets were mixed with 1mL (75% chloroform, 25% methanol) and sonicated for 5 min followed by 10 min centrifugation (17,900 rcf). Organic extracts were resuspended in 1ml methanol (4ºC) and aliquoted into vials. The results are presented for the organic extracts (not the aqueous extracts).

Samples obtained from these animals were measured by LC/MS using a quadrupole ion trap-time of flight mass spectrometer (LC-MS-IT-TOF, Shimadzu Corporation) using data dependent acquisitions in electrospray ionization (ESI) in both positive and negative mode. To identify biologically significant components, high mass accuracy MS and MSn fragment ion information was used to identify the most likely candidate formula. A pooled biological QC sample was injected throughout the sample batch analysis (interspersed between every 5 samples) and the sample batch run time was approximately 60 hours (run time 40 minutes for each sample; 90 samples injected as one batch).

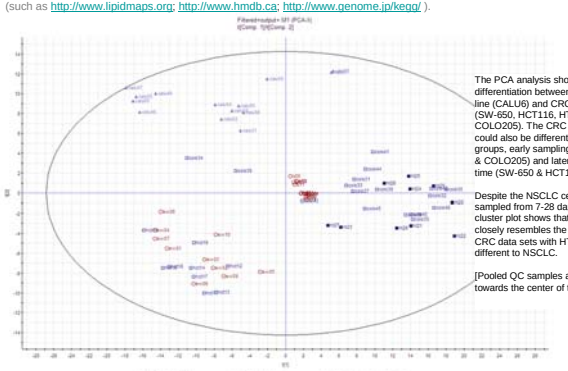
Name	GPCho	Tyrosine	Phenylalanine	Tryptophan	Sphinganine	GPCho (14:0/0:0)	GPCho (16:0/0:0)	GPEtn (20:4/0:0)	GPEtn (18:1/0:0)
m/z (M+H)+	258.1125	182.0812	166.0863	205.0972	302.3054	468.3085	496.3398	502.2928	480.3085
RT (mins)	1.16	1.31	2.47	3.32	14.3	19.194	22.232	20.493	22.307
Formula	C8H20NO6P	C9H11NO3	C9H11NO2	C11H12N2O2	C18H39NO2	C22H46NO7P	C24H50NO7P	C25H44NO7P	C23H46NO7P
Run time [hrs]									
00:00:00	175,819,677	35,698,287	96,218,492	25,878,560	9,194,575	8,448,625	157,126,935	117,357,371	106,998,130
00:41:00	175,078,416	32,519,921	100,719,378	27,604,047	9,279,610	8,993,876	157,403,460	120,276,858	108,122,038
01:22:00	173,098,282	35,313,628	100,435,274	26,414,098	9,680,927	9,076,358	156,599,918	116,883,754	106,000,715
02:03:00	175,959,779	34,103,598	91,884,479	25,668,259	8,995,969	9,162,069	154,948,808	121,239,218	110,062,146
02:44:13	177,614,189	31,986,367	90,325,202	25,396,012	9,333,772	8,681,956	156,338,729	119,311,707	105,670,008
03:25:08	175,518,060	32,901,960	93,706,828	25,070,923	8,876,362	8,747,578	155,074,774	118,248,157	106,381,031
04:06:00	177,490,486	33,320,722	91,516,570	25,109,231	9,294,091	8,836,448	153,564,504	118,161,015	108,360,995
04:46:00	175,165,388	33,639,346	92,666,447	25,219,130	9,087,959	8,630,074	155,206,964	118,320,958	106,042,626
05:27:00	171,682,634	32,652,048	94,473,274	24,671,561	9,128,927	8,692,762	152,199,413	118,537,088	105,491,046
06:08:00	172,879,873	32,369,036	90,554,498	24,419,534	9,161,753	8,974,752	155,375,378	117,794,714	106,248,288
10:14:00	171,400,811	32,430,634	91,681,086	24,243,356	9,029,694	8,326,164	153,993,427	117,056,493	105,117,762
14:19:00	173,067,774	30,962,515	84,469,780	23,741,476	9,054,149	8,348,209	150,417,611	118,165,368	105,557,225
18:24:00	173,987,719	31,396,459	87,424,412	22,567,950	8,885,352	8,510,806	152,655,451	121,074,206	107,117,165
22:30:00	173,459,811	29,610,907	82,972,674	23,397,470	8,563,786	8,747,903	152,614,351	114,907,738	104,123,707
26:35:00	174,414,334	29,913,803	82,333,777	22,031,268	8,706,700	8,467,973	151,043,333	119,310,739	100,763,221
30:41:00	171,888,307	31,527,927	86,637,580	23,619,888	9,493,587	8,921,377	150,572,771	122,103,629	108,537,163
34:46:00	172,230,206	30,248,585	89,220,271	22,226,224	9,188,171	8,686,985	149,327,341	121,165,271	105,690,346
38:52:00	172,542,997	29,530,487	83,852,259	21,752,308	9,081,559	8,443,643	151,468,624	122,802,189	105,022,569
42:57:00	173,550,284	30,949,871	89,359,561	23,153,813	9,177,307	9,203,614	152,139,705	123,005,926	109,773,520
47:02:00	175,709,791	30,138,434	93,070,580	23,641,628	9,880,487	8,770,527	151,067,427	124,481,110	107,578,192
%RSD	1.064	5.576	5.704	6.369	3.115	3.01	1.556	2.037	1.949
Maximum	177,614,189	35,698,287	100,719,378	27,604,047	9,880,487	9,203,614	157,403,460	124,481,110	110,062,146
Minimum	171,400,811	29,530,487	82,333,777	21,752,308	8,563,786	8,326,164	149,327,341	114,907,738	100,763,221
Std. Dev.	1,853,338.08	1,787,810.26	5,171,768.16	1,547,052.85	303,505.90	262,851	2,448,193	2,434,369	2,074,769
Average	174,127,941	32,060,727	90,676,121	24,291,337	9,154,737	8,733,585	153,456,946	119,510,175	106,432,895

Table 1.
Pooled QC sample analysis was used to assess the performance of the system by repeatedly injecting the QC sample throughout the analytical run over a 47 hour period. The table highlights the response of several metabolites following repeated injection, this includes amino acids (for example tyrosine, phenylalanine, tryptophan) and lipid signals (GPCho; GPCho [14:0/0:0], GPCho [16:0/0:0], GPCho [20:4/0:0], GPEtn [20:4/0:0], GPEtn [18:1/0:0]).

Results

m/z (M+H)+	Ion RT	Metabolite	Avg SW	Avg HCT	Avg HT	Avg COLO	Avg Calu
150.0583	1.30	Methionine	2,802,593	3,550,220	11,424,851	6,675,120	4,376,629
165.0546	1.31	5-hydroxy-7Z-Nonene-3,5-diynoic acid	4,584,292	5,449,732	16,980,057	10,075,245	6,075,314
166.0863	2.47	Phenylalanine	36,821,650	51,552,829	143,667,334	92,345,081	55,244,622
175.1190	1.32	Arginine	986,797	256,557	2,834,609	3,307,231	2,111,481
182.0812	1.31	Tyrosine	14,287,778	16,885,789	49,119,906	31,818,527	19,150,160
188.0706	3.32	Indoleacrylic acid	6,891,651	9,887,687	29,833,194	20,422,123	10,926,326
205.0972	3.32	Tryptophan	9,855,816	13,643,754	40,736,133	27,846,806	14,547,551
220.1179	3.32	Pantothenic acid	1,968,194	158,879	4,045,000	6,744,707	2,306,090
258.1101	1.16	sn-Glycero-3-phosphorylcholine	14,829,958	1,201,059	204,699,454	167,991,089	125,010,370
268.1040	1.36	Adenosine	28,309,461	15,234,981	11,668,430	8,841,507	12,837,523
282.2791	1.03	9Z-octadecenamide	1,085,113	0	812,105	73,596	6,753,668
284.0989	1.89	Guanosine	0	0	1,972,395	2,236,582	552,241
291.1299	1.06	N-(L-arginino)succinate	1,048,514	960,940	6,602,083	3,850,328	2,365,672
298.0968	3.28	5'-methylthioadenosine	1,022,641	893,474	5,106,193	4,845,628	2,796,139
302.3054	14.30	Sphinganine	8,448,576	7,595,759	8,567,921	7,795,346	9,868,218
310.1133	1.61	N-acetylneuraminic acid	605,980	71,639	9,566,799	4,559,409	1,148,276
400.3421	1.59	O-Palmitoyl-R-carnitine	416,549	91,683	7,581,863	3,582,780	920,778
480.3085	1.65	1-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine	627,315	251,763	2,033,967	1,204,991	1,056,461
496.3398	25.44	1-Palmitoyllysophosphatidylcholine	180,527	604,338	1,394,431	1,534,697	391,022
502.2928	24.95	Phosphoethanolamine	47,719,221	49,893,245	61,051,126	51,484,131	59,509,071
522.3554	22.99	1-(1Z-hexadecenyl)-2-acetyl-sn-glycero-3-phosphocholine	16,207,533	8,171,116	23,736,112	14,151,014	5,642,148
524.3711	26.39	1-hexadecyl-2-acetyl-glycero-3-phosphocholine	2,534,233	1,709,715	5,576,523	4,216,229	1,682,107

Table 2.
Dependent upon the tumor cell line, the levels of several endogenous metabolites changed significantly. The table above shows a number of amino acids and lipids which change dependent on the cancer model and cell line [key; Calu – corresponds to a non small cell lung cancer ; SW, HCT, HT and COLO are colorectal tumors]. The identification of each component was verified using MS and MSn data to correlate mass accuracy and isotopic patterns with external data bases (such as http://www.lipidmaps.org; http://www.hmdb.ca; http://www.genome.jp/kegg/).



The PCA analysis shows clear differentiation between NSCLC cell line (CALU6) and CRC cell lines (SW-650, HCT116, HT-29 and COLO205). The CRC cell lines could also be differentiated into two groups, early sampling time (HT-29 & COLO205) and later sampling time (SW-650 & HCT116).

Despite the NSCLC cell line being sampled from 7-28 days the PCA cluster plot shows that it more closely resembles the later sampled CRC data sets with HT-29 most different to NSCLC.

[Pooled QC samples are clustered towards the center of the PCA plot.]

Figure 1.
Principal component plot for the electrospray positive ion data (MS). Ion signals (or detected features) which resulted in a peak area variance of less than 30% were considered in this analysis (338 ion signals in total). [All results presented are for the organic extract].

Results

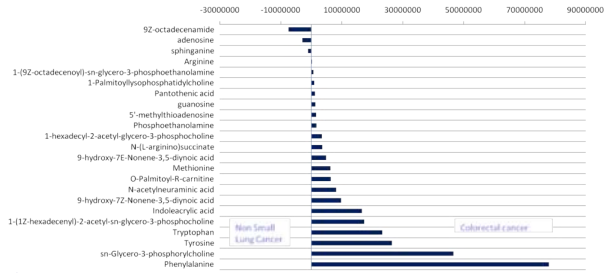


Figure 2.
Levels of endogenous metabolites in the tumor extracts were compared between 2 different tumor bearing mice models (colorectal cancer [cell line HT-29; a cultured human colon cancer cell line] and non small lung cancer [cell line CALU6]). The differences are expressed as an average peak area value between the 2 groups.

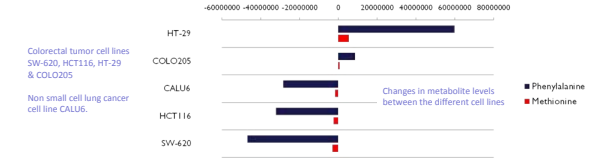


Figure 3.
Relative changes in the levels of phenylalanine and methionine for each cell line. To help visualize the changes in metabolite levels between each cell line the response has been normalized to the pooled QA sample. In the case of cell line HT29 the levels of phenylalanine and methionine were markedly higher than the other cell lines.

Discussion and Conclusion

- Untargeted global metabolite profiling has been applied to analysis of endogenous metabolite levels in colorectal and non small cell lung cancer tumors implanted in mice. The changes in amino acid and lipid levels provide a useful framework to differentiate the human cell line tumors.
- Endogenous metabolite levels have been measured and identified using high accuracy MS2 data acquired on a LCMS-IT-TOF system and verified by reference to internal and external databases ((http://www.lipidmaps.org; http://www.hmdb.ca; http://www.genome.jp/kegg/).
- The use of pooled samples in quality control has been recognized for some time but it lends itself well to profiling studies and PCA interpretation. In this study the pooled QC sample was used to characterize the reproducibility of the 'system'. Whilst the FDA suggests that variability of ±15% of the nominal value represents an acceptable degree of reproducibility, in long term profiling studies the tolerance is often relaxed to between 20-40%. In this study, ion signals which resulted in a relative standard deviation (RSD) <30% and retention time RSD<1% were considered in subsequent principal component analysis. (338 ion signals in ESI positive data and 467 ions were detected in negative ion).