Phenotypic Characterization Analysis of Human Hepatocarcinoma by Urine Metabolomics Approach

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Table 1. Clinical characteristics of the subjects at baseline.

Samples	HCC	Control
Sample No.	25	12
Age	38±4	40±3
Sex (F/M)	12/13	6/6
BMI(kg/m2)	21.77±2.56	22.25±1.91
ALT (U/L)	110.46±49.35	56.78±27.13
AST (U/L)	90.10±52.45	37.07±26.92
GGT(U/L)	76.18±17.72	34.27±9.34
Median tumor size (cm)	<2 cm	-
AFP(ng/mL)	109.22±3.76	1.64±0.89

Table 2. Primary metabolites differentially expressed in urine of HCC and normal control patients.

No.	VIP	Rt	$[M+H]^+$	$[M-H]^-$	Formula	Metabolites	HMDB	PubChem
1	10.3182	4.64	257.0823		$C_{16}H_{34}O_2$	Palmitic acid	HMDB00220	985
2	9.55195	3.84	265.1887		$C_{13}H_{16}N_2O_4$	Alpha-N-Phenylacetyl-L-glutamine	HMDB06344	92258
3	7.5964	5.22	318.2927		$C_{18}H_{40}NO_3$	Phytosphingosine	HMDB04610	122121
4	7.18613	3.81	304.1372		$C_{15}H_{19}N_3O_4$	Indoleacetyl glutamine	NA	NA
5	6.88439	8.46	466.3082		$C_{26}H_{44}NO_6$	Glycocholic acid	HMDB00138	439604
6	6.68089	3.83	179.0514		$C_9H_9NO_3$	Hippurate	HMDB00714	464
7	6.51659	4.29	220.9994		$C_{11}H_{14}N_2O_3$	5-Hydroxy-L-tryptophan	HMDB00472	144
8	6.33054	2.61	144.0076		$C_5H_5O_5$	2-Oxopentanedioate	HMDB00208	51
9	6.25576	4.53	516.2921		$C_{26}H_{46}NO_7S$	Taurocholic acid	HMDB00036	440567
10	6.07649	4.43	393.2948		$C_{24}H_{41}O_4$	Chenodeoxycholic acid	HMDB00518	10133
11	8.5538	6.5		539.2488	$C_{27}H_{40}O_{11}$	Cortolone-3-glucuronide	HMDB10320	125853
12	7.1982	7.19	285.0062		$C_{19}H_{24}O_2$	Androstenedione	HMDB00053	6128
13	6.4612	5.24	252.1392		$C_{13}H_{17}NO_4$	3-Indolecarboxylic acid	HMDB03320	69867
14	6.1047	6.66	541.2601		$C_{27}H_{42}O_{11}$	Cortolone-3-glucuronide	HMDB10320	125853
15	6.0566	2.44	195.0496		$C_{10}H_{12}O_4$	Homoveratric acid	HMDB00434	7139

Table 3: Pathway-associated metabolite sets for HCC from Metabolite Set Enrichment Analysis.

No.		Total	Expected	Hits	Raw p	p-value
1	Bile acid biosynthesis	49	0.772	3	0.037	0.00
2	Citric acid cycle	23	0.362	1	0.309	0.01
3	Tryptophan metabolism	34	0.536	1	0.424	0.02
4	Urea cycle	20	0.315	1	0.275	0.04
5	Phenylacetate metabolism	4	0.063	1	0.062	0.06
6	Alanine metabolism	6	0.095	1	0.091	0.06
7	Malate-aspartate shuttle	8	0.126	1	0.120	0.09
8	Glucose-alanine cycle	12	0.189	1	0.175	0.12
9	Glycerolipid metabolism	13	0.205	1	0.188	0.12
10	Androgen and estrogen metabolism	17	0.268	1	0.239	0.17
11	Ammonia recycling	18	0.284	1	0.251	0.19
12	Glutamate metabolism	18	0.284	1	0.251	0.22
13	Insulin signalling	19	0.299	1	0.263	0.28
14	Fatty acid elongation in mitochondria	26	0.410	1	0.343	0.38
15	Gluconeogenesis	27	0.425	1	0.353	0.47
16	Fatty acid metabolism	29	0.457	1	0.374	0.47

Table 4: Location-based metabolite sets for HCC from Metabolite Set Enrichment Analysis.

No.		Total	Expected	Hits	Raw p	p-value
1	Liver	234	4.06	4	0.618	0.01
2	Hepatocyte	1	0.02	1	0.017	0.03
3	Fibroblasts	183	3.17	4	0.395	0.03
4	Kidney	164	2.84	2	0.815	0.05
5	Platelet	108	1.87	2	0.580	0.12
6	Epidermis	100	1.73	2	0.535	0.13
7	Thyroid gland	12	0.21	1	0.191	0.14
8	Myelin	26	0.45	1	0.370	0.22
9	Skeletal muscle	45	0.78	1	0.556	0.33
10	Membrane	50	0.87	1	0.595	0.37
11	Endoplasmic reticulum	53	0.92	1	0.617	0.42
12	Bladder	92	1.59	1	0.820	0.48
13	Muscle	101	1.75	1	0.850	0.60
14	Brain	122	2.11	1	0.903	0.62
15	Intestine	145	2.51	1	0.940	0.78

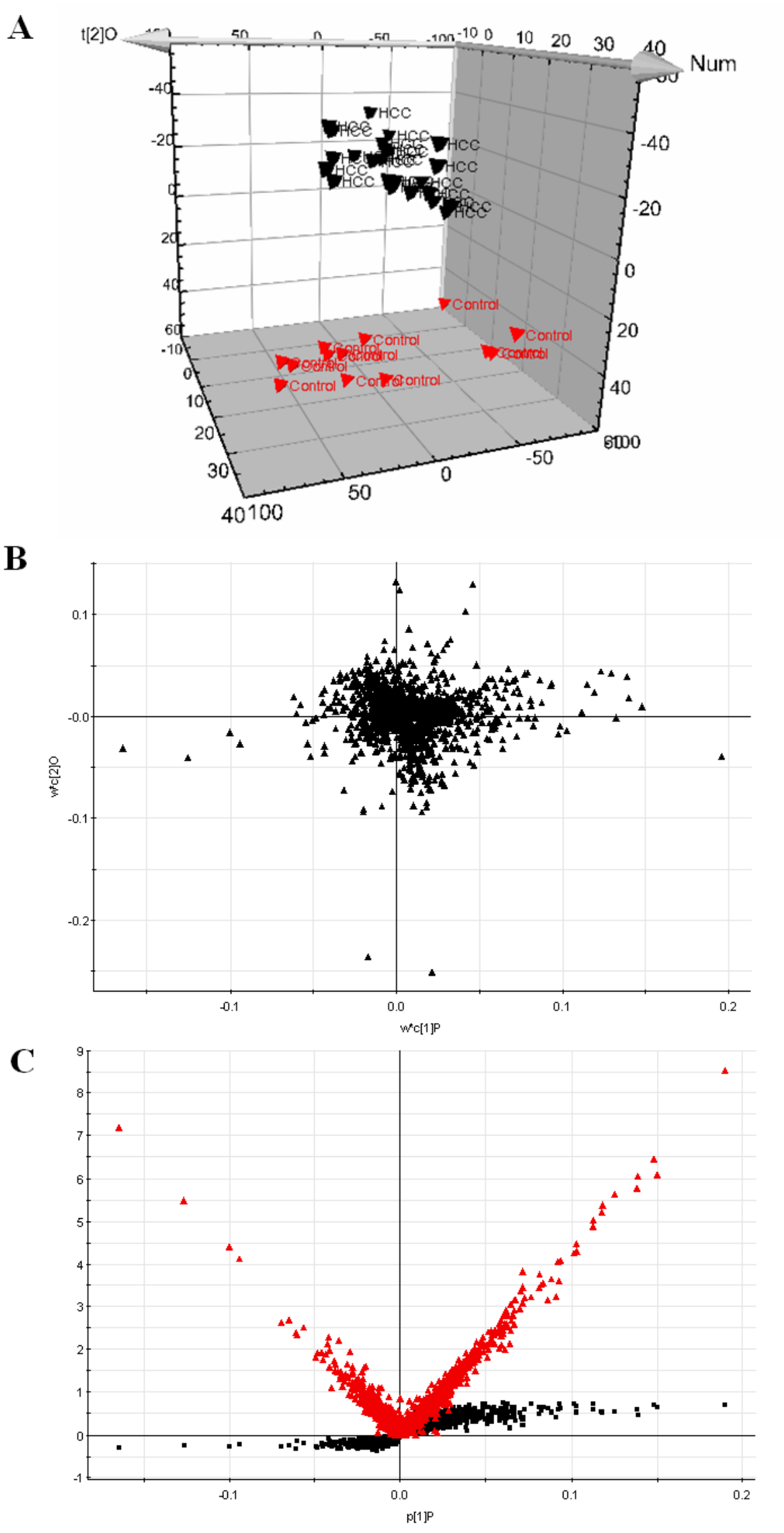

Fig. S1. Metabolomic profiling of HCC in positive mode. 3-D of PCA model for HCC group (A). Loading plot of OPLS-DA of HCC in positive mode (B). Panel C shows the combination of S- and VIP-score plots constructed from the supervised OPLS analysis of urine (ESI^- mode) mode (C).