SUPPLEMENTAL DATA

Integration of metabolomics and transcriptomics reveals major metabolic pathways and potential biomarker involved in prostate cancer

Shancheng Ren[1♠], Yaping Shao[2♠], Xinjie Zhao[2], Christopher S. Hong[3], Fubo Wang[1], Xin Lu[2], Jia Li [2], Guozhu Ye[2], Min Yan[2], Zhengping Zhuang[3], Chuanliang Xu[1], Guowang Xu[2*], Yinghao Sun[1]*

[1] Department of Urology, Shanghai Changhai Hospital, Second Military Medical University, Shanghai, China

[2] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 116023 Dalian, China.

[3] Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA.

♠: equal contribution.

*: To whom correspondence should be addressed.

Guowang Xu: Tel/Fax: +86-411-84379530. xugw@dicp.ac.cn.

Yinghao Sun: Tel/Fax: +86-021-65566429. sunyh@medmail.com.cn

Table S1. Preparation of 30 mL methanol with isotope standards.

Isotope standard	Stock concentration (mg/ml)	Volume of stock solution (μL)	Final concentration (μg/mL)
L-leucine-5,5,5-d3	0.267	37.5	0.333
L-phenyl-d5-alanine	1.00	5.00	0.167
L-tryptophan-d5	0.500	10.0	0.167
Acetyl-d3-L-carnitine	0.0114	132	0.0500
Decanoyl-d3-carnitine	0.00208	192	0.0133
Palmitoyl-d3-carnitine	0.0234	21.4	0.0167
Palmitic acid-16,16,16-d3	1.00	15.0	0.500
Stearic acid-18,18,18-d3	0.3330	45.0	0.500
Cholic acid-2,2,4,4-d4	0.0125	80.0	0.0333
Chenodeoxycholic acid-2,2,4,4-d4	0.0125	16.0	0.00667
LysoPC(19:0)	1.000	20.0	0.667

Table S2. Differential metabolites in discovery cohorts.

Metabolites name	ESI mode	m/z	t_R/min	RSD in QC	PCT/ANT ratio	*p* value	Metabolic Pathway
5-methylthioadenosine	ESI+	298.09	2.25	4.35%	2.2	0.0032	Cysteine and methionine metabolism
Adenosine	ESI+	268.10	0.86	18.6%	0.6	0.0019	Purine metabolism
Alanyl-glycine	ESI+	147.07	0.72	3.10%	1.5	0.0422	
Alanyl-leucine	ESI+	203.13	0.79	2.55%	0.7	0.0080	
Bilirubin	ESI+	585.26	18.0	2.62%	0.5	0.0004	Porphyrin and chlorophyll metabolism
Biliverdine	ESI+	584.26	18.0	16.8%	0.4	0.0004	Porphyrin and chlorophyll metabolism
Choline	ESI+	104.10	0.71	4.71%	1.3	0.0149	Glycerophospholipid metabolism; Glycine, serine and threonine metabolism
Choline glycerophosphate	ESI+	258.11	0.72	3.04%	4.5	0.0009	Glycerophospholipid metabolism; Ether lipid metabolism
CDP-choline	ESI+	489.11	0.73	7.09%	2.6	0.0002	Glycerophospholipid metabolism
GlcN-6-P	ESI+	260.04	0.73	2.92%	1.9	0.0016	Amino sugar and nucleotide sugar metabolism
Glycated valine	ESI+	296.06	0.71	3.72%	4.2	0.0004	
Glycyl-tyrosine	ESI+	239.10	0.86	5.26%	0.7	0.0058	
Homoserine	ESI+	120.06	0.73	5.65%	1.3	0.0094	Cysteine and methionine metabolism; Lysine biosynthesis; Glycine, serine and threonine metabolism
L-homoserine lactone	ESI+	102.05	0.72	3.97%	1.4	0.0005	Cysteine and methionine metabolism; Lysine biosynthesis; Glycine, serine and threonine metabolism
GlcNAc	ESI+	222.09	0.76	3.49%	2.5	<0.0001	Amino sugar and nucleotide sugar metabolism
N-Acetyl-L-methionine	ESI+	192.06	2.91	2.53%	0.6	0.0128	
NMN	ESI+	335.06	0.74	7.44%	2.2	0.0005	Nicotinate and nicotinamide metabolism
2-Aminoadipic acid	ESI+	162.07	0.81	1.75%	1.8	0.0149	Lysine biosynthesis;Lysine degradation
Nε,Nε,Nε-Trimethyl-lysine	ESI+	189.15	0.66	6.11%	2.4	0.0080	Lysine degradation
Phenylacetyl-L-glutamine	ESI+	265.11	3.48	2.47%	0.5	0.0480	Phenylalanine metabolism
SAM	ESI+	399.14	0.64	17.2%	6.8	<0.0001	Cysteine and methionine metabolism; Arginine and proline metabolism
Trigonelline	ESI+	138.04	0.82	2.33%	1.2	0.0199	Nicotinate and nicotinamide metabolism
Tryptophan	ESI+	205.09	2.46	3.85%	1.2	0.0324	Tryptophan Metabolism
UDP-GlcNAc	ESI+	608.09	0.8	4.73%	4.9	0.0004	Amino sugar and nucleotide sugar metabolism
uric acid	ESI+	169.03	0.79	3.09%	0.8	0.0094	Purine metabolism
uridine	ESI+	245.07	0.85	8.14%	1.5	0.0000	Pyrimidine metabolism
CDCA	ESI-	391.28	18.5	11.4%	0.7	0.0042	Primary bile acid biosynthesis
DHEA sulfate	ESI-	367.15	14.4	4.66%	0.7	0.0264	Steroid hormone biosynthesis

D-Pantothenic acid	ESI-	218.10	2.51	4.63%	1.6	0.0014	Pantothenate and CoA biosynthesis; beta-Alanine metabolism
Etiocholanolone sulfate	ESI-	369.17	15.8	5.50%	0.7	0.0160	Steroid hormone biosynthesis
Fumaric acid	ESI-	115.00	0.83	6.13%	1.7	0.0049	Nicotinate and nicotinamide metabolism; Alanine,aspartate and glutamate metabolism; Phenylalanine metabolism
GCDCA	ESI-	448.30	18.1	4.21%	1.4	0.0264	Primary bile acid biosynthesis
Glycerol 3-phosphate	ESI-	171.00	0.84	1.65%	3.0	0.0005	Glycerophospholipid metabolism; Glycerolipid metabolism
Sphingosine	ESI+	300.29	13.34	2.43%	2.6	0.0007	Sphingolipid metabolism
Lactic acid	ESI-	89.02	0.95	1.60%	1.3	0.0102	Pyruvate metabolism; TCA cycle; Propanoate metabolism
L-Saccharopine	ESI-	275.12	0.88	4.39%	3.0	0.0007	Lysine biosynthesis; Lysine degradation
GlcNAc-6-P	ESI-	300.04	0.86	2.32%	2.1	0.0247	Amino sugar and nucleotide sugar metabolism
N-Acetylglutamic acid	ESI-	188.05	0.85	3.38%	0.4	0.0021	
N-Acetylaspartic acid	ESI-	174.04	0.85	3.42%	2.0	0.0016	Alanine,aspartate and glutamate metabolism
NAD	ESI-	662.10	3.02	57.3%	27.3	0.0019	Nicotinate and nicotinamide metabolism
NADP	ESI-	744.08	1.12	8.66%	5.1	0.0029	Nicotinate and nicotinamide metabolism
Nonanoic acid	ESI-	157.12	14.0	3.21%	1.3	0.0199	
O-succinylhomoserine	ESI-	218.06	0.88	6.38%	1.7	0.0422	Cysteine and methionine metabolism; Sulfur metabolism
p-Cresol sulfate	ESI-	187.00	7.10	3.55%	0.8	0.0396	
SAH	ESI-	383.11	4.53	2.02%	1.4	0.0005	Cysteine and methionine metabolism
Succinic acid	ESI-	117.02	0.84	3.26%	2.2	0.0214	Alanine,aspartate and glutamate metabolism; (TCA cycle); Phenylalanine metabolism; Propanoate metabolism; Butanoate metabolism
UDP-glucose	ESI-	565.04	0.89	9.30%	3.0	0.0214	Glycerolipid metabolism; Amino sugar and nucleotide sugar metabolism, Starch and sucrose metabolism
carnitine	ESI+	162.1128	0.72	3.65%	2.0	0.0025	Lysine biosynthesis; Lysine degradation; Fatty acid β-oxidation
carnitine C10:0	ESI+	316.2468	10.1	4.47%	0.3	0.0005	Fatty acid β-oxidation
carnitine C14:1	ESI+	370.2951	12.8	5.22%	0.6	0.0032	Fatty acid β-oxidation
carnitine C14:2	ESI+	368.2801	11.9	2.46%	0.3	0.0004	Fatty acid β-oxidation
carnitine C16:1	ESI+	398.3277	14.1	8.77%	0.8	0.0283	Fatty acid β-oxidation
carnitine C16:2	ESI+	396.3094	13.3	6.01%	0.4	0.0004	Fatty acid β-oxidation
carnitine C18:0	ESI+	428.375	16.3	4.01%	3.0	0.0068	Fatty acid β-oxidation
carnitine C4:0-OH	ESI+	248.1489	0.86	10.7%	1.9	0.0006	Fatty acid β-oxidation

carnitine C5:0-OH	ESI+	262.1651	0.87	16.1%	4.7	0.0013	Fatty acid β-oxidation
carnitine C6:0	ESI+	260.186	5.09	3.86%	0.7	0.0019	Fatty acid β-oxidation
carnitine C6:0-OH	ESI+	276.1818	2.70	2.72%	4.0	0.0002	Fatty acid β-oxidation
carnitine C8:0	ESI+	288.2169	7.92	2.73%	0.3	<0.0001	Fatty acid β-oxidation
carnitine C8:1	ESI+	286.2017	6.62	1.73%	0.6	<0.0001	Fatty acid β-oxidation

Table S3. Significantly altered genes in cysteine and methionine metabolism, NAD metabolism, hexosamine pathway, lysine degradation and fatty acid β-oxidation.

Genes	*p*	PCT/ANT ratio	Protein	Metabolic pathway	Target microRNA
DNMT3A	0.0299	1.3	DNA (cytosine-5-)-methyltransferase 3 alpha	Cysteine and methionine metabolism	hsa-miR-29c-3p ↑, hsa-miR-143-3p↓
DNMT1	0.0455	1.2	DNA (cytosine-5-)-methyltransferase 1	Cysteine and methionine metabolism	hsa-miR-152↓, hsa-miR-26a-5p↓, hsa-miR-148a-3p↑, hsa-miR-100-5p↓
DNMT3B	0.0004	1.7	DNA (cytosine-5-)-methyltransferase 3 beta	Cysteine and methionine metabolism	hsa-miR-29c-3p ↑, hsa-miR-375↑, hsa-miR-148a-3p↑, hsa-miR-93-5p↑
AHCY	0.0011	1.8	Adenosylhomocysteinase	Cysteine and methionine metabolism	hsa-miR-26a-5p↓
CBS	<0.0001	3.3	Cystathionine-beta-synthase	Cysteine and methionine metabolism	hsa-miR-615-3p↑, hsa-miR-106b-3p↑
GNMT	0.0006	6.4	Glycine N-methyltransferase	Cysteine and methionine metabolism	hsa-miR-100-5p↓
PNP	0.0129	1.8	Purine nucleoside phosphorylase	Cysteine and methionine metabolism	hsa-miR-1↓
CHDH	0.0222	1.6	Choline dehydrogenase	Cysteine and methionine metabolism	
ALDH7A1	0.0017	1.9	Aldehyde dehydrogenase 7 family, member A1	Cysteine and methionine metabolism	hsa-let-7a-5p↑
BHMT2	0.0003	0.5	Betaine-homocysteine S-methyltransferase 2	Cysteine and methionine metabolism	
NAMPT	0.0240	1.9	Nicotinamide phosphoribosyltransferase	NAD metabolism	
NADK2	0.0004	1.6	NAD kinase 2, mitochondrial	NAD metabolism	
HK2	0.0012	1.9	Hexokinase 2	Hexosamine pathway	hsa-miR-143-3p↓
GFPT1	0.0002	2.0	Glutamine-fructose-6-phosphate transaminase 1	Hexosamine pathway	
GNPNAT1	0.0001	2.3	Glucosamine-phosphate N-acetyltransferase 1	Hexosamine pathway	hsa-miR-1↓
PGM3	0.0177	1.6	Phosphoglucomutase 3	Hexosamine pathway	hsa-miR-125a-5p↓, hsa-miR-455-3p↓
UAP1	<0.0001	3.4	UDP-N-acetylglucosamine pyrophosphorylase 1	Hexosamine pathway	
OGT	0.0039	1.5	O-linked N-acetylglucosamine transferase	Hexosamine pathway	hsa-miR-26a-5p↓, hsa-miR-125a-5p↓
AASS	0.0014	1.4	Aminoadipate-semialdehyde synthase	Lysine degradation	hsa-let-7d-5p↓, hsa-miR-944↓, hsa-miR-7-1-3p↑, hsa-let-7e-5p↓
AASDH	0.0015	1.5	Aminoadipate-semialdehyde dehydrogenase	Lysine degradation	
AADAT	0.0018	2.1	Aminoadipate aminotransferase	Lysine degradation	
TMLHE	0.0072	0.7	Trimethyllysine hydroxylase, epsilon	Lysine degradation	
ACAD8	0.0002	2.9	Acyl-CoA dehydrogenase family, member 8	Fatty acid β-oxidation	hsa-let-7a-5p↑
ACAD9	0.0101	1.5	Acyl-CoA dehydrogenase family, member 9	Fatty acid β-oxidation	
ACAD10	0.0455	1.3	Acyl-CoA dehydrogenase family, member 10	Fatty acid β-oxidation	
ACADSB	0.0032	1.8	Acyl-CoA dehydrogenase, short/branched chain	Fatty acid β-oxidation	
CPT2	0.0101	1.5	Carnitine palmitoyltransferase 2	Fatty acid β-oxidation	hsa-miR-378a-3p↓
CPT1A	0.0093	1.6	Carnitine palmitoyltransferase 1A	Fatty acid β-oxidation	
CPT1C	0.0039	0.6	Carnitine palmitoyltransferase 1C	Fatty acid β-oxidation	hsa-miR-615-3p↓
ACAT1	0.0015	2.2	Acetyl-CoA acetyltransferase 1	Fatty acid β-oxidation	hsa-miR-23b-3p↓

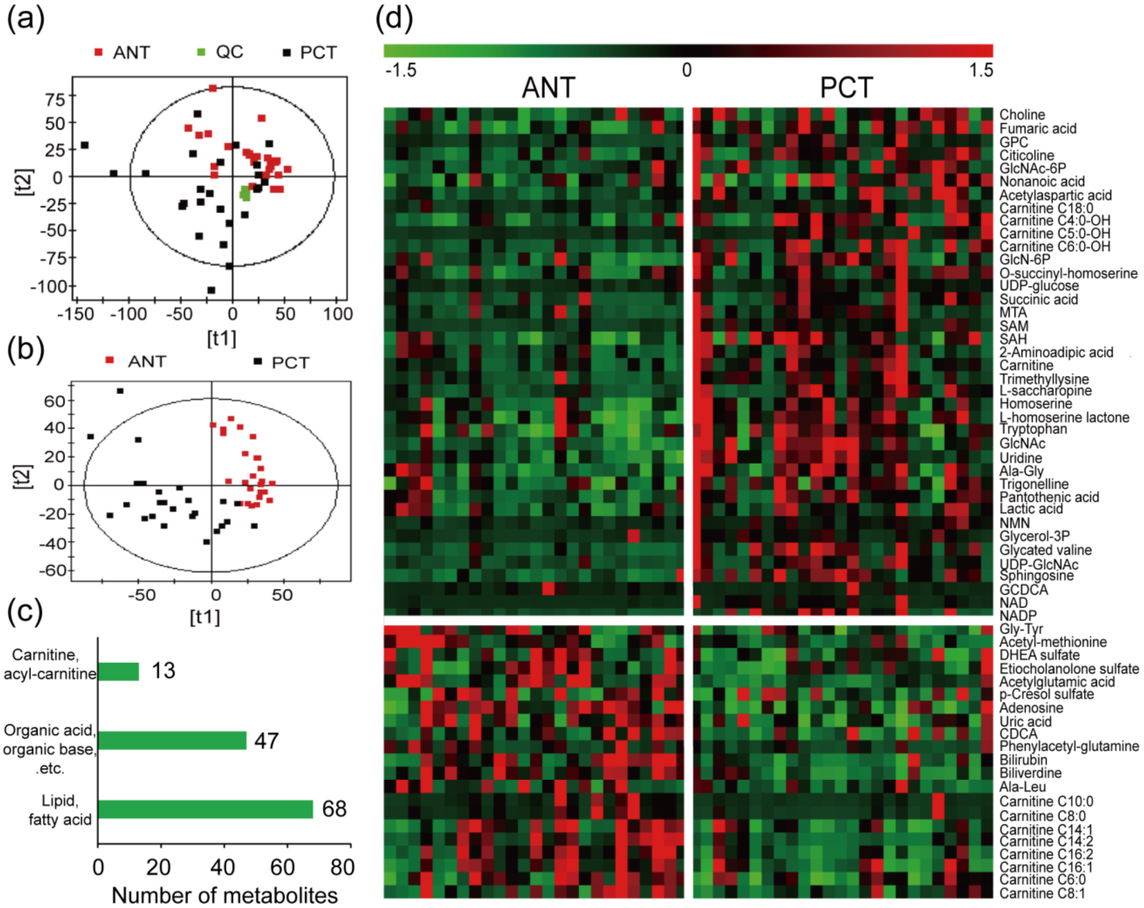

Figure S1. Multivariate statistical analysis and differential metabolites of prostate cancer tissue and noncancerous tissue. (a) Score scatter plot of PCA model. R2X=0.272, Q2=0.154. (b) Score scatter plot of PLS-DA model. R2X=0.237, R2Y=0.768, Q2=0.567. (c) Distribution of all of differential metabolites in various kinds of compounds. (d) Relative level of differential metabolites in PCT and ANT. PCT: Prostate cancer tissue, ANT: Adjacent noncancerous tissue.

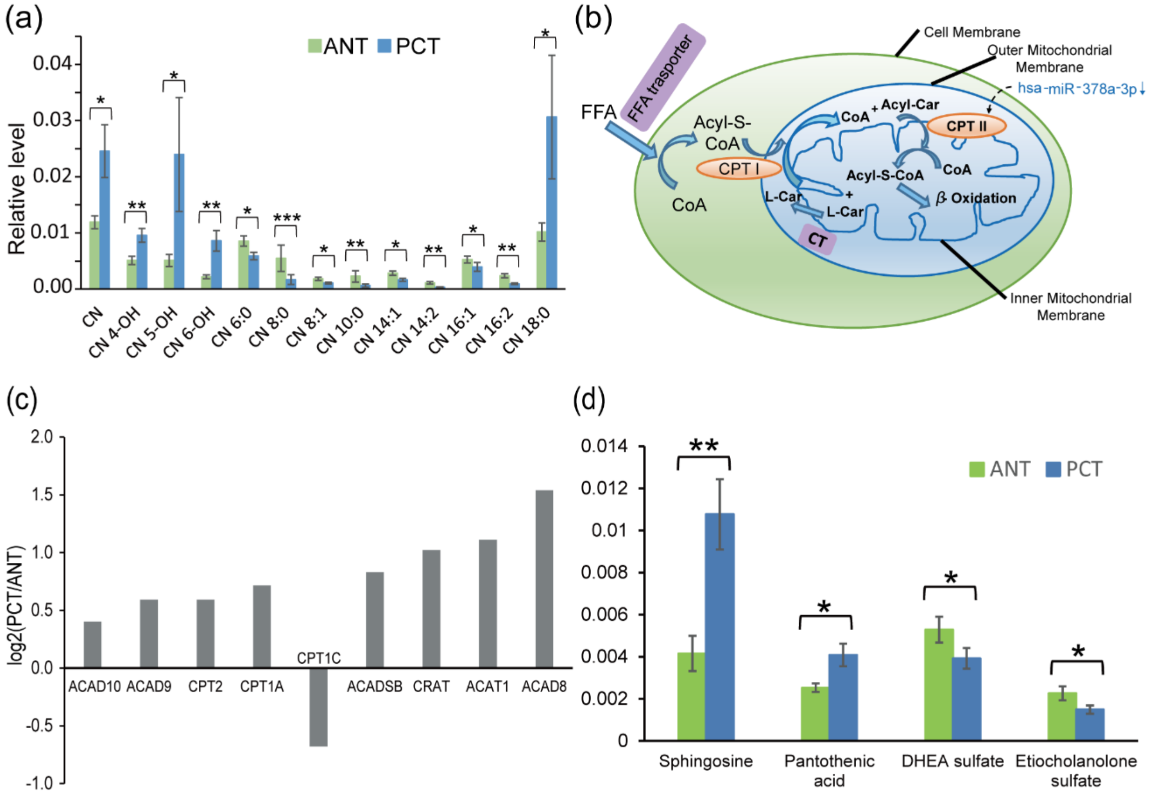

Figure S2. Molecular alterations in prostate cancer. (a) Relative levels of carnitine and acylcarnitine in PCT and ANT. *: a significant difference, $0.001<p<0.05$, **: $0.0001<p<0.001$, ***: $p<0.0001$. (b) The important role of acylcarnitine in fatty acid β-oxidation. (c) The alteration of genes associated with fatty acid β-oxidation. (d) Relative levels of sphingosine, pantothenic acid, DHEA sulfate and etiocholanolone sulfate in PCT versus ANT.