

Rhein Improves Renal Fibrosis by Restoring Cpt1a-Mediated Fatty Acid Oxidation through SirT1/STAT3/Twist1 Pathway

Xianrui Song ¹, Zesen Du ¹, Zhenqi Yao ¹, Xiaoyan Tang ¹ and Mian Zhang ^{1,2,*}

¹ School of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing 211198, China

² Mian Zhang, School of Traditional Chinese Medicine, China Pharmaceutical University, No.639 Longmian Road, Nanjing 211198, China

* herbs@cpu.edu.cn; Tel.: +86-2586185137

Supplemental materials

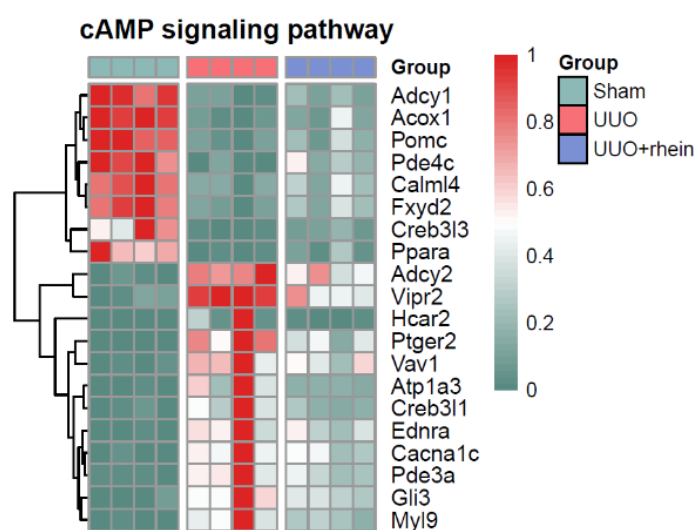


Figure S1. Heatmap of RNA sequencing genes associated with cAMP signaling pathway ($n = 4$).

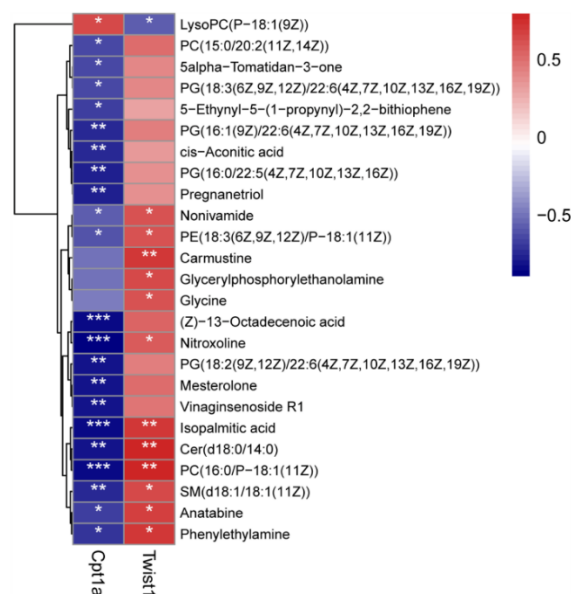


Figure S2. Heatmap of the relationship between Twist1 & Cpt1a genes and differential metabolites.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, gene correlated metabolites.

Table S1. Primers for q-PCR

Primers	Direction	Sequences
Acot1	Forward	5'-ACCGCTTTTGGTTTACA-3'
	Reverse	5'-GATTGAACCCAGGAACTT-3'
Acta2	Forward	5'-CACTACCATGTACCCAGGCA-3'
	Reverse	5'-CATATAGCGGTGGACAATGGA-3'
Col1a1	Forward	5'-CAGGCTGGTGTGATGGGATT-3'
	Reverse	5'-CACCTCGTTCTCCAGCCTTT-3'
Col2a1	Forward	5'-CGCTCAAGTCGCTGAACAACCA-3'
	Reverse	5'-ACCAGTTCTTCCGACGCACAGT-3'
Col3a1	Forward	5'-AGTGGCCATAATGGGGAACG-3'
	Reverse	5'-CAG GGTTCCTATCCCTTCCG-3'
Col4a1	Forward	5'-GTGCGGTTTGTGAAGCACCG-3'
	Reverse	5'-GTTCTTCTCATGCACACTTC-3'
Cpt1a	Forward	5'-ACCCAGTCAGATTCCAACCA-3'
	Reverse	5'-TCAAGAAAGGCACTCCCCT-3'
Cpt1b	Forward	5'-CATGTATCGCCGCAAACCTGG-3'
	Reverse	5'-CCTGGGATGCGTGTAGTGTT-3'
Cpt1c	Forward	5'-GGATGGCACTGAAGAGGAAA-3'
	Reverse	5'-TCCTGGAAAAGGCATCTCTC-3'
Cpt2	Forward	5'-GCCCCAAACCCCATTTTCTA-3'
	Reverse	5'-TAGGCAGAGGCAGAAGACAGCA-3'
Decr1	Forward	5'-GTACGGCAATGGCTTTATCA-3'
	Reverse	5'-CAATCCCCTCCTGCAACTT-3'
E-cadherin	Forward	5'-AGGTCGGTGCCCGTATTG-3'
	Reverse	5'-TGGTCTTGGGGTCTGTGATG-3'
Fn	Forward	5'-GTTGGTTGCCCTGTTCTGC-3'
	Reverse	5'-GGCTACCTGTGTTTCCCTTTG-3'
Twist1	Forward	5'-CGCACCCAGTCGCTGAACG-3'
	Reverse	5'-CCGCCAGTTTGAGGGTCTGAATC-3'
TGF- β	Forward	5'-CATGGAGCTGGTGAAACGGAAG-3'
	Reverse	5'-GACTGGCGAGCCTTAGTTTGGAC-3'
Vimentin	Forward	5'-TCAGACAGGATGTTGACAAT-3'
	Reverse	5'-GACATGCTGTTCTGAATCT-3'
β -actin	Forward	5'-CCGAGGGCCCACTAAAGG-3'
	Reverse	5'-GCTGTTGAAGTCACAGGAGACAA-3'

Table S2. KEGG analysis of transcriptome data

Description	q-value
Cytokine-cytokine receptor interaction	7.43E-21
Viral protein interaction with cytokine and cytokine receptor	2.92E-14
Cell adhesion molecules	6.10E-13
PI3K-Akt signaling pathway	2.90E-12
Hematopoietic cell lineage	5.95E-11
Chemokine signaling pathway	1.49E-09
Dilated cardiomyopathy	1.81E-09
ECM-receptor interaction	2.37E-09
Osteoclast differentiation	5.60E-09
Hypertrophic cardiomyopathy	1.57E-08

TNF signaling pathway	2.37E−08
MAPK signaling pathway	2.74E−08
Arrhythmogenic right ventricular cardiomyopathy	2.90E−08
Toxoplasmosis	6.04E−08
JAK-STAT signaling pathway	7.69E−08
Amoebiasis	1.05E−07
Complement and coagulation cascades	1.46E−07
Epstein-Barr virus infection	1.82E−07
Human papillomavirus infection	1.86E−07
Focal adhesion	2.93E−07
Rap1 signaling pathway	3.17E−07
Platelet activation	4.43E−07
Human T-cell leukemia virus 1 infection	1.61E−06
NF-kappa B signaling pathway	1.61E−06
Malaria	1.99E−06
Leishmaniasis	2.09E−06
Measles	2.53E−06
Small cell lung cancer	3.69E−06
AGE-RAGE signaling pathway in diabetic complications	3.93E−06
Th17 cell differentiation	3.93E−06
Chagas disease	3.93E−06
Coronavirus disease – COVID-19	7.08E−06
Kaposi sarcoma-associated herpesvirus infection	8.51E−06
Th1 and Th2 cell differentiation	8.51E−06
Ras signaling pathway	1.21E−05
Pertussis	1.21E−05
Viral myocarditis	1.49E−05
Tuberculosis	1.49E−05
Protein digestion and absorption	1.63E−05
Rheumatoid arthritis	2.00E−05
Legionellosis	2.11E−05
Inflammatory bowel disease	2.32E−05
Valine, leucine and isoleucine degradation	2.80E−05
Phagosome	3.75E−05
Human cytomegalovirus infection	3.75E−05
Relaxin signaling pathway	4.06E−05
Yersinia infection	4.82E−05
Salmonella infection	5.62E−05
Leukocyte transendothelial migration	5.62E−05
PD-L1 expression and PD-1 checkpoint pathway in cancer	1.17E−04
Parathyroid hormone synthesis, secretion and action	1.41E−04
Influenza A	1.41E−04
Toll-like receptor signaling pathway	1.48E−04
Primary immunodeficiency	1.60E−04
Glyoxylate and dicarboxylate metabolism	1.70E−04
C-type lectin receptor signaling pathway	2.06E−04
Circadian entrainment	2.39E−04
Transcriptional misregulation in cancer	2.81E−04
Gap junction	3.37E−04
Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan	3.49E−04

sulfate	
Axon guidance	3.49E−04
T cell receptor signaling pathway	4.68E−04
Tryptophan metabolism	5.49E−04
Hepatitis B	5.61E−04
Hepatitis C	6.71E−04
Lipid and atherosclerosis	6.80E−04
Apoptosis	8.34E−04
Proteoglycans in cancer	8.34E−04
Intestinal immune network for IgA production	9.20E−04
NOD-like receptor signaling pathway	9.25E−04
cAMP signaling pathway	9.52E−04
Cysteine and methionine metabolism	9.77E−04
Regulation of actin cytoskeleton	1.09E−03
Oxytocin signaling pathway	1.18E−03
Prostate cancer	1.18E−03
Staphylococcus aureus infection	1.22E−03
Glycine, serine and threonine metabolism	1.62E−03
Lysine degradation	1.78E−03
Adrenergic signaling in cardiomyocytes	1.96E−03
Fc gamma R-mediated phagocytosis	1.96E−03
Alcoholic liver disease	2.09E−03
Natural killer cell mediated cytotoxicity	2.26E−03
B cell receptor signaling pathway	2.53E−03
Amphetamine addiction	2.82E−03
Viral carcinogenesis	3.18E−03
Cocaine addiction	3.18E−03
Breast cancer	3.22E−03
Cell cycle	5.23E−03
Hepatocellular carcinoma	5.23E−03
Glutamatergic synapse	5.69E−03
Notch signaling pathway	6.45E−03
Insulin secretion	6.87E−03
Cellular senescence	7.09E−03
Phospholipase D signaling pathway	7.17E−03
Morphine addiction	7.18E−03
Cholinergic synapse	8.19E−03
Glioma	1.04E−02
Vascular smooth muscle contraction	1.08E−02
IL-17 signaling pathway	1.09E−02
cGMP-PKG signaling pathway	1.09E−02
Acute myeloid leukemia	1.13E−02
ABC transporters	1.15E−02
Cushing syndrome	1.25E−02
Fluid shear stress and atherosclerosis	1.25E−02
Other types of O-glycan biosynthesis	1.26E−02
Colorectal cancer	1.32E−02
Mucin type O-glycan biosynthesis	1.33E−02
Thyroid hormone synthesis	1.51E−02
EGFR tyrosine kinase inhibitor resistance	1.58E−02

Endocrine resistance	1.69E−02
Inflammatory mediator regulation of TRP channels	1.73E−02
DNA replication	1.73E−02
Human immunodeficiency virus 1 infection	1.75E−02
GABAergic synapse	1.75E−02
Salivary secretion	1.81E−02
Apelin signaling pathway	1.88E−02
Chronic myeloid leukemia	1.91E−02
Cortisol synthesis and secretion	1.94E−02
Melanogenesis	1.99E−02
Gastric acid secretion	1.99E−02
Pancreatic cancer	1.99E−02
Adherens junction	2.10E−02
One carbon pool by folate	2.10E−02
2-Oxocarboxylic acid metabolism	2.10E−02
Basal cell carcinoma	2.10E−02
Estrogen signaling pathway	2.14E−02
Fatty acid degradation	2.16E−02
Antifolate resistance	2.16E−02
Calcium signaling pathway	2.32E−02
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Gastric cancer	2.52E−02

Table S3. Thirty-four differential metabolites identified in positive and negative mode.**In positive mode**

NO	[M+H] ⁺ (m/z)	Delta (ppm)	Formula	Metabolites	VIP	P
1	99.0650	−9.33	C ₅ H ₁₀ NO	2-Piperidinone	1.7567385022	0.0128659156
2	101.1195	−9.43	C ₆ H ₁₆ N	Hexylamine	2.0379096022	0.0023337061
3	111.0427	−4.94	C ₄ H ₆ N ₃ O	Cytosine	2.0418150497	0.0010972827
4	121.0883	−7.34	C ₈ H ₁₂ N	Phenylethylamine	1.9814597721	0.0024521583
5	160.0991	−6.23	C ₁₀ H ₁₃ N ₂	Anatabine	1.7979543685	0.0053108897
6	162.0671	−6.28	C ₁₀ H ₁₁ O ₂	4,5-Dihydro-1-benzoxepin-3(2H)-one	2.3499237288	0.0000092619
7	188.1285	6.10	C ₇ H ₁₇ N ₄ O ₂	L-Targinine	1.6074156932	0.0156388593
8	190.0366	−6.65	C ₉ H ₇ N ₂ O ₃	Nitroxoline	1.2314601400	0.0471657634
9	215.0550	−3.86	C ₅ H ₁₅ NO ₆ P	Glycerylphosphorylethanolamine	1.6064902294	0.0165914871
10	220.1089	−4.70	C ₁₃ H ₁₇ O ₃	1-(5-Acetyl-2-hydroxyphenyl)-3-methyl-1-butanone	2.3521427735	0.0000073518
11	293.1979	−3.96	C ₁₇ H ₂₈ NO ₃	Nonivamide	2.4139510116	0.0000004355
12	336.2651	−3.91	C ₂₁ H ₃₇ O ₃	Pregnanetriol	1.6838470507	0.0054941678
13	413.3291	−0.56	C ₂₇ H ₄₄ NO ₂	5alpha-Tomatidan-3-one	1.5118790876	0.0139911382
14	505.3514	−3.65	C ₂₆ H ₅₃ NO ₆ P	LysoPC(P-18:1(9Z))	1.9615013500	0.0004286134
15	511.4947	−3.32	C ₃₂ H ₆₆ NO ₃	Cer(d18:0/14:0)	1.3676883747	0.0433282614
16	545.3443	−7.11	C ₂₈ H ₅₃ NO ₇ P	LysoPC(20:3(5Z,8Z,11Z))	1.8926985265	0.0011333993
17	723.5180	−3.16	C ₄₁ H ₇₅ NO ₇ P	PE(18:3(6Z,9Z,12Z)/P-18:1(11Z))	2.3330819395	0.0000051317
18	728.5802	−4.09	C ₄₁ H ₈₂ N ₂ O ₆ P	SM(d18:1/18:1(11Z))	1.4632555185	0.0264426424
19	743.5798	−4.12	C ₄₂ H ₈₃ NO ₇ P	PC(16:0/P-18:1(11Z))	1.5551483320	0.0170014236
20	771.5752	−3.44	C ₄₃ H ₈₃ NO ₈ P	PC(15:0/20:2(11Z,14Z))	1.9614160117	0.0008837868

In negative mode

NO	[M-H] [−] (m/z)	Delta (ppm)	Formula	Metabolites	VIP	P
1	774.5362	−6.28	C ₄₂ H ₇₈ O ₁₀ P	PG(18:0/18:2(9Z,12Z))	1.9623653742	0.0009273760
2	282.2534	−8.81	C ₁₈ H ₃₃ O ₂	(Z)-13-Octadecenoic acid	1.9108915324	0.0010083556
3	792.4869	−9.14	C ₄₄ H ₇₂ O ₁₀ P	PG(16:1(9Z)/22:6 (4Z,7Z,10Z,13Z,16Z,19Z))	2.0001715309	0.0010713927
4	912.5654	−8.14	C ₄₉ H ₈₄ O ₁₃ P	PI(18:0/22:5(4Z,7Z,10Z,13Z,16Z))	2.0956677860	0.0002078235
5	796.5192	−7.89	C ₄₄ H ₇₅ O ₁₀ P	PG(16:0/22:5(4Z,7Z,10Z,13Z,16Z))	1.4884584386	0.0174221265
6	842.5034	0.77	C ₄₄ H ₇₃ O ₁₅	Vinaginsenoside R1	1.5139760286	0.0193148061
7	816.4863	−9.61	C ₄₆ H ₇₂ O ₁₀ P	PG(18:3(6Z,9Z,12Z)/22:6 (4Z,7Z,10Z,13Z,16Z,19Z))	1.7761854290	0.0031726938
8	228.0051	−7.22	C ₁₃ H ₇ S ₂	5-Ethynyl-5'-(1-propynyl)- 2,2'-bithiophene	1.2755898001	0.0495846592
9	304.2378	−8.00	C ₂₀ H ₃₁ O ₂	Mesterolone	1.3987605779	0.0261059783
10	174.0170	3.40	C ₆ H ₅ O ₆	cis-Aconitic acid	1.4947798644	0.0234651146
11	213.0073	0.71	C ₅ H ₈ Cl ₂ N ₃ O ₂	Carmustine	1.4687272903	0.0179947660
12	818.5037	−7.38	C ₄₆ H ₇₄ O ₁₀ P	PG(18:2(9Z,12Z)/22:6 (4Z,7Z,10Z,13Z,16Z,19Z))	1.5661563684	0.0119455349
13	111.0427	−4.94	C ₂ H ₄ NO ₂	Glycine	1.4715910009	0.0206321526
14	256.2379	−8.97	C ₁₆ H ₃₁ O ₂	Isopalmitic acid	1.4663757188	0.0240594117