A Multi-Omics Study Revealing the Metabolic Effects of Estrogen in Liver Cancer Cells HepG2

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Supplemental Tables

Supplemental Tables 1-5. Effects of estradiol, $ER\alpha$ agonist PPT, and $ER\beta$ agonist DPN on gene expression detected via RNA sequencing and KEGG pathway analysis.

Genome functional	Group	P Value	Adjusted	# Total	#	Associated Genes
pathways			P Value	Hits	Hits	
Carbohydrate digestion	Group	3.7E-8	1.1E-7	15	13	[AMY1A, AMY1B, AMY1C, AMY2A,
and absorption	3					AMY2B, ATP1A1, ATP1B2, FXYD2, G6PC,
						HK1, MGAM, MGAM2, SLC2A5]
Starch and sucrose					11	[AMY1A, AMY1B, AMY1C, AMY2A,
metabolism						AMY2B, G6PC, GBE1, GYS1, HK1,
						MGAM, MGAM2]
Complement and	Group	3.2E-6	6.5E-6	14	14	[C3, C4A, C4B, C7, C8B, F11, F5, ITGB2,
coagulation cascades	2					KLKB1, PLG, SERPINE1, SERPING1, VTN,
						VWF]
HIF-1 signaling pathway	Group	5.9E-5	5.9E-5	14	14	[CAMK2A, EDN1, EGFR, EGLN1, EGLN3,
	1					ENO2, ENO3, EPO, HK1, IGF1R, PDK1,
						PFKFB3, SERPINE1, VEGFA]

Supplemental Table 1. Upregulated genes and pathways by estradiol comparing to control treatment.

Supplemental Table 2. Downregulated genes and pathways by estradiol comparing to control treatment.

Genome functional pathways	Group	P Value	Adjuste d P Value	# Total Hits	# Hits	Associated Genes
Ascorbate and aldarate metabolism	Group 4	2.7E-10	1.0E-9	23	13	[UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B41
Pentose and glucuronate interconversions	_				14	[AKR1B10, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Retinol metabolism	_				17	[ADH4, ADH6, ALDH1A1, RDH5, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Metabolism of xenobiotics by cytochrome P450	_				17	[ADH4, ADH6, GSTA1, GSTA2, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Drug metabolism	_				14	[TK1, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Steroid hormone biosynthesis	_				15	[AKR1D1, SULT1E1, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Chemical carcinogenesis	_				17	[ADH4, ADH6, GSTA1, GSTA2, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Porphyrin and chlorophyll metabolism	_				13	[UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B11, UGT2B28, UGT2B4]
Cell cycle	Group3	1.7E-4	3.4E-4	14	12	[BUB1, CCNA2, CCNB1, CCNB2, CDC20, CDC25A, CDC25C, CDK1, CDKN2C, PLK1, PTTG1, TTK]
Progesterone-mediated oocyte maturation	_				9	[BUB1, CCNA2, CCNB1, CCNB2, CDC25A, CDC25C, CDK1, PIK3R3, PLK1]
Oocyte meiosis					10	[AURKA, BUB1, CCNB1, CCNB2, CDC20, CDC25C, CDK1, FBXO5, PLK1, PTTG1]
Glycine, serine and threonine metabolism	Group2	2.1E-5	6.5E-5	7	7	[BPGM, CTH, DAO, GLDC, PHGDH, PSAT1, SHMT2]
p53 Signaling pathway	Group1	7.4E-4	7.4E-4	7	7	[CCNB1, CCNB2, CDK1, GTSE1, RRM2, SESN2, SESN3]

Genome functional pathways	Group	P Value	Adjuste d P Value	# Total Hits	# Hits	Associated Genes
Steroid hormone	Group	1 3E-6	9 7E-6	18	15	[AKR1D1, CYP17A1, CYP7A1, SUILT1E1, UGT1A1,
biosynthesis	8	1.01 0)., <u>L</u> 0	10	10	UGT1A10. UGT1A3. UGT1A4. UGT1A5. UGT1A6.
biosyntheois	0					UGT1A7, UGT1A8, UGT1A9, UGT2B10, UGT2B111
Ascorbate and					11	[UGT1A1_UGT1A10_UGT1A3_UGT1A4_UGT1A5
aldarate						UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10
metabolism						UGT2B11]
Pentose and					11	[UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5,
glucuronate						UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B10,
interconversions						UGT2B111
Drug metabolism					12	ITK1 LIGT1A1 LIGT1A10 LIGT1A3 LIGT1A4
Drug metabolism					12	LIGT1A5 LIGT1A6 LIGT1A7 LIGT1A8 LIGT1A9
						UGT2B10 UGT2B111
Porphyrin and					11	[UGT1A1 UGT1A10 UGT1A3 UGT1A4 UGT1A5
chlorophyll					11	UGT1A6 UGT1A7 UGT1A8 UGT1A9 UGT2B10
metabolism						UGT2B111
Retinol					12	IRDH5 LIGT1A1 LIGT1A10 LIGT1A3 LIGT1A4
metabolism					14	UGT1A5 UGT1A6 UGT1A7 UGT1A8 UGT1A9
inclubonom						UGT2B10, UGT2B111
Metabolism of					12	IGSTM2, UGT1A1, UGT1A10, UGT1A3, UGT1A4
xenobiotics by						UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9,
cvtochrome P450						UGT2B10, UGT2B111
<u>Chemical</u>					12	IGSTM2 UGT1A1 UGT1A10 UGT1A3 UGT1A4
carcinogenesis						UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9,
						UGT2B10, UGT2B111
Cell cycle	Group7	3.8E-11	3.0E-10	25	22	[BUB1, BUB1B, CCNA2, CCNB1, CCNB2, CDC20,
5	1					CDC25C, CDC45, CDK1, CDKN2C, CHEK1, E2F1,
						MAD2L1, MCM2, MCM5, ORC1, PCNA, PLK1, PTTG1,
						SKP2, TGFB3, TTK]
Oocyte meiosis					12	[AURKA, BUB1, CCNB1, CCNB2, CDC20, CDC25C,
,						CDK1, FBXO43, MAD2L1, PLK1, PTTG1, SGO1]
Progesterone-					8	[BUB1, CCNA2, CCNB1, CCNB2, CDC25C, CDK1,
mediated oocyte						MAD2L1, PLK1]
maturation						
Butanoate	Group6	3.1E-3	3.1E-3	4	4	[ACSM2A, ACSM2B, ACSM3, HMGCS2]
metabolism	_					
Primary bile acid	Group5	4.4E-4	1.7E-3	4	4	[AKR1D1, BAAT, CYP7A1, CYP8B1]
biosynthesis						
Complement and	Group4	8.6E-6	5.1E-5	10	10	[C6, C7, C8A, C8B, CFI, CPB2, F13B, MBL2, SERPINA5,
coagulation						SERPIND1]
cascades						
p53 signaling	Group3	1.9E-5	9.6E-5	9	9	[CCNB1, CCNB2, CDK1, CHEK1, DDB2, GTSE1,
pathway						RRM2, SESN3, TP73]

Supplemental Table 3. Downregulated genes and pathways by PPT comparing to control treatment.

Fanconi anemia	Group2 1.	.2E-3	2.5E-3	6	6	[BLM, BRIP1, FANCB, FANCD2, RAD51, RMI2]
pathway						
PPAR signaling pathway	Group1 1.	.0E-3	3.1E-3	7	7	[ACSL6, ANGPTL4, CYP7A1, CYP8B1, HMGCS2, SORBS1, UCP1]

Supplemental Table 4. Upregulated genes and pathways by DPN comparing to control treatment.

Genome functional	Group	Р	Adjuste	#	#	Associated Genes
pathways		Value	d P	Total	Hits	
			Value	Hits		
Hematopoietic cell lineage	Group	1.9E-3	3.9E-3	6	6	[CD22, CD9, IL11, IL6R, ITGA2,
	3					ITGA3]
Complement and	Group	6.8E-4	2.0E-3	6	6	[C5AR1, C8G, ITGAX, PROCR,
coagulation cascades	2					SERPINE1, SERPING1]
HIF-1 signaling pathway	Group	2.4E-3	2.4E-3	6	6	[EGFR, EGLN3, IL6R, NOS3,
	1					SERPINE1, TIMP1]

Genome functional	Group	Group	Adjuste	#	#	Associated Genes
pathways		Р	d P	Total	Hits	
		Value	Value	Hits		
DNA replication	Group	2.9E-6	1.7E-5	9	6	[LIG1, MCM2, MCM5, MCM7,
	_7					POLD1, POLE]
Base excision repair					4	[LIG1, NEIL3, POLD1, POLE]
Nucleotide excision					4	[DDB2, LIG1, POLD1, POLE]
repair						
Mismatch repair					3	[EXO1, LIG1, POLD1]
Cell cycle	Group	9.1E-17	6.3E-16	26	21	[BUB1, BUB1B, CCNA2, CCNB1,
	6					CCNB2, CDC20, CDC25B,
						CDC25C, CDC45, CDK1, CDKN2C,
						E2F1, ESPL1, MAD2L1, MCM2,
						MCM5, MCM7, ORC1, PLK1,
						PTTG1, TTK]
Oocyte meiosis					14	[AURKA, BUB1, CCNB1, CCNB2,
						CDC20, CDC25C, CDK1, ESPL1,
						FBXO43, FBXO5, MAD2L1, PLK1,
						PTTG1, SGO1]
Progesterone-mediated					9	[BUB1, CCNA2, CCNB1, CCNB2,
oocyte maturation						CDC25B, CDC25C, CDK1,
						MAD2L1, PLK1]
Homologous	Group	8.2E-5	3.2E-4	7	5	[BLM, BRIP1, POLD1, RAD51,
recombination	_5					RAD54L]
Fanconi anemia					5	[BLM, BRIP1, FANCD2, RAD51,
pathway						RMI2]
p53 Signaling pathway	Group	2.8E-5	1.4E-4	7	7	[CCNB1, CCNB2, CDK1, DDB2,
	4					GTSE1, RRM2, TP73]
Bladder cancer	Group	1.6E-2	1.6E-2	3	3	[E2F1, RPS6KA5, UPK3A]
	3					
HTLV-I infection	Group	1.1E-4	3.3E-4	12	12	[BUB1B, CCNB2, CDC20,
	2					CDKN2C, E2F1, IL15, MAD2L1,
						MYBL1, MYBL2, POLD1, POLE,
						PTTG1]
Steroid hormone	Group	6.7E-3	1.3E-2	4	4	[AKR1D1, CYP17A1, HSD11B2,
biosynthesis	1					SULT1E1]

Supplemental Table 5. Downregulated genes and pathways by DPN comparing to control treatment.

Supplemental Tables 6-8. Effects of estradiol, $ER\alpha$ specific agonist PPT, and $ER\beta$ specific agonist DPN on metabolites detected via metabolite profiling and KEGG pathway analysis.

			<u> </u>	Adjusted P	1 0	
Metabolic pathways	Total	Hits	P Value	value	FDR	Impact
Tryptophan metabolism	41	8	8.09E-05	3.56E-03	3.05E-03	0.3450
Taurine and hypotaurine metabolism	8	3	1.91E-04	8.21E-03	3.05E-03	0.7140
Histidine metabolism	16	2	3.46E-04	1.42E-02	3.05E-03	0.3110
Tyrosine metabolism	42	10	3.47E-04	1.42E-02	3.05E-03	0.3460
Purine metabolism	65	5	5.52E-04	2.15E-02	3.70E-03	0.0968
Amino sugar and nucleotide sugar						
metabolism	37	5	6.76E-04	2.57E-02	3.70E-03	0.1160
One carbon pool by folate	9	1	7.56E-04	2.80E-02	3.70E-03	0.7920
Glycine, serine and threonine metabolism	33	9	8.80E-04	3.08E-02	3.87E-03	0.1290
Pyruvate metabolism	22	4	1.39E-03	4.73E-02	4.55E-03	0.2380
Citrate cycle (TCA cycle)	20	4	1.39E-03	4.73E-02	4.55E-03	0.1200
Arginine and proline metabolism	38	7	1.41E-03	4.73E-02	4.55E-03	0.1700
Glycolysis / Gluconeogenesis	26	2	1.45E-03	4.73E-02	4.55E-03	0.2060

Supplemental Table 6. Altered metabolites and pathways by estradiol comparing to control treatment.

				Adjusted P		
Metabolic pathways	Total	Hits	P Value	value	FDR	Impact
Tyrosine metabolism	42	10	1.04E-11	4.57E-10	4.57E-10	0.3460
Glycerophospholipid						
metabolism	36	4	2.77E-08	1.19E-06	4.03E-07	0.0872
Taurine and hypotaurine						
metabolism	8	3	3.66E-08	1.50E-06	4.03E-07	0.7143
Amino sugar and nucleotide						
sugar metabolism	37	5	2.42E-07	9.69E-06	2.13E-06	0.1159
Vitamin B6 metabolism	9	3	7.61E-07	2.97E-05	5.58E-06	0.6471
Pyrimidine metabolism	39	6	1.81E-06	6.89E-05	1.14E-05	0.2146
Tryptophan metabolism	41	8	1.03E-05	3.81E-04	3.74E-05	0.3448
Cysteine and methionine						
metabolism	33	4	1.07E-05	3.81E-04	3.74E-05	0.3272
Purine metabolism	65	5	1.07E-05	3.81E-04	3.74E-05	0.0968
Pyruvate metabolism	22	4	1.10E-05	3.81E-04	3.74E-05	0.2379
Citrate cycle (TCA cycle)	20	4	1.10E-05	3.81E-04	3.74E-05	0.1203
Histidine metabolism	16	2	1.89E-05	5.66E-04	5.54E-05	0.3115
Nicotinate and nicotinamide						
metabolism	15	3	2.21E-05	6.41E-04	6.08E-05	0.5671
Glycine, serine and threonine						
metabolism	33	9	4.39E-05	1.19E-03	1.07E-04	0.1288
Glycolysis / Gluconeogenesis	26	2	5.65E-05	1.47E-03	1.27E-04	0.2059
Glutathione metabolism	28	5	6.40E-05	1.53E-03	1.30E-04	0.0872
Arginine and proline						
metabolism	38	7	6.50E-05	1.53E-03	1.30E-04	0.1701
Aminoacyl-tRNA biosynthesis	48	2	9.00E-05	1.98E-03	1.72E-04	0.0556
beta-Alanine metabolism	21	3	9.43E-05	1.98E-03	1.73E-04	0.1045
Glyoxylate and dicarboxylate						
metabolism	32	4	2.30E-04	4.38E-03	3.90E-04	0.2990
Alanine, aspartate and						
glutamate metabolism	28	4	3.65E-04	6.37E-03	5.56E-04	0.0889
Pentose and glucuronate						
interconversions	18	1	3.66E-04	6.37E-03	5.56E-04	0.0781
Starch and sucrose metabolism	18	2	6.68E-04	1.00E-02	9.80E-04	0.4304
Arginine biosynthesis	14	3	7.79E-04	1.09E-02	1.11E-03	0.2893
Caffeine metabolism	10	2	1.81E-03	2.17E-02	2.41E-03	0.6923

Supplemental Table 7. Altered metabolites and pathways by PPT comparing to control treatment.

				Adjusted		
Metabolic pathways	Total	Hits	P Value	P value	FDR	Impact
Tyrosine metabolism	42	10	2.75E-11	1.18E-09	6.06E-10	0.3460
Glycerophospholipid metabolism	36	4	1.59E-10	6.69E-09	2.34E-09	0.0872
Taurine and hypotaurine metabolism	8	3	2.97E-10	1.22E-08	3.27E-09	0.7143
Tryptophan metabolism	41	8	5.50E-09	2.20E-07	4.56E-08	0.3448
Amino sugar and nucleotide sugar						
metabolism	37	5	6.22E-09	2.43E-07	4.56E-08	0.1159
Purine metabolism	65	5	1.06E-08	4.04E-07	6.56E-08	0.0968
Vitamin B6 metabolism	9	3	1.19E-08	4.41E-07	6.56E-08	0.6471
Glutathione metabolism	28	5	2.04E-08	7.34E-07	9.37E-08	0.0872
beta-Alanine metabolism	21	3	2.13E-08	7.45E-07	9.37E-08	0.1045
Cysteine and methionine metabolism	33	4	2.83E-08	9.61E-07	1.13E-07	0.3272
Arginine and proline metabolism	38	7	8.17E-08	2.61E-06	2.63E-07	0.1701
Pyrimidine metabolism	39	6	9.76E-08	2.93E-06	2.81E-07	0.2146
Aminoacyl-tRNA biosynthesis	48	2	1.02E-07	2.96E-06	2.81E-07	0.0556
Glycolysis / Gluconeogenesis	26	2	1.87E-07	5.25E-06	4.85E-07	0.2059
Glycine, serine and threonine						
metabolism	33	9	2.11E-07	5.69E-06	5.15E-07	0.1288
Pyruvate metabolism	22	4	3.62E-07	9.40E-06	7.96E-07	0.2379
Citrate cycle (TCA cycle)	20	4	3.62E-07	9.40E-06	7.96E-07	0.1203
Nicotinate and nicotinamide metabolism	15	3	5.11E-07	1.23E-05	1.07E-06	0.5671
Arginine biosynthesis	14	3	5.61E-07	1.29E-05	1.12E-06	0.2893
Starch and sucrose metabolism	18	2	8.88E-07	1.87E-05	1.63E-06	0.4304
Alanine, aspartate and glutamate						
metabolism	28	4	1.89E-06	3.59E-05	3.20E-06	0.0889
Histidine metabolism	16	2	2.37E-06	4.18E-05	3.72E-06	0.3115
Glyoxylate and dicarboxylate						
metabolism	32	4	3.92E-06	5.90E-05	5.75E-06	0.2990
Caffeine metabolism	10	2	6.22E-06	8.70E-05	8.82E-06	0.6923
Pentose and glucuronate						
interconversions	18	1	7.58E-06	9.86E-05	1.04E-05	0.0781
One carbon pool by folate	9	1	1.68E-05	1.70E-04	2.06E-05	0.7921
Inositol phosphate metabolism	30	1	7.15E-05	5.01E-04	8.07E-05	0.1294
Biotin metabolism	10	1	2.17E-04	8.67E-04	2.32E-04	0.2000

Supplemental Table 8. Altered metabolites and pathways by DPN comparing to control treatment.

Supplemental Figures

Supplemental Figure S1. Western blot analysis detected protein expression of ER α (66 kDa), ER β (56 kDa), and a housekeeping protein β -actin (45 kDa) in HepG2 cells used in this study. The gel blot image shows protein bands and a protein ladder as the molecular size marker.



Supplemental Figure S2.

Representative images of HepG2 cells treated with 2-deoxy-D-glucose, oxamate, and oligomycin. HepG2 cells treated with vehicle DMSO (Control), 2-deoxy-D-glucose (5 and 10 mM), oxamate (5, 10, and 50 mM), or oligomycin (0.1, 0.5, and 1.0 µg/ml) were evaluated using light microscopy (10 × magnification). Bars = 50 µm.



Control



Oxamate 5 mM





Oxamate 10 mM



2-deoxy-D-glucose 5 mM 2-deoxy-D-glucose 10 mM



Oxamate 50 mM



Oligomycin 0.1 µg/ml



Oligomycin 0.5 µg/ml



Oligomycin 1.0 µg/ml

Supplemental Figure S3. Heatmap presentation of metabolic profiles comparing relative concentrations of detected 174 metabolites from HepG2 cells treated with control, estradiol (E2), ER α specific agonist PPT, and ER β specific agonist DPN. Each column represents one biological replicate, and each row represents one targeted metabolite detected.



Supplemental Figure 4. Gene-metabolite interaction maps.

Gene-metabolite interaction maps showing the effect of estradiol (S4A), $ER\alpha$ specific agonist PPT (S4B), and $ER\beta$ specific agonist DPN (S4C) on the transcriptome and metabolome of HepG2 cells using significantly differentially expressed genes identified by RNA-Seq analysis and their significantly altered metabolites detected by targeted metabolomics. Each square note represents one metabolite, and each round note represents one gene. The size of the nodes is proportional to their degree values, signifying the number of connections it has to other nodes. Nodes with higher node degree act as hubs in a network. The color of nodes reflects their betweenness centrality values, the number of shortest paths, or interactions going through the node.

Supplemental Figure 4A. Gene-metabolite interaction maps showing the effect of estradiol.



Supplemental Figure 4B. Gene-metabolite interaction maps showing the effect of $ER\alpha$ specific agonist PPT.



Supplemental Figure 4C. Gene-metabolite interaction maps showing the effect of $ER\beta$ specific agonist DPN.

