

Supplementary Information

Enriched Environment Inhibits Mouse Pancreatic Cancer Growth and Down-regulates the Expression of Mitochondria-related Genes in Cancer Cells

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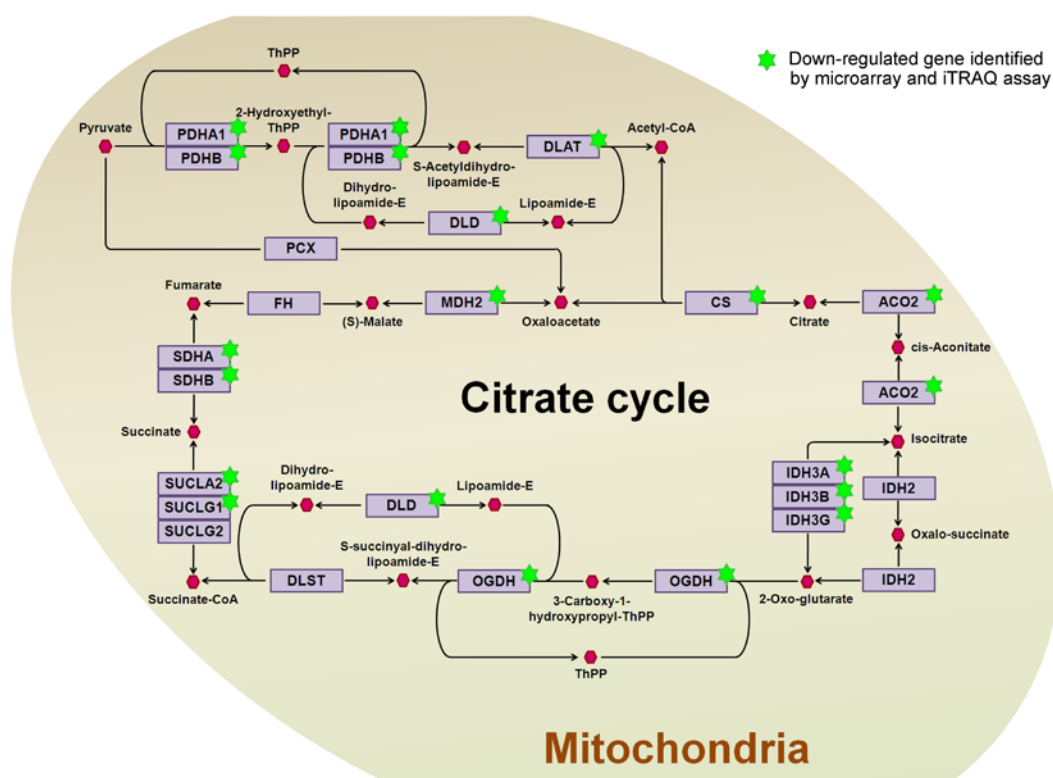
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Supplementary Figure S1 | Summary of the down-regulated genes in the citrate cycle pathway induced by enriched environment housing. The green hexagrams indicate the down-regulated genes identified both by the microarray and by the iTRAQ assay in this study.

Supplementary Table S1 | Differentially expressed proteins identified by iTRAQ assay

Supplementary Table S2 | Fold-changes for the differentially expressed genes involved in the citrate cycle and oxidative phosphorylation pathways

Gene symble	Gene name	Pathway	Microarray ratio (EE:SE)	iTRAQ ratio (EE:SE)
Idh3b	Isocitrate dehydrogenase 3 (NAD ⁺) beta	Citrate cycle	0.156	0.099
Idh3g	Isocitrate dehydrogenase 3 (NAD ⁺), gamma	Citrate cycle	0.361	0.099
Suclg1	Succinate-CoA ligase, GDP-forming, alpha subunit	Citrate cycle	0.359	0.103
Dlat	Dihydrolipoamide S-acetyltransferase	Citrate cycle	0.328	0.107
Pdha1	Pyruvate dehydrogenase E1 alpha 1	Citrate cycle	0.332	0.114
Aco2	Aconitase 2, mitochondrial	Citrate cycle	0.283	0.117
Idh3a	Isocitrate dehydrogenase 3 (NAD ⁺) alpha	Citrate cycle	0.230	0.128
Sucla2	Succinate-Coenzyme A ligase, ADP-forming, beta subunit	Citrate cycle	0.227	0.136
Ogdh	Oxoglutarate dehydrogenase (lipoamide)	Citrate cycle	0.281	0.142
Cs	Citrate synthase	Citrate cycle	0.362	0.146
Dld	Dihydrolipoamide dehydrogenase	Citrate cycle	0.452	0.211
Pdhb	Pyruvate dehydrogenase (lipoamide) beta	Citrate cycle	0.380	0.236
Mdh2	Malate dehydrogenase 2, NAD (mitochondrial)	Citrate cycle	0.393	0.261
Mdh1	Malate dehydrogenase 1, NAD (soluble)	Citrate cycle	0.489	0.575
Aco1	Aconitase 1	Citrate cycle	0.334	0.603
Sdha	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	Citrate cycle & OXPHOS	0.329	0.131
Sdhb	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	Citrate cycle & OXPHOS	0.219	0.560
Ndufs2	NADH dehydrogenase (ubiquinone) Fe-S protein 2	OXPHOS	0.420	0.043
Ndufab1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1	OXPHOS	0.318	0.069
Uqcrc2	Ubiquinol cytochrome c reductase core protein 2	OXPHOS	0.409	0.072
Uqcrb	Ubiquinol-cytochrome c reductase binding protein	OXPHOS	0.413	0.104

Supplementary Table S2 | Fold-changes for the differentially expressed genes involved in the citrate cycle and oxidative phosphorylation pathways (continued)

Gene symble	Gene name	Pathway	Microarray ratio (EE:SE)	iTRAQ ratio (EE:SE)
Uqcrh	Ubiquinol-cytochrome c reductase hinge protein	OXPHOS	0.494	0.177
Ndufs1	NADH dehydrogenase (ubiquinone) Fe-S protein 1	OXPHOS	0.348	0.256
Uqcrc1	Ubiquinol-cytochrome c reductase core protein 1	OXPHOS	0.294	0.283
Ndufv2	NADH dehydrogenase (ubiquinone) flavoprotein 2	OXPHOS	0.335	0.296
Cox5a	Cytochrome c oxidase, subunit Va	OXPHOS	0.339	0.302
Ndufa9	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9	OXPHOS	0.444	0.310
Ndufs3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	OXPHOS	0.356	0.313
Atp5a1	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	OXPHOS	0.454	0.356
Atp5b	ATP synthase, H ⁺ transporting mitochondrial F1 complex, beta subunit	OXPHOS	0.414	0.373
Ndufs6	NADH dehydrogenase (ubiquinone) Fe-S protein 6	OXPHOS	0.383	0.429
Cox5b	Cytochrome c oxidase, subunit Vb	OXPHOS	0.414	0.437
Uqcrrf1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	OXPHOS	0.256	0.453
Ndufa10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	OXPHOS	0.37	0.565
Cox6c	Cytochrome c oxidase, subunit VIc	OXPHOS	0.496	0.592
Ndufv1	NADH dehydrogenase (ubiquinone) flavoprotein 1	OXPHOS	0.336	0.625

EE: enriched environment; OXPHOS: oxidative phosphorylation; SE: standard environment.

Supplementary Table S3 | Primers used in quantitative RT-PCR experiments

Gene symbol	Full name	Primers
Postn	Periostin, osteoblast specific factor	Forward: 5'-ACTCTGAGCATGGACCAACG Reverse: 5'-CTGCTTGCTTCCTCTCACCA
Anxa1	Annexin A1	Forward: 5'-ACTCCAGCTTTCTTTGCCGA Reverse: 5'-CAATTTCCGAACGGGAGACC
Krt8	Keratin 8	Forward: 5'-GGTGTCTGGGGGCATCACAG Reverse: 5'-CCACTTGGTCTCCAGCATCTT
Col6a3	Collagen, type VI, alpha 3	Forward: 5'-CAGGCATTGCCACGACTCAT Reverse: 5'-TTCCCAGCACTCCAAGAGGA
Uqcrc1	Ubiquinol-cytochrome c reductase core protein 1	Forward: 5'-TCAGCATCTTGGACAACGGG Reverse: 5'-CAGTCTCATAGCGACTGCCA
Idh3b	Isocitrate dehydrogenase 3 (NAD+) beta	Forward: 5'-GAGCAGGTGCTGAGTTCCAT Reverse: 5'-TGTGCCGAGTCTTGTATCCAG
Pdha1	Pyruvate dehydrogenase E1 alpha 1	Forward: 5'-AAGATGCTTGCCGCTGTATCC Reverse: 5'-ATCCTCTCTGGTGAGCACTGT
Suclg1	Succinate-CoA ligase, GDP-forming, alpha subunit	Forward: 5'-TCAGCAGGCTTTGGAGTACG Reverse: 5'-AGCCGTTGCTCCTGTTTTCT
Hk2	Hexokinase 2	Forward: 5'-GGTTTCTCTATTTGGCCCCGA Reverse: 5'-CATCTGAGAGACGCATGTGGT
Acaa2	Acetyl-Coenzyme A acyltransferase 2	Forward: 5'-GGACTTCTCTGCCACCGATT Reverse: 5'-CCAGGTATGCCGCATCTGAA
Sdha	Succinate dehydrogenase complex, subunit A,	Forward: 5'-ATGGAAAATGGGGAGTGCCG Reverse: 5'-ACAGCTGAAGTAGGTTCCGC
Actb	Actin, beta	Forward: 5'-ATGTGGATCAGCAAGCAGGA Reverse: 5'-AAAGGGTGTAACGCAGCTC