

Beyond fatty acid oxidation: Comprehensive functional profiling reveals FABP12-driven mitochondrial remodeling in prostate cancer

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Introduction

Mitochondrial metabolism has re-emerged as a central determinant of cancer progression, therapeutic resistance, and metastatic potential [1]. Although oxidative phosphorylation (OXPHOS) drives mitochondrial energy production, its supporting pathways are integrated with the broader metabolic networks influencing biosynthetic precursor generation, redox homeostasis, and signaling. Yet, functional characterization of mitochondrial metabolism remains largely reductionist. Most studies interrogate limited mitochondrial pathways or rely on transcriptomic or metabolomic signatures to infer function. While informative, these approaches may fail to capture metabolic heterogeneity across cancer models, clones, tumor regions, and patients. High-resolution respirometry (HRR) can bridge this gap by directly assessing multiple oxidation pathways that define the mitochondrial metabolic phenotype.

As a proof-of-concept, we applied this strategy to metastatic prostate cancer (PCa) cells expressing fatty acid-binding protein 12 (FABP12), which is preferentially amplified and/or overexpressed in metastatic tumors and xenograft models [2]. FABP12 promotes epithelial-to-mesenchymal transition, cell motility, and invasion while increasing fatty acid uptake and lipid accumulation. Measurements in intact cells, including inhibition with etomoxir, further suggested that FABP12 promotes fatty acid β -oxidation (FAO) through PPAR γ activation [2]. We therefore used permeabilized cells and an expanded range of substrate protocols to uncover previously hidden effects of FABP12 on mitochondrial metabolism.

Methods

A comprehensive panel of high-resolution respirometry protocols was used to interrogate major mitochondrial entry pathways contributing to OXPHOS. Experiments were performed using PC3 stable clonal populations of pREP4 cells and two pREP4-FABP12-expressing clones [2]. Given the prominent role of lipid metabolism in PCa [3,4], particular emphasis was placed on FAO. Unless otherwise specified, conditions for measurement followed [5]. Substrate-specific protocols included pyruvate (5 mM) with malate (2 mM, PM); glutamate (10 mM) alone (G) or with malate (2 mM, GM);

malate (2 mM, M); 2-oxoglutarate (10 mM, Og); glycerophosphate (10 mM, Gp); acetylcarnitine (5 mM, ALCAR); octanoate (2 mM, C8:0); octanoyl-CoA (120 μ M, C8:0-CoA); octanoylcarnitine (0.2 mM, C8:0-car); palmitoyl-CoA (20 μ M, C16:0-CoA); palmitoylcarnitine (10 μ M, C16:0-car); arachidonoylcarnitine (5 μ M, C20:4-car). Acyl-CoA substrates were tested with carnitine (0.5 mM) and M (0.1 mM); and acylcarnitines and octanoate with M (0.1 mM). For each protocol, ROUTINE respiration was measured in intact cells before addition of the pathway-specific substrate(s) and plasma permeabilization with digitonin (5 μ g $\cdot$ 10⁻⁶ cells). LEAK respiration was measured in the absence of adenylates, followed by ADP-induced substrate-specific OXPHOS. Respiration was then complemented sequentially with NADH-linked substrates (malate, pyruvate, glutamate) and succinate (10 mM). Rotenone (0.5 μ M) and antimycin A (2.5 μ M) were subsequently added to determine residual oxygen consumption. Cytochrome c oxidase (Complex IV; CIV) was assessed with ascorbate (2 mM) and *N,N,N',N'*-Tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD; 0.5 mM), followed by azide (100 mM) to determine the chemical background. Citrate synthase activity was measured as a marker of mitochondrial content [2]. RNA sequencing was performed and processed by Novogene Corporation Inc.

Results and discussion

FABP12 expression increased OXPHOS capacity per cell together with mitochondrial content. To distinguish pathway utilization from mitochondrial abundance, respiration was expressed as flux control ratios (FCRs) relative to maximal OXPHOS capacity. Comprehensive substrate profiling revealed strongly substrate-dependent remodeling beyond FAO (Fig. 1). OXPHOS FCRs increased in both FABP12 clones with GM, Og, ALCAR, C8:0, C8:0-car, and C16:0-car, with similar trends for M and G. LEAK also increased with M, PM, G, Og, ALCAR, C8:0, C8:0-car, and C16:0-car. Importantly, OXPHOS increased to an equivalent or greater extent than LEAK, indicating that enhanced proton conductance alone cannot explain the OXPHOS phenotype.

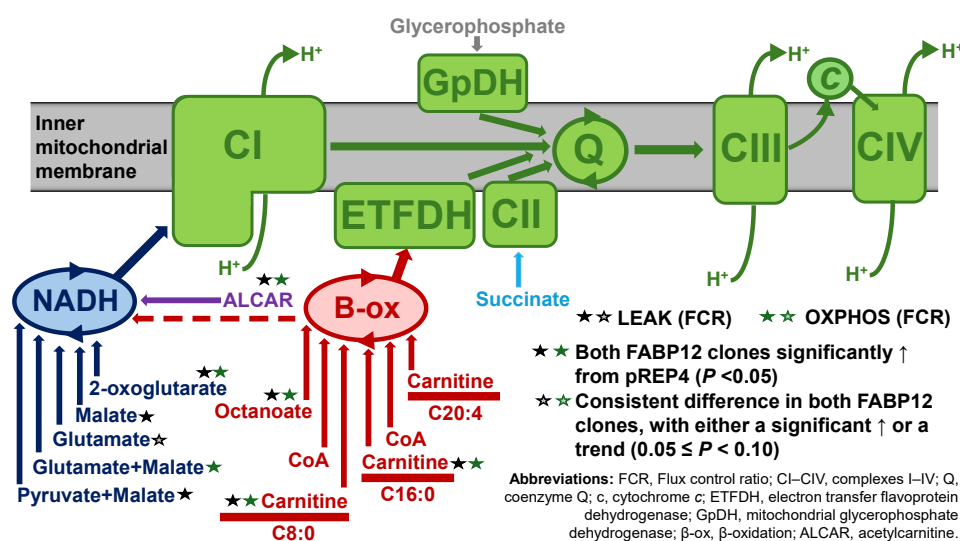


Fig. 1. FABP12-associated changes in mitochondrial respiration. Substrate-specific differences in LEAK and OXPHOS respiration, expressed as FCRs, are mapped to their respective entry pathways. One-way ANOVA with Holm-Sidak or and Kruskal-Wallis with Dunn's test; $n = 3-9$.

Increased respiration across substrates entering mitochondrial metabolism through distinct routes indicates broader remodeling of substrate handling and matrix oxidative metabolism rather than a uniform increase in respiratory capacity. Increased G/GM/Og oxidation points toward enhanced glutamate/2-oxoglutarate metabolism, potentially involving mitochondrial transport, transamination/GDH, and/or OGDH control, despite unchanged OGDH expression. Increased respiration with C16:0-car but not C16:0-CoA suggests enhanced capacity downstream of CPT1 that remains masked when CPT1-dependent entry is required. Similarly, increased respiration with C8:0 and C8:0-car, but not C8:0-CoA, implicates substrate delivery, activation, or compartmentation. Unchanged C20:4-car further demonstrates that FABP12 does not uniformly enhance acylcarnitine or FAO utilization.

Greater substrate oxidation may also contribute to the increased LEAK. Enhanced uncoupling alongside increased oxidative capacity could limit excessive proton-motive force and redox pressure under elevated electron supply, potentially constraining ROS formation. Consistent with this possibility, RNA-sequencing identified increased UCP2 expression in FABP12 cells, whereas adenine nucleotide translocases and the 2-oxoglutarate carrier were unchanged. Whether UCP2 contributes to this substrate-dependent coupling remains to be established.

Together, these findings extend the established role of FABP12 in lipid metabolism [2] to selective remodeling of mitochondrial substrate oxidation beyond FAO. Given the link between FABP12 and PPAR γ activation [2], FABP12 may drive broader mitochondrial metabolic reprogramming, integrating pathway-specific changes in substrate oxidation with altered respiratory coupling.

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Keywords

FABP12; prostate cancer; mitochondrial metabolism; high-resolution respirometry; OXPHOS pathways

Author contributions

HL and RG conceived and designed the study. NW, ML, and CB performed the experiments. NW, ML, CB, and HL analyzed the data. HL prepared the figure and wrote the manuscript. RG reviewed the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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