

# **The role of PGC-1 $\alpha$ in breast cancer liver metastasis**

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## List of Abbreviations

| Abbreviation         | Full Term                                                    |
|----------------------|--------------------------------------------------------------|
| 2792                 | Liver-tropic murine breast cancer cell line                  |
| <i>3830403N18Rik</i> | Uncharacterized predicted gene (mouse genome)                |
| 4T1                  | Murine mammary carcinoma cell line (ATCC CRL-2539)           |
| <i>Adgrl3</i>        | Adhesion G Protein-Coupled Receptor L3                       |
| Alb-Cre <sup>+</sup> | Albumin-Cre positive (hepatic Cre expression)                |
| Alb-Cre <sup>-</sup> | Albumin-Cre negative (no hepatic Cre expression; control)    |
| AMP                  | Adenosine monophosphate                                      |
| AMPK                 | AMP-activated protein kinase                                 |
| <i>Apc2</i>          | APC Regulator of WNT Signaling Pathway 2                     |
| <i>Arhgdib</i>       | Gene encoding Rho GDP-dissociation inhibitor beta            |
| ATP                  | Adenosine triphosphate                                       |
| AUP                  | Animal Use Protocol                                          |
| BALB/c               | Inbred albino mouse strain                                   |
| BAM15                | Mitochondria-targeted protonophore (mitochondrial uncoupler) |
| BCAA                 | Branched-chain amino acids                                   |
| β-oxidation          | Fatty acid beta-oxidation (see also FAO)                     |
| BRAF                 | B-Raf proto-oncogene, serine/threonine kinase                |
| CaMKIV               | Calcium/calmodulin-dependent protein kinase IV               |
| cAMP/PKA             | Cyclic adenosine monophosphate/protein kinase A              |
| <i>Cask</i>          | Calcium/calmodulin-dependent serine protein kinase 1         |
| <i>Cav1</i>          | Caveolin-1                                                   |
| CCAC                 | Canadian Council on Animal Care                              |
| <i>Ccnd2</i>         | Cyclin D2                                                    |
| CDK4/6               | Cyclin-dependent kinase 4 and 6                              |
| cDNA                 | Complementary DNA                                            |
| clusterProfiler      | R package for gene set enrichment analysis                   |
| CO <sub>2</sub>      | Carbon dioxide                                               |
| CoA                  | Coenzyme A                                                   |

|              |                                                                                           |
|--------------|-------------------------------------------------------------------------------------------|
| CREB         | cAMP response element-binding protein                                                     |
| CTCs         | Circulating tumor cells                                                                   |
| DAPI         | 4',6-diamidino-2-phenylindole                                                             |
| DESeq2       | Differential expression analysis package                                                  |
| DMEM         | Dulbecco's Modified Eagle's Medium                                                        |
| dMRM         | Dynamic multiple reaction monitoring                                                      |
| Dsp          | Desmoplakin                                                                               |
| ECAR         | Extracellular acidification rate                                                          |
| EMT          | Epithelial-mesenchymal transition                                                         |
| ER           | Estrogen receptor                                                                         |
| ERBB2        | Erb-b2 receptor tyrosine kinase 2 (human gene encoding the HER2 protein)                  |
| ErbB2/Neu    | Erb-b2 receptor tyrosine kinase 2 / Neu oncogene (murine mammary tumor model designation) |
| ERR $\alpha$ | Estrogen-related receptor alpha                                                           |
| ESI          | Electrospray ionization                                                                   |
| ETC          | Electron transport chain                                                                  |
| EV           | Empty vector                                                                              |
| FACC         | Facility Animal Care Committee                                                            |
| FAO          | Fatty acid oxidation (see also $\beta$ -oxidation)                                        |
| FastQC       | Sequence quality control software                                                         |
| FBS          | Fetal bovine serum                                                                        |
| FCCP         | Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone                                      |
| FDR          | False discovery rate                                                                      |
| FOXO1        | Forkhead box protein O1                                                                   |
| GCN5         | General control non-derepressible 5 (histone acetyltransferase; also known as KAT2A)      |
| GeneRatio    | Proportion of gene set members detected at maximum enrichment score                       |
| GFP          | Green fluorescent protein                                                                 |

|                               |                                                      |
|-------------------------------|------------------------------------------------------|
| Gls2                          | Glutaminase 2                                        |
| GLUT3 (SLC2A3)                | Glucose transporter 3                                |
| GO: BP                        | Gene Ontology Biological Process                     |
| GRCm39                        | Genome Reference Consortium Mouse Build 39           |
| GSEA                          | Gene Set Enrichment Analysis                         |
| H&E                           | Hematoxylin and eosin                                |
| H <sub>2</sub> O              | Water                                                |
| H <sub>2</sub> O <sub>2</sub> | Hydrogen peroxide                                    |
| HCl                           | Hydrochloric acid                                    |
| HEPES                         | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid   |
| HER2                          | Human epidermal growth factor receptor 2             |
| HIF-1 $\alpha$                | Hypoxia-inducible factor 1-alpha                     |
| HMDB                          | Human Metabolome Database                            |
| HNF4 $\alpha$                 | Hepatocyte nuclear factor 4 alpha                    |
| HR <sup>+</sup>               | Hormone receptor-positive                            |
| HSCs                          | Hepatic stellate cells                               |
| IACS-010759                   | Mitochondrial Complex I inhibitor                    |
| J-ATP                         | ATP production rate (J-ATP-OXPHOS, J-ATP-Glycolysis) |
| KEGG                          | Kyoto Encyclopedia of Genes and Genomes              |
| KO                            | Knockout                                             |
| LC-MS/MS                      | Liquid Chromatography–Tandem Mass Spectrometry       |
| LSECs                         | Liver sinusoidal endothelial cells                   |
| MAPK                          | Mitogen-activated protein kinase                     |
| MDA-MB-231                    | Human breast cancer cell line                        |
| MFP                           | Mammary fat pad                                      |
| min                           | Minutes                                              |
| MITF                          | Microphthalmia-associated transcription factor       |
| MMPs                          | Matrix metalloproteinases                            |
| MOI                           | Multiplicity of infection                            |
| mpH                           | Milli-pH units (ECAR unit)                           |

|                  |                                                                                    |
|------------------|------------------------------------------------------------------------------------|
| mRNA             | Messenger RNA                                                                      |
| MSEA             | Metabolite Set Enrichment Analysis                                                 |
| MTHFD            | Methylenetetrahydrofolate dehydrogenase                                            |
| N <sub>2</sub>   | Molecular nitrogen                                                                 |
| NaCl             | Sodium chloride                                                                    |
| Na <sup>+</sup>  | Sodium ion                                                                         |
| NAD <sup>+</sup> | Nicotinamide adenine dinucleotide (oxidized form)                                  |
| NADH             | Nicotinamide adenine dinucleotide (reduced form)                                   |
| NADPH            | Nicotinamide adenine dinucleotide phosphate                                        |
| NBF              | Neutral buffered formalin                                                          |
| Ndufa9           | NADH: ubiquinone oxidoreductase subunit A9 (Complex I)                             |
| Ndufb5           | NADH: ubiquinone oxidoreductase subunit B5 (Complex I)                             |
| NF-κB            | Nuclear factor kappa-light-chain-enhancer of activated B cells                     |
| NK cells         | Natural killer cells                                                               |
| NRF1             | Nuclear respiratory factor 1                                                       |
| NRF2             | Nuclear factor erythroid 2-related factor 2                                        |
| NRF2/GABPA       | Nuclear respiratory factor 2/GA-binding protein transcription factor subunit alpha |
| OCR              | Oxygen consumption rate                                                            |
| OE               | Overexpression                                                                     |
| OXPHOS           | Oxidative phosphorylation                                                          |
| p38 MAPK         | p38 mitogen-activated protein kinase                                               |
| PBS              | Phosphate-buffered saline                                                          |
| PC1, PC2         | Principal component 1 / 2                                                          |
| PCA              | Principal component analysis                                                       |
| PCR              | Polymerase chain reaction                                                          |
| PDK1             | Pyruvate dehydrogenase kinase 1                                                    |
| PGC-1α           | Peroxisome proliferator-activated receptor gamma coactivator 1-alpha               |
| <i>PPRC1</i>     | Human PRC-encoding gene                                                            |
| <i>PPARGC1A</i>  | Human PGC-1α-encoding gene                                                         |

|                                |                                                                                  |
|--------------------------------|----------------------------------------------------------------------------------|
| <i>PPARGC1B</i>                | Human PGC-1 $\beta$ -encoding gene                                               |
| <i>Pparg1a</i>                 | Mouse PGC-1 $\alpha$ -encoding gene                                              |
| pH                             | Measure of hydrogen ion concentration                                            |
| PHGDH                          | Phosphoglycerate dehydrogenase                                                   |
| PPAR $\gamma$                  | Peroxisome proliferator-activated receptor gamma                                 |
| <i>Pparg1a<sup>fl/fl</sup></i> | <i>Pparg1a</i> gene with both alleles flanked by loxP sites                      |
| PR                             | Progesterone receptor                                                            |
| PRC                            | PGC-1-related coactivator                                                        |
| QuPath                         | Digital pathology software                                                       |
| RNA-seq                        | RNA sequencing                                                                   |
| ROS                            | Reactive oxygen species                                                          |
| Rotenone/Antimycin A           | Complex I inhibitor (Rotenone) combined with Complex III inhibitor (Antimycin A) |
| RT-qPCR                        | Reverse transcription quantitative PCR                                           |
| SAM                            | S-adenosylmethionine                                                             |
| SGOC                           | Serine-glycine one-carbon (metabolic pathway)                                    |
| SHMT                           | Serine hydroxymethyltransferase                                                  |
| SIRT1                          | Sirtuin 1 (NAD-dependent protein deacetylase)                                    |
| <i>Slc14a1</i>                 | Solute carrier family 14 member 1 (urea transporter)                             |
| <i>Slc7a1</i>                  | Solute carrier family 7 member 1 (cationic amino acid transporter 1)             |
| <i>Sod1</i>                    | Superoxide dismutase 1 (cytoplasmic copper-zinc)                                 |
| <i>Sod2</i>                    | Superoxide dismutase 2 (mitochondrial antioxidant)                               |
| SOD2                           | Superoxide dismutase 2 (protein)                                                 |
| SPF                            | Specific pathogen-free                                                           |
| SRC                            | Spare respiratory capacity                                                       |
| STAR                           | Spliced Transcripts Alignment to a Reference                                     |
| TCA                            | Tricarboxylic acid (cycle)                                                       |
| <i>Tfpi</i>                    | Tissue Factor Pathway Inhibitor                                                  |
| <i>TRIzol</i>                  | RNA isolation reagent                                                            |
| TNF- $\alpha$                  | Tumor necrosis factor alpha                                                      |

|                       |                                                                                   |
|-----------------------|-----------------------------------------------------------------------------------|
| TNBC                  | Triple-negative breast cancer                                                     |
| Trypsin-EDTA          | Trypsin combined with ethylenediaminetetraacetic acid (cell dissociation reagent) |
| UHPLC                 | Ultra-high-performance liquid chromatography                                      |
| <i>Uqcrg</i>          | Ubiquinol-cytochrome c reductase subunit Q (Complex III)                          |
| <i>Uqcrc1</i>         | Ubiquinol-cytochrome c reductase subunit Q1 (Complex III)                         |
| VEGF                  | Vascular endothelial growth factor                                                |
| VIP                   | Variable Importance in Projection                                                 |
| VLDL                  | Very low-density lipoprotein                                                      |
| Wnt/ $\beta$ -catenin | Wingless-related integration site/ $\beta$ -catenin signaling pathway             |
| WT                    | Wild type                                                                         |

## ABSTRACT

Breast cancer liver metastasis is a clinically devastating event associated with poor prognosis and limited treatment options, yet the metabolic determinants that govern whether disseminated breast cancer cells can successfully colonize the hepatic microenvironment remain poorly understood. Liver-tropic breast cancer cells exhibit unexpectedly low expression of PGC-1 $\alpha$ , a master regulator of mitochondrial biogenesis and oxidative phosphorylation (OXPHOS), and instead rely predominantly on a glycolytic metabolic program. Whether this glycolytic phenotype constitutes a metabolic optimum for hepatic colonization, or alternatively reflects a suboptimal default state whose reprogramming through PGC-1 $\alpha$  gain-of-function could enhance metastatic fitness, had not been directly tested. Equally unresolved is whether the PGC-1 $\alpha$ -dependent oxidative metabolic state of host hepatocytes independently influences the permissiveness of the hepatic microenvironment for metastatic colonization.

This thesis addressed these questions through a dual-compartment seed-and-soil experimental strategy. Stable PGC-1 $\alpha$ -overexpressing and matched empty-vector control variants of the liver-tropic 2792 murine breast cancer cell line were generated by lentiviral transduction and characterized by Seahorse extracellular flux analysis, genome-wide RNA sequencing, and targeted LC-MS/MS metabolomics. Hepatocyte-specific *Ppargc1a* knockout (KO) mice were generated using Alb-Cre-mediated loxP recombination and validated by PCR genotyping. Metastatic colonization efficiency was assessed using orthotopic mammary fat pad implantation and intrasplenic portal vein injection with blinded digital quantification of hepatic and pulmonary metastatic burden.

PGC-1 $\alpha$  overexpression in liver-tropic breast cancer cells significantly increased basal and maximal mitochondrial ATP production rates and enlarged spare respiratory capacity, while concurrently reducing maximal glycolytic output. PGC-1 $\alpha$  selectively increased the expression of *Sod2* (manganese superoxide dismutase) and *Uqcrc1* (respiratory Complex III subunit). Genome-wide RNA sequencing identified "Oxidative phosphorylation" as the overwhelmingly dominant activated KEGG pathway. Targeted metabolomics confirmed enrichment of serine-glycine one-carbon metabolism, tricarboxylic acid cycle (TCA), and OXPHOS metabolite sets, with O-phosphoserine and adenosine as the two metabolites elevated in PGC-1 $\alpha$ -overexpressing cells, providing multi-omic convergence on the same core biological programs. In vivo, PGC-1 $\alpha$

overexpression had no effect on orthotopic primary tumor growth yet increased pulmonary metastatic area more than threefold following spontaneous dissemination. In the intrasplenic liver colonization model, PGC-1 $\alpha$  overexpression dramatically increased hepatic lesion number. In striking contrast, hepatocyte-specific *Ppargc1a* deletion produced no significant change in hepatic metastatic colonization efficiency across all host genotypes.

These findings establish that PGC-1 $\alpha$ -driven metabolism in the tumor cell is a potent, cell-autonomous amplifier of breast cancer hepatic metastatic colonization competence, operating through enhanced mitochondrial bioenergetic reserve, antioxidant capacity, and serine-one-carbon metabolic flux. The metabolic state of the host hepatocyte is dispensable as a determinant of colonization efficiency, demonstrating that in the seed (breast cancer cell)–soil (liver tissue) PGC-1 $\alpha$ -dependent metabolic duality, the seed's PGC-1 $\alpha$  metabolic programming is the decisive variable. This work resolves a previously unaddressed biological paradox, identifies PGC-1 $\alpha$ -driven metabolic pathways as targetable vulnerabilities in metastatic breast cancer, and provides a molecularly grounded rationale for stratifying patients with PGC-1 $\alpha$ -high metastatic disease for metabolically targeted therapeutic intervention.

**Keywords:** PGC-1 $\alpha$ ; breast cancer; liver metastasis; oxidative phosphorylation; mitochondrial biogenesis; metabolic reprogramming; seed and soil hypothesis.

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This work is dedicated to everyone who has ever believed that understanding the biology of cancer, one carefully designed experiment at a time, is a worthy and meaningful way to spend a life.

# CHAPTER 1

## INTRODUCTION

### PART I

#### **Foundational Concepts: Cancer Metastasis, the Metastatic Cascade, and Metabolic Reprogramming**

##### **1. Cancer Metastasis as the Central Clinical Challenge**

Cancer remains one of the foremost causes of morbidity and mortality worldwide, representing an immense burden on global public health. Despite transformative advances in early detection, molecular diagnostics, surgical techniques, radiotherapy, endocrine therapy, chemotherapy, and molecularly targeted therapeutics, metastatic disease continues to account for the overwhelming majority of cancer-related deaths (Steeg, 2006; Gupta and Massagué, 2006; Canadian Cancer Statistics Advisory Committee, 2025). In many solid malignancies, primary tumors can be locally controlled or surgically resected with curative intent; however, once tumor cells disseminate and establish secondary lesions in distant organs, the therapeutic landscape changes dramatically and curative treatment becomes substantially more difficult. Accordingly, metastasis represents the most clinically consequential stage of cancer progression and remains one of the most deeply unresolved problems in modern oncology (Steeg, 2016; Li et al., 2025).

The lethality of metastatic disease derives from several interrelated factors. First, metastatic lesions frequently arise within vital organs such as liver, lung, brain, and bone, where progressive tumor burden disrupts essential physiological functions in ways that cannot be surgically remedied. Second, metastatic tumors exhibit markedly increased phenotypic and molecular heterogeneity relative to the primary tumor, reflecting clonal evolution, selection pressures, and adaptive plasticity acquired during dissemination through diverse tissue environments (Steeg, 2006; Nguyen and Massagué, 2007; Liu et al., 2024; Li et al., 2025). Third, and perhaps most critically from a therapeutic perspective, metastatic disease commonly exhibits reduced sensitivity to systemic therapy through mechanisms encompassing altered drug penetration, immune evasion, lineage plasticity, metabolic rewiring, and microenvironment-mediated resistance (Steeg, 2016; Li et al., 2025). As a result, patients with advanced metastatic disease frequently experience relapse even

after initial treatment responses, underscoring the profound unmet need for mechanistic insights into metastatic biology.

Historically, cancer research focused heavily on tumor initiation and primary tumor growth, generating transformative insights into oncogenic signaling, tumor suppressor biology, and precision medicine. Yet it is increasingly recognized that metastasis is not simply an extension of primary tumor growth. Instead, metastatic progression demands additional, and often entirely distinct, biological capabilities: the capacity to detach from the primary tumor, invade surrounding tissue, intravasate into blood or lymphatic vessels, survive hostile conditions in circulation, extravasate at distant capillary beds, and successfully colonize a foreign organ microenvironment (Gupta and Massagué, 2006; Li et al., 2025). These demands impose profound selective bottlenecks, such that only a minority fraction of disseminated tumor cells ultimately form macroscopic, clinically detectable metastases (Luzzi et al., 1998; Nguyen and Massagué, 2007; Welch and Hurst, 2019; Liu et al., 2024).

Importantly, metastatic competence is shaped by both tumor cell-intrinsic properties and host-derived factors within target organs. Tumor cells must acquire invasive, migratory, and stress-resistant phenotypes; yet they must also successfully engage with stromal cells, vascular networks, immune populations, and organ-specific metabolic resources (Liu et al., 2024). This dual dependence confers enduring relevance upon Paget's classical "seed and soil" hypothesis, which proposed that successful metastasis depends fundamentally on compatibility between disseminating tumor cells (the "seed") and the distant organ microenvironment (the "soil") (Paget, 1889). Originally conceptual, this framework has since gained extensive molecular support and remains among the most influential paradigms in modern metastasis biology (Fidler, 2003; Wang et al., 2024). For these reasons, understanding metastasis requires an integrative perspective combining cell biology, systems metabolism, immunology, and tissue ecology, an approach particularly relevant to studies examining metabolic regulators that may simultaneously influence tumor fitness and the permissiveness of organ-specific niches.

## **2. The Metastatic Cascade: A Multistep and Highly Inefficient Biological Process**

Metastasis is not a singular event but rather a sequential, multistep biological process, often referred to as the metastatic cascade that is remarkable for its inefficiency (Luzzi et al., 1998; Wang et al.,

2024). This cascade encompasses local invasion from the primary tumor, intravasation into blood or lymphatic vessels, survival of circulating tumor cells, arrest at distant capillary beds, extravasation into parenchymal tissue, adaptation and persistence as disseminated or dormant cells, and eventual colonization into macroscopic metastatic lesions (Gupta and Massagué, 2006; Steeg, 2006; Nguyen and Massagué, 2007; Liu et al., 2024; Li et al., 2025). Each step imposes unique biological constraints and represents a distinct selective bottleneck, such that only a very small minority of tumor cells that enter the metastatic cascade will complete it successfully (Liu et al., 2024).

## **2.1 Local Invasion and Intravasation**

The earliest stages of metastatic departure require tumor cells to breach tissue boundaries and invade the surrounding stroma. This process frequently involves epithelial-to-mesenchymal transition (EMT)-like transcriptional programs, remodeling of cell adhesion molecules, acquisition of increased motility, and the secretion of matrix-degrading proteases, including matrix metalloproteinases (MMPs). Tumor-associated fibroblasts, macrophages, and inflammatory infiltrates further facilitate invasion by remodeling extracellular matrix architecture and secreting pro-migratory cytokines and chemokines (Szabo et al., 2023; Youssef et al., 2024). Intravasation subsequently allows invasive cells to penetrate nearby blood vessels or lymphatics, a process enhanced by aberrant tumor angiogenesis, increased vascular permeability, and reciprocal signaling between tumor cells and endothelial cells. Upon entering the vasculature, tumor cells are immediately subjected to intense biomechanical, oxidative, and immune challenges (Strilic et al., 2017).

## **2.2 Survival in Circulation**

Circulating tumor cells (CTCs) face a formidable gauntlet of stresses, including fluid shear forces, anoikis-inducing loss of matrix attachment, and cytotoxic attack by natural killer cells and other innate immune effectors. Only a small fraction survive transit (Pantel and Brakenhoff, 2004; Strilic and Offermanns, 2017). Tumor cells may improve intravascular survival by forming multicellular clusters, recruiting a platelet coating that provides immune camouflage, or activating cellular stress-response programs (Gu et al., 2024). These adaptations already impose a substantial metabolic requirement: detached cells must preserve ATP production and maintain redox balance

under nutrient-limiting and mechanically challenging conditions, representing an early manifestation of the metabolic selectivity that shapes metastatic competence (Vander Heiden et al., 2017).

### **2.3 Extravasation and the Critical Bottleneck of Colonization**

After arresting in distant capillary beds, tumor cells extravasate across endothelial barriers into parenchymal tissue. However, extravasation alone is far from sufficient to establish clinically meaningful metastatic disease. The most stringent and rate-limiting step for many cancers is metastatic colonization, the process by which disseminated cells must adapt to the new organ microenvironment and initiate sustained, self-renewing proliferation. This adaptation requires simultaneously overcoming innate and adaptive immune defenses, competing for nutrients against highly active resident parenchymal cells, resisting oxidative injury, and recruiting local stromal support (Nguyen and Massagué, 2007; Massagué and Obenauf, 2016; Liu et al., 2024). Clinically, disseminated tumor cells may remain dormant for months or even years before reactivation, contributing to the phenomenon of delayed relapse that is well-characterized in breast cancer and other solid malignancies. This latency underscores that the completion of colonization, rather than initial seeding, is the truly limiting event in metastatic disease (Strilic et al., 2017).

### **2.4 Organotropism: The Non-Random Nature of Metastatic Spread**

A striking and clinically important feature of metastasis is its non-randomness. Different cancer types preferentially colonize specific organs, a phenomenon designated organotropism, that reflects deep compatibility between tumor cell-intrinsic programs and organ-specific microenvironments. Breast cancer commonly metastasizes to bone, lung, liver, and brain; colorectal cancer preferentially disseminates to the liver; and prostate cancer shows a particular predilection for bone (Nguyen and Massagué, 2007; Li et al., 2025). Landmark gene-expression studies have identified molecular signatures that direct site-selective metastasis, demonstrating that breast cancer cells expressing distinct transcriptional programs preferentially colonize bone, lung, or brain depending on their gene expression state (Minn et al., 2005; Bos et al., 2009; Gan et al., 2024).

Beyond signaling determinants, however, there is growing recognition that metabolic compatibility between tumor cells and target organ environments constitutes a critical, and comparatively understudied, dimension of organotropic selectivity (Obenauf and Massagué, 2015; Li et al., 2025).

### **3. Metabolic Reprogramming as a Hallmark of Cancer**

Among the most influential conceptual advances in oncology over the past two decades has been the recognition that altered cellular metabolism is not merely a passive consequence of malignant transformation, but an active and essential contributor to cancer progression. Hanahan and Weinberg formally incorporated deregulated cellular energetics into the expanded hallmarks of cancer framework, acknowledging that metabolic reprogramming represents a fundamental capability acquired during tumorigenesis (Hanahan and Weinberg, 2011). Normal cells coordinate nutrient uptake and metabolic flux according to tissue function, homeostatic demands, and growth factor signaling. By contrast, cancer cells systematically rewire these pathways to support uncontrolled proliferation, survival under stress, immune evasion, and adaptation to variable microenvironmental nutrient availability (DeBerardinis and Chandel, 2016).

#### **3.1 The Warburg Effect and the Modern View of Cancer Metabolism**

Otto Warburg first described the striking observation that many tumors convert glucose to lactate even in the presence of adequate oxygen, a phenomenon subsequently designated aerobic glycolysis or the Warburg effect, observations that trace back to his original reports in the 1920s (Warburg, 1924, 1925, 1956).

Although historically interpreted as evidence of mitochondrial dysfunction, it is now well established that most tumors retain structurally and functionally competent mitochondria (Vander Heiden et al., 2009). Rather than reflecting metabolic failure, aerobic glycolysis represents an adaptive strategy: it rapidly regenerates  $\text{NAD}^+$  for sustained glycolytic flux, generates biosynthetic intermediates for anabolic growth, and produces lactate that can acidify the tumor microenvironment in ways that promote invasion and immune suppression. Critically, glycolysis and oxidative phosphorylation (OXPHOS) are not mutually exclusive metabolic states in cancer; instead, they frequently coexist and cooperate, with the balance between them shifted according to nutrient availability, oncogenotype, lineage, and microenvironmental context (Pavlova and Thompson, 2016; DeBerardinis and Chandel, 2016).

### **3.2 Biosynthesis, Nutrient Scavenging, and Redox Control**

The metabolic reprogramming of cancer extends well beyond glucose utilization. Rapidly dividing cells require abundant carbon and nitrogen for the synthesis of nucleotides, lipids, and proteins, and cancer cells accordingly upregulate pathways that direct metabolic flux toward biosynthetic precursors. Glutamine addiction, the dependence of many cancer cells on exogenous glutamine as a carbon and nitrogen donor for anaplerosis, nucleotide biosynthesis, and lipogenesis via reductive carboxylation, represents one of the most pervasive metabolic alterations in cancer (Pavlova and Thompson, 2016; McGuirk et al., 2020). Additionally, cancer cells co-opt fatty acid oxidation as an alternative energy source during nutrient deprivation or metabolic stress, and frequently upregulate de novo lipogenesis to support membrane expansion. One-carbon metabolism and serine biosynthesis contribute to nucleotide production and methylation reactions (Audet-Walsh et al., 2016). Simultaneously, cancer cells must manage the elevated oxidative stress inherent to rapid proliferation and mitochondrial activity by generating reducing equivalents, principally NADPH and glutathione, through the pentose phosphate pathway, folate metabolism, and mitochondrial one-carbon pathways (DeBerardinis and Chandel, 2016; Pavlova and Thompson, 2016; Li et al., 2025). This balance between biosynthetic output and redox buffering is critical to tumor survival and therapeutic resistance (Audet-Delage et al., 2023).

### **3.3 Metabolism as a Regulatory and Epigenetic Signaling System**

Beyond its role in ATP production and anabolic biosynthesis, cellular metabolism has emerged as a powerful regulator of gene expression and cell identity. Metabolites including acetyl-CoA,  $\alpha$ -ketoglutarate, succinate, fumarate, NAD<sup>+</sup>, and S-adenosylmethionine (SAM) function as co-substrates and allosteric regulators of chromatin-modifying enzymes, linking the metabolic state of a cell directly to its epigenetic landscape and transcriptional programs (Yang et al., 2013; DeBerardinis and Chandel, 2016). Mutations in metabolic enzymes, such as IDH1/2, succinate dehydrogenase (SDH), and fumarate hydratase (FH), cause oncometabolite accumulation that reshapes the epigenome and drives aberrant differentiation states in cancer (Yang et al., 2013; Lanzetti, 2024). This broader perspective positions metabolism not only as a bioenergetic system but as a regulatory platform through which environmental signals are transduced into durable changes in cell fate, phenotypic plasticity, and therapeutic response, all properties directly relevant to the adaptive requirements of metastatic progression.

#### 4. Metabolic Adaptation During Metastatic Progression

Although metabolic reprogramming is well-characterized in the context of primary tumor growth, metastasis imposes a distinct and continuously shifting set of metabolic constraints. As tumor cells navigate the metastatic cascade, they repeatedly encounter microenvironments that differ dramatically from the primary tumor, varying in nutrient composition, oxygen tension, stromal interactions, immune pressure, and biomechanical forces. The ability to adaptively reprogram metabolism in response to these changing conditions may therefore constitute a critical determinant of metastatic success, distinct from the metabolic requirements for primary tumor proliferation (Strilic et al., 2017; Andrzejewski et al., 2017; McGuirk et al., 2020).

At the stage of detachment and anoikis resistance, cancer cells transitioning to anchorage-independence must rely on antioxidant pathways, including increased NADPH production and glutathione recycling, to neutralize the elevated reactive oxygen species (ROS) generated in the absence of integrin-mediated survival signals. Circulating tumor cells additionally require metabolic flexibility to sustain ATP production under transient nutrient deprivation and to withstand the energetic cost of immune evasion. Upon arrival in distant organs, disseminated tumor cells must rapidly exploit locally available substrates, whether lipids in adipose-adjacent niches, glucose in highly perfused tissues, or amino acids released by stromal remodeling, to fuel the energy-intensive processes of colonization and proliferative outgrowth. Mitochondrial function appears to be especially important during metastatic colonization. Multiple studies have documented that highly metastatic cells display increased respiratory capacity, enhanced mitochondrial biogenesis, and greater OXPHOS activity compared with their poorly metastatic counterparts, suggesting that mitochondrial fitness confers a selective advantage during dissemination and early establishment at distant sites (LeBleu et al., 2014; Andrzejewski et al., 2017; Strilic and Offermanns, 2017; McGuirk et al., 2020; Liu et al., 2024).

However, metastatic metabolism is profoundly context-dependent. Compelling evidence indicates that the metabolic requirements for successful colonization differ substantially across target organs. Breast cancer cells that preferentially colonize the lung or bone display relatively high expression of the mitochondrial master regulator PGC-1 $\alpha$  and rely heavily on OXPHOS, whereas liver-tropic breast cancer cells exhibit a distinct metabolic phenotype characterized by increased glycolytic flux and reduced mitochondrial respiration (Dupuy et al., 2015; Andrzejewski et al., 2017). This organ-

selective metabolic divergence strongly implies that target organ environments impose distinct metabolic selective pressures, and that tumor cells must align their metabolic programs with those demands to achieve successful colonization. These observations support a central conceptual principle of this thesis: that successful metastasis may depend less on any single fixed metabolic pathway and more on the capacity for flexible metabolic reprogramming in response to organ-specific challenges, a property that may be coordinately governed by upstream metabolic regulators such as PGC-1 $\alpha$ .

Having established metastasis as the dominant clinical problem in oncology and adaptive metabolic reprogramming as a core requirement for metastatic competence, the following sections will focus specifically on breast cancer metastasis, liver tropism, and the unique biology of the hepatic metastatic niche, before turning to the PGC-1 $\alpha$  biology that provides the mechanistic foundation for this thesis.

## PART II

### PGC-1 $\alpha$ Biology, Mitochondrial Control, and Emerging Roles in Cancer Progression

#### 5. The PGC-1 Family: Master Regulators of Cellular Metabolic Plasticity

The capacity of cells to adaptively reprogram their metabolism in response to changes in energy demand, nutrient availability, hormonal signals, and environmental stress is fundamental to life. A central molecular system governing this capacity is the peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) family of transcriptional coactivators, comprising PGC-1 $\alpha$  (encoded by *PPARGC1A*), PGC-1 $\beta$  (*PPARGC1B*), and PGC-1-related coactivator (PRC, encoded by *PPRC1*). Unlike classical sequence-specific DNA-binding transcription factors, PGC-1 family members function through direct protein-protein interactions with multiple transcription factors and chromatin-modifying complexes, enabling them to dramatically amplify and reshape transcriptional programs governing mitochondrial and metabolic function without themselves having intrinsic DNA-binding activity (Puigserver and Spiegelman, 2003; Lin et al., 2005).

Among the three family members, PGC-1 $\alpha$  is the most extensively characterized and is now widely regarded as the master regulator of mitochondrial biogenesis and oxidative metabolism across a broad spectrum of physiological contexts. Its activity is particularly prominent in tissues with high energetic demands or the need for rapid metabolic adaptation, including skeletal muscle, cardiac muscle, liver, brown adipose tissue, and brain. Since its discovery, PGC-1 $\alpha$  has been shown to play pivotal roles in skeletal muscle fiber-type adaptation and exercise physiology, hepatic gluconeogenesis and fasting responses, cardiac energetic homeostasis, adaptive thermogenesis in brown adipose tissue, and neuronal stress responses (Puigserver et al., 1998; Handschin and Spiegelman, 2006; Qian et al., 2024). The extraordinary functional breadth of PGC-1 $\alpha$  reflects its ability to integrate extracellular metabolic signals with transcriptional networks that control substrate utilization, ATP production, redox balance, and organelle maintenance, positioning it at the interface between environmental sensing and metabolic plasticity.

#### 6. Discovery and Canonical Functions of PGC-1 $\alpha$

PGC-1 $\alpha$  was first identified in 1998 by Puigserver, Spiegelman, and colleagues during studies of adaptive thermogenesis in brown adipose tissue (Puigserver et al., 1998). Cold exposure was found

to induce a novel coactivator that interacted with PPAR $\gamma$  to stimulate uncoupling protein 1 (UCP1) expression and amplify mitochondrial gene programs, enabling thermogenic heat production. This discovery rapidly expanded into a broader recognition that PGC-1 $\alpha$  serves as a conserved orchestrator of mitochondrial biogenesis across multiple tissue types. Subsequent landmark studies demonstrated that PGC-1 $\alpha$  drives the expression of nuclear-encoded mitochondrial genes through coactivation of key transcription factors including nuclear respiratory factor 1 (NRF1), NRF2/GABPA, estrogen-related receptor alpha (ERR $\alpha$ ; originally identified by Giguère et al., 1988; shown to mediate PGC-1 $\alpha$ -dependent OXPHOS by Mootha et al., 2004), and mitochondrial transcription factor A (TFAM), the last of which directly activates mitochondrial DNA (mtDNA) transcription and replication, thereby increasing both the number and functional capacity of mitochondria (Wu et al., 1999; Scarpulla, 2011).

## **7. Molecular Mechanisms of PGC-1 $\alpha$ Action**

PGC-1 $\alpha$  exerts its broad transcriptional influence through an extensive network of protein-protein interactions, enabling it to coordinate diverse metabolic outputs in a highly cell-type- and context-specific manner.

### **7.1 Interaction with Nuclear Receptors and Key Transcription**

PGC-1 $\alpha$  coactivates a wide array of transcription factors that collectively govern the major metabolic processes of the cell. Through its interaction with PPAR $\alpha$  and PPAR $\delta$ , PGC-1 $\alpha$  promotes fatty acid oxidation and lipid homeostasis (Puigserver and Spiegelman, 2003; Lin et al., 2005). Its partnership with ERR $\alpha$ , which shares considerable target gene overlap with the estrogen receptor but operates independently of classical estrogen ligands, is particularly important for mitochondrial respiration, OXPHOS gene expression, and nutrient sensing (Giguère et al., 1988; Mootha et al., 2004). Coactivation of NRF1 and NRF2/GABPA drives expression of components of the mitochondrial electron transport chain (ETC) and the mitochondrial import machinery (Wu et al., 1999; Scarpulla, 2011). Through FOXO1, PGC-1 $\alpha$  supports hepatic gluconeogenesis and stress adaptation, while its interaction with HNF4 $\alpha$  coordinates the hepatic metabolic gene network (Puigserver and Spiegelman, 2003; Lin et al., 2005). The breadth of these partnerships allows PGC-1 $\alpha$  to generate tissue-specific metabolic responses through combinatorial transcription factor assembly, while utilizing a common coactivator platform.

## 7.2 Chromatin Remodeling and Transcriptional Amplification

Mechanistically, PGC-1 $\alpha$  functions as a transcriptional amplifier by recruiting chromatin-modifying complexes, including the histone acetyltransferases CBP/p300, the Mediator complex, and SRC-family coactivators, that increase local chromatin accessibility, enhancer-promoter looping, and transcriptional output at target loci (Wallberg et al., 2003; Puigserver and Spiegelman, 2003). This mechanism enables PGC-1 $\alpha$  to reinforce and sustain transcriptional programs initiated by its partner transcription factors, effectively providing a signal integration and amplification function that links upstream metabolic sensing to durable changes in gene expression (Puigserver and Spiegelman, 2003). As a result, PGC-1 $\alpha$  should be understood not as a single-pathway regulator, but as a systems-level coordinator of metabolic state capable of simultaneously modulating multiple interdependent metabolic circuits.

## 7.3 Alternative Isoforms and Contextual Complexity

The *PPARGC1A* locus generates multiple distinct transcripts and protein isoforms through the use of alternative promoters, alternative splicing, and post-transcriptional regulatory mechanisms. These isoforms exhibit distinct tissue distributions, interaction partners, and transcriptional targets, expanding the functional versatility of the PGC-1 $\alpha$  system beyond what a single canonical form could achieve (Martínez-Redondo et al., 2015; Qian et al., 2024). Recent work has further demonstrated that certain alternative isoforms play non-redundant physiological roles, particularly in the context of metabolic adaptation to exercise and environmental stress, indicating that different biological stimuli engage distinct arms of the PGC-1 $\alpha$  system (Nomura et al., 2024). This isoform diversity raises the important possibility that different disease contexts, or different stages of metastatic progression, may be governed by distinct PGC-1 $\alpha$  isoforms, a possibility that remains underexplored in cancer biology (Martínez-Redondo et al., 2015; Qian et al., 2024).

## 8. Upstream Regulation of PGC-1 $\alpha$ : Integration of Metabolic Stress Signals

Because cellular metabolic demands fluctuate continuously in response to nutrient availability, hormonal signals, and exercise or stress, PGC-1 $\alpha$  itself is subject to stringent multilayered regulation at transcriptional, post-transcriptional, and post-translational levels, enabling rapid and reversible tuning of its activity in response to environmental conditions (Puigserver and Spiegelman, 2003; Lin et al., 2005; Qian et al., 2024).

Two key energy-sensing pathways converge on PGC-1 $\alpha$  to link cellular bioenergetic status to metabolic gene programs. AMP-activated protein kinase (AMPK), the master sensor of cellular ATP reduction, directly phosphorylates PGC-1 $\alpha$  at conserved serine residues, increasing its transcriptional activity and promoting mitochondrial biogenesis as an adaptive response to energy deficit (Zong et al., 2002; Jäger et al., 2007). Sirtuin 1 (SIRT1), a NAD<sup>+</sup>-dependent protein deacetylase, deacetylates and activates PGC-1 $\alpha$  in response to elevated cellular NAD<sup>+</sup> levels during fasting or nutrient deprivation, coupling redox and nutritional status to transcriptional metabolic reprogramming (Rodgers et al., 2005; Gerhart-Hines et al., 2007). The p38 MAPK pathway also phosphorylates and stabilizes PGC-1 $\alpha$  during cellular stress responses, relieving inhibitory interactions and enhancing its coactivator activity (Puigserver et al., 2001; Akimoto et al., 2005). Transcriptionally, PGC-1 $\alpha$  expression is induced by cAMP/PKA signaling through CREB (activated by glucagon and  $\beta$ -adrenergic agonists), by calcium-dependent pathways including CaMKIV and calcineurin in exercising muscle, and by reactive oxygen species in certain contexts (Herzig et al., 2001; Handschin et al., 2003; Liang et al., 2010).

Conversely, PGC-1 $\alpha$  activity is suppressed by inflammatory signaling (notably TNF- $\alpha$  and NF- $\kappa$ B), nutrient excess (through acetylation and decreased SIRT1 activity), and specific oncogenic programs, creating an intricate balance of activating and repressive inputs that reflects the net metabolic state of the cell (Dominy et al., 2010; LaGory et al., 2015; Rius-Pérez et al., 2020). This multilayered regulatory system is of particular relevance in cancer, where oncogenic signaling, chronic inflammatory stress, nutrient fluctuations, and therapeutic pressure coexist and interact to collectively determine PGC-1 $\alpha$  activity and its downstream consequences for tumor cell biology.

## **9. PGC-1 $\alpha$ as a Coordinator of Mitochondrial Fitness and Resilience**

Mitochondria perform biological functions that extend far beyond ATP generation: they serve as hubs for biosynthetic reactions (including the tricarboxylic acid cycle (TCA) that generates precursors for amino acid, lipid, and nucleotide biosynthesis), regulate apoptotic signaling, maintain cellular calcium homeostasis, participate in iron-sulfur cluster assembly, and produce ROS that serve both as damaging agents and as signaling molecules. PGC-1 $\alpha$  enhances multiple dimensions of mitochondrial fitness simultaneously: it generally promotes mitochondrial biogenesis, though the magnitude varies by context, augments the capacity of the ETC and ATP synthase, enhances fatty acid import and oxidation, stimulates antioxidant defense systems

including superoxide dismutase 2 (SOD2) and glutathione peroxidase, and maintains mitochondrial membrane potential and respiratory chain coupling (Lin et al., 2005; Scarpulla, 2011). Importantly, PGC-1 $\alpha$ -driven mitochondrial enhancement confers not merely increased respiratory rate, but increased respiratory reserve capacity, the ability to rapidly increase ATP production in response to acute energy demand, and greater metabolic flexibility in fuel selection (Marchetti et al., 2020). These properties may be especially advantageous under conditions of metabolic stress such as nutrient deprivation, hypoxia-reoxygenation, matrix detachment, or therapeutic perturbation, precisely the conditions encountered by disseminated tumor cells during metastatic transit and colonization (LeBleu et al., 2014; McGuirk et al., 2020; Qian et al., 2024).

## **10. Emerging Roles of PGC-1 $\alpha$ in Cancer: Context-Dependence and Dichotomy**

Given PGC-1 $\alpha$ 's central role in metabolic adaptation, it has attracted considerable and growing attention in cancer research over the past decade. The conditions confronting cancer cells *in vivo*, fluctuating nutrient availability, hypoxia, oxidative stress, immune pressure, and therapeutic challenge, parallel the physiological stress states in which PGC-1 $\alpha$  is normally activated, raising the compelling hypothesis that a regulator of mitochondrial flexibility and stress resilience could provide selective advantages to cancer cells navigating these challenges.

However, the role of PGC-1 $\alpha$  in cancer is not unidirectional. In many tumor contexts, PGC-1 $\alpha$  drives increased OXPHOS, mitochondrial biogenesis, and antioxidant defense in ways that support aggressive behavior, drug resistance, stem-like persistence, and metastasis (Haq et al., 2013; Vazquez et al., 2013; LeBleu et al., 2014; Andrzejewski et al., 2017; McGuirk et al., 2021). In a minority of tumor contexts, PGC-1 $\alpha$ -mediated mitochondrial enhancement may suppress glycolytic growth programs, promote differentiation, or restrict proliferation (LaGory et al., 2015; Torrano et al., 2016; Gravel, 2018; Wang et al., 2024). This paradox is a manifestation of an increasingly recognized principle in cancer metabolism: the functional consequence of activating or suppressing a metabolic regulator depends critically on tumor lineage, oncogenotype, microenvironment, and stage of disease progression (LeBleu et al., 2014; Gravel, 2018; Bost and Kaminski, 2019; Wang et al., 2024).

Altered PGC-1 $\alpha$  expression has been documented across a broad range of tumor types, including melanoma, prostate cancer, colorectal cancer, ovarian cancer, and hematologic malignancies

(Tennakoon et al., 2014; Gravel, 2018; Wang et al., 2024; Zhang et al., 2024). In melanoma, two complementary studies illuminated a complex, context-dependent relationship between PGC-1 $\alpha$  and BRAF signaling: in 2013, Haq et al. demonstrated that oncogenic BRAF suppresses PGC-1 $\alpha$  and oxidative metabolism through MITF, promoting glycolysis, and that BRAF inhibition de-represses PGC-1 $\alpha$ , rendering tumor cells dependent on OXPHOS as a compensatory survival mechanism (Haq et al., 2013). In parallel, Vazquez et al. showed that a subset of human melanomas with high MITF-driven PGC-1 $\alpha$  expression displays elevated mitochondrial capacity and resistance to oxidative stress, demonstrating a pro-survival function of PGC-1 $\alpha$  in the melanocyte lineage independent of BRAF status (Vazquez et al., 2013). In prostate cancer, elevated PGC-1 $\alpha$  has been linked to castration resistance and increased mitochondrial biogenesis (Tennakoon et al., 2014). These examples collectively illustrate that PGC-1 $\alpha$  may function as a context- and lineage-dependent metabolic regulator whose oncological consequences depend on the specific oncogenomic background and selective pressures present. Importantly, while exceptions exist, the large majority of evidence positions PGC-1 $\alpha$  as a pro-tumorigenic factor: tumor-suppressive or anti-metastatic effects have been documented in only a limited subset of cancer contexts, and PGC-1 $\alpha$  should therefore be understood primarily as an amplifier of cancer aggressiveness, mitochondrial fitness, and metastatic competence (Torrano et al., 2016; Gravel, 2018; Bost and Kaminski, 2019; Wang et al., 2024).

## **11. PGC-1 $\alpha$ , Cancer Cell Plasticity, and Therapeutic Resistance**

A recurring and clinically critical theme in cancer biology is phenotypic plasticity, the ability of cancer cells to reversibly transition between distinct functional states including proliferative, invasive, dormant, stem-like, and therapy-resistant phenotypes (Youssef et al., 2024; Li et al., 2025). Each of these states carries distinct energetic and biosynthetic demands, creating a fundamental link between metabolic flexibility and cell state transitions. PGC-1 $\alpha$ , as a coordinator of mitochondrial outputs across multiple metabolic circuits, is a plausible molecular enabler of such metabolic state transitions. Invasive cells engaged in cytoskeletal remodeling and active migration require high-amplitude ATP production for motor protein activity and membrane dynamics (LeBleu et al., 2014). Dormant disseminated tumor cells may require efficient low-level oxidative maintenance to survive without active proliferative signaling. Re-entering proliferation at a distant metastatic site demands rapid biosynthetic upscaling. Drug-resistant cancer cells

surviving under therapeutic pressure have been found to depend on elevated mitochondrial respiration and antioxidant capacity, both of which are enhanced by PGC-1 $\alpha$  (Pavlova and Thompson, 2016; McGuirk et al., 2021). In a particularly relevant study from the St-Pierre laboratory, McGuirk et al. demonstrated that resistance to anthracycline chemotherapy in breast cancer cells is associated with upregulation of PGC-1 $\alpha$  and ERR $\alpha$ , driving metabolic reprogramming toward glutathione metabolism and OXPHOS, identifying PGC-1 $\alpha$  as a mediator of acquired therapy resistance (McGuirk et al., 2021). Thus, PGC-1 $\alpha$  may function as a molecular enabler of adaptive state transitions across the metastatic cascade, rather than as a fixed regulator of a single phenotypic program.

## PART III

### **Breast Cancer Metastasis, Liver Tropism, and the Hepatic Metastatic Niche**

#### **12. Breast Cancer Metastasis: Clinical Landscape and Molecular Heterogeneity**

Breast cancer is the most frequently diagnosed malignancy among women worldwide and a leading cause of cancer-related death globally (Bray et al., 2024; Siegel et al., 2024; Canadian Cancer Statistics Advisory Committee, 2025). Although outcomes for patients with localized disease have improved substantially through advances in screening, surgery, radiotherapy, endocrine therapy, HER2-targeted agents, immune checkpoint inhibitors, and antibody-drug conjugates, metastatic breast cancer (MBC) remains largely incurable. Once distant dissemination occurs, treatment is aimed at disease control, prolongation of survival, and preservation of quality of life rather than cure (Waks and Winer, 2019). Approximately 6–10% of patients present with de novo metastatic disease, while a substantial proportion of patients initially diagnosed with localized disease will eventually develop metastatic recurrence, sometimes decades after primary treatment. The persistent lethality of metastatic breast cancer underscores the urgent translational importance of understanding the biology of metastatic progression (Huber et al., 2026).

Breast cancer is not a single disease but a collection of molecularly and clinically distinct subtypes with differing therapeutic vulnerabilities, recurrence patterns, and organotropic behaviors (Perou et al., 2000; Kennecke et al., 2010). Broadly, tumors are classified according to the expression of hormone receptors (estrogen receptor [ER] and/or progesterone receptor [PR]) and HER2 (human epidermal growth factor receptor 2) amplification status. Hormone receptor-positive (HR<sup>+</sup>) tumors are the most prevalent subtype and are managed with endocrine therapies, often in combination with targeted agents such as CDK4/6 inhibitors; HR<sup>+</sup> tumors tend to recur late and most frequently metastasize to bone. HER2-positive (HER2<sup>+</sup>) tumors, characterized by ERBB2 gene amplification, are treated with HER2-directed therapies and are more prone to early visceral metastasis, including brain and liver. Triple-negative breast cancer (TNBC), defined by the absence of ER, PR, and HER2 amplification, is the most genomically complex and clinically aggressive subtype, with a propensity for early recurrence and visceral metastasis to the lung, liver, and brain (Kennecke et al., 2010). This subtype-dependent heterogeneity in metastatic organotropism indicates that metastatic site preference is fundamentally linked to tumor cell-intrinsic biology rather than being

a stochastic event, a concept with direct implications for the metabolic determinants of organ-selective colonization.

### **13. Organotropism in Breast Cancer: Molecular Determinants of Site-Selective Metastasis**

Breast cancer exhibits reproducible and clinically predictable patterns of organ-selective spread, most commonly involving bone, lung, liver, and brain. This non-random tropism has long served as biological evidence that metastatic success depends on compatibility between tumor cell programs and the specific biology of host tissues, the molecular elaboration of Paget's seed and soil hypothesis (Paget, 1889; Fidler, 2003; Obenauf and Massagué, 2015; Li et al., 2025). Distinct metastatic sites impose distinct biological barriers and opportunities: bone provides a growth factor-rich mineralized matrix and dynamic reciprocal interactions with osteoclasts and osteoblasts; the lung offers a highly vascularized capillary network with distinctive immune cell populations; the brain presents the specialized blood-brain barrier together with a lipid-rich and neurotransmitter-associated metabolic environment; and the liver, as discussed in detail below, functions as the body's metabolic hub and presents a uniquely dynamic, nutrient-rich, and immunologically complex ecological niche.

The molecular basis of organotropism has been substantially illuminated by functional genomic studies. Minn and colleagues demonstrated that breast cancer cells expressing a specific gene signature, including mediators of vascular remodeling, cell adhesion, and inflammatory signaling, were selectively competent to colonize the lung (Minn et al., 2005). Similarly, Bos et al. identified a distinct transcriptional program mediating brain-tropic metastasis in breast cancer, encompassing genes regulating blood-brain barrier transmigration and neuronal microenvironment exploitation (Bos et al., 2009). These findings established the principle that organotropism is at least partly encoded within tumor cell gene expression programs and is subject to selection and amplification during metastatic progression.

An emerging and comparatively underexplored dimension of organotropic selectivity is metabolic compatibility between tumor cells and target organ environments. Tumor cells capable of exploiting the specific nutrient landscape, oxygen gradients, redox milieu, and stromal ecology of a given tissue may possess a selective advantage in that organ that is independent of, or complementary to, their signaling competence. Indeed, compelling evidence has now established

that breast cancer cells destined for different organs display fundamentally distinct metabolic phenotypes (Dupuy et al., 2015; Andrzejewski et al., 2017). This metabolic specification, governed by upstream regulators such as PGC-1 $\alpha$ , suggests that metastatic organotropism is shaped not only by adhesion molecule expression and chemokine receptor engagement, but also by inherent metabolic compatibility with the biochemical environment of the target organ.

#### **14. Liver Metastasis in Breast Cancer: Clinical Significance and Biological Challenges**

Although hepatic metastasis is most classically associated with gastrointestinal malignancies such as colorectal and pancreatic cancers, liver involvement represents a clinically significant and biologically important metastatic site in advanced breast cancer. Liver metastasis occurs in approximately 15–25% of patients with metastatic breast cancer and is particularly frequent in HER2-positive and triple-negative subtypes (Kennecke et al., 2010). Its occurrence is associated with an aggressive disease course, progressive impairment of hepatic function, including hyperbilirubinemia, coagulopathy, and synthetic dysfunction, and markedly reduced survival compared to patients with non-visceral (e.g., bone-only) metastases (Waks and Winer, 2019). Despite advances in systemic therapies, durable eradication of established liver metastases remains uncommon, highlighting the need for mechanistic understanding of the biological processes that enable breast cancer cells to colonize this organ.

The liver's unique anatomy and physiology make it particularly accessible to circulating tumor cells: it receives approximately 75% of its blood supply from the portal vein, which drains the gastrointestinal tract and delivers gut-derived nutrients, metabolites, and potentially disseminated tumor cells directly into the hepatic sinusoidal network. Its dense and fenestrated sinusoidal endothelium facilitates intimate contact between circulating cells and hepatocytes, a feature that contributes to the high seeding efficiency observed in colorectal cancer liver metastasis (Mielgo and Schmid, 2020; Xu et al., 2025). However, mere exposure of the liver to circulating tumor cells does not explain metastatic success, as many disseminated cells that arrive at the liver fail to survive or establish proliferative foci (Luzzi et al., 1998). Thus, hepatic colonization requires specialized adaptive mechanisms that allow tumor cells to persist and expand within the highly specific and demanding hepatic environment, mechanisms that are only beginning to be understood at the molecular and metabolic levels.

## 15. The Liver as a Metabolic and Cellular Ecosystem

The liver is the central organ of systemic metabolic homeostasis, performing functions that are remarkable in both their diversity and their regulatory precision. Hepatocytes, the dominant parenchymal cells of the liver, comprising approximately 70–80% of liver mass, regulate glucose production, glycogen storage, lipid synthesis and oxidation, amino acid catabolism, ketogenesis, cholesterol biosynthesis, bile acid production, plasma protein secretion, and xenobiotic detoxification (Trefts et al., 2017). To sustain this extraordinary metabolic throughput, hepatocytes contain a substantial mitochondrial complement that supports continuous oxidative metabolism, though mitochondrial content is highest in tissues with the greatest continuous energetic demands, such as cardiac muscle and brown adipose tissue. Unlike most tissues, the liver experiences continuous and substantial fluctuations in substrate availability depending on feeding state, fasting duration, hormonal milieu, circadian rhythm, and the composition of gut-derived metabolites delivered through the portal vein (Geisler et al., 2016; Tognini et al., 2017; Trefts et al., 2017; Muñoz et al., 2025). As a result, the hepatic microenvironment is metabolically dynamic rather than static, a property with important implications for tumor cells attempting to colonize it.

A particularly important and frequently overlooked feature of liver biology is its spatial metabolic heterogeneity, or metabolic zonation. Hepatocytes positioned in the periportal zone (nearest to the portal triad where oxygenated blood and nutrient-rich portal flow first enter) are enriched for gluconeogenesis, fatty acid oxidation ( $\beta$ -oxidation), amino acid catabolism, and urea cycle activity. By contrast, hepatocytes in the pericentral zone (adjacent to the central vein where blood has traversed the sinusoid and oxygen tension is lower) preferentially engage glycolysis, de novo lipogenesis, glutamine synthesis, and xenobiotic biotransformation (Trefts et al., 2017). This zoned architecture is maintained by gradients of oxygen, Wnt/ $\beta$ -catenin signaling, hormones, and metabolite concentrations that establish fundamentally distinct biochemical microdomains within a single organ. From the perspective of a disseminated tumor cell, this spatial metabolic heterogeneity means that colonization of the liver is not a uniform biological challenge: tumor cells arresting in periportal versus pericentral regions will encounter profoundly different nutrient landscapes, substrate availabilities, and competition with resident hepatocytes, selective pressures that may favor distinct metabolic phenotypes depending on anatomical location within the liver (Trefts et al., 2017; Guo et al., 2026).

## **15.1 Cellular Composition of the Hepatic Metastatic Niche**

Beyond hepatocytes, the liver contains a rich and functionally specialized complement of non-parenchymal cells that collectively shape the hepatic microenvironment and profoundly influence metastatic colonization. Liver sinusoidal endothelial cells (LSECs), which line the hepatic sinusoids and are characterized by their fenestrated morphology, mediate exchange between the circulation and hepatocytes, regulate immune cell trafficking, and express adhesion molecules and chemokines that may influence early tumor cell arrest and extravasation. Kupffer cells, the liver-resident macrophages, represent a first line of immune defense and can phagocytose circulating tumor cells and initiate cytotoxic immune responses; however, under certain conditions, tumor-educated Kupffer cells and recruited myeloid populations may paradoxically promote metastasis through immunosuppressive cytokine secretion, matrix remodeling, and angiogenic support. Hepatic stellate cells (HSCs), normally quiescent vitamin A-storing pericytes, become activated in response to liver injury and generate extracellular matrix components, inflammatory mediators, and growth factors that can reshape tissue architecture and create pro-metastatic stromal niches, with increased matrix stiffness also activating mechanotransduction pathways that may influence tumor cell fate. The liver additionally harbors a distinctive immune-tolerant environment, shaped by continuous exposure to gut-derived antigens via the portal circulation. (Trefts et al., 2017; Lee et al., 2019; Williamson et al., 2019; Mielgo and Schmid, 2020; Liu et al., 2023). Collectively, these diverse cellular components establish that the hepatic metastatic niche is defined not by hepatocytes alone, but by coordinated interactions among parenchymal, stromal, vascular, and immune compartments, each of which can be modulated by metabolic states, both within tumor cells and within the host liver itself.

## **16. Metabolic Pressures Imposed on Tumor Cells in the Hepatic Niche**

For a disseminated breast cancer cell arriving in the liver, successful colonization requires overcoming a constellation of simultaneously acting metabolic pressures that are qualitatively distinct from those encountered at the primary tumor site or during intravascular transit. Chief among these is the intense metabolic competition imposed by hepatocytes themselves: as the most metabolically active parenchymal cell type in the body, hepatocytes maintain high rates of glucose uptake and processing, fatty acid oxidation, and amino acid catabolism, potentially limiting the substrate availability accessible to infiltrating tumor cells. The dynamic cycling of hepatic

metabolism between fed and fasted states, driven by oscillating insulin, glucagon, and glucocorticoid signals, subjects disseminated cells to periodic fluctuations in glucose and lipid availability that demand metabolic flexibility rather than dependence on a single carbon source. (Pavlova and Thompson, 2016; Trefts et al., 2017; Mielgo and Schmid, 2020; Faubert et al., 2020; Liu et al., 2024; Lin et al., 2024; Tzouanas et al., 2026).

## **17. The Seed and Soil Hypothesis Revisited: Metabolic Co-Adaptation as a New Dimension**

Stephen Paget's seminal 1889 observation that metastatic spread follows non-random patterns of organ preference, determined by biological compatibility between disseminating tumor cells and distant tissue environments (Paget, 1889), initiated a conceptual framework that has proven remarkably durable and continues to guide metastasis research over a century later (Fidler, 2003). Modern research has provided rich molecular validation of this paradigm through studies of adhesion molecules, chemokine receptor-ligand axes, extracellular matrix composition, vascular architecture, and immune cell interactions as determinants of organ-selective colonization (Nguyen and Massagué, 2007; Massagué and Obenauf, 2016).

An additional and comparatively underexplored dimension of the seed-soil relationship is metabolic compatibility. Tumor cells and host tissues must not only communicate through shared signaling pathways but must also coexist within shared metabolic constraints that include nutrient availability, oxygen gradients, redox balance, and bioenergetic demands. Emerging evidence increasingly supports the concept that metastatic success depends on whether tumor cell metabolic programs are compatible with the metabolic landscape of the target organ. In this expanded framework, the liver represents an exceptionally powerful model system for interrogating the metabolic basis of the seed-soil interaction: its "soil" is one of the most metabolically specialized, spatially organized, and experimentally tractable environments in the body, while the "seed", disseminating breast cancer cells, can be engineered, traced, and profiled using robust in vivo models (Trefts et al., 2017; Wang et al., 2024; Liu et al., 2024;).

This expanded metabolic interpretation of the seed-soil framework directly motivates a central unresolved question in the field: Is metastatic success in the liver primarily dictated by tumor cell-intrinsic metabolic fitness, the seed's own metabolic programming, by the metabolic state of the host hepatic environment, the soil, or by reciprocal metabolic interactions between both

compartments? The liver's unique architecture, its inducible genetic tractability through mouse models, and its deep metabolic specialization make it ideally positioned to address this question. Providing a mechanistic answer to this question is the central ambition of this thesis.

## PART IV

### **PGC-1 $\alpha$ in Breast Cancer, the Unresolved Seed–Soil Metabolic Question, and Thesis Rationale**

#### **18. PGC-1 $\alpha$ in Breast Cancer: From Tumor Growth to Metastatic Competence**

Breast cancer is characterized by marked molecular, phenotypic, and metabolic heterogeneity, and this diversity extends to the role of mitochondrial metabolism in disease progression (Pavlova and Thompson, 2016; McGuirk et al., 2020). While glycolytic reprogramming is prominent across many breast cancer subtypes, a substantial body of evidence indicates that mitochondrial oxidative metabolism, and specifically PGC-1 $\alpha$ -regulated programs, plays important and subtype-specific roles in breast cancer biology (Klimcakova et al., 2012; McGuirk et al., 2013; Audet-Walsh et al., 2016; Andrzejewski et al., 2017; McGuirk et al., 2021).

The St-Pierre laboratory was among the first to systematically explore PGC-1 $\alpha$  function in breast cancer. In a foundational study, Klimcakova et al. demonstrated that ectopic expression of PGC-1 $\alpha$  in a murine model of ErbB2/Neu-driven breast cancer enhanced mitochondrial metabolism, stimulated tumor vascularization through upregulation of VEGF, and ultimately promoted primary tumor growth by improving nutrient supply to the tumor (Klimcakova et al., 2012). This work established that PGC-1 $\alpha$  can function as a pro-tumorigenic factor in the specific context of HER2-positive breast cancer. Extending this line of investigation, McGuirk et al. further demonstrated that the PGC-1 $\alpha$ /ERR $\alpha$  transcriptional axis serves as a central regulator of glutamine metabolism in ERBB2-positive breast cancer, coordinately upregulating glutamine transport, catabolism, and reductive carboxylation for lipogenic purposes, and that elevated glutamine pathway gene expression correlates with reduced survival in ERBB2+ breast cancer patients (McGuirk et al., 2013). Together, these studies revealed that PGC-1 $\alpha$  rewires multiple intersecting metabolic circuits, not only mitochondrial respiration, but also glutamine anaplerosis and lipid biosynthesis, in ways that collectively support aggressive tumor behavior in HER2-positive breast cancer.

The connection between PGC-1 $\alpha$  and metastatic behavior of breast cancer cells was further strengthened by two landmark studies. LeBleu and colleagues demonstrated, in a broad analysis of cancer cell lines and clinical specimens, that invasive cancer cells with high metastatic potential upregulate PGC-1 $\alpha$  to drive mitochondrial biogenesis and increase oxygen consumption rates;

crucially, genetic suppression of PGC-1 $\alpha$  impaired metastatic dissemination without substantially affecting primary tumor growth, establishing a functional dissociation between PGC-1 $\alpha$ 's contributions to primary tumor proliferation and to the metastatic program (LeBleu et al., 2014). This was a conceptually important finding, as it implied that PGC-1 $\alpha$ 's relevance may be particularly, and perhaps selectively, acute during the energetically demanding stages of dissemination and colonization. Notably, these findings are not contradictory but rather reflect the context-dependence of PGC-1 $\alpha$  function: Klimcakova et al. (2012) demonstrated a pro-growth effect in the specific context of HER2-driven mammary tumors, whereas LeBleu et al. (2014) observed no broad effect on primary tumor growth across diverse cancer cell lines, while both studies converged on a pro-metastatic function for PGC-1 $\alpha$ . This context-dependence underscores the importance of interpreting PGC-1 $\alpha$  biology within specific oncogenomic settings.

In parallel, the St-Pierre laboratory conducted a systematic analysis of PGC-1 $\alpha$  expression across breast cancer cell line variants with defined organ-specific metastatic tropisms (Andrzejewski et al., 2017). Using murine 4T1 variants that selectively colonize the bone, lung, or liver, as well as human MDA-MB-231 variants with defined organotropic preferences, this study revealed that PGC-1 $\alpha$  expression and mitochondrial respiratory capacity were highest in lung-metastatic cells and substantially lower in liver-tropic cells. PGC-1 $\alpha$  overexpression in breast cancer cells promoted migration, invasion, and lung metastasis in vivo (Andrzejewski et al., 2017). However, liver-tropic breast cancer variants expressed relatively low levels of PGC-1 $\alpha$ , consistent with earlier findings by Dupuy et al. (2015), published prior to Andrzejewski et al. (2017), who showed that liver-tropic breast cancer cells display a metabolic program characterized by increased glycolytic flux and reduced mitochondrial respiration, inversely to what is seen in lung-metastatic cells, a switch driven in part by PDK1-mediated pyruvate dehydrogenase inhibition (Dupuy et al., 2015). These findings generated a controversial and important conceptual paradox: if PGC-1 $\alpha$  promotes overall metastatic competence and mitochondrial fitness, why do liver-tropic breast cancer cells appear to downregulate it? Does reduced PGC-1 $\alpha$  represent a metabolic adaptation specifically favorable for hepatic colonization, perhaps because the demanding oxidative and immune environment of the liver selects for glycolytic tumor cells better adapted to exploit specific metabolic substrates? Alternatively, forced elevation of PGC-1 $\alpha$  in liver-metastatic cells may nevertheless enhance their colonization capacity by providing mitochondrial resilience against the oxidative and nutrient pressures of the hepatic niche.

## **19. The Critical Knowledge Gap: PGC-1 $\alpha$ and Breast Cancer Liver Metastasis**

Despite the body of evidence linking PGC-1 $\alpha$  to breast cancer metastatic competence, its specific role in hepatic colonization, the biologically and clinically distinct process of establishing metastatic disease within the liver, remains critically underexplored. Prior studies have predominantly focused on general metastatic potential or lung-specific metastasis models, leaving the following fundamental questions unresolved:

**Does PGC-1 $\alpha$  functionally regulate breast cancer colonization of the liver?** Given the observation that liver-tropic variants express low endogenous PGC-1 $\alpha$ , it is unknown whether experimental elevation of PGC-1 $\alpha$  in breast cancer cells promotes or impairs their ability to establish metastatic foci specifically within the liver, as opposed to other organs.

**What is the relative contribution of tumor cell-intrinsic versus host hepatocyte PGC-1 $\alpha$  to metastatic success?** The liver's PGC-1 $\alpha$  activity, which is low at baseline and regulated by nutrient and hormonal status (Leone et al., 2005; Rodgers et al., 2005), means that the metabolic state of the host hepatic tissue, regulated in large part by hepatocyte PGC-1 $\alpha$ , could independently shape the permissiveness of the hepatic niche for tumor cell colonization. Whether host hepatic PGC-1 $\alpha$  represents an enabling or restricting factor for incoming tumor cells has never been directly tested.

**Which specific metabolic programs downstream of PGC-1 $\alpha$  are critical for hepatic metastatic fitness?** PGC-1 $\alpha$  coordinates multiple metabolic outputs simultaneously; defining which specific downstream pathways, OXPHOS, fatty acid oxidation, antioxidant defense, TCA flux, or glutamine metabolism, are most critical for hepatic colonization would provide mechanistic specificity and potentially therapeutically actionable vulnerabilities.

**Can PGC-1 $\alpha$ -regulated metabolic programs be therapeutically targeted in hepatic metastasis?** If PGC-1 $\alpha$ -driven metabolism confers a colonization advantage in the liver, metastatic breast cancer cells with elevated PGC-1 $\alpha$  activity might harbor specific vulnerabilities to inhibitors of specific metabolic pathways, a translational implication that would require mechanistic substantiation.

Addressing these questions requires both tumor-cell-directed and host-directed experimental approaches in models specifically designed to interrogate liver colonization, rather than general metastatic competence.

## 20. Central Hypothesis and Experimental Framework

The paradoxical observation that liver-tropic breast cancer cells express relatively low endogenous PGC-1 $\alpha$ , despite evidence that PGC-1 $\alpha$  generally promotes metastatic competence and that the liver imposes severe metabolic demands on colonizing tumor cells, provides the central intellectual tension driving this thesis. This paradox raises two distinct and tractable experimental questions. First, does the low-PGC-1 $\alpha$ , glycolytic phenotype of liver-tropic cells represent a metabolic optimum for hepatic colonization, such that elevating PGC-1 $\alpha$  might be limiting rather than advantageous, or does elevating PGC-1 $\alpha$  provide mitochondrial resilience that enhances colonization capacity? Second, does the PGC-1 $\alpha$ -dependent oxidative metabolic program of the host hepatocytes independently modulate the permissiveness of the hepatic niche for incoming tumor cells?

**Central Hypothesis:** We hypothesized that elevated PGC-1 $\alpha$  in liver-tropic breast cancer cells may limit their hepatic metastatic capacity, given that these cells naturally adopt a low-PGC-1 $\alpha$ , glycolytic phenotype that may represent a metabolic state favorable for liver colonization. Separately, we hypothesized that hepatocyte-specific loss of PGC-1 $\alpha$  would limit breast cancer metastatic colonization by reducing the metabolic flexibility of the liver tissue and its capacity to sustain tumor outgrowth. Together, these two hypotheses framed a dual seed-and-soil experimental strategy in which PGC-1 $\alpha$  activity was independently manipulated in the tumor cell and in the host hepatocyte to determine its contribution to hepatic colonization efficiency from each compartment.

To directly test these hypotheses, this thesis employed a complementary dual-compartment experimental strategy. In the tumor cell compartment (seed), breast cancer cells with defined liver-metastatic tropism were engineered to overexpress PGC-1 $\alpha$  and evaluated in an orthotopic spontaneous metastasis model *in vivo*. These cells were also subjected to intrasplenic portal vein injection, which selectively seeds the liver via portal circulation, allowing direct quantification of hepatic metastatic burden as a function of tumor-cell PGC-1 $\alpha$  status. In the host hepatocyte compartment (soil), liver-specific *Ppargc1a* knockout (KO) mice were generated using Alb-Cre-

mediated deletion of floxed *Ppargc1a* alleles to determine whether loss of hepatocyte PGC-1 $\alpha$  alters the liver's capacity to support or restrict metastatic colonization. Transcriptomic and metabolomic analyses of liver-metastatic breast cancer cells were incorporated to define the downstream PGC-1 $\alpha$ -regulated programs associated with metastatic fitness. This integrated design allowed for a direct and simultaneous comparison of seed-intrinsic and soil-intrinsic PGC-1 $\alpha$  contributions to breast cancer liver metastasis.

## **21. Conceptual and Translational Significance of This Work**

The results emerging from this thesis carry significance at multiple interconnected levels. Conceptually, they refine and molecularly specify the metabolic dimension of the seed-soil hypothesis, allowing for a direct experimental answer to the question of whether metastatic success in the liver is primarily determined by tumor cell-intrinsic metabolic programming, by the metabolic state of the host organ, or by reciprocal interactions between both. Biologically, this work identifies mitochondrial regulatory programs as critical determinants of organ-specific metastatic outgrowth, extending the known functions of PGC-1 $\alpha$  beyond general metastatic biology to the context of hepatic colonization specifically. Translationally, the demonstration that PGC-1 $\alpha$ -dependent metabolic programs influence metastatic fitness in the liver has direct implications for the therapeutic targeting of oxidative metabolism in metastatic breast cancer. Metastatic cells that rely on PGC-1 $\alpha$ -driven mitochondrial programs for survival within the hepatic niche may harbor specific vulnerabilities to inhibitors of the ETC, mitochondrial biogenesis, or fatty acid oxidation, vulnerabilities that might be exploited selectively in the metastatic setting while sparing normal tissues. Regarding the hepatic soil compartment, the experimental results presented in this thesis demonstrate that hepatocyte-specific PGC-1 $\alpha$  status does not significantly alter hepatic metastatic colonization efficiency under the conditions studied, indicating that therapeutic efforts should be directed at the tumor cell rather than the hepatic metabolic environment.

## **22. Conclusion: A Mechanistic Framework for Metabolic Regulation of Organ-Specific Metastasis**

In summary, this thesis is motivated by the convergence of three major lines of evidence and reasoning: the clinical urgency of metastatic breast cancer and the particular lethality of hepatic

involvement; the emerging principle that metabolic plasticity, governed by upstream regulators such as PGC-1 $\alpha$ , is a critical and understudied determinant of organ-specific metastatic colonization; and the unresolved paradox that liver-tropic breast cancer cells express low PGC-1 $\alpha$  despite the demanding hepatic environment. By dissecting the role of PGC-1 $\alpha$  in both the tumor cell and the host hepatocyte compartment, this work aims to provide a mechanistic framework linking mitochondrial regulatory programs to the biology of breast cancer liver metastasis, to define which cellular compartment, seed or soil, is the primary metabolic determinant of hepatic colonization, and to identify molecular vulnerabilities in the metastatic process that might be therapeutically exploited. The findings reported herein advance our fundamental understanding of metastatic metabolism and contribute to the broader goal of developing rational, mechanism-based strategies to prevent and treat the most lethal stage of breast cancer progression.

## CHAPTER 2

### **PGC-1 $\alpha$ -Driven Oxidative Metabolic Reprogramming in Tumor Cells, Not Host Hepatocytes, Governs Breast Cancer Liver Metastasis**

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The authors declare that they have no conflicts of interest with the contents of this article.

## ABSTRACT

Liver metastasis affects 15–25% of patients with metastatic breast cancer and carries poor prognosis, yet the metabolic determinants governing hepatic colonization remain poorly understood. Liver-tropic breast cancer cells express paradoxically low levels of PGC-1 $\alpha$ , the master transcriptional coactivator of mitochondrial biogenesis and oxidative phosphorylation (OXPHOS), and instead rely predominantly on glycolysis. We hypothesized that this low-PGC-1 $\alpha$  state represents a metabolic adaptation favorable for hepatic colonization, such that elevating PGC-1 $\alpha$  might limit colonization competence, and that hepatocyte-specific *Ppargc1a* loss would impair colonization by reducing hepatic metabolic flexibility. To test both hypotheses, we employed a dual-compartment seed-and-soil experimental strategy combining gain-of-function and conditional tissue-specific loss-of-function models with in vivo metastasis assays, genome-wide transcriptomics, and targeted metabolomics. Contrary to the seed hypothesis, PGC-1 $\alpha$  overexpression in liver-tropic 2792 murine breast cancer cells repositioned bioenergetics from glycolytic-dominant to oxidative-dominant, amplifying mitochondrial ATP production while reducing glycolytic output, and selectively induced *Sod2* and *Uqcrc1*. RNA sequencing identified "Oxidative phosphorylation" as the overwhelmingly dominant activated KEGG pathway, and metabolomics confirmed multi-omic convergence on OXPHOS, serine-glycine one-carbon, and TCA cycle programs. In vivo, PGC-1 $\alpha$  overexpression had no effect on primary tumor growth yet dramatically increased hepatic lesion number and metastatic area in the intrasplenic colonization model. In striking contrast, hepatocyte-specific *Ppargc1a* deletion produced no significant change in hepatic colonization efficiency. These findings establish tumor cell-intrinsic PGC-1 $\alpha$  as a potent and unexpected amplifier of breast cancer hepatic colonization, while the hepatic microenvironment is dispensable, revealing the metabolic seed as the decisive variable.

**Keywords:** PGC-1 $\alpha$ ; breast cancer; liver metastasis; oxidative phosphorylation; mitochondrial biogenesis; metabolic reprogramming; seed and soil; hepatic colonization.

## INTRODUCTION

Metastatic breast cancer remains the dominant cause of breast cancer mortality, with systemic dissemination accounting for the majority of cancer-related deaths despite transformative advances in targeted therapeutics, immune checkpoint inhibitors, and antibody-drug conjugates (Bray et al., 2024; Siegel et al., 2024; Canadian Cancer Statistics Advisory Committee, 2025). Hepatic involvement occurs in approximately 15–25% of patients with metastatic breast cancer, is particularly frequent in HER2-positive and triple-negative subtypes, and carries especially poor prognosis, with median survival often measured in months and therapeutic options remaining largely palliative (Kennecke et al., 2010; Waks and Winer, 2019). For a disseminated tumor cell arriving in the liver, successful colonization requires simultaneously withstanding cytotoxic immune pressure from Kupffer cells and liver-resident natural killer cells (Liu et al., 2023), resisting anoikis in a novel adhesive context, navigating the specific metabolic landscape of the hepatic environment including its distinct substrate composition, oxygen gradients, and sinusoidal architecture (Trefts et al., 2017; Mielgo and Schmid, 2020), and transitioning from a stress-resistant survival state to biosynthetically intensive proliferative outgrowth. These demands collectively implicate tumor cell bioenergetic capacity and metabolic flexibility as critical determinants of hepatic colonization competence.

An emerging and comparatively underexplored dimension of metastatic organotropism is metabolic compatibility between tumor cells and the specific bioenergetic ecology of target organs (Obenauf and Massagué, 2015; Li et al., 2025). Breast cancer cells with defined organ-specific tropisms display fundamentally distinct metabolic phenotypes. Lung-tropic variants express elevated PGC-1 $\alpha$  (encoded by *PPARGC1A*), the master transcriptional coactivator of mitochondrial biogenesis and OXPHOS (OXPHOS), and rely substantially on mitochondrial respiration (Andrzejewski et al., 2017). In sharp contrast, liver-tropic breast cancer cells express paradoxically low PGC-1 $\alpha$  and instead adopt a metabolic program characterized by elevated glycolytic flux and reduced mitochondrial respiration, a phenotypic switch driven in part by HIF-1 $\alpha$ -mediated upregulation of pyruvate dehydrogenase kinase 1 (PDK1) (Dupuy et al., 2015; Andrzejewski et al., 2017). This divergence raises a central and unresolved question: does the low-PGC-1 $\alpha$ , glycolytic state of liver-tropic cells represent a metabolic adaptation specifically

favorable for hepatic colonization, such that artificially elevating PGC-1 $\alpha$  might limit their metastatic capacity in the liver?

PGC-1 $\alpha$  has emerged as a context-dependent regulator of metastatic behavior across multiple cancer types. LeBleu et al. demonstrated that invasive cancer cells with high metastatic potential upregulate PGC-1 $\alpha$  to drive mitochondrial biogenesis, that PGC-1 $\alpha$  facilitates cancer cell survival during intravascular transit, and that genetic suppression of PGC-1 $\alpha$  impairs metastatic dissemination without substantially affecting primary tumor growth, establishing a functional dissociation between PGC-1 $\alpha$ 's contributions to primary tumor proliferation and the metastatic program (LeBleu et al., 2014). In breast cancer specifically, foundational work from the St-Pierre laboratory demonstrated that PGC-1 $\alpha$  promotes primary tumor growth in HER2-positive disease (Klimcakova et al., 2012), regulates glutamine metabolism through the PGC-1 $\alpha$ /ERR $\alpha$  axis (McGuirk et al., 2013), and mediates therapeutic resistance to anthracycline chemotherapy (McGuirk et al., 2021). A systematic analysis of organ-tropic 4T1 and MDA-MB-231 variants established that PGC-1 $\alpha$  expression and OXPHOS capacity are highest in lung-tropic cells, that PGC-1 $\alpha$  overexpression promotes lung metastasis in vivo, and that liver-tropic variants maintain endogenously low PGC-1 $\alpha$  (Andrzejewski et al., 2017). Given that liver-tropic cells naturally adopt a low-PGC-1 $\alpha$ , glycolytic phenotype, we hypothesized that this state may represent a metabolic adaptation favorable for hepatic colonization, such that experimentally elevating PGC-1 $\alpha$  in these cells might limit their capacity to establish metastatic foci in the liver.

An equally important and unresolved question concerns the host liver compartment. The liver's PGC-1 $\alpha$  activity is low at baseline and dynamically regulated by nutritional and hormonal status, rising during fasting to support gluconeogenesis and fatty acid oxidation (Leone et al., 2005; Rodgers et al., 2005). Whether the PGC-1 $\alpha$ -dependent metabolic state of host hepatocytes independently shapes the liver's permissiveness for metastatic colonization has never been tested. We hypothesized that hepatocyte-specific loss of *Ppargc1a* would limit breast cancer metastatic colonization by reducing the metabolic flexibility of the hepatic tissue and its capacity to sustain tumor outgrowth.

Here we employ a systematic dual-compartment seed-and-soil experimental strategy, independently manipulating PGC-1 $\alpha$  activity in liver-tropic tumor cells and in host hepatocytes through complementary gain-of-function and loss-of-function models combined with in vivo

metastasis assays, genome-wide transcriptomics, and targeted metabolomics. Contrary to our seed hypothesis, PGC-1 $\alpha$  overexpression dramatically amplifies rather than limits hepatic colonization efficiency, while the soil hypothesis was equally unsupported, as hepatocyte-specific *Ppargc1a* deletion had no effect on colonization. Together these results establish that the low endogenous PGC-1 $\alpha$  state of liver-tropic breast cancer cells is not a metabolic adaptation optimized for hepatic colonization but rather a suboptimal default state that actively limits their colonization potential, and that the tumor cell's own metabolic programming, not the hepatic microenvironment, is the decisive determinant of metastatic success in the liver. This finding reframes how we understand metabolic organotropism, provides a mechanistic basis for the recently established observation that liver-tropic and lung-tropic breast cancer cells display fundamentally distinct metabolic phenotypes, and identifies tumor cell-intrinsic PGC-1 $\alpha$ -driven oxidative programs as both markers of metastatic aggressiveness and candidate vulnerabilities for therapeutic intervention in breast cancer liver metastasis.

## MATERIALS AND METHODS

### 1. Cell Lines and Standard Culture Conditions

Two murine mammary carcinoma cell lines served as the primary in vitro experimental systems for this study. The 4T1 cell line (ATCC CRL-2539) is a highly aggressive, poorly differentiated triple-negative breast cancer line syngeneic to BALB/c mice that spontaneously metastasizes to the lungs, liver, bone, and brain following orthotopic implantation, faithfully recapitulating stage IV human breast cancer. The 2792 cell line is a liver-tropic variant selected for preferential hepatic colonization and characterized by relatively low endogenous PGC-1 $\alpha$  expression, the metabolic phenotype that constitutes the central paradox motivating this study (Andrzejewski et al., 2017). By engineering matched gain-of-function PGC-1 $\alpha$  variants in both lines, these cells provided complementary tools to dissect how augmenting mitochondrial capacity affects breast cancer biology specifically in the context of liver-directed metastasis.

All cell lines were cultured at 37°C under a humidified atmosphere of 5% CO<sub>2</sub> in Dulbecco's Modified Eagle's Medium (DMEM; Wisent Inc., Saint-Bruno, QC) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 20 mM HEPES, and 1% penicillin-streptomycin. Stable lentiviral transductants (see Section 2) were maintained under continuous puromycin selection (6  $\mu$ g/mL; Sigma-Aldrich) throughout all experiments to prevent transgene silencing. Cells were passaged at 70-80% confluency and were confirmed mycoplasma-free by PCR-based testing (Lookout Mycoplasma PCR Detection Kit, Sigma-Aldrich) prior to all in vitro and in vivo experiments. Cell identity was authenticated by Short Tandem Repeat (STR) profiling before the commencement of this study.

### 2. Generation of Stable PGC-1 $\alpha$ -Overexpressing Breast Cancer Cell Lines by Lentiviral Transduction

To directly test the functional consequences of PGC-1 $\alpha$  activation in liver-tropic breast cancer cells, stable populations constitutively overexpressing full-length murine PGC-1 $\alpha$  were generated. The murine *Ppargc1a* coding sequence was cloned into the lentiviral expression vector pLX\_307, and a matched empty-vector (EV) construct was prepared in parallel as a control. Custom lentiviral particles were produced by Sigma-Aldrich. Cells were seeded at  $5 \times 10^4$  cells per well 24 hours prior to transduction. Lentiviral particles were added at a multiplicity of infection (MOI) of 5 in

the presence of 8 µg/ml polybrene to enhance transduction efficiency. Medium was replaced 24 hours post-transduction, and puromycin selection (5 µg/ml) was initiated 72 hours post-transduction and maintained to eliminate non-transduced cells. Stably transduced populations were subsequently subjected to single-cell clonal isolation to generate isogenic clonal lines used in all subsequent experiments.

### **3. RNA Extraction and Quantitative Reverse-Transcription PCR (RT-qPCR)**

Total RNA was extracted from sub-confluent cell monolayers using the PureLink RNA Mini Kit (Thermo Fisher Scientific) following the manufacturer's protocol, including an on-column PureLink DNase I digestion step to eliminate genomic DNA contamination. RNA concentration was quantified by spectrophotometry (NanoDrop One; Thermo Fisher Scientific), and purity was assessed by the  $A_{260}/A_{280}$  ratio (acceptable range: 2-2.50). First-strand complementary DNA (cDNA) was synthesized from 1000 ng of total RNA per reaction using the High-Capacity cDNA reverse transcription kit with RNase Inhibitor Master Mix (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's protocol. The cDNA product was diluted to the concentration of 10 ng /µL in RNase-free water prior to use.

Quantitative PCR was performed on an Applied Biosystems QuantStudio 3 Real-Time PCR System in a total reaction volume of 20 µL per well, containing 18 µL Taqman master mix, 20X of each gene-specific primer, and 20 ng of diluted cDNA template. Standard thermocycling conditions were: initial denaturation at 95°C for 30 seconds; 40 amplification cycles of 95°C for 5 seconds and 60°C for 30 seconds, followed by melt-curve analysis from 65°C to 95°C in 0.5°C increments to verify the specificity of each amplicon. All reactions were run in technical triplicate. Gene-specific primers were designed to span exon-exon junctions to preclude amplification of genomic DNA, and were validated for amplification efficiency between 90% and 110% by serial dilution standard curve analysis prior to use. Relative mRNA expression was calculated by the  $2^{-\Delta\Delta C_t}$  method, normalized to the average mean of the house keeping gene (*Tbp*) selected based on expression stability analysis across all experimental conditions. A minimum of three independent biological replicates were used for all RT-qPCR experiments.

#### **4. Cell Proliferation Assay**

To determine whether PGC-1 $\alpha$  overexpression influences the intrinsic proliferative capacity of breast cancer cells, growth kinetics were quantified over a five-day time course by direct cell counting. Cells were seeded at 7,500 cells per well in six-well plates in 2 mL complete growth medium. At each designated time point, days 2, 3, 4, and 5 post-seeding, adherent cells were detached by trypsinization with 0.25% trypsin-EDTA (WISENT) and resuspended in complete medium. Live cell counts were determined using a Countess II Automated Cell Counter (ThermoFisher Scientific) with Trypan Blue exclusion to identify and exclude non-viable cells. Only viability-corrected live cell counts were used for growth curve construction. Experiments were performed in independent biological triplicate across a minimum of three separate passages, and growth curves were compared between PGC-1 $\alpha$ -overexpressing and empty-vector control lines.

#### **5. Real-Time Mitochondrial and Glycolytic Bioenergetic Profiling by Extracellular Flux Analysis**

To characterize the bioenergetic phenotype conferred by PGC-1 $\alpha$  overexpression, oxygen consumption rate (OCR), a real-time surrogate of mitochondrial oxidative phosphorylation, and extracellular acidification rate (ECAR), a surrogate of net glycolytic proton extrusion, were measured simultaneously and dynamically using the Seahorse XFe96 Extracellular Flux Analyzer (Agilent Technologies). Accordingly, the Seahorse XF Cell Mito Stress Test was employed to resolve key bioenergetic parameters and to calculate absolute rates of mitochondrial and glycolytic ATP production.

##### **5.1 Cell Seeding and Pre-assay Equilibration**

Twenty-four hours prior to the assay, 7,500 cells per well were seeded in Seahorse XFe96 Cell Culture Microplates (Agilent Technologies) in 200  $\mu$ L of their standard growth medium. On the day of the assay, 170  $\mu$ L of growth medium was aspirated, washed three times with 200  $\mu$ L XF DMEM Assay media, followed by adding 150  $\mu$ L of pre-warmed Seahorse XF DMEM Assay Medium to a final total volume of 180  $\mu$ L (pH 7.40; Agilent Technologies) supplemented with 1 M glucose (Agilent Technologies), 100 mM pyruvate (Agilent Technologies), and 200 mM glutamine (Agilent Technologies), each added from concentrated stock solutions and place in the

water bath (37°C) before use. Plates were subsequently transferred to a CO<sub>2</sub>-free incubator at 37°C for 60 minutes to establish equilibration prior to loading. The Seahorse sensor cartridge was hydrated overnight in XF Calibrant solution (Agilent Technologies) at 37°C in a non-CO<sub>2</sub> incubator according to the manufacturer's instructions.

## **5.2 Modified Mitochondrial Stress Test Protocol with BAM15 Uncoupling**

Baseline OCR and ECAR were measured over three measurement cycles (each cycle: 3 minutes mix, 3 minutes measure). Four pharmacological agents were then delivered through the instrument's integrated port system with three measurement cycles acquired following each injection. The injection sequence was as follows:

(i) Oligomycin (2.5 µM; Sigma-Aldrich): an inhibitor of the mitochondrial F<sub>0</sub>F<sub>1</sub>-ATP synthase (Complex V). The resulting suppression of OCR defines the ATP-linked respiration rate, and the residual OCR represents proton leak across the inner mitochondrial membrane. The concomitant increase in ECAR after oligomycin treatment reflects the compensatory upregulation of glycolysis in response to suppressed mitochondrial ATP synthesis.

(ii) BAM15 (2.5 µM; Sigma-Aldrich): a mitochondria-targeted protonophore that selectively dissipates the inner mitochondrial membrane electrochemical gradient to drive maximal electron transport chain (ETC) activity. BAM15 was employed in preference to the conventional carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) uncoupler because BAM15 does not depolarize the plasma membrane at standard concentrations, avoiding the artifactual suppression of OCR caused by FCCP-induced plasma membrane ion transport in many cancer cell lines (Kenwood et al., 2013). The OCR following BAM15 injection defines maximal respiratory capacity, and the difference between maximal and basal OCR defines spare respiratory capacity, a metric of bioenergetic reserve.

(iii) Rotenone (2.5 µM) + Antimycin A (2.5 µM) (Sigma-Aldrich): added simultaneously to inhibit mitochondrial Complex I and Complex III, respectively, thereby completely abolishing mitochondrial electron transfer. The residual OCR following this injection defines non-mitochondrial oxygen consumption. Subtracting this value from all preceding OCR measurements yields mitochondria-specific OCR.

(iv) Monensin (36.08  $\mu\text{M}$ ; Sigma-Aldrich) + Hoechst 33342 (20  $\mu\text{M}$ ; Thermo Fisher Scientific): monensin is an electroneutral  $\text{Na}^+/\text{H}^+$  ionophore that drives maximal glycolytic acidification rate by stimulating  $\text{Na}^+$ -coupled transport and osmotic stress. Hoechst 33342 was co-injected in the final port to stain nuclei for post-assay cell-count normalization.

### **5.3 Post-assay Imaging, Normalization, and Bioenergetic Calculations**

Immediately following completion of all flux measurements, the Seahorse microplate was imaged on an EVOS FL Auto Cell Imaging System (Thermo Fisher Scientific) using a 10 $\times$  objective and the DAPI fluorescence channel. Automated nuclei counting was performed using ImageJ (NIH) with the Analyze Particles module applied to thresholded DAPI images to determine the number of live cells per well. All raw OCR and ECAR values were normalized to well-specific nuclei counts to correct for differential seeding efficiency and any growth occurring during the overnight seeding period.

Data analysis and result reporting of the XF Cell Mito Stress tests were performed using the Agilent XF Seahorse Analytics, a web-based software platform that automatically calculate the key parameters and provides relative graphs (<https://seahorseanalytics.agilent.com>). From the normalized kinetic traces, the following bioenergetic parameters were computed: basal respiration; ATP-linked respiration; proton leak; maximal respiratory capacity; spare respiratory capacity; and non-mitochondrial oxygen consumption. Absolute rates of mitochondrial ATP production ( $J_{\text{ATP,ox}}$ ; in pmol ATP/min/cell) and glycolytic ATP production ( $J_{\text{ATP,glyc}}$ ; in pmol ATP/min/cell) were calculated from the normalized OCR and ECAR traces according to the stoichiometric framework established by Mookerjee et al., 2017. All Seahorse experiments were performed in ten technical replicates per condition and independently replicated on three separate occasions.

## **6. Targeted Metabolomic Profiling by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)**

To define the intracellular metabolic reprogramming induced by PGC-1 $\alpha$  overexpression and to identify specific alterations in central carbon metabolic pathways relevant to metastatic fitness, targeted quantitative metabolomic profiling was performed by LC-MS/MS. This platform simultaneously quantified over 100 metabolites spanning glycolysis, the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle, the urea cycle, nucleotide metabolism, and amino

acid metabolism. To minimize ex vivo metabolite degradation, all extraction steps involving labile phosphorylated species were performed with strict temperature control, and all solvents were of LC-MS/MS grade pre-equilibrated to  $-20^{\circ}\text{C}$ .

### **6.1 Cell Seeding and Metabolite Extraction**

Cells were seeded in six-well plates and cultured for 48 hours under standard conditions to allow establishment of metabolic steady state. At the time of harvest, growth medium was rapidly aspirated, and cell monolayers were immediately washed three times with 1 mL of ice-cold 150 mM ammonium formate (pH 7.4) to quench metabolism and remove extracellular metabolites without perturbing intracellular pools. Cells were lysed by addition of 380  $\mu\text{M}$  ice-cold 50% (v/v) LCMS-grade methanol directly to the plate, and cell lysates were transferred by scraping into pre-chilled 2 mL microcentrifuge tubes containing six ceramic beads (1.4 mm), pre-cooled on dry ice. 220  $\mu\text{M}$  Ice-cold LCMS-grade acetonitrile was added to achieve a final extraction solvent. Samples were vortexed for 10 seconds, then subjected to two rounds of bead-beating lysis at 2,000 rpm for 60 seconds each using a MagNA Lyser instrument (Roche Life Science) to ensure complete mechanical disruption of cells and quantitative metabolite release.

Following lysis, 600  $\mu\text{L}$  ice-cold LCMS-grade dichloromethane and 300  $\mu\text{L}$  LC-MS grade water were added to create a biphasic liquid-liquid extraction system separating polar metabolites (upper aqueous phase) from lipophilic species (lower organic phase). Samples were vortexed for 60 seconds with periodic vortexing to facilitate partitioning, then samples were incubated on ice for 10 minutes, then centrifuged at 10000 rpm for 10 minutes at  $4^{\circ}\text{C}$ . The upper polar aqueous phase (550  $\mu\text{L}$ ) was carefully transferred to a new pre-chilled tube and dried to completeness using a refrigerated CentriVap Vacuum Concentrator at  $-4^{\circ}\text{C}$  (Labconco Corporation, Kansas City, USA).

### **6.2 Chromatographic Separation**

Samples were resuspended in water and run on an Agilent 6470A tandem quadrupole mass spectrometer equipped with a 1290 Infinity II ultra-high performance LC (Agilent Technologies) utilizing the Metabolomics Dynamic Multiple Reaction Monitoring Database and Method (Agilent), which uses an ion-pairing reverse phase chromatography (Agilent Technologies). This method was further optimized for phosphate-containing metabolites with the addition of 5  $\mu\text{M}$

InfinityLab deactivator (Agilent) to mobile phases A and B, which requires decreasing the backflush acetonitrile to 90%.

Reversed phase separation was obtained using the ZORBAX RRHD Extend-C18 column (2.1 × 150 mm, 1.8 micron, Agilent technologies) coupled to a guard column, ZORBAX Extend Fast Guards for UHPLC, Extend-C18, (2.1 mm, 1.8 micron). The mobile phase A and B are water and methanol respectively and both containing 10mM tributylamine and 5 µM Agilent's InfinityLab deactivator solution. The auto-sampler and column compartment temperatures were maintained at 4°C and 35°C respectively. Samples were analyzed using 250 µL/min flow rate with following gradient: 0-2.5 min 100%A, 2.5-7.5 min 100%-80% A; 7.5-13 min 80%-55% A, 13-20 min 55%-1% A, 20-24 min holding at 1% A, 24.05-31.5 min backflushing the column with 90% Acetonitrile using channel C and then 32.25-40 min equilibrating the column with 100% A.

### **6.3 Tandem Mass Spectrometry Detection**

Mass spectrometry detection settings included: N<sub>2</sub> drying gas temperature 150°C; N<sub>2</sub> drying gas flow 13 L/min; nebulizer pressure 45 psig, sheath gas temperature 325°C, sheath gas flow 12L/min, capillary voltage 2000 V, and Nozzle voltage of 500 V. Samples were injected in negative ESI mode. Dynamic multiple reaction monitoring (dMRM) transitions were optimized using authentic standards and quality control samples.

### **6.4 Quantification and Data Processing**

Metabolites were quantified by integrating the area under the curve of each compound using external standard calibration curves with Mass Hunter Quant software (Agilent technologies). No corrections for ion suppression or enhancement were performed, as such, uncorrected metabolite concentrations are presented. Metabolomics analyses were performed at the University of Ottawa Metabolomics Core Facility, which is supported by the Terry Fox Foundation and the University of Ottawa.

## **7. Genome-Wide Transcriptomic Profiling by RNA Sequencing (RNA-seq)**

To comprehensively characterize the transcriptional programs induced by PGC-1 $\alpha$  overexpression in breast cancer cells and to identify pathway-level alterations with functional relevance to metastatic biology, strand-specific polyA-selected bulk RNA sequencing was performed.

## 7.1 RNA Isolation and Quality Control

Cells were harvested directly into TRIzol reagent (600 µL per well of a six-well plate; Thermo Fisher Scientific) and stored at  $-80^{\circ}\text{C}$  until extraction. Total RNA was extracted using the TRIzol Plus RNA Purification Kit (Thermo Fisher Scientific), incorporating an on-column PureLink DNase I digestion step to eliminate residual genomic DNA. RNA concentration was determined by fluorometry using the Qubit RNA Broad Range Assay Kit (Thermo Fisher Scientific), and RNA integrity was assessed using the Agilent 2100 Bioanalyzer with the RNA 6000 Nano Kit (Agilent Technologies). Only samples with an RNA Integrity Number (RIN)  $\geq 8.0$  were advanced to library preparation, ensuring that sequencing data accurately reflected steady-state transcript abundances rather than degradation artefacts.

## 7.2 Library Preparation and Next-Generation Sequencing

All library preparation and sequencing procedures were performed by the Genomics Platform of the Institute for Research in Immunology and Cancer (IRIC) at the Université de Montréal. Strand-specific mRNA sequencing libraries were prepared from 500 ng of total RNA per sample using the KAPA RNA HyperPrep Kit (Roche) with polyA mRNA enrichment performed using oligo-dT magnetic beads (Thermo Fisher Scientific) to selectively capture and sequence polyadenylated transcripts. Library quality and average fragment size were assessed using the Agilent 2100 Bioanalyzer with the High Sensitivity DNA Kit prior to sequencing. Next-generation sequencing was performed on the Illumina NextSeq 2000 platform, generating approximately 30 million single-end reads of 101 base pairs per sample, a depth sufficient for robust differential expression analysis and pathway-level interpretation.

## 7.3 Bioinformatic Processing Pipeline

Raw sequencing read quality was assessed using FastQC (version 0.12.1). Adapter sequences and polyA tails were trimmed from raw reads using fastp (version 1.0.1) with default parameters and automatic adapter detection. Quality-controlled reads were aligned to the *Mus musculus* reference genome assembly GRCm39 (Ensembl release 109; Ensembl.org) using STAR (version 2.7.11b) in two-pass alignment mode: a first-pass alignment was performed on a randomly sampled subset of reads across all samples to discover novel splice junctions with high sensitivity, and these junctions were incorporated into the genome index for the second-pass, definitive alignment of all reads

across all samples. This two-pass strategy substantially improves the accuracy of splice-site detection and read alignment at unannotated junctions.

Gene-level read counts were generated from coordinate-sorted BAM alignment files using the `featureCounts` function of the `Rsubread` package (version 2.26.0; Bioconductor), with parameters set for strand-specific libraries (`strandness = 1`) and counting of reads mapping unambiguously to annotated exonic regions, using the Ensembl GRCm39 release 109 GTF annotation file. Multi-mapping reads and reads mapping to multiple features were excluded from count matrices.

#### **7.4 Differential Gene Expression Analysis**

All downstream statistical analyses were conducted in R (version 4.6.0). Raw count matrices were imported into DESeq2 (version 1.52.0; Bioconductor) for differential gene expression analysis using the negative binomial generalized linear model framework. Prior to analysis, lowly expressed genes were removed by applying a minimum count threshold of 10 reads in at least the number of samples corresponding to the smallest experimental group, reducing noise from genes with insufficient statistical power for reliable differential testing. Size factor normalization was performed using DESeq2's median-of-ratios method to correct for differences in library sequencing depth. Dispersion was estimated using the DESeq2 empirical Bayes shrinkage procedure. Differential expression was assessed using the Wald test with  $\log^2$  fold change shrinkage applied using the `apecglm` estimator to produce stable effect size estimates for low-count genes. Genes were classified as significantly differentially expressed at an adjusted *P*-value (Benjamini–Hochberg false discovery rate; FDR) < 0.05.

#### **7.5 Gene Set Enrichment Analysis (GSEA)**

To identify coordinated transcriptional programs and biological pathways enriched in PGC-1 $\alpha$ -overexpressing cells relative to empty-vector controls, Gene Set Enrichment Analysis (GSEA) was performed using the `clusterProfiler` package (version 4.20.0; Bioconductor). Analyses were conducted using a pre-ranked gene list ordered by the signed  $\log_2$ FoldChange, which simultaneously encodes both the magnitude and direction of expression change and preserves the full transcriptome-wide ranking for unbiased enrichment testing. Two complementary curated gene set collections were queried in parallel: (i) Gene Ontology Biological Process (GO: BP) terms and (ii) KEGG Metabolic and Signaling Pathway gene sets, providing independent orthogonal

perspectives on pathway-level enrichment. Gene sets with fewer than 10 or more than 800 member genes were excluded. Enrichment significance was assessed by permutation testing (1,000 permutations) and reported at  $FDR < 0.05$ . Results were visualized as ranked dot plots for the top enriched and depleted terms using ggplot2 and enrichplot packages in R. Enrichment score plots were generated for pathways of primary mechanistic interest.

## **8. Ethical Statement and Animal Husbandry**

All animal experiments were designed and performed in strict accordance with the guidelines of the Canadian Council on Animal Care (CCAC) and were approved by the Facility Animal Care Committee (FACC) of McGill University under Animal Use Protocol number MCGL-5129. Animals were housed under specific pathogen-free (SPF) conditions on a 12-hour light-dark cycle. The facility operates within institutionally specified environmental set points of 18–24 °C and 30–70% relative humidity; room temperature and humidity were maintained by the facility within these limits but were not recorded as experimental variables in this study. Breeding cages were maintained as pairs (one male and one female per cage). Stock and experimental animals were group-housed at a maximum of five mice per cage. Animals were provided with ad libitum access to standard irradiated rodent chow and sterile water. Cages, bedding, and enrichment items were autoclaved regularly. All procedures were performed by personnel holding appropriate institutional FACC and CCAC certification. Animal numbers per experiment were determined by power analysis based on pilot data, with group sizes selected to achieve 80% statistical power at  $\alpha = 0.05$ , subject to CCAC 3Rs principles (Replacement, Reduction, and Refinement) to minimize animal use.

## **9. Mouse Models**

### **9.1 Syngeneic Immunocompetent Host: BALB/c Mice**

Female BALB/c mice (4 weeks of age) were purchased from Charles River Laboratories (strain code 028, Saint-Constant, QC, Canada) and acclimatized in the Goodman Cancer Institute Animal Facility for a minimum of 7 days prior to experimental procedures. The BALB/c strain is the syngeneic immunocompetent host for both 4T1 and 2792 murine breast cancer cell lines, enabling tumor engraftment and metastatic progression within the context of a fully intact innate and adaptive immune system. This immunological authenticity is critical for producing an

experimentally and clinically relevant model of breast-to-liver metastasis, as immune surveillance, Kupffer cell biology, and the inflammatory state of the hepatic microenvironment all contribute to the biology of hepatic colonization (Mielgo & Schmid, 2020).

## 9.2 Liver-Specific *Ppargc1a* Knockout Mice (Liver PGC-1 $\alpha$ KO)

To interrogate the contribution of host hepatocyte PGC-1 $\alpha$ , and by extension the metabolic competence of the hepatic soil, to metastatic colonization efficiency, a liver-specific *Ppargc1a* knockout (KO) model was generated using Cre-loxP-mediated recombination. Mice homozygous for the floxed *Ppargc1a* allele (*Ppargc1a*<sup>fl/fl</sup>), in which loxP sites flank the genomic region encoding the transcriptional activation domain of PGC-1 $\alpha$ , were crossed to mice expressing Cre recombinase under the control of the hepatocyte-specific albumin (*Alb*) gene (*Alb*-Cre). Both mouse lines were originally received from Dr. Jennifer Estall's lab at the Institut de Recherches Cliniques de Montréal of the University of Montreal. *Alb*-Cre drives hepatocyte-specific Cre expression beginning early in postnatal liver development, achieving highly efficient and selective recombination in hepatocytes throughout the adult liver with minimal ectopic expression in other tissues. Breeding was performed as pairs, in which *Ppargc1a*<sup>fl/fl</sup> or *Ppargc1a*<sup>fl/+</sup> females were crossed with hemizygous *Alb*-Cre males. Experimental animals were drawn from a pooled cohort generated across these contemporaneous crosses. Only *Ppargc1a*<sup>fl/fl</sup>; *Alb*-Cre<sup>+</sup> (Liver PGC-1 $\alpha$  KO) and *Ppargc1a*<sup>fl/fl</sup>; *Alb*-Cre<sup>-</sup> (floxed control) animals were assigned to experimental groups; heterozygous floxed animals arising from these crosses were maintained for colony propagation and were not included in the study. Because the KO and floxed control groups were generated from the same crosses and co-housed under identical conditions, the primary comparison is internally controlled for genetic background, housing, and microbiome. Wild-type BALB/c mice (Charles River Laboratories) were included as an independent reference group to confirm normal tumor cell engraftment and outgrowth in an unmanipulated syngeneic host; as these animals were commercially sourced rather than colony-bred, they are not background-matched to the floxed lines and were not used for that purpose.

## 10. Mouse Genotyping

### 10.1 Genomic DNA Extraction from Tail Biopsies

Genomic DNA for routine postnatal genotyping was extracted from tail biopsy tissue (<2 mm) collected at weaning age (3 weeks). Tissue was placed in 500  $\mu$ L of tail lysis buffer (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM EDTA pH 8.0, 0.5% SDS, 0.2 mg/mL Proteinase K; all from Sigma-Aldrich) in 1.5 mL microcentrifuge tubes and digested overnight at 55°C. The following morning, 150  $\mu$ L of 5 M NaCl was added, and samples were vortexed vigorously for 20 seconds to facilitate protein precipitation. After centrifugation at maximum speed for 5 minutes, 500  $\mu$ L of the cleared supernatant was transferred to a fresh tube. An equal volume (500  $\mu$ L) of 100% ethanol was added, and samples were re-centrifuged at maximum speed for 10 minutes to pellet DNA. The supernatant was aspirated, and the pellet was washed with 500  $\mu$ L of 70% ethanol, then re-centrifuged at maximum speed for 10 minutes. Following complete aspiration of the supernatant, tubes were inverted on absorbent paper and allowed to air-dry for 15 minutes. DNA pellets were resuspended in 100  $\mu$ L of sterile nuclease-free water and stored at -20°C until use.

### 10.2 PCR Genotyping Strategy

The genotype of each mouse was determined using three independent PCR assays: (1) detection of the Alb-Cre transgene; (2) detection and zygosity assessment of the floxed *Ppargc1a* allele; and (3) end-point verification of successful hepatocyte-specific Cre-mediated excision of the floxed *Ppargc1a* exons in liver tissue. All PCR reactions were performed in a total volume of 20  $\mu$ L with the components described in the Supplementary Table 1. Thermocycling conditions were: initial denaturation at 95°C for 3 minutes; 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds; final extension at 72°C for 5 minutes; hold at 4°C. PCR products were resolved on 2% agarose gels stained with ethidium bromide (0.5  $\mu$ g/mL) and visualized under UV transillumination.

Primer sequences, concentrations, and expected amplicons for each genotyping assay are provided in the Supplementary Table 2. For Alb-Cre detection, a three-primer multiplex reaction (Primers G, H, I) was used, generating a ~390 bp Cre transgene-specific amplicon and a ~351 bp internal control amplicon confirming DNA template quality.

Detection of the floxed *Ppargc1a* allele utilized Primers E and F, which flank the upstream loxP insertion site and produce distinct amplicons from wild-type (unfloxed) and floxed alleles. End-point excision confirmation was performed on DNA from 5 mm<sup>3</sup> pieces of liver tissue (target organ; expected to show excision in KO mice) and kidney tissue (control organ; no Alb-Cre expression; expected to retain wild-type allele) from all experimental animals, using Primers C and D for the excised allele and Primers A and B for the wild-type allele.

## **11. Orthotopic Primary Tumor Model: Mammary Fat Pad (MFP) Injection**

To evaluate the effect of PGC-1 $\alpha$  overexpression on primary tumor growth and to generate a clinically relevant model of spontaneous metastatic dissemination from an orthotopic mammary site, 4T1 and 2792 PGC-1 $\alpha$ -overexpressing and empty-vector control cells were implanted into the fourth (inguinal) mammary fat pad (MFP) of female BALB/c mice. MFP implantation recapitulates the natural tissue microenvironment of breast cancer, supports efficient tumor engraftment, and gives rise to spontaneous metastases via both lymphatic and hematogenous routes, consistent with the clinical trajectory of metastatic breast cancer.

### **11.1 Cell Preparation and Injection Protocol**

On the day of injection, cells were trypsinized, washed twice with ice-cold PBS to remove serum and trypsin, and assessed for viability by Trypan Blue exclusion (minimum 95% viable). Cells were resuspended at a final concentration of 20,000 cells/mL in a 1:1 (v/v) mixture of ice-cold sterile phosphate-buffered saline (PBS) and growth factor-reduced phenol red-free Matrigel Basement Membrane Matrix (Corning), prepared and maintained on ice to prevent premature gelation. The final injection volume was 50  $\mu$ L per mouse (containing 1 000 cells), delivered as a single orthotopic implant into the fourth inguinal MFP under isoflurane anesthesia (2.5% in 100% O<sub>2</sub>). The inclusion of Matrigel in the cell suspension promotes local engraftment by providing a provisional extracellular matrix scaffold, resulting in more consistent and reproducible tumor take rates compared with PBS-only injections. Injections were performed using a 27-gauge, 1/2-inch needle.

### **11.2 Primary Tumor Monitoring and Resection**

Tumor formation was monitored by bilateral caliper measurements every 2-3 days beginning from day 7 post-injection. Tumor volume was calculated using the modified ellipsoid formula:  $V = (\pi/6) \times L \times W^2$ , where L and W are the longest and shortest orthogonal diameters, respectively, measured with calipers. Animals were monitored daily for signs of distress, rapid weight loss (>20% baseline body weight), tumor ulceration, or impaired ambulation, which constituted humane endpoints requiring immediate euthanasia irrespective of tumor size. Primary tumors were surgically resected under isoflurane anesthesia when they reached a threshold volume of approximately 500 mm<sup>3</sup>, at which point sufficient primary tumor burden had been established to generate detectable metastatic seeding while remaining within ethical size limits and avoiding confounders from necrotic tumor cores. Following resection, wounds were closed with wound clips, and animals were monitored during recovery. Resected tumor tissue was fixed 10% neutral buffered formalin (NBF) for downstream histopathological examination. Mice were maintained for two weeks post-resection to allow development of macroscopic metastatic lesions before terminal organ collection.

## **12. Experimental Liver Metastasis Model: Intrasplenic Portal Vein Injection**

To selectively and directly interrogate the capacity of PGC-1 $\alpha$ -overexpressing versus control breast cancer cells to colonize and establish metastatic outgrowth within the liver, independent of the upstream metastatic cascade steps of local invasion, intravasation, and intravascular survival, an intrasplenic injection experimental metastasis model was employed. In this well-established model, tumor cells introduced into the splenic parenchyma enter the splenic vasculature, drain through the portal vein, and are delivered directly to the hepatic sinusoidal network, faithfully recapitulating the portal-vein-mediated route of hepatic seeding relevant to breast cancer liver metastasis. By isolating the colonization and outgrowth phases of metastasis, this model provided a precise, experimentally controlled readout of hepatic colonization competence as a function of PGC-1 $\alpha$  status.

### **12.1 Surgical Procedure**

Cells were prepared as described in Section 11.1 and resuspended in 100  $\mu$ L of sterile PBS (without Matrigel) per mouse. Under isoflurane anesthesia (2-3% induction, 2% maintenance), a small (approximately 1 cm) left lateral flank incision was made through the skin and peritoneum to expose the spleen. Cells were injected slowly over approximately 60 seconds into the splenic

parenchyma using a 27-gauge, ½-inch needle. Gentle pressure was applied to the injection site with sterile gauze for 60 seconds to ensure hemostasis and to prevent reflux of cells into the peritoneal cavity. Immediately following confirmed hemostasis, the spleen was surgically removed by suture ligation of the splenic hilum followed by excision, to prevent the development of a primary intrasplenic tumor mass that would obscure the assessment of true hepatic metastatic burden and confound the interpretation of results. The peritoneum was closed with a single absorbable suture, and the skin was closed with wound clips. Animals were placed on a warm pad during recovery and monitored until fully ambulatory.

## **12.2 Endpoint and Organ Collection**

Mice were maintained for two weeks following intrasplenic injection before terminal organ collection, based on pilot experiments establishing this as the timepoint at which macroscopic hepatic metastatic foci are consistently detectable with minimal animal morbidity. At endpoint, mice were euthanized as described in Section 8, and the liver was collected. Organs were fixed in 10% NBF for 24 hours prior to paraffin embedding and histological processing.

## **13. Tissue Processing, Histological Sectioning, and Hematoxylin & Eosin Staining**

Following overnight fixation in 10% NBF organs were transferred to 70% ethanol for processing. Tissue processing through graded dehydration (70%, 95%, and two changes of 100% ethanol), clearing in xylene (two changes), and paraffin infiltration was performed using an automated tissue processor (Leica ASP300S) with standard overnight processing cycle settings. Tissue blocks were cast in paraffin using a Leica EG1150 embedding center. Paraffin blocks were allowed to equilibrate overnight at 4°C prior to sectioning.

Serial sections were cut at a uniform thickness of 4 µm using a Leica RM2245 rotary microtome. For the liver, one representative section per animal was analyzed, taken from a mid-axial plane of the organ to ensure systematic and reproducible sampling across animals. For the lungs, two sections per animal separated by 250 µm (step-section approach) were analyzed to account for the three-dimensional distribution of pulmonary micrometastatic foci and to minimize under-sampling of smaller lesions throughout the lung parenchyma. Sections were mounted on positively charged glass slides (Superfrost Plus, Thermo Fisher Scientific), baked at 60°C for 30 minutes, de-

paraffinized in three changes of xylene, rehydrated through a descending series of ethanol concentrations (100%, 95%, 80%, 70%), and rinsed in distilled water prior to staining.

Hematoxylin and Eosin (H&E) staining was performed on an automated stainer using the following protocol: Mayer's hematoxylin (5 minutes); differentiation in 1% acid ethanol (5 seconds); bluing in Scott's tap water substitute (1 minute); eosin-Y (1 minute); dehydration through ascending ethanols; clearing in xylene; and coverslipping with DPX permanent mounting medium. Stained sections were reviewed by a qualified observer to confirm tissue quality and identify metastatic tumor foci prior to digital quantification.

#### **14. Quantification of Metastatic Tumor Burden by Digital Pathology**

H&E-stained histological sections were digitized at 20× magnification using a whole-slide scanner (Aperio AT2; Leica Biosystems) to produce high-resolution digital slide images. Metastatic tumor burden was quantified using QuPath open-source digital pathology software (version 0.4.x; University of Edinburgh). For each digital slide, the total tissue area was annotated as the region of interest. Metastatic tumor foci were identified and annotated based on established histopathological criteria: nuclear pleomorphism, high nuclear-to-cytoplasmic ratio, loss of normal tissue architecture, and formation of cohesive tumor cell clusters distinct from surrounding hepatic parenchyma or pulmonary alveolar tissue.

Tumor area was quantified using a pixel classifier trained in QuPath to distinguish tumor from non-tumor tissue based on nuclear and cellular morphology features. Metastatic burden was expressed as the percentage of total annotated tissue area occupied by tumor foci (% tumor area). In addition, the total number of discrete metastatic foci per section and the mean foci area were recorded, enabling distinction between differences in initial seeding and arrest efficiency (reflected by foci number) versus post-colonization proliferative outgrowth (reflected by foci size). All quantification was performed by a single trained investigator blinded to experimental group assignment to eliminate observer bias.

#### **15. Statistical Analysis**

All statistical analyses were performed using GraphPad Prism (version 9.5 or later; GraphPad Software Inc., San Diego, CA, USA). Data are presented as mean  $\pm$  standard error of the mean

(SEM) unless otherwise specified. The specific statistical tests employed and the number of biological replicates for each experiment are detailed in the corresponding figure legends.

For pairwise comparisons between two groups, an unpaired, two-tailed Student's *t*-test was applied when data satisfied the assumptions of normality (assessed by Shapiro–Wilk test,  $\alpha = 0.05$ ) and equal variance (assessed by Levene's test). When normality assumptions were not met, the non-parametric Mann–Whitney U test was used. For experiments comparing more than two groups, one-way analysis of variance (ANOVA) with Tukey's multiple comparisons post hoc correction was used for normally distributed data, or the Kruskal–Wallis test with Dunn's post hoc correction for non-normal data. A *P*-value  $< 0.05$  was considered statistically significant throughout. Exact *P*-values are reported where feasible, with significance thresholds denoted as:  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ ;  $****P < 0.0001$ ; ns, not significant.

For metabolomic data, group differences in individual metabolite levels were assessed by unpaired *t*-test with Benjamini–Hochberg FDR correction applied across the full metabolite panel to control the false discovery rate arising from multiple simultaneous comparisons; both uncorrected and FDR-adjusted *P*-values are reported. For RNA-seq differential expression and GSEA statistical frameworks, see Sections 7.4 and 7.5.

## RESULTS

### 1. PGC-1 $\alpha$ Overexpression Elevates Mitochondrial Oxidative ATP Production While Suppressing Glycolytic Capacity in Liver-Tropic Breast Cancer Cells

A central and motivating hypothesis of this study is that liver-tropic breast cancer cells, characterized by endogenously low PGC-1 $\alpha$  expression, reside in a predominantly glycolytic metabolic state. This state, while adequate for survival in the primary tumor context, may be suboptimal for the demanding and oxidatively challenging hepatic sinusoidal environment. To determine whether restoring mitochondrial capacity through PGC-1 $\alpha$  gain-of-function could fundamentally reprogram the bioenergetics of these cells in a manner predicted to confer hepatic colonization advantage, comprehensive real-time bioenergetics analyses of PGC-1 $\alpha$ -overexpressing cells and EV cells were performed.

Continuous monitoring of the oxygen consumption rate (OCR), the real-time readout of mitochondrial electron transport chain (ETC) activity, revealed that PGC-1 $\alpha$ -overexpressing cells maintained a modestly elevated basal respiratory rate under resting conditions relative to EV controls (Figure 1A). Upon BAM15-mediated uncoupling, which removes the thermodynamic constraint imposed by ATP synthase and thereby unmasks the true electron transport capacity of the mitochondria, the difference between the two populations became dramatically apparent: PGC-1 $\alpha$ -overexpressing cells achieved a substantially higher maximal OCR of approximately 146 pmol/min per  $10^3$  cells, compared with approximately 119 pmol/min per  $10^3$  cells in EV controls (Figure 1A). This enhanced respiratory capacity was fully abolished by subsequent co-injection of the Complex I inhibitor rotenone and the Complex III inhibitor antimycin A, confirming that the elevated signal was entirely mitochondria-specific. Non-mitochondrial oxygen consumption was equivalent between groups at both basal ( $\sim 11$  pmol/min/ $10^3$  cells) and maximal ( $\sim 41$  pmol/min/ $10^3$  cells) conditions (Figure 1F), underscoring that all observed bioenergetic differences between the two populations reside exclusively within the mitochondrial compartment.

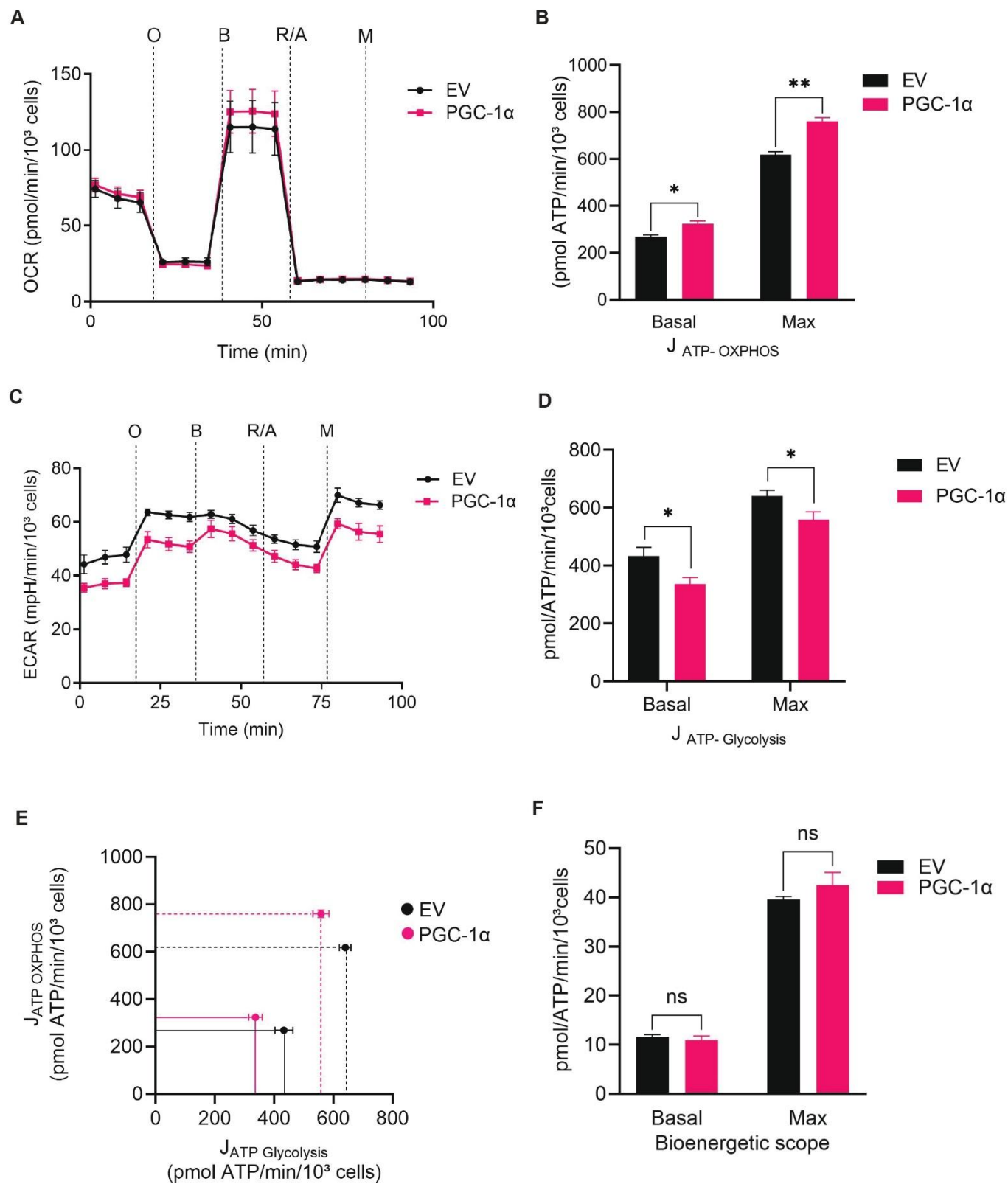
To convert these respiratory measurements into biological interpretation, absolute rates of mitochondrial ATP production from oxidative phosphorylation (OXPHOS;  $J_{\text{ATP-OXPHOS}}$ ) were calculated. This analysis revealed that PGC-1 $\alpha$  overexpression significantly increased basal oxidative ATP production from approximately 269 pmol ATP/min/ $10^3$  cells in EV controls to

approximately 324 pmol ATP/min/ $10^3$  cells in PGC-1 $\alpha$ -overexpressing cells ( $p < 0.05$ ; Figure 1B). More strikingly, at maximal oxidative ATP production, PGC-1 $\alpha$ -overexpressing cells achieved an oxidative ATP production rate of approximately 760 pmol ATP/min/ $10^3$  cells, compared with approximately 618 pmol ATP/min/ $10^3$  cells in EV controls, a significant and substantial amplification ( $p < 0.001$ ; Figure 1B). The proportionally greater expansion of maximal relative to basal oxidative ATP production demonstrates that PGC-1 $\alpha$  overexpression substantially enlarges the spare respiratory capacity (SRC) of these cells, that is, the bioenergetic reserve that can be instantaneously recruited in response to energetic stress. This property is mechanistically significant in the context of hepatic colonization, where newly arrested tumor cells must rapidly meet the energetic demands of immune evasion, resistance to anoikis, and initiation of proliferative signaling under conditions of acute metabolic challenge.

In striking reciprocal contrast, real-time monitoring of the extracellular acidification rate (ECAR), reflecting net glycolytic proton production, revealed that PGC-1 $\alpha$ -overexpressing cells consistently maintained lower glycolytic activity than EV controls throughout the entire measurement period (Figure 1C). This suppression of glycolytic acidification was evident both at baseline and following oligomycin-mediated ATP synthase inhibition, a condition that normally forces glycolytic upregulation to compensate for reduced oxidative ATP supply. Upon maximal monensin-stimulated challenge, EV cells reached approximately 68 mpH/min per  $10^3$  cells, whereas PGC-1 $\alpha$ -overexpressing cells plateaued at approximately 61 mpH/min per  $10^3$  cells, indicating a reduced glycolytic ceiling rather than robust suppression of glycolytic compensation (Figure 1C). Quantification of absolute glycolytic ATP production rates ( $J_{\text{ATP-Glycolysis}}$ ) confirmed that PGC-1 $\alpha$ -overexpressing cells trended toward lower basal glycolytic ATP output ( $\sim 337$  vs  $\sim 433$  pmol ATP/min/ $10^3$  cells in EV;  $*P < 0.05$ ; Figure 1D) and exhibited a statistically significant reduction in maximal glycolytic ATP production capacity ( $\sim 558$  vs  $\sim 640$  pmol ATP/min/ $10^3$  cells in EV;  $p < 0.05$ ; Figure 1D). These data establish a clear and reciprocal bioenergetic rebalancing: PGC-1 $\alpha$  overexpression amplifies mitochondrial oxidative ATP production rather than glycolytic capacity, shifting the energetic architecture of these liver-tropic cells towards increased OXPHOS.

The integrated bioenergetic scope representation, in which each cell population is mapped as a two-dimensional coordinate defined by its glycolytic (x-axis) and oxidative (y-axis) ATP production rates at basal and maximal conditions, provided the most direct and compelling visualization of

this metabolic rebalancing (Figure 1E). At baseline, EV cells occupied a position reflecting higher glycolytic and lower oxidative ATP output ( $J_{\text{ATP-Glycolysis}} \sim 433$ ,  $J_{\text{ATP-OXPHOS}} \sim 269$  pmol ATP/min/ $10^3$  cells), consistent with the predominantly glycolytic phenotype expected of liver-tropic cells with low endogenous PGC-1 $\alpha$ . By contrast, PGC-1 $\alpha$  cells at baseline occupied a position shifted toward higher oxidative and reduced glycolytic output ( $J_{\text{ATP-Glycolysis}} \sim 337$ ,  $J_{\text{ATP-OXPHOS}} \sim 324$  pmol ATP/min/ $10^3$  cells), representing a discrete repositioning in bioenergetic scope. At maximum capacity, this divergence was most pronounced along the OXPHOS axis: PGC-1 $\alpha$ -overexpressing cells reached approximately 760 pmol ATP/min/ $10^3$  cells oxidatively compared with approximately 618 pmol ATP/min/ $10^3$  cells for EV controls, while maximal glycolytic capacities remained comparable ( $\sim 558$  vs  $\sim 640$  pmol ATP/min/ $10^3$  cells, respectively). Collectively, the bioenergetic scope analysis demonstrates that PGC-1 $\alpha$  overexpression repositions liver-tropic cells from a glycolytic-dominant toward an oxidative-dominant bioenergetic state, providing the metabolic preconditions hypothesized to confer selective fitness advantages within the energetically demanding and oxidatively challenging hepatic metastatic niche.



**Figure 1. PGC-1 $\alpha$  overexpression shifts liver-tropic breast cancer cells toward a mitochondrial oxidative bioenergetic state with enhanced ATP-linked respiration and reduced glycolytic capacity.** (A) Representative kinetic trace of the oxygen consumption rate (OCR; pmol/min/10<sup>3</sup> cells) over the course of the Mito Stress Test after injection of oligomycin (O), BAM15 (B), rotenone/antimycin A (R/A), and monensin (M). Time points at which each compound was injected are indicated by dashed vertical lines. Data points represent the mean of replicate wells per condition per time point. (B) Basal and maximal rates of mitochondrial ATP production from oxidative phosphorylation ( $J_{\text{ATP-OXPHOS}}$ ; pmol ATP/min/10<sup>3</sup> cells) derived from the OCR kinetic trace. Basal  $J_{\text{ATP-OXPHOS}}$  was calculated from pre-oligomycin OCR measurements; maximal  $J_{\text{ATP-OXPHOS}}$  was derived from BAM15-stimulated OCR measurements. \* $P < 0.05$ , \*\* $P < 0.01$  by unpaired two-tailed t-test. (C) Representative kinetic trace of the extracellular acidification rate (ECAR; mpH/min/10<sup>3</sup> cells) over the course of the assay, reflecting real-time glycolytic proton production after injection of oligomycin (O), BAM15 (B), rotenone/antimycin A (R/A), and monensin (M). (D) Basal and maximal rates of glycolytic ATP production ( $J_{\text{ATP-Glycolysis}}$ ; pmol ATP/min/10<sup>3</sup> cells) derived from ECAR measurements. Basal  $J_{\text{ATP-Glycolysis}}$  was calculated from pre-oligomycin ECAR; maximal  $J_{\text{ATP-Glycolysis}}$  was derived from monensin-stimulated ECAR. \* $P < 0.05$ , by unpaired two-tailed t-test. (E) Bioenergetic scope plot displaying each cell population as a coordinate defined by its glycolytic ATP production rate (x-axis;  $J_{\text{ATP-Glycolysis}}$ ) and oxidative ATP production rate (y-axis;  $J_{\text{ATP-OXPHOS}}$ ). Data points with dashed lines depict maximal ATP production rates, whereas data points with solid lines depict ATP production rates under basal conditions. The areas delimited by the dashed and solid lines represent the maximal and basal bioenergetic scope, respectively. Each point represents the mean basal or maximal  $J_{\text{ATP}}$  value for the indicated cell population. The plot illustrates the net bioenergetic repositioning of PGC-1 $\alpha$  cells toward greater oxidative and reduced glycolytic ATP production relative to EV cells. (F) Calculated bioenergetic scope values (pmol ATP/min/10<sup>3</sup> cells) at basal (post-BAM15) and maximum (post-rotenone/antimycin A) conditions. ns, nonsignificant, by unpaired two-tailed t-test. All Seahorse experiments were performed with 10 technical replicate wells per condition per assay and independently repeated across at 3 biological replicates. Representative data from a single experiment are shown in (A) and (C); bar graphs in (B), (D), and (F) and the bioenergetic scope in (E) represent summary statistics across independent experiments. Data are presented as mean  $\pm$  SEM. ns, nonsignificant.

## **2. PGC-1 $\alpha$ Overexpression in Liver Tropic Breast Cancer Cells Selectively Induces the Expression of Antioxidant and Electron Transport Chain Components**

Before interpreting the phenotypic consequences of PGC-1 $\alpha$  overexpression in vitro and in vivo, it was essential to confirm that the lentiviral transduction system delivered robust, reproducible, and biologically meaningful transgene expression, and that the expected downstream PGC-1 $\alpha$  transcriptional programs were engaged in transduced cells relative to matched empty-vector controls. Quantitative reverse-transcription PCR demonstrated that PGC-1 $\alpha$  cells harbored a dramatic and highly significant elevation in *Ppargc1a* transcript abundance compared with EV controls, with PGC-1 $\alpha$ -overexpressing cells showing overwhelmingly higher *Ppargc1a* mRNA expression at the scale of the assay ( $p < 0.0001$ ; Figure 2A). This statistically compelling difference confirmed successful, high-level, and constitutive PGC-1 $\alpha$  expression in the lentiviral population, validating the experimental system.

To determine which downstream effectors of the PGC-1 $\alpha$  transcriptional network displayed increased expression in these cells, we profiled a curated panel of canonical PGC-1 $\alpha$  target genes encompassing mitochondrial metabolism, ETC subunit expression, and mitochondrial antioxidant defense (Figure 2B). *Sod2* was the most robustly induced target, with a highly significant increase in transcript abundance in PGC-1 $\alpha$ -overexpressing cells relative to EV controls ( $p < 0.01$ ; Figure 2B). The expression of *Uqcrc1* (complex II subunit) was also increased ( $p < 0.05$ ; Figure 2B). The selective and strong induction of *Sod2*, encoding the primary mitochondrial superoxide-scavenging manganese superoxide dismutase and one of the most conserved PGC-1 $\alpha$ -responsive antioxidant targets, is of particular significance: it directly links PGC-1 $\alpha$  overexpression to reinforced mitochondrial redox homeostasis, a property highly related to tumor cell survival within the oxidatively hostile hepatic sinusoidal environment. The concurrent upregulation of *Uqcrc1*, a structural subunit of the cytochrome bc1 complex, provides corroborating evidence for enhanced respiratory chain assembly in PGC-1 $\alpha$ -overexpressing cells, consistent with the higher maximal OCR observed in bioenergetic profiling.

## **3. PGC-1 $\alpha$ Overexpression in Liver Tropic Breast Cancer Cells Does Not Accelerate Primary Tumor Growth In Vivo**

Given the metabolic rebalancing induced by PGC-1 $\alpha$  overexpression, we next asked whether this bioenergetic shift was sufficient to alter the intrinsic growth capacity of liver-tropic breast cancer

cells under standard culture conditions. Growth curve analysis over five days demonstrated largely parallel kinetics between PGC-1 $\alpha$  and EV cells for the first four days of culture (Figure 2C). From day 5 onward, PGC-1 $\alpha$ -overexpressing cells began to outpace EV controls, accumulating approximately  $3.5 \times 10^6$  live cells per well by day 6 compared with approximately  $2.5 \times 10^6$  cells in EV controls (Figure 2C). This difference reached statistical significance at the measured day-6 endpoint, with PGC-1 $\alpha$  cells maintaining approximately  $3.5 \times 10^5$  viable cells compared with approximately  $2.5 \times 10^5$  for EV controls ( $p < 0.05$ ; Figure 2D).

Despite this modest in vitro growth advantage, primary tumor development following orthotopic MFP implantation in immune-intact syngeneic BALB/c hosts was strikingly equivalent between conditions (Figure 2E, F). Both PGC-1 $\alpha$  and EV tumors grew along essentially similar growth trajectories, reaching approximately 400–450 mm<sup>3</sup> by day 20–22 post-implantation with equivalent times to reach the surgical resection threshold of approximately 500 mm<sup>3</sup> (Figure 2F). This result establishes a functional dissociation between PGC-1 $\alpha$ 's influence on cell-intrinsic proliferation in two-dimensional culture and its impact on tumor growth kinetics in the complex immunological and stromal microenvironment of an orthotopic mammary tumor. This dissociation is consistent with the established notion that the selective fitness advantages conferred by enhanced metabolic capacity emerges preferentially under conditions of metabolic stress as well as mechanical and immune challenges, conditions more representative of dissemination and colonization than of primary tumor expansion.

#### **4. PGC-1 $\alpha$ Overexpression in Liver Tropic Breast Cancer Cells Significantly Amplifies Metastatic Burden Following Spontaneous Dissemination from the Orthotopic Primary Tumor**

Following primary tumor resection at approximately 500 mm<sup>3</sup>, mice were maintained for two weeks to permit metastatic outgrowth before terminal organ collection, histological processing, and blinded digital quantification of metastatic burden in the lungs and liver. This post-resection window was designed to capture the formation of macroscopic metastatic lesions that had already seeded prior to surgical intervention, providing a biologically realistic assessment of spontaneous metastatic capacity.

Histological examination of pulmonary sections revealed a quantitatively significant increase in metastatic burden in animals that had PGC-1 $\alpha$ -overexpressing tumors (Figure 2G). Lungs from mice that had EV tumors harbored sparse, small, and individually identifiable metastatic foci interspersed within largely preserved alveolar parenchyma. In contrast, lungs from mice that had PGC-1 $\alpha$ -overexpressing tumors displayed extensively colonized sections dominated by large, densely cellular, confluent tumor nodules that replaced substantial volumes of pulmonary tissue. While the number of discrete visible lesions trended toward a greater count in animals that had PGC-1 $\alpha$ -overexpressing tumors than those that had EV tumors, this comparison did not reach statistical significance owing to the substantial inter-animal variability inherent to spontaneous metastasis models ( $p = \text{ns}$ ; Figure 2H). In contrast, total pulmonary metastatic area, a continuous and more statistically robust metric reflecting total tumor cell accumulation, was significantly greater in animals that had PGC-1 $\alpha$ -overexpressing tumors: tumor cells occupied approximately 38% of total lung cross-sectional area compared with approximately 12% in animals that had EV tumors, representing a greater than threefold increase in pulmonary metastatic burden ( $p < 0.01$ ; Figure 2I). This finding is consistent with the established pro-metastatic role of PGC-1 $\alpha$  in breast cancer reported by Andrzejewski et al. (2017) and demonstrates that elevated mitochondrial capacity facilitates metastatic outgrowth in the oxygen-rich pulmonary environment.

The hepatic metastatic burden in mice bearing orthotopic MFP tumors presented a distinctly different and important pattern (Figures 2J–2L). H&E analysis of liver sections from both experimental groups revealed predominantly normal hepatic architecture with minimal tumor involvement, and neither the number of hepatic lesions (Figure 2K) nor the hepatic metastatic area (Figure 2L) differed significantly between groups, though a non-significant trend toward greater hepatic involvement was observed in PGC-1 $\alpha$  animals. The low absolute level of hepatic metastatic burden in the MFP model reflects that the orthotopic primary site produces predominantly pulmonary metastases rather than the portal-vein-mediated hepatic seeding. To directly and quantitatively investigate hepatic colonization efficiency, we performed intrasplenic injection experiments described below.

## **5. Direct Portal Vein Delivery by Intrasplenic Injection Reveals That PGC-1 $\alpha$ Overexpression in Liver Tropic Breast Cancer Cells Promotes Liver Metastasis**

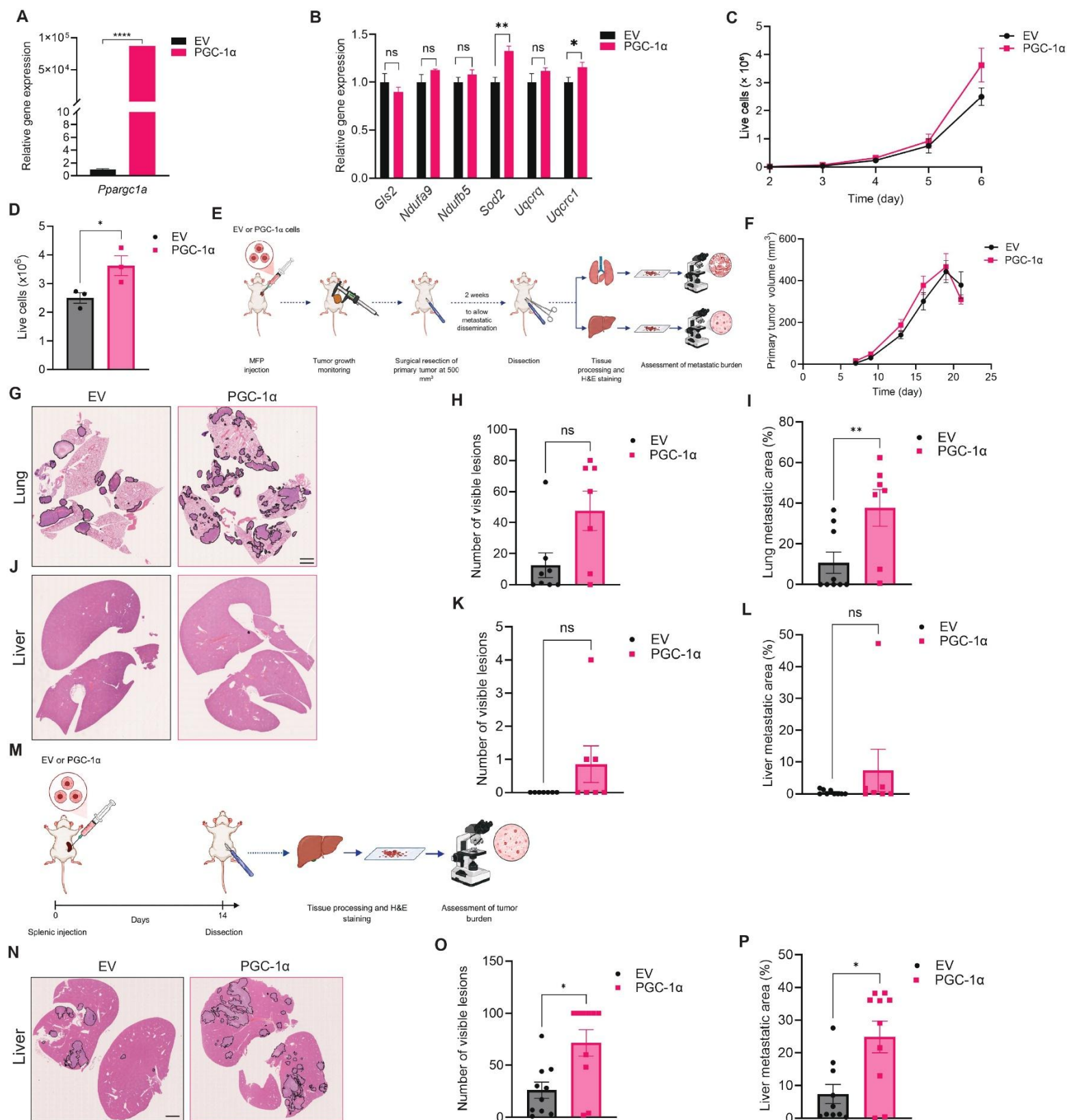
The intrasplenic portal vein injection experimental metastasis model provides the most direct and controlled test of hepatic colonization competence by delivering tumor cells quantitatively and reproducibly to the hepatic sinusoidal network via the portal circulation. This model recapitulates the physiological route of hepatic seeding in breast cancer, while isolating the upstream steps of primary tumor invasion and intravasation (Figure 2M).

The histological contrast between the two experimental groups in this model was unambiguous and striking (Figure 2N). Liver sections from animals injected with EV liver-tropic breast cancer cells harbored scattered, predominantly small and isolated tumor foci distributed sparsely within the hepatic parenchyma, with large zones of intact hepatic architecture separating individual lesions. Livers from animals injected with PGC-1 $\alpha$ -overexpressing liver-tropic breast cancer cells presented a qualitatively transformed metastatic landscape: expanded, confluent tumor masses occupied substantial proportions of both hepatic lobes, with individual foci that were not only more numerous but also considerably larger in area, indicating both superior initial colonization efficiency and more vigorous post-colonization proliferative outgrowth within the hepatic environment.

The number of discrete visible liver lesions was significantly greater in animals injected with PGC-1 $\alpha$ -overexpressing liver-tropic breast cancer cells, averaging approximately 70 metastatic foci per liver section compared with approximately 22 for those injected with EV controls ( $p < 0.05$ ; Figure 2O). Total hepatic metastatic area was similarly and significantly increased: PGC-1 $\alpha$ -overexpressing cells colonized approximately 25% of liver cross-sectional area, compared with approximately 8% in animals injected with EV cells ( $p < 0.05$ ; Figure 2P). This approximately threefold amplification of hepatic metastatic burden by PGC-1 $\alpha$  represents a biologically meaningful effect size, reflecting the difference between a liver with largely preserved functional parenchyma and one approaching the threshold of metastasis-related organ compromise.

These findings demonstrate that tumor cell–intrinsic PGC-1 $\alpha$  expression is a significant and potent determinant of breast cancer liver metastatic colonization. This finding is particularly significant given that these liver-tropic breast cancer cells express low PGC-1 $\alpha$  endogenously (Andrzejewski

et al., 2017). This demonstrates that low-PGC-1 $\alpha$  and the associated high-glycolysis state in these cells is not a metabolic requirement for hepatic colonization.



**Figure 2. PGC-1 $\alpha$  overexpression in liver-tropic breast cancer cells activates antioxidant and respiratory chain target genes, enhances proliferation, and potently promotes metastatic colonization of the lung and liver.** Molecular validation of PGC-1 $\alpha$  overexpression and functional characterization of PGC-1 $\alpha$  and EV breast cancer cells in vitro, followed by assessment of metastatic burden in syngeneic immunocompetent BALB/c mice using orthotopic mammary fat pad (MFP) implantation and intrasplenic portal vein injection models. **(A)** Relative *Ppargc1a* mRNA expression in EV and PGC-1 $\alpha$  cells quantified by quantitative reverse-transcription PCR (RT-qPCR) using the  $2^{-\Delta\Delta C_t}$  method normalized to the geometric mean of *Tbp* reference genes. Unpaired two-tailed t-test; \*\*\*\*P < 0.0001. n = 3 independent biological replicates. **(B)** Relative mRNA expression of canonical PGC-1 $\alpha$  transcriptional target genes in EV (black) and PGC-1 $\alpha$  (pink) cells: *Gls2* (glutaminase 2), *Ndufa9* (NADH:ubiquinone oxidoreductase subunit A9; Complex I), *Ndufb5* (NADH:ubiquinone oxidoreductase subunit B5; Complex I), *Sod2* (superoxide dismutase 2; mitochondrial antioxidant defense), *Uqcrcq* (ubiquinol-cytochrome c reductase subunit Q; Complex III), and *Uqcrc1* (ubiquinol-cytochrome c reductase subunit Q1; Complex III). Expression was normalized as described in (A). Unpaired two-tailed t-test per gene; \*\*P < 0.01 (*Sod2*), \*P < 0.05 (*Uqcrc1*); all other comparisons, ns, nonsignificant. n = 3 independent biological replicates. Data are presented as mean  $\pm$  SEM. **(C)** Cell proliferation time course of EV and PGC-1 $\alpha$  cells seeded at 7,500 cells per well in six-well plates and counted on days 2, 3, 4, 5, and 6 post-seeding using a Countess II Automated Cell Counter with Trypan Blue exclusion to determine live cell number. Data are presented as mean  $\pm$  SEM of n = 3 independent biological replicates. **(D)** Endpoint live cell counts from the proliferation assay shown in (C). Individual biological replicate values are plotted. \*P < 0.05 by unpaired two-tailed t-test. n = 3 independent biological replicates. Data are presented as mean  $\pm$  SEM. **(E)** Schematic illustration of the orthotopic MFP experimental metastasis model. EV or PGC-1 $\alpha$  cells were injected into the MFP of female BALB/c mice (6 weeks of age; 20,000 cells in 50  $\mu$ L of 1:1 PBS: Matrigel per mouse). Primary tumors were monitored by caliper measurement every 2–3 days and surgically resected under isoflurane anesthesia at approximately 500 mm<sup>3</sup>. Mice were maintained for 2 weeks post-resection to allow spontaneous metastatic dissemination, then euthanized for organ collection. Lungs and livers were fixed, sectioned (lungs: two-step sections 250  $\mu$ m apart; liver: one mid-axial section), H&E stained, and digitally imaged for blinded metastatic burden quantification using QuPath software. [Created with BioRender.com.] **(F)** Orthotopic primary tumor growth curves for mice bearing EV or PGC-

1 $\alpha$  mammary tumors. Tumor volume was measured every 2–3 days by digital caliper and calculated as  $V = (\pi/6) \times \text{length} \times \text{width}^2$ . Tumors were resected upon reaching approximately 500 mm<sup>3</sup>. **(G)** Representative whole-section H&E micrographs of lung tissue from mice bearing EV (left) or PGC-1 $\alpha$  (right) primary mammary tumors, collected 2 weeks after tumor resection. Dark-staining tumor nodules are readily visible throughout the PGC-1 $\alpha$  lung section. Scale bar, 2 mm. **(H)** Number of visible metastatic lesions per lung step-section (two sections per animal, 250  $\mu$ m apart) counted by blinded digital pathology analysis in QuPath. Individual animal values are plotted. Statistical significance was assessed using a two-tailed Mann–Whitney U test, ns, nonsignificant. Data are presented as mean  $\pm$  SEM. EV (n = 8) and PGC-1 $\alpha$  (n = 7). **(I)** Pulmonary metastatic area expressed as a percentage of total lung cross-sectional area, quantified by blinded pixel classifier analysis in QuPath. Individual animal values are plotted. Statistical significance was assessed using a two-tailed Mann–Whitney U test, \*\*P < 0.01. Data are presented as mean  $\pm$  SEM. EV (n = 9) and PGC-1 $\alpha$  (n = 7). **(J)** Representative whole-section H&E micrographs of liver tissue from mice bearing EV (left) or PGC-1 $\alpha$  (right) mammary tumors, collected 2 weeks after tumor resection. Liver sections show predominantly preserved hepatic architecture with minimal tumor involvement in both groups. **(K)** Number of visible metastatic lesions per liver section (one mid-axial section per animal) in the MFP model, quantified by blinded QuPath analysis. Individual animal values are plotted. Statistical significance was assessed using a two-tailed Mann–Whitney U test, ns, nonsignificant. Data are presented as mean  $\pm$  SEM. EV (n = 7) and PGC-1 $\alpha$  (n = 7). **(L)** Hepatic metastatic area as a percentage of total liver cross-sectional area in the MFP model, quantified by blinded QuPath analysis. Individual animal values are plotted. Statistical significance was assessed using a two-tailed Mann–Whitney U test, ns, nonsignificant. Data are presented as mean  $\pm$  SEM. EV (n = 9) and PGC-1 $\alpha$  (n = 7). **(M)** Schematic illustration of the intrasplenic portal vein injection experimental liver metastasis model. EV or PGC-1 $\alpha$  cells were injected into the splenic parenchyma of female BALB/c mice (100,000 cells in 100  $\mu$ L sterile PBS per mouse) under isoflurane anesthesia. Mice were maintained for 14 days post-injection, then euthanized for liver collection. Livers were fixed, sectioned (one mid-axial section per animal, 4  $\mu$ m thickness), H&E stained, and subjected to blinded digital quantification of hepatic metastatic burden using QuPath software. [Created with BioRender.com.] **(N)** Representative whole-section H&E micrographs of liver tissue from mice injected intrasplenically with EV (left) or PGC-1 $\alpha$  (right) cells, collected 14 days post-injection. Confluent dark-staining metastatic masses are visible throughout both hepatic

lobes in the PGC-1 $\alpha$  condition, in contrast to the sparse, small foci in EV livers. Scale bar, 2 mm. **(O)** Number of visible metastatic lesions per liver section in the intrasplenic injection model, quantified by blinded digital pathology analysis in QuPath. Individual animal values are plotted. Statistical significance was assessed using a two-tailed Mann–Whitney U test, \*P < 0.05. Data are presented as mean  $\pm$  SEM. EV (n = 10) and PGC-1 $\alpha$  (n = 10). **(P)** Hepatic metastatic area as a percentage of total liver cross-sectional area in the intrasplenic injection model, quantified by blinded pixel classifier analysis in QuPath. Individual animal values are plotted. Statistical significance was assessed using a two-tailed Mann–Whitney U test, \*P < 0.05. Data are presented as mean  $\pm$  SEM. EV (n = 10) and PGC-1 $\alpha$  (n = 10).

For all in vivo MFP experiments (panels F–L), female BALB/c mice (6 weeks of age; Charles River) were used. For all in vivo intrasplenic experiments (panels N–P), female BALB/c mice (6 weeks of age; Charles River) were used. All animal experiments were conducted under AUP MCGL-5129 approved by the McGill University FACC. Euthanasia: isoflurane inhalation followed by CO<sub>2</sub> asphyxiation and cervical dislocation.

## 6. Generation and Genomic Validation of a Hepatocyte-Specific *Ppargc1a* Knockout (KO) Mouse Model

The striking pro-metastatic effect of tumor cell-intrinsic PGC-1 $\alpha$  established the seed component of the seed-and-soil duality. The second and equally critical question of this study is: does the metabolic state of the hepatic soil itself, governed by hepatocyte PGC-1 $\alpha$ , independently influence the liver's permissiveness for tumor cell colonization? To address this, a hepatocyte-specific *Ppargc1a* knockout (KO) model was generated by crossing *Ppargc1a*<sup>fl/fl</sup> mice with albumin promoter-driven Alb-Cre transgenic mice, yielding experimental *Ppargc1a*<sup>fl/fl</sup>; Alb-Cre<sup>+</sup> (KO) animals and *Ppargc1a*<sup>fl/fl</sup>; Alb-Cre<sup>-</sup> littermate floxed controls (Figure 3A).

Robust genotypic verification was performed using a three-tier PCR strategy that independently confirmed the Alb-Cre transgene, the floxed *Ppargc1a* allele, and, critically, the organ-specific occurrence of Cre-mediated recombination at the experimental endpoint (Figure 3B). The Alb-Cre multiplex PCR assay (Primers G, H, I) discriminated Cre-positive animals by a ~390 bp transgene-specific amplicon alongside a ~351 bp internal control band, with clear differentiation of Cre-positive from Cre-negative littermates across all three genotypic classes (Alb-Cre; *Ppargc1a*<sup>+/-</sup>, Alb-Cre; *Ppargc1a*<sup>-/-</sup>, and *Ppargc1a*<sup>+/+</sup>; Figure 3B, upper gel panels). The *Ppargc1a* locus PCR (Primers E and F) distinguished the floxed allele (~400 bp) from the wild-type allele (~360 bp), confirming floxed allele zygosity in each animal (Figure 3B, middle gel panel). Most importantly, the endpoint liver-specific excision assay (Primers A, B, C, D) demonstrated that the Cre-mediated excision amplicon (~450 bp) was present exclusively in liver tissue from Alb-Cre-positive animals, while kidney tissue from the same animals retained the wild-type non-excised band (~200 bp), formally proving that *Ppargc1a* deletion was complete, hepatocyte-specific, and absent from non-hepatic tissues (Figure 3B, lower gel panels). Alb-Cre-negative floxed controls showed only the wild-type non-excised band in both liver and kidney tissue, as expected. This three-tier genomic verification provided molecular confirmation of the specificity of the model.

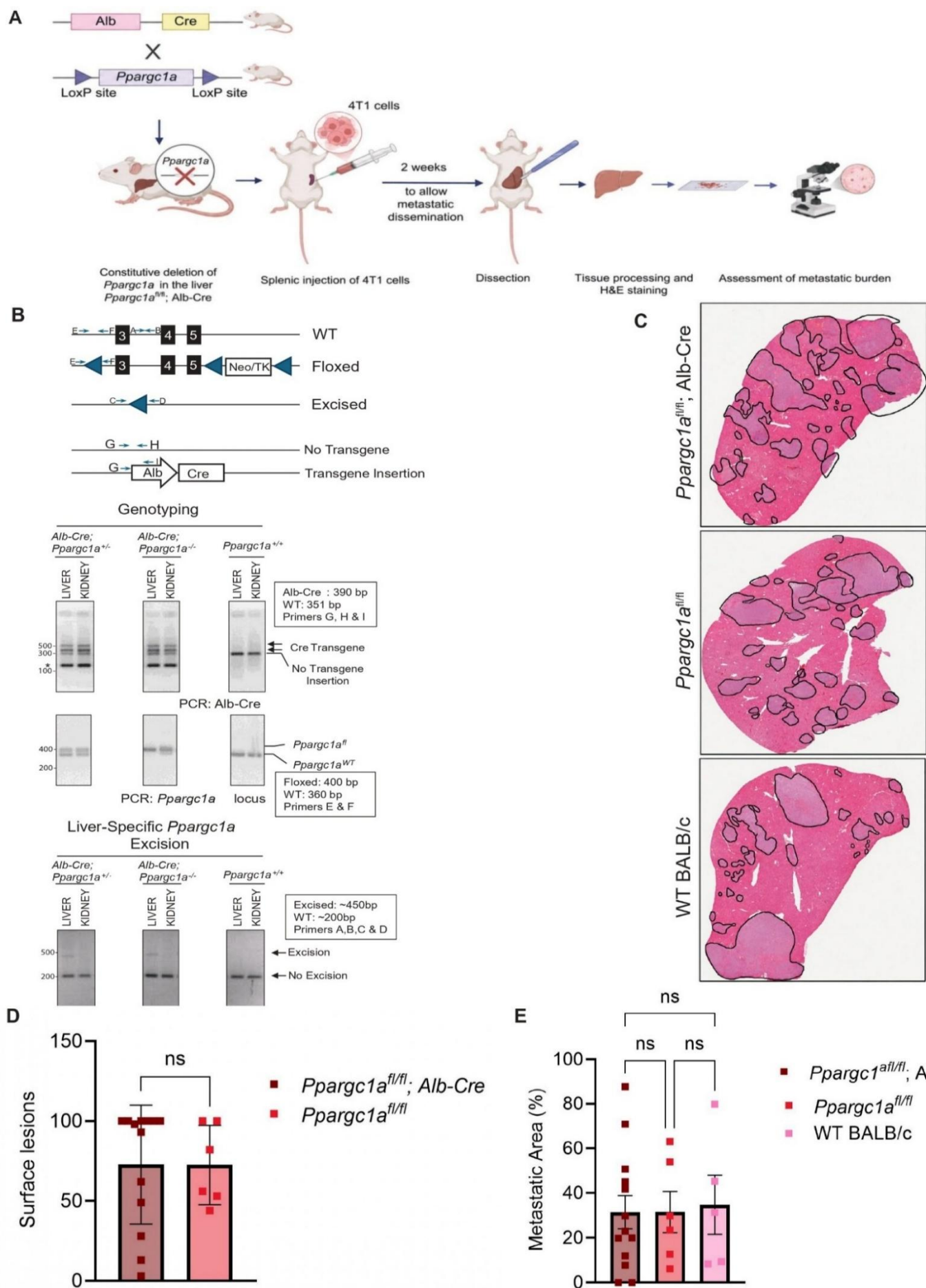
## 7. Hepatocyte-Specific Deletion of PGC-1 $\alpha$ Does Not Significantly Alter the Liver's Capacity to Support Metastatic Colonization

To directly interrogate the contribution of the hepatic soil to metastatic permissiveness, 4T1 breast cancer cells were delivered via intrasplenic portal vein injection into *Ppargc1a*-KO mice,

*Ppargc1a*-floxed littermate controls, and wild-type BALB/c animals. Livers were collected 14 days post-injection and processed for H&E histology and blinded digital quantification of metastatic burden (Figure 3A). This design directly tests both competing soil hypotheses: if high hepatocyte PGC-1 $\alpha$  activity restricts colonization, KO mice would exhibit greater metastatic burden than controls; if hepatocyte PGC-1 $\alpha$  loss reduces colonization by limiting tissue metabolic flexibility, KO mice would exhibit reduced burden.

Representative whole-section H&E micrographs from all three genotypes revealed dark-staining tumor foci distributed throughout the hepatic parenchyma at broadly comparable density across *Ppargc1a*-KO, floxed control, and wild-type BALB/c livers, with no visually discernible differences in lesion number, size, or distribution between groups (Figure 3C). This histological equivalence was confirmed by quantitative analysis. Hepatic metastatic lesion number was essentially identical across all three host conditions ( $p = \text{ns}$ ; Figure 3D). Total hepatic metastatic area, the more sensitive and continuous measure of colonization success, was similarly indistinguishable: *Ppargc1a*-KO mice averaged approximately 30% metastatic area, floxed controls approximately 32%, and wild-type BALB/c mice approximately 34%, with no statistically significant difference across any pairwise comparison ( $p = \text{ns}$ ; Figure 3E).

The absence of a significant impact of hepatocyte-specific PGC-1 $\alpha$  loss on metastatic colonization efficiency establishes unambiguously that the PGC-1 $\alpha$ -dependent metabolic programs of hepatocytes is dispensable as a determinant of the liver's receptivity to incoming tumor cells. Reducing the metabolic fitness of the hepatic soil by deleting a master metabolic regulator neither impairs the liver's capacity to support incoming tumor cell colonization nor creates a competitive vacuum that tumor cells exploit, nor does it create an environment less favorable to their survival. The critical metabolic driver of breast cancer hepatic metastatic competence in this system is clearly the seed, not the soil. This finding narrows the therapeutic inference arising from this work with precision: strategies aimed at exploiting PGC-1 $\alpha$ -driven metabolism in the setting of breast cancer liver metastasis should be directed at the tumor cell itself.



**Figure 3. Generation and validation of a liver-specific *Ppargc1a* knockout mouse model reveals that the hepatocyte metabolic state is dispensable for breast cancer hepatic metastatic colonization.** Generation, genomic validation, and functional assessment of hepatocyte-specific *Ppargc1a* knockout (KO) mice to interrogate the contribution of the host hepatic metabolic “soil” to breast cancer liver metastasis. **(A)** Schematic overview of the experimental workflow. Liver-specific PGC-1 $\alpha$  KO mice (*Ppargc1a*<sup>fl/fl</sup>; Alb-Cre) were generated and subjected to splenic injection of 4T1 breast cancer cells to establish experimental liver metastasis. After 2 weeks, livers were collected, processed for H&E staining, and metastatic burden was assessed. **(B)** Top: Schematic diagram of the *Ppargc1a* genomic locus illustrating the wild-type (WT), floxed (FLX), and Cre-excised (Excised) allele configurations. LoxP sites (triangles) flank exons 3-5 of the *Ppargc1a* gene encoding the N-terminal transcriptional activation domain. The Alb-Cre transgene locus and relative positions of genotyping primers (A–I) are shown. Primer pairs used for each assay: Alb-Cre detection, Primers G + H + I (three-primer multiplex); floxed *Ppargc1a* allele detection, Primers E + F; liver-specific excision confirmation, Primers A–D. Middle panels: Representative PCR genotyping agarose gels for three genotypic classes, Alb-Cre; *Ppargc1a*<sup>+/-</sup> (heterozygous floxed, Cre-positive), Alb-Cre; *Ppargc1a*<sup>-/-</sup> (homozygous floxed, Cre-positive), and *Ppargc1a*<sup>+/+</sup> (wild-type, Cre-negative), using liver and kidney genomic DNA. PCR: Alb-Cre (Primers G, H, I): Alb-Cre transgene band, ~390 bp; wild-type (no transgene insertion) band, ~351 bp. PCR: *Ppargc1a* locus (Primers E, F): floxed (*Ppargc1a*<sup>fl</sup>) allele band, ~400 bp; wild-type (*Ppargc1a*<sup>WT</sup>) allele band, ~360 bp. Bottom panel: Liver-specific *Ppargc1a* excision confirmation (Primers A, B, C, D) performed at experimental endpoint on DNA extracted from liver and kidney tissue of each genotypic class. Excision-specific amplicon: ~450 bp (present exclusively in liver tissue from Alb-Cre-positive animals). Wild-type (non-excised) amplicon: ~200 bp (present in kidney of all animals and liver of Cre-negative controls). Note the liver-specific excision band in Alb-Cre; *Ppargc1a*<sup>+/-</sup> and Alb-Cre; *Ppargc1a*<sup>-/-</sup> mice in liver but not kidney, confirming hepatocyte specificity of Cre-mediated recombination. Molecular weight markers (bp) are indicated on the left. **(C)** Representative H&E-stained whole-section liver micrographs from three host genotypes: *Ppargc1a*<sup>fl/fl</sup>; Alb-Cre (*Ppargc1a*-KO), *Ppargc1a*<sup>fl/fl</sup> (floxed control), and wild-type BALB/c (WT), collected 14 days following intrasplenic portal vein injection of 4T1 breast cancer cells. Dark-staining metastatic tumor foci are visible in all three groups at broadly comparable density and distribution throughout the hepatic parenchyma, consistent with the

quantitative data shown in panels D and E. All tumor lesion annotations use a single uniform color throughout all three images. Scale bar, 2 mm. **(D)** Number of visible hepatic surface metastatic lesions per liver section (4  $\mu$ m, one section per animal) following intrasplenic portal vein injection of 4T1 breast cancer cells into *Ppargc1a*<sup>fl/fl</sup>; Alb-Cre (KO, n = 13) or *Ppargc1a*<sup>fl/fl</sup> (floxed control, n = 6) mice. Individual animal values are plotted. Unpaired t-test; ns, nonsignificant. Data are presented as mean  $\pm$  SEM. **(E)** Hepatic metastatic area (%) per liver section following intrasplenic portal vein injection of 4T1 breast cancer cells into three host genotypes: *Ppargc1a*<sup>fl/fl</sup>; Alb-Cre (dark red, n = 13), *Ppargc1a*<sup>fl/fl</sup> (light red, n = 6), and WT BALB/c mice (pink, n = 5). Metastatic area was quantified by blinded pixel classifier analysis in QuPath. Individual animal values are plotted. One-way ANOVA with Tukey's multiple comparisons post hoc test; all pairwise comparisons ns; global test ns nonsignificant. Data are presented as mean  $\pm$  SEM.

All mice used in this figure were female and 6 weeks of age at the time of intrasplenic injection. *Ppargc1a*<sup>fl/fl</sup>; Alb-Cre<sup>+</sup> (KO) and *Ppargc1a*<sup>fl/fl</sup> (floxed control) animals were generated in-house from the breeding crosses described in the Methods; wild-type BALB/c mice were purchased from Charles River Laboratories. Animals were housed and experiments conducted at the Goodman Cancer Institute Animal Facility, McGill University, under AUP MCGL-5129.

## 8. Genome-Wide RNA Sequencing Identifies a Dominant OXPHOS and Mitochondrial Biogenesis Transcriptional Program in Liver Tropic Breast Cancer Cells Overexpressing PGC-1 $\alpha$

We performed transcriptomic analyses to uncover the impact of PGC-1 $\alpha$  overexpression on the global expression profile of liver-tropic breast cancer cells (Figure 4A). Volcano plot visualization revealed a rich and asymmetric differential expression landscape with numerous genes reaching significant statistical confidence (Figure 4A). As expected, *Ppargc1a* itself was the most significantly upregulated gene, exhibiting the highest statistical confidence in the dataset (log2 fold change exceeding +10), providing a powerful internal validation of the transcriptomic dataset. Among the most significantly upregulated genes beyond the transgene were *Dsp* (desmoplakin), *Cask* (calcium/calmodulin-dependent serine protein kinase 1), and *Apc2* (APC Regulator of WNT Signaling Pathway 2, a negative regulator of the Wnt/ $\beta$ -catenin signaling pathway). Regarding the most significantly and strongly downregulated genes were *Arhgdib* (Rho GDP-dissociation inhibitor beta, a regulator of Rho GTPase cycling and cell motility), *3830403N18Rik*, *Slc14a1* (urea transporter), *Adgrl3* (Adhesion G Protein-Coupled Receptor L3, also known as Latrophilin-3, a cell-adhesion receptor involved in synaptogenesis), and *Tfpi* (Tissue Factor Pathway Inhibitor, a serine protease inhibitor that regulates the extrinsic coagulation cascade). An additional notably downregulated gene was *Cav1* (caveolin-1), a glycolysis-promoting membrane scaffold protein whose suppression is consistent with the reduced glycolytic output documented by Seahorse analysis (Figure 1).

At single-gene resolution, the heatmap of top differentially expressed genes revealed two clearly defined co-expression clusters separating PGC-1 $\alpha$ -overexpressing from EV cells (Figure 4B). Among the most biologically meaningful upregulated genes were *Ppargc1a* itself (confirming robust transgene expression), *Sod1* (cytoplasmic copper-zinc superoxide dismutase, a key antioxidant enzyme), and *Slc7a1* (a cationic amino acid transporter mediating arginine uptake, consistent with the "Arginine and Proline Metabolism" pathway enrichment identified by MSEA). Among the most significantly downregulated genes were *Cav1* (caveolin-1, a membrane scaffold protein associated with aerobic glycolysis, whose suppression is consistent with the reduced glycolytic ceiling documented by Seahorse ECAR measurements) and *Ccnd2* (cyclin D2, a positive regulator of G1/S cell cycle progression). The suppression of *Ccnd2* is particularly noteworthy as

it suggests a potential metabolic-proliferative trade-off in which cells allocating greater energetic resources toward mitochondrial oxidative programs divert them from active cell cycle progression, consistent with the modest in vitro proliferative difference and the absence of a primary tumor growth advantage in vivo.

Gene Set Enrichment Analysis (GSEA) was performed using pre-ranked gene lists against both GO Biological Process and KEGG pathway databases to identify the gene expression programs most coherently activated or suppressed by PGC-1 $\alpha$  overexpression (Figures 4C, 4D). GO: BP analysis identified a significant and specific activation of mitochondrial oxidative metabolic programs (Figure 4C). The highest-ranked activated biological process term was "oxidative phosphorylation" (GeneRatio approximately 0.47; FDR < 0.010), establishing OXPHOS as the most coherently and significantly upregulated GO: BP program in PGC-1 $\alpha$ -overexpressing cells. Notably, this mirrors the top KEGG pathway hit (Figure 4D), providing multi-omic convergence on the same core biological program. "Respiratory electron transport chain" and "electron transport chain" followed immediately (GeneRatio approximately 0.43–0.45; FDR < 0.010), together with "aerobic respiration" (GeneRatio approximately 0.33), all achieving the highest GeneRatios and lowest adjusted P-values among all assessed GO: BP terms. A coherent cluster of nucleotide catabolic and diphosphate metabolic terms followed at intermediate GeneRatios (approximately 0.20–0.22), including "nucleotide catabolic process," "nucleoside phosphate catabolic process," "purine nucleoside diphosphate catabolic process," "diphosphate catabolic process," and "purine ribonucleoside diphosphate catabolic process," collectively reflecting enhanced purine nucleotide turnover consistent with the elevated ATP synthesis rates documented by Seahorse analysis. "Adipose tissue development" also reached enrichment significance (GeneRatio approximately 0.28; FDR approximately 0.030), a term whose enrichment reflects the shared transcriptional targets between PGC-1 $\alpha$ -driven thermogenic adipose programs and the mitochondrial bioenergetic programs activated in these cells. At the lowest GeneRatio in the activated panel, "keratinocyte differentiation" appeared (GeneRatio approximately 0.07; FDR approximately 0.300), representing a marginally enriched term of uncertain biological relevance in the breast cancer cell context. The suppressed GO: BP panel revealed a distinct and biologically coherent cluster of developmental and reproductive programs. The most significantly and highly enriched suppressed terms included "sperm DNA condensation" (GeneRatio approximately 0.40; FDR approximately 0.020), "regulation of vesicle fusion" (GeneRatio approximately 0.28), "embryonic heart tube

morphogenesis" (GeneRatio approximately 0.25), "postsynaptic specialization organization," "spermatid nucleus differentiation," "iron ion transmembrane transport," "maintenance of synapse structure," "locomotion involved in locomotory behavior," "negative regulation of reproductive process," and "trophoblast cell migration." These suppressed terms are dominated by spermatogenic and reproductive developmental programs, neurological and synaptic specialization processes, and cell migration programs. While several of these terms (particularly those related to spermatogenesis) likely reflect incidental transcriptional suppression of gene sets not functionally active in the breast cancer cell context, the consistent suppression of developmental and differentiation-associated programs is consistent with PGC-1 $\alpha$  redirecting transcriptional resources toward oxidative metabolic programs at the expense of differentiation-associated gene networks. The suppression of "iron ion transmembrane transport" is particularly noteworthy in the context of hepatic colonization, as iron homeostasis is critically regulated in the liver and alterations in iron transport may reflect metabolic remodeling relevant to the hepatic niche.

The KEGG pathway GSEA uncovered "Oxidative phosphorylation" as the most significantly enriched KEGG term, achieving the highest gene ratio of all assessed pathways (approximately 0.52), the largest gene set representation (>50 genes), and an adjusted P-value approaching  $10^{-4}$ , far exceeding all other enriched terms in both statistical confidence and biological representation (Figure 4D). These results highlight that PGC-1 $\alpha$  overexpression in liver-tropic breast cancer cells drives a coherent and dominant OXPHOS gene expression program. Additional enriched KEGG pathways included "Thermogenesis", consistent with shared PGC-1 $\alpha$ -regulated gene networks between mitochondrial biogenesis and thermogenic programs; and "Antigen processing and presentation" and "Arrhythmogenic right ventricular cardiomyopathy", the latter two reflecting partial overlap with ETC gene sets rather than direct immunological or cardiac biological relevance.

## **9. Targeted Metabolomic Profiling Uncovers OXPHOS, Antioxidant Defense, and Broad Amino Acid and Nucleotide Metabolic Reprogramming in Liver Tropic Breast Cancer Cells Overexpressing PGC-1 $\alpha$**

Transcriptional programs translate their biological effects through altered enzyme levels and metabolic flux. To determine whether the oxidative and mitochondrial gene signature identified by transcriptomics was reflected in measurable changes in the intracellular metabolite landscape, we

performed targeted quantitative LC-MS/MS metabolomic profiling of PGC-1 $\alpha$  and EV cells, covering more than 100 metabolites across central carbon metabolism, including intermediates from the TCA cycle, amino acid metabolism, nucleotide biosynthesis, the pentose phosphate pathway, and antioxidant pathways.

Principal component analysis (PCA) of the complete metabolomic dataset show that PGC-1 $\alpha$  overexpression produces a profoundly distinct intracellular metabolic state (Figure 4E). The scores plot revealed clear and complete separation of EV and PGC-1 $\alpha$ -overexpressing cell populations. Cells within each group formed tight, compact, and well-defined clusters along both PC1 (45.8%) and PC2 (18.2%), confirming high biological reproducibility within experimental conditions. The magnitude and completeness of the inter-group separation along PC1 relative to the within-group scatter demonstrates that PGC-1 $\alpha$  overexpression is a powerful and consistent driver of intracellular metabolic state in these cells.

Variable Importance in Projection (VIP) scoring from Partial Least Squares–Discriminant Analysis (PLS-DA) identified the individual metabolites contributing most powerfully to group discrimination (Figure 4F). Critically, only two metabolites were elevated in PGC-1 $\alpha$ -overexpressing (OE) cells: O-phosphoserine (VIP  $\sim$ 3.7), the highest-ranking discriminating metabolite, and adenosine (VIP  $\sim$ 2.5). O-phosphoserine is the phosphorylated intermediate in the de novo serine biosynthesis pathway (the phosphoglycerate dehydrogenase branch of upper glycolysis); its accumulation indicates enhanced channeling of glycolytic intermediates into serine biosynthesis, a pathway that fuels one-carbon metabolism, NADPH generation, nucleotide biosynthesis, and glutathione regeneration. The elevation of adenosine in OE cells is consistent with increased mitochondrial purine nucleotide turnover accompanying higher rates of ATP synthesis and utilization. In contrast, all other high-VIP metabolites were enriched in EV control cells, including cysteine (VIP  $\sim$ 2.3),  $\gamma$ -glutamylcysteine ( $\gamma$ -Glu-Cys, the immediate dipeptide precursor of glutathione), lactic acid, malonic acid, N-acetylmethionine, guanine, gluconic acid, N-acetylserine, N-formylmethionine, N-acetylalanine, aminoadipic acid, erythronic acid, and proline. The higher levels of cysteine and  $\gamma$ -Glu-Cys in EV cells likely reflect accumulation of these precursors when oxidative demand and antioxidant consumption are low, rather than active antioxidant deployment. This interpretation is consistent with the transcriptional upregulation of *Sod2* (by RT-qPCR) and *Sod1* (by RNA-seq) in OE cells, both encoded by the official mouse gene

names in lowercase italic format, which reflects enhanced enzymatic superoxide scavenging capacity independently of glutathione precursor pool levels. The apparent discrepancy between lower cysteine/ $\gamma$ -Glu-Cys in OE cells and the Metabolite Set Enrichment Analysis (MSEA) enrichment of “Glutathione Metabolism” in OE cells is addressed in the following paragraph. The enrichment of proline and other amino acids in EV cells may reflect proline oxidase-mediated catabolism as a compensatory stress response in cells with lower intrinsic oxidative metabolic capacity.

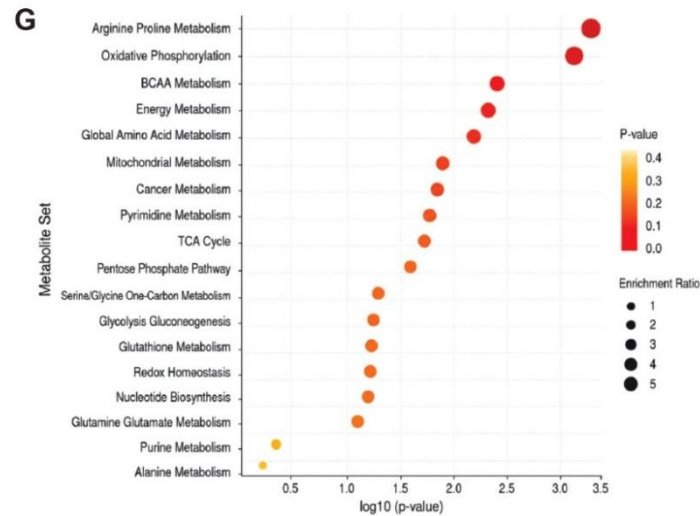
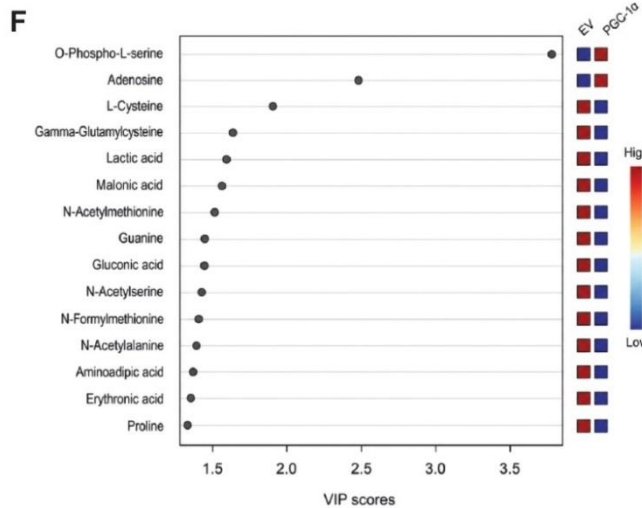
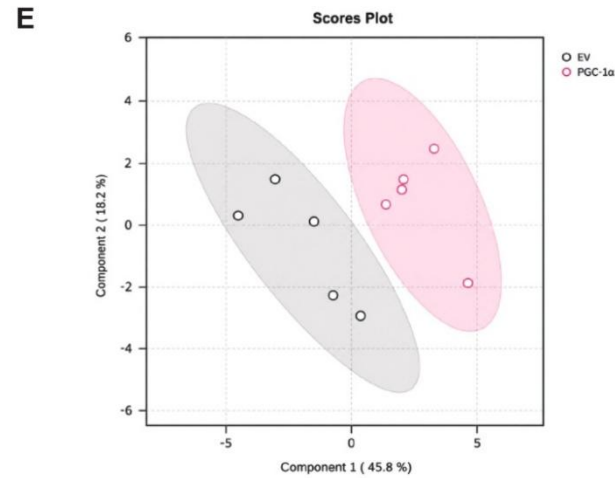
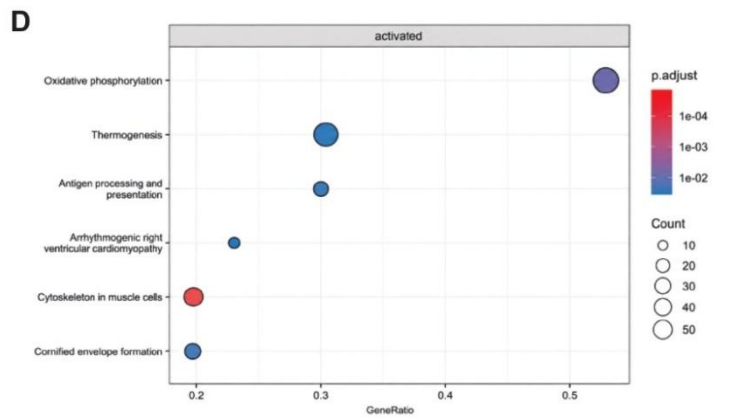
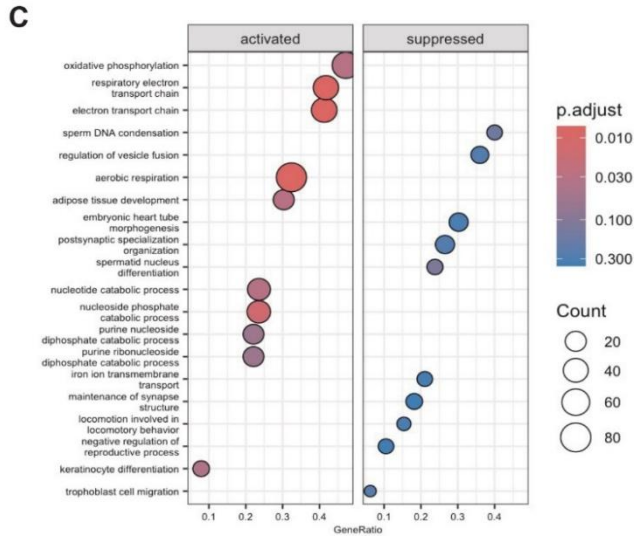
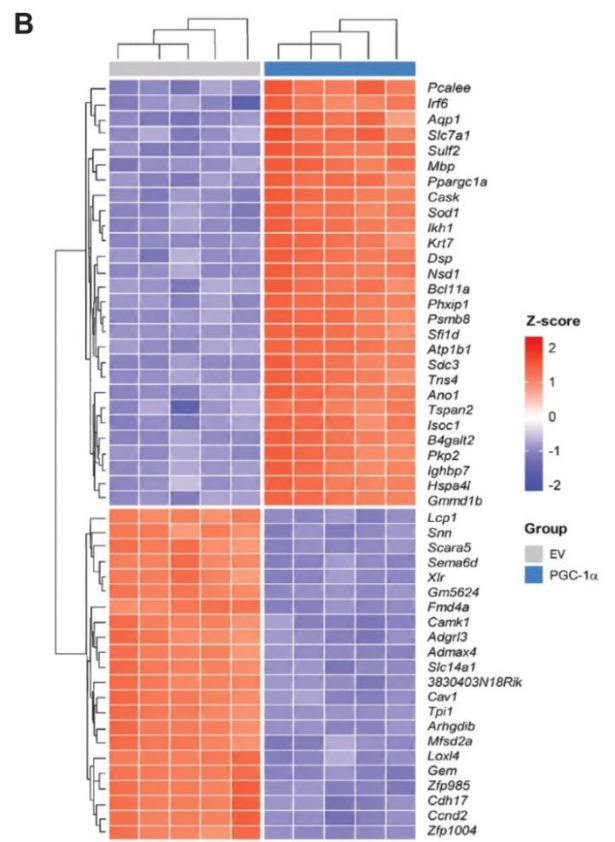
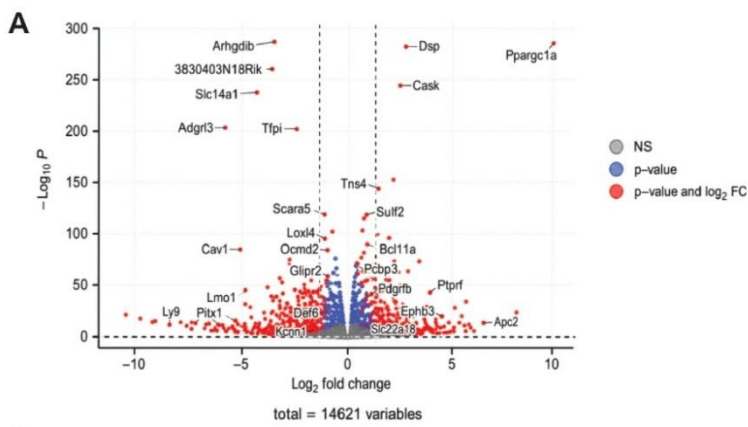
MSEA provided pathway-level interpretation of the metabolomic differences that converged with the transcriptomic findings (Figure 4G). The most strongly enriched metabolite set in PGC-1 $\alpha$ -overexpressing cells was “Arginine and Proline Metabolism” (enrichment ratio  $\sim 5$ ;  $-\log^{10}(p) \sim 3.4$ ), consistent with the transcriptional upregulation of the cationic amino acid transporter *Slc7a1* and the GO: BP arginine metabolic pathway enrichment identified by RNA-seq. “Oxidative Phosphorylation” was the second most enriched metabolite set (enrichment ratio  $\sim 4$ ;  $-\log^{10}(p) \sim 3.2$ ), providing significant multi-omic convergence: the same biological pathway emerged as the most dominant activated program by both unbiased transcriptomic GSEA (KEGG) and metabolomic MSEA analyses. This convergence highlights that OXPHOS is a key metabolic pathway upregulated at both gene expression and metabolite levels in liver-tropic breast cancer cells overexpressing PGC-1 $\alpha$ .

“BCAA Metabolism” (branched-chain amino acid catabolism providing acetyl-CoA and succinyl-CoA for TCA cycle anaplerosis, “Energy Metabolism,” “Global Amino Acid Metabolism,” and “Mitochondrial Metabolism” also displayed high enrichment ratios, together reflecting the broader anabolic and anaplerotic remodeling that sustains the expanded mitochondrial TCA cycle activity of PGC-1 $\alpha$ -overexpressing cells. Other metabolite sets of most direct relevance to the hepatic metastatic context, “Glutathione Metabolism” and “Redox Homeostasis” were both significantly enriched, supporting the gene expression data showing that PGC-1 $\alpha$  activates antioxidant defense networks in these cells.

“TCA Cycle” and “Serine/Glycine One-Carbon Metabolism” were also significantly enriched, converging with the VIP data showing elevated O-phosphoserine and linking PGC-1 $\alpha$  overexpression to one-carbon metabolism, NADPH production, and nucleotide precursor biosynthesis. “Nucleotide Biosynthesis,” “Pyrimidine Metabolism,” “Pentose Phosphate

Pathway,” and “Purine Metabolism” were additional enriched sets, suggesting that PGC-1 $\alpha$ -overexpressing cells maintain a more abundantly stocked nucleotide precursor pool, a property that would support the biosynthetic demands of metastatic outgrowth once initial colonization is established.

In conclusion, the convergence of transcriptomic and metabolomic data on the same core set of pathways, OXPHOS, mitochondrial metabolism, and antioxidant defense, suggest that PGC-1 $\alpha$ -overexpressing cells are better equipped to withstand oxidative stress, compete effectively for metabolic substrates, and sustain the biosynthetic demands of proliferative outgrowth within the hepatic microenvironment, collectively supporting their enhanced hepatic colonization efficiency.



**Figure 4. Integrated transcriptomic and metabolomic profiling identifies OXPHOS, antioxidant defense, and broad amino acid and nucleotide metabolic reprogramming as the dominant molecular signatures of PGC-1 $\alpha$  overexpression in liver-tropic breast cancer cells.**

Genome-wide RNA sequencing (RNA-seq) and targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) metabolomic profiling of EV and PGC-1 $\alpha$  (OE) breast cancer cells to define the transcriptional and metabolic programs regulated by PGC-1 $\alpha$ . **(A)** Volcano plot of differential gene expression between PGC-1 $\alpha$  and EV cells assessed by DESeq2 analysis of RNA-seq data (total 14,621 genes assessed; n = 5 independent biological replicates per condition). The x-axis represents the log<sub>2</sub> fold change (PGC-1 $\alpha$  vs EV) and the y-axis represents  $-\log_{10}(\text{adjusted P-value; Benjamini-Hochberg FDR correction})$ . Dashed vertical lines indicate  $|\log_2 \text{FC}| = 0.5$  thresholds; dashed horizontal line indicates FDR = 0.05. Genes colored red meet both significance (FDR < 0.05) and fold-change ( $|\log_2 \text{FC}| \geq 0.5$ ) thresholds; genes colored blue meet the significance threshold only; genes colored gray are non-significant (NS). Representative genes of biological interest are labeled. Log<sub>2</sub> fold change shrinkage was applied using the apegglm estimator. Total number of genes assessed is indicated below the x-axis. **(B)** Heatmap of Z-score-normalized expression values for the top differentially expressed genes between EV (gray column annotation) and PGC-1 $\alpha$  (blue column annotation) cells. Rows (genes) and columns (samples) were subjected to unsupervised hierarchical clustering using Euclidean distance and complete linkage. The color scale represents row-wise Z-score (red, high relative expression; blue, low relative expression). Selected genes of biological interest are labeled on the right. n = 5 independent biological replicates per condition. **(C)** Gene Set Enrichment Analysis (GSEA) dot plot of significantly enriched Gene Ontology Biological Process (GO: BP) gene sets in PGC-1 $\alpha$ -overexpressing cells relative to EV cells, displayed separately for activated (left) and suppressed (right) gene sets. GSEA was performed using clusterProfiler (v4.20.0) on a pre-ranked gene list ordered by the signed DESeq2 Wald test statistic. Dot size represents the number of genes in the gene set contributing to enrichment (gene count; scale shown at right). Dot color indicates the Benjamini-Hochberg FDR-adjusted P-value (p.adjust; color scale shown at right, ranging from p.adjust < 0.010 in red to p.adjust approximately 0.300 in dark blue). Gene sets are ordered by GeneRatio (the proportion of gene set members detected at the point of maximum enrichment score in the ranked gene list). The top activated terms are dominated by mitochondrial OXPHOS and ETC programs, with "oxidative phosphorylation" achieving the highest GeneRatio (approximately 0.47) and lowest adjusted P-

value among all assessed terms. The suppressed panel is dominated by reproductive developmental, spermatogenic, neurological, and synaptic gene programs. All gene sets shown achieved nominal enrichment; statistical significance ( $p_{\text{adjust}} < 0.05$ ) is indicated by red-to-pink coloring, while terms with  $p_{\text{adjust}} > 0.05$  are shown in blue for completeness. **(D)** GSEA dot plot of significantly enriched KEGG pathway gene sets in PGC-1 $\alpha$  cells relative to EV cells (activated pathways only; no KEGG pathways were significantly suppressed at  $FDR < 0.05$ ). Analysis parameters and dot aesthetics are as described for (C).  $p_{\text{adjust}}$  color scale is shown at right (range  $10^{-4}$  to  $10^{-2}$ ). The “Oxidative phosphorylation” KEGG pathway (GeneRatio  $\approx 0.52$ ; gene count  $> 50$ ;  $p_{\text{adjust}} \approx 10^{-4}$ ) represents the most significantly and broadly enriched activated pathway. **(E)** Principal component analysis (PCA) scores plot of LC-MS/MS targeted metabolomic data from EV (gray) and PGC-1 $\alpha$  (pink) cells. Each data point represents one independent biological replicate. Ellipses represent 95% confidence regions for each group. The percentage of total metabolomic variance explained by each principal component is shown in parentheses on the respective axis (PC1, 45.8%; PC2, 18.2%). Metabolites were extracted from cells 48 hours post-seeding and quantified by LC-MS/MS as described in the Methods. Metabolomics analyses were performed at the University of Ottawa Metabolomics Core Facility. **(F)** Variable Importance in Projection (VIP) scores derived from Partial Least Squares-Discriminant Analysis (PLS-DA) of the metabolomics dataset. Metabolites with VIP score  $> 1.5$  are displayed in descending order of discriminating power. VIP score  $> 1.0$  is generally considered to indicate a meaningful contribution to group discrimination; higher values indicate greater discriminating power. The paired color blocks to the right of each metabolite name indicate the relative abundance (high = red; low = blue) of each metabolite in the EV and PGC-1 $\alpha$  (OE) groups, respectively, providing directional information about which group has higher levels of each discriminating metabolite. **(G)** Metabolite Set Enrichment Analysis (MSEA) bubble plot showing metabolite sets significantly enriched in PGC-1 $\alpha$  relative to EV cells. The x-axis represents  $-\log_{10}(\text{P-value})$ ; bubble size represents the enrichment ratio (observed/expected number of metabolites in the set above a significance threshold); bubble color indicates the P-value (orange-red gradient; brighter red indicates lower P-value, as indicated by the color scale at right). Metabolite sets are ordered from highest to lowest enrichment ratio. The metabolite set library used was the Human Metabolome Database (HMDB) or equivalent curated metabolite set collection.

For RNA-seq: Total RNA was extracted from three independent biological replicate cultures of EV and PGC-1 $\alpha$  cells per condition and subjected to polyA-selected strand-specific library preparation

(KAPA RNA HyperPrep; Roche) and sequencing on the Illumina NextSeq 500 platform (~30 million single-end 75 bp reads per sample) at the IRIC Genomics Platform (Université de Montréal). Bioinformatic processing: FastQC (v0.12.1), fastp (v1.0.1), STAR two-pass alignment to GRCm39/Ensembl release 109 (v2.7.11b), UMI-tools (v1.1.6), featureCounts (Rsubread v2.26.0; stranded), DESeq2 (v1.52.0), clusterProfiler (v4.20.0), R (v4.6.0). For targeted metabolomics: cells were seeded in six-well plates, extracted at 48 hours by methanol/acetonitrile/dichloromethane biphasic extraction, and analyzed on an Agilent 6470A triple quadrupole mass spectrometer coupled to an Agilent 1290 Infinity II UHPLC system in negative ESI mode using the Agilent Metabolomics Dynamic MRM Database and Method (Application Note 5991-6482EN) as described in the Methods.

## DISCUSSION

### 1. Principal Findings

This study addressed a precisely defined and biologically compelling paradox at the intersection of mitochondrial biology and breast cancer liver metastasis. Liver-tropic breast cancer cells express endogenously low PGC-1 $\alpha$  and adopt a predominantly glycolytic metabolic phenotype (Dupuy et al., 2015; Andrzejewski et al., 2017), yet the liver is among the most metabolically demanding and oxidatively hostile microenvironments that disseminating tumor cells encounter. We asked whether this apparently unexpected phenotypic state represents a metabolic necessity for hepatic colonization, or whether it is instead a metabolic baseline that, when exceeded through PGC-1 $\alpha$  gain-of-function, yields superior colonization capacity. Equally, we asked whether the PGC-1 $\alpha$ -dependent oxidative metabolic state of hepatocytes in the host liver, the metabolic “soil”, itself influences metastatic permissiveness independently of the tumor cell.

Four principal and interconnected findings emerge from the experimental work presented in this study:

First, constitutive PGC-1 $\alpha$  overexpression in liver-tropic 2792 breast cancer cells produce a profound and reciprocal bioenergetic rebalancing: mitochondrial oxidative ATP production is significantly elevated at both basal and maximal capacity, while glycolytic ATP production capacity was concurrently reduced. The bioenergetic state of PGC-1 $\alpha$ -overexpressing cells is repositioned from glycolytic-dominant toward oxidative-dominant in bioenergetic space, and these cells acquire substantially larger spare respiratory capacity, the bioenergetic reserve deployable under acute metabolic challenge.

Second, at the molecular level, this bioenergetic shift is accompanied by selective upregulation of the canonical PGC-1 $\alpha$  transcriptional targets *Sod2* (mitochondrial superoxide dismutase 2) and *Uqcrc1* (a respiratory Complex III subunit). At the genomic scale, we found a sweeping and clear upregulation of the oxidative phosphorylation (OXPHOS) gene program that is confirmed simultaneously by both transcriptomic (RNA-seq) and metabolomic (LC-MS/MS) analyses, with multi-omic convergence on the same core pathways constituting the most compelling possible evidence of critical pathway activation.

Third, PGC-1 $\alpha$  overexpression in liver-tropic breast cancer cells, while modestly enhancing in vitro proliferation and significantly increasing pulmonary metastatic burden following spontaneous dissemination, most significantly promotes hepatic metastatic colonization efficiency in the direct intrasplenic portal vein injection model: the number of liver metastatic foci increased approximately threefold and hepatic metastatic area increased from approximately 8% to 25%, demonstrating that PGC-1 $\alpha$  is a cell-autonomous amplifier of hepatic colonization competence.

Fourth, and perhaps most conceptually striking, hepatocyte-specific deletion of *Ppargc1a* in the host liver, which removes the PGC-1 $\alpha$ -dependent oxidative metabolic program from the hepatic parenchyma, does not alter the efficiency of metastatic colonization by injected 4T1 breast tumor cells, with colonization burden indistinguishable across knockout (KO), floxed control, and wild-type BALB/c hosts. The metabolic state of the hepatic soil is therefore dispensable; the critical metabolic determinant of hepatic breast cancer colonization is the seed.

Together, these findings provide a mechanistic, quantitative, and multi-compartmental answer to the central question of this study: PGC-1 $\alpha$ -driven oxidative metabolic programming in the tumor cell, not in the hepatocyte, is the dominant metabolic determinant of hepatic metastatic colonization efficiency in this experimental model.

## **2. The Low-PGC-1 $\alpha$ Phenotype of Liver-Tropic Cells Is a Suboptimal State, not a Metabolic Requirement**

One competing hypothesis was that elevating PGC-1 $\alpha$  in liver-tropic cells might limit their hepatic metastatic capacity, given that these cells naturally adopt a low-PGC-1 $\alpha$ , glycolytic state that could represent a metabolic optimum for liver colonization. The present results are inconsistent with this possibility: experimentally elevating PGC-1 $\alpha$  in liver-tropic cells dramatically increased their hepatic colonization efficiency, directly resolving the central conceptual tension that motivated this study. Prior work from the St-Pierre laboratory had established that breast cancer cell line variants with defined liver-metastatic tropism express significantly lower PGC-1 $\alpha$  compared with lung-metastatic counterparts, and that liver-tropic cells adopt a metabolic program characterized by elevated glycolytic flux, increased HIF-1 $\alpha$  activity, and PDK1-mediated suppression of pyruvate oxidation (Dupuy et al., 2015; Andrzejewski et al., 2017). These observations implied either that glycolytic dominance provides a survival advantage in the hepatic environment, or at minimum,

that PGC-1 $\alpha$ -driven oxidative metabolism is neither required nor advantageous for liver colonization. The present results overturn this inference with direct functional evidence. Furthermore, LeBleu et al. (2014) demonstrated that elevated PGC-1 $\alpha$  facilitates cancer cell survival during intravascular transit, suggesting that PGC-1 $\alpha$  may serve important functions at the circulating tumor cell stage of the metastatic cascade. It has also been proposed that PGC-1 $\alpha$  levels could be reduced once tumor cells colonize a new tissue site (LeBleu et al., 2014). The present work demonstrates that maintaining high PGC-1 $\alpha$  is not detrimental and is in fact advantageous for hepatic establishment, indicating that downregulation of PGC-1 $\alpha$  upon liver arrival is not required for successful colonization.

The data demonstrate clearly that the low-PGC-1 $\alpha$ , glycolytic-dominant state of liver-tropic cells, represents a metabolic baseline that, when elevated by PGC-1 $\alpha$  gain-of-function, yields dramatically superior colonization outcomes. This is conceptually important for the following reason: it implies that the glycolytic phenotype of liver-tropic cells is not a positive metabolic adaptation evolved specifically for optimal hepatic colonization, but rather a suboptimal default state, likely reflecting the metabolic inheritance of the primary tumor microenvironment or the selective pressures encountered during earlier stages of dissemination, that does not represent the bioenergetic optimum for navigating the hepatic metastatic niche. In this framing, the low endogenous PGC-1 $\alpha$  of liver-tropic cells identifies a metabolic bottleneck that limits their colonization potential, and PGC-1 $\alpha$  overexpression overcomes this bottleneck by providing the mitochondrial capacity, antioxidant resilience, and bioenergetic flexibility required to thrive within the liver.

A parallel and broadly consistent observation from the lung context supports this interpretation. Andrzejewski et al. (2017) demonstrated that lung-metastatic breast cancer cells express higher endogenous PGC-1 $\alpha$  and rely more heavily on OXPHOS, and PGC-1 $\alpha$  overexpression promoted lung metastasis in that study. Our own results confirm a significant increase in pulmonary metastatic burden in PGC-1 $\alpha$ -overexpressing cells in the mammary fat pad model. Together with the liver colonization data from the intrasplenic model, these findings suggest that PGC-1 $\alpha$  is a broadly pro-metastatic factor across multiple organ colonization contexts, but that the magnitude of its advantage is superimposed on, and amplified by, the particularly challenging oxidative and

nutrient-competitive microenvironment of the liver, where the bioenergetic resilience conferred by PGC-1 $\alpha$  is most acutely needed.

Importantly, the PDK1/HIF-1 $\alpha$  axis previously identified by Dupuy et al. (2015) as necessary for glycolytic reprogramming and efficient liver metastasis in breast cancer cells is not contradicted by the present findings, rather, the two lines of evidence are reconciled within a coherent framework. PDK1-driven glycolytic reprogramming may represent the minimal metabolic threshold required for baseline liver colonization under physiological conditions; PGC-1 $\alpha$ -driven oxidative enhancement then provides an additional and superior layer of bioenergetic fitness on top of this glycolytic baseline, yielding substantially amplified colonization efficiency. In this view, these pathways are not alternative but complementary metabolic solutions for hepatic metastatic success, operating at different levels of metastatic efficiency.

### **3. Enhanced Spare Respiratory Capacity as a Survival Mechanism Under the Metabolic Demands of Hepatic Colonization**

The bioenergetic profiling data reveal that the most consequential bioenergetic change induced by PGC-1 $\alpha$  overexpression is not simply an increase in basal respiratory rate, but rather the dramatically enlarged spare respiratory capacity (SRC), the difference between maximal and basal mitochondrial respiration. While basal oxidative ATP production increased modestly but significantly in PGC-1 $\alpha$ -overexpressing cells (~310 vs ~255 pmol ATP/min/10k cells;  $p < 0.05$ ), maximal oxidative ATP production increased far more dramatically (~760 vs ~615 pmol ATP/min/10k cells;  $p < 0.0001$ ), indicating a proportionally larger expansion of the respiratory reserve than of the basal rate. This enlarged SRC has been specifically highlighted as a critical parameter of bioenergetic resilience that enables cells to rapidly deploy additional mitochondrial ATP production in response to acute energetic stress, thereby buffering against ATP crises during cellular demand spikes (Marchetti et al., 2020).

In the specific context of hepatic metastatic colonization, disseminated tumor cells likely depend on rapid utilization of bioenergetic reserve capacity during the earliest and most vulnerable hours and days following arrest in the liver, when they must withstand profound metabolic and environmental stress to survive and initiate metastatic outgrowth. Tumor cells must: (i) withstand cytotoxic attack by Kupffer cells and liver-resident NK cells, immune effector functions that

generate reactive oxygen species and demand active membrane repair, stress-response protein synthesis, and anti-apoptotic signaling, all of which are energetically costly; (ii) maintain redox homeostasis against the substantial oxidative tone of the hepatic sinusoidal environment generated by high-activity hepatocyte electron transport chains (ETC) and lipid beta-oxidation; (iii) resist anoikis-inducing loss of integrin engagement during transit from the sinusoidal lumen to the hepatic parenchyma; and (iv) initiate the biosynthetically intensive transition from survival mode to active proliferative outgrowth. Each of these demands creates transient spikes in cellular ATP and NADPH requirements that can be far better met by cells with a large mitochondrial bioenergetic reserve than by those operating near their respiratory ceiling. The enlarged SRC of PGC-1 $\alpha$ -overexpressing cells thus provides a direct and mechanistically coherent explanation for their superior survival and colonization efficiency within the hepatic niche.

This interpretation is broadly supported by the growing literature demonstrating context-specific dependence on mitochondrial respiratory function. A landmark CRISPRi screen by Bennett et al. (2022) in lung cancer models demonstrated that primary tumors and metastatic tumors exhibit divergent systems-level dependencies on mitochondrial respiratory chain genes: while primary tumor growth showed relatively low sensitivity to disruption of specific respiratory chain components, metastatic tumor growth was more substantially impaired, indicating that the metabolic demands of metastatic colonization impose greater reliance on sustained mitochondrial function than does primary tumor growth in an established vascular niche (Bennett et al., 2022). This principle, that the selective pressure for functional mitochondria is most acute during the metastatic phase of disease, aligns precisely with the functional dissociation observed in the present study between PGC-1 $\alpha$ 's lack of effect on primary tumor growth and its powerful amplification of hepatic colonization efficiency.

#### **4. SOD2 Induction and Antioxidant Defense as a Specific Adaptation to the Hepatic Oxidative Microenvironment**

Among the canonical PGC-1 $\alpha$  downstream targets profiled by RT-qPCR, *Sod2* emerged as the most robustly and significantly induced gene in PGC-1 $\alpha$ -overexpressing cells, with significant transcript upregulation ( $p < 0.01$ ). *Sod2* encodes mitochondrial manganese superoxide dismutase (SOD2 protein; MnSOD), the primary enzymatic defense against superoxide anion radicals generated as byproducts of mitochondrial electron transport at Complexes I and III. Its induction

by PGC-1 $\alpha$  has been established as a conserved transcriptional response coupling mitochondrial biogenesis with antioxidant defense, demonstrated in vascular endothelial cells by Valle et al. (2005) and confirmed as a broad, physiologically required ROS-protective mechanism across multiple cell types by St-Pierre et al. (2006), who showed PGC-1 $\alpha$  is required for the induction of key ROS-detoxifying enzymes including GPx1 and SOD2 in response to oxidative stress (Valle et al., 2005; St-Pierre et al., 2006; Rius-Pérez et al., 2020). The genome-wide RNA-seq data further confirmed upregulation of *Sod1* (encoding cytoplasmic copper-zinc SOD) in the heatmap of top differentially expressed genes. MSEA identified enrichment of "Glutathione Metabolism" and "Redox Homeostasis" in PGC-1 $\alpha$ -overexpressing cells; this pathway-level enrichment is supported by the transcriptional upregulation of *Sod2* and *Sod1*, confirming enhanced enzymatic antioxidant capacity at the gene expression level. Together, these data indicate that PGC-1 $\alpha$  activates a coherent and multilayered antioxidant defense program in liver-tropic breast cancer cells.

The biological significance of this antioxidant upregulation specifically in the hepatic colonization context is profound. Kupffer cells, the liver-resident macrophages lining the hepatic sinusoids, represent the first immune barrier encountered by circulating tumor cells arriving in the liver, and their cytotoxic capability includes the production of superoxide via NADPH oxidase and nitric oxide via inducible NOS, generating reactive oxygen and nitrogen species that can kill arrested tumor cells within minutes of arrival (Liu et al., 2023). Tumor cells with insufficient antioxidant capacity are highly vulnerable to this Kupffer cell-mediated oxidative burst. By upregulating both enzymatic (SOD1, SOD2) and metabolic (glutathione, cysteine) antioxidant defenses, PGC-1 $\alpha$ -overexpressing cells are substantially better equipped to neutralize Kupffer cell-generated superoxide and H<sub>2</sub>O<sub>2</sub>, survive the earliest and most lethal phase of hepatic innate immune challenge, and proceed toward colonization. This mechanism provides a direct, mechanistically grounded, and liver-specific explanation for the colonization advantage that goes beyond generic bioenergetic considerations.

An important additional consideration is that SOD2, while primarily a mitochondrial antioxidant enzyme, also generates H<sub>2</sub>O<sub>2</sub> as its catalytic product, and H<sub>2</sub>O<sub>2</sub> can serve as a signaling molecule at low, controlled concentrations. The dual role of SOD2 as both a cytoprotective enzyme and a mediator of downstream H<sub>2</sub>O<sub>2</sub>-dependent signaling events, including the activation of transcription factors governing cell survival, migration, and angiogenesis, may contribute additional layers to

the SOD2-mediated metastatic advantage in the liver beyond simple ROS neutralization (Miriya et al., 2011). Future studies examining the relative contributions of enzymatic antioxidant activity versus H<sub>2</sub>O<sub>2</sub>-mediated signaling in PGC-1 $\alpha$ -overexpressing cells within the hepatic niche would provide valuable mechanistic refinement.

## **5. Transcriptomic and Metabolomic Multi-Omic Convergence Confirms the Mechanistic Architecture**

The genome-wide RNA-seq and targeted metabolomics analyses independently and convergently identified OXPHOS as the dominant activated program in PGC-1 $\alpha$ -overexpressing cells, with the KEGG "Oxidative phosphorylation" pathway achieving the highest gene ratio (~0.52) and most significant adjusted *P*-value among all enriched pathways by transcriptomic GSEA, and the metabolite set "Oxidative Phosphorylation" achieving the second highest enrichment ratio by metabolomic MSEA. This convergence across independent analytical platforms and complementary data types provides exceptionally strong evidence that OXPHOS is genuinely and substantially upregulated at the systems level, not merely in a subset of genes or metabolites, but as a consistent, coherent, and biologically crucial transcriptional and metabolic program. Importantly, GO: BP analysis independently confirmed "oxidative phosphorylation" as the top activated biological process term (GeneRatio approximately 0.47; FDR < 0.010), providing convergence between the GO: BP and KEGG analyses on the same core biological program.

Several additional multi-omic observations deserve specific discussion. The strong enrichment of "Arginine and Proline Metabolism" as the top metabolomic pathway (enrichment ratio ~5), alongside transcriptional upregulation of *Slc7a1* (the cationic amino acid transporter responsible for cellular arginine uptake), points toward a coordinated increase in arginine metabolic flux in PGC-1 $\alpha$ -overexpressing cells. Arginine is the substrate for both nitric oxide synthase, generating nitric oxide that can serve as a signaling molecule in immune evasion, and arginase, which catabolizes arginine to ornithine and polyamines critical for cell proliferation. Additionally, arginine can be converted to proline, which serves as a structural amino acid and is metabolized by proline oxidase in mitochondria to generate ATP, a function that links arginine-proline metabolism directly to oxidative bioenergetics. The concurrent upregulation of arginine import machinery (*Slc7a1*), arginine-proline metabolic pathway activity (MSEA), and mitochondrial bioenergetics (OXPHOS, TCA cycle) suggests a coherent metabolic network in which PGC-1 $\alpha$  orchestrates

enhanced amino acid catabolism as an additional fuel source feeding the expanded respiratory capacity. Of note, the GO: BP analysis (Figure 4C) confirms enrichment of "nucleotide catabolic process" and related nucleotide diphosphate catabolic terms in PGC-1 $\alpha$ -overexpressing cells, consistent with the elevated purine nucleotide turnover accompanying increased ATP synthesis rates.

The metabolomic and transcriptomic evidence for enhanced serine-glycine one-carbon (SGOC) metabolism, reflected by the highest VIP discriminating metabolite O-phosphoserine (VIP ~3.7), "Serine/Glycine One-Carbon Metabolism" enrichment by MSEA, and "Nucleotide Biosynthesis" pathway enrichment by both RNA-seq GSEA and MSEA, is equally noteworthy. O-phosphoserine is the penultimate intermediate in the de novo serine biosynthesis pathway (the PHGDH-PSAT1-PSPH axis), and its accumulation in PGC-1 $\alpha$ -overexpressing cells is consistent with a diversion of glycolytic 3-phosphoglycerate into this anabolic branch. De novo serine biosynthesis fuels multiple intersecting metabolic demands that are particularly relevant during metastatic colonization: it supplies one-carbon units to the folate cycle for nucleotide biosynthesis, drives NADPH production through serine hydroxymethyltransferase (SHMT) and methylenetetrahydrofolate dehydrogenase (MTHFD) reactions, and provides glycine as a glutathione precursor (Locasale, 2013; Lee et al., 2024). The co-enrichment of serine/glycine one-carbon metabolism with glutathione metabolism and redox homeostasis by MSEA therefore suggests an integrated metabolic network in which PGC-1 $\alpha$  coordinates enhanced serine biosynthesis, one-carbon folate cycling, NADPH regeneration, and glutathione synthesis, collectively fortifying the antioxidant capacity of these cells at the metabolite level in a manner fully consistent with the transcriptional *Sod2/Sod1* data.

The downregulation of *Cav1* (caveolin-1) in PGC-1 $\alpha$ -overexpressing cells, which appears prominently in the volcano plot and heatmap, also merits discussion. Caveolin-1 has been shown to promote aerobic glycolysis in colon cancer by upregulating GLUT3 (*SLC2A3*) glucose transporter expression through HMGA1-dependent transcriptional activation (Ha et al., 2012, 2012); in breast cancer stem cells, caveolin-1 loss is conversely associated with a switch from mitochondrial respiration to aerobic glycolysis (Zhang et al., 2020). The reciprocal relationship between caveolin-1 and glycolytic activity suggests that its transcriptional downregulation in PGC-1 $\alpha$ -overexpressing cells may represent a mechanistic contributor to the reduced glycolytic ceiling

observed in Seahorse ECAR measurements, reinforcing the OXPHOS-dominant state at the gene expression level through suppression of a pro-glycolytic scaffold protein.

The suppression of *Ccnd2* (cyclin D2) in PGC-1 $\alpha$ -overexpressing cells adds an important dimension to the interpretation of the in vitro proliferation data and the in vivo primary tumor null result. Cyclin D2 is a key positive regulator of G1/S cell cycle progression, and its downregulation implies that cells are less actively cycling under basal conditions when PGC-1 $\alpha$  is elevated, consistent with the hypothesis that cells allocating greater energetic resources toward mitochondrial biogenesis and antioxidant maintenance at baseline divert them partially from active proliferative cycling (Zhang et al., 2025). This metabolic-proliferative trade-off may explain why the in vitro proliferative advantage emerges only at high cell density (day 5–6), when local metabolic stress amplifies the bioenergetic advantage of oxidative cells, and why the in vivo primary tumor growth difference is absent entirely in the nutrient- and oxygen-replete context of an established mammary tumor.

## **6. PGC-1 $\alpha$ Selectively Amplifies Metastatic Competence Without Affecting Primary Tumor Growth: Mechanistic Insights and Broader Context**

One of the most conceptually important observations in this study is the clear dissociation between PGC-1 $\alpha$ 's effect on in vitro proliferation and in vivo primary tumor growth on the one hand, and its dramatic pro-metastatic effect on the other. PGC-1 $\alpha$  overexpression produced only a modest and late-emerging in vitro growth advantage and produced no detectable acceleration of orthotopic mammary tumor growth in syngeneic BALB/c hosts. Yet the same PGC-1 $\alpha$  overexpression almost tripled hepatic metastatic colonization efficiency in the direct intrasplenic liver seeding model. This dissociation is not unique to the present work: LeBleu et al. similarly observed that genetic silencing of PGC-1 $\alpha$  impaired metastatic dissemination without affecting primary tumor growth in breast cancer models, establishing PGC-1 $\alpha$  as a factor with preferential functional significance during metastatic progression relative to primary tumor expansion (LeBleu et al., 2014).

The mechanistic basis for this stage-selective advantage can be rationalized from the distinct metabolic environments encountered at each disease stage. Primary mammary tumors, once established and vascularized, exist in a relatively nutrient-replete, oxygen-accessible environment in which rapid glucose uptake and glycolytic ATP production are sufficient to sustain the high rates

of biosynthesis and proliferation required for tumor expansion. In this setting, the additional oxidative capacity conferred by PGC-1 $\alpha$  may offer no net advantage over the more ATP-efficient glycolytic program for the purpose of cell division, and the metabolic-proliferative trade-off reflected in *Ccnd2* downregulation may actually slightly restrain cell cycling. By contrast, the hepatic colonization environment confronts disseminated tumor cells with a series of demands, oxidative stress from Kupffer cell immune effector functions, nutrient competition from metabolically hyperactive hepatocytes, anoikis resistance during circulation, and the biosynthetically demanding transition to proliferative outgrowth in an immunologically challenging niche, for which bioenergetic resilience, antioxidant capacity, and metabolic flexibility are far more determinative of survival and colonization success than the raw rate of cell division only.

This stage-selective advantage aligns with the broader principle, now supported by multiple independent experimental systems, that primary tumor growth and metastatic colonization have fundamentally distinct and partially non-overlapping metabolic requirements (McGuirk et al., 2020; Bennett et al., 2022). The present findings extend this principle specifically to the liver, an organ whose biology makes mitochondrial resilience especially critical for colonization, and identify PGC-1 $\alpha$  as the master transcriptional regulator mediating this stage-selective metabolic advantage. Clinically, this dissociation carries important implications: it suggests that targeting PGC-1 $\alpha$ -driven oxidative metabolism would not be predicted to impair primary tumor growth (a property that might limit anti-metastatic utility in the adjuvant setting), while potentially providing a selective window against the metastatic process specifically.

## **7. Hepatocyte PGC-1 $\alpha$ Deletion Does Not Alter Metastatic Permissiveness: The Metabolic Soil Is Dispensable**

The finding that liver-specific deletion of *Ppargc1a* in hepatocytes does not alter the efficiency of breast cancer hepatic metastatic colonization is, in the context of this study, a result of equal conceptual importance to the positive colonization data. Negative results in strong, carefully controlled experimental systems are often the most mechanistically informative outcomes in biology, and this is particularly true here, because the null result definitively answers a question that could not be answered by studying only the tumor cell compartment (the seed).

The liver-specific knockout model was rigorously validated by three independent PCR genotyping assays confirming hepatocyte-specific, tissue-restricted excision of the floxed *Ppargc1a* allele, and histological examination confirmed that liver architecture was grossly intact at baseline under the experimental housing conditions employed. This baseline histological normalcy is consistent with published reports of liver-specific PGC-1 $\alpha$  KO models, which show that hepatocyte-specific PGC-1 $\alpha$  loss produces primarily metabolic phenotypes, impaired fasting-induced gluconeogenesis, reduced fatty acid oxidation capacity, and exaggerated diet-induced steatosis, rather than overt structural liver pathology under standard feeding conditions (Leone et al., 2005; Besse-Patin et al., 2019). This implies that the KO livers, while metabolically altered with respect to their oxidative program, maintained the structural, cellular, and immunological architecture needed to function as a permissive or restrictive niche for metastatic colonization.

Three non-mutually exclusive interpretations can be offered for the null result of the soil experiment:

First, the metabolic consequences of hepatocyte PGC-1 $\alpha$  deletion may be functionally compensated by alternative metabolic programs, particularly PGC-1 $\beta$ , which shares overlapping target gene networks and metabolic functions with PGC-1 $\alpha$  in the liver, as demonstrated by studies of the liver-specific PGC-1 $\beta$  KO (Bhatt et al., 2012). PGC-1 $\beta$  may partially sustain hepatic fatty acid oxidation and oxidative capacity in the absence of PGC-1 $\alpha$ , buffering the metabolic perturbation to an extent insufficient to alter the tumor-hepatocyte metabolic interaction landscape. Future experiments employing dual liver-specific PGC-1 $\alpha/\beta$  knockout models would directly address this possibility.

Second, and perhaps most important mechanistically, the null result may indicate that the metabolic interactions governing metastatic colonization in the liver are predominantly tumor cell-intrinsic rather than dependent on paracrine metabolic provision or competitive substrate dynamics with hepatocytes. In this view, the tumor cells' own metabolic program is the decisive variable, and the availability or composition of hepatocyte-derived metabolic substrates within the range modulated by PGC-1 $\alpha$  deletion does not constitute a rate-limiting constraint on colonization. This interpretation is supported by the observation that, even under normal conditions, the metabolic demands of early-stage colonization, survival, anoikis resistance, immune evasion, are met primarily by the tumor cell's own ATP-generating machinery rather than by nutrient transfer from

adjacent parenchymal cells. Newly arrived tumor cells in the sinusoidal space are not immediately in intimate metabolic exchange with hepatocytes; their initial fate is determined by their own bioenergetic reserves and stress-response capacity.

Third, the two-week colonization window employed in these experiments may not be sufficient to reveal potential metabolic soil effects that could emerge over longer time scales, particularly at the stage of macrometastatic outgrowth when established tumor colonies must compete for nutrients and growth factors with the surrounding hepatic parenchyma at larger spatial scales. The present experiments definitively establish that the seed metabolic state governs early colonization efficiency; whether hepatocyte PGC-1 $\alpha$  shapes the outgrowth of established macrometastases over longer periods remains an open question. Similarly, the model employs 4T1-lineage cells that may differ from other breast cancer subtypes in their degree of metabolic dependence on hepatocyte-derived substrates; other breast cancer models, particularly those with higher intrinsic dependence on lipid oxidation (a function strongly regulated by hepatocyte PGC-1 $\alpha$ /PPA $\alpha$  activity), might show greater sensitivity to hepatocyte PGC-1 $\alpha$  status.

Regardless of the mechanistic interpretation, the conclusion from the seed-soil dissection is clear and clinically actionable: within the experimental system studied, the PGC-1 $\alpha$ -dependent metabolic program of the hepatic soil does not limit or enable metastatic colonization efficiency. Therapeutic strategies aimed at disrupting PGC-1 $\alpha$ -driven metabolic advantages in breast cancer liver metastasis should therefore be directed at the tumor cell rather than the hepatic microenvironment.

## **8. Placing This Work Within the PGC-1 $\alpha$ in Cancer Field**

The context-dependent and apparently paradoxical role of PGC-1 $\alpha$  in cancer has been recognized for over a decade (Gravel, 2018; Wang et al., 2024; Zhang et al., 2024). In some cancers and contexts, PGC-1 $\alpha$  suppresses tumorigenicity by restraining glycolytic growth programs and promoting differentiation, a pattern observed in hepatocellular carcinoma and certain colorectal cancer models. In others, elevated PGC-1 $\alpha$  supports metastatic aggressiveness by providing mitochondrial resilience and bioenergetic flexibility under stress, the pattern observed in invasive breast cancer, melanoma, and the current work. This context-dependence can now be rationalized

more precisely in terms of the metabolic environment in which each tumor evolves, and the specific metabolic demands imposed by the cancer's stage of progression.

The present study adds three specific insights to this landscape. First, it resolves the apparent paradox of organ-selective organotropism and PGC-1 $\alpha$  expression by demonstrating that liver-tropic cells carrying low endogenous PGC-1 $\alpha$  are metabolically suboptimal for their intended target organ, and that elevating PGC-1 $\alpha$  substantially enhances their fitness for that organ. This implies that the low PGC-1 $\alpha$  state of liver-tropic cells, rather than being actively selected for hepatic colonization, may instead reflect selective pressures at earlier stages of the metastatic cascade, perhaps during primary tumor growth (where the glycolytic phenotype is advantageous) or during intravascular survival (where reduced mitochondrial activity may lower ROS generation and reduce oxidative cytotoxicity risk). Second, it provides the first direct experimental test of whether host hepatocyte PGC-1 $\alpha$  influences metastatic permissiveness, a question that had never been addressed in the context of breast cancer liver metastasis, and establishes a clear negative answer within the conditions studied. Third, through comprehensive multi-omic profiling, it identifies the specific downstream programs, OXPHOS, SOD2/glutathione-mediated antioxidant defense, serine-one-carbon metabolism, and arginine-proline metabolism, through which PGC-1 $\alpha$  confers hepatic colonization advantage, providing a molecular roadmap for future mechanistic and therapeutic investigations.

## **9. Contextualizing the Results Within the Metabolic Seed-Soil Hypothesis**

The theoretical framework of the seed-and-soil hypothesis, expanded here to encompass a metabolic dimension, provides the conceptual architecture for interpreting all results of this study. The classical seed-soil framework, elaborated by Fidler (Fidler, 2003) and extensively refined by Massagué and colleagues (Nguyen & Massagué, 2007; Massagué & Obenauf, 2015), has emphasized signaling-based compatibility between tumor cell programs and organ microenvironmental features as the determinant of organotropic success. The metabolic extension of this framework declares that bioenergetic compatibility between the tumor cell metabolic state and the organ's metabolic ecology constitutes an additional and potentially independent dimension of organotropic selection.

The present data provide a higher resolution of the metabolic seed-soil question specifically for breast cancer liver metastasis: the dominant determinant of colonization efficiency is seed-intrinsic, not soil-intrinsic. This finding does not invalidate the concept of metabolic soil regulation in principle; rather, it defines the specific conditions under which the seed metabolic program is the limiting variable. It is plausible that in other metastatic contexts, particularly for cancers with higher dependence on hepatocyte-derived lipid metabolites (e.g., hepatic VLDL-derived fatty acids that could be oxidized by tumor cells with high FAO activity, a function regulated by hepatocyte PPAR $\alpha$  coactivated by PGC-1 $\alpha$ ), or for models employing alternative tumor cell lines with distinct metabolic dependencies, the soil metabolic state would exert a more substantial influence. The present work establishes the methodological template, dual-compartment genetic manipulation with controlled portal vein delivery, for testing these possibilities systematically.

## **10. OXPHOS as a Therapeutic Vulnerability in PGC-1 $\alpha$ -High Metastatic Breast Cancer**

The robust upregulation of OXPHOS at both transcriptomic and metabolomic levels in PGC-1 $\alpha$ -overexpressing cells, combined with the demonstration that this program is functionally important for hepatic colonization efficiency, immediately raises the translational question of whether OXPHOS-dependent metastatic cells harbor specific pharmacological vulnerabilities that could be exploited therapeutically. This question has gained considerable clinical urgency as a growing body of evidence has identified OXPHOS dependence as a metabolic vulnerability in multiple cancer contexts, including therapy-resistant metastatic disease.

A landmark study by Tosi et al. demonstrated that a fraction of endocrine therapy- and palbociclib-resistant metastatic estrogen receptor-positive breast cancers display high OXPHOS gene expression and are exquisitely sensitive to IACS-010759, a potent and selective inhibitor of mitochondrial Complex I, both in patient-derived xenograft models and in molecular analyses of patient tumor biopsies (Tosi et al., 2023). IACS-010759 strongly inhibited tumor growth in these OXPHOS-dependent PDX models and simultaneously reduced metabolites of the glutathione, glycogen, and pentose phosphate pathways, pathways that our own metabolomic data identify as enriched in PGC-1 $\alpha$ -overexpressing cells, raising the important possibility that PGC-1 $\alpha$ -high metastatic cells may share the OXPHOS-dependent metabolic vulnerability identified in treatment-resistant ER<sup>+</sup> tumors. More broadly, the use of Complex I inhibitors including IACS-010759 and phenformin has been shown to impair metastasis formation in preclinical models of multiple cancer

types (Pujalte-Martin et al., 2024). However, clinical development of IACS-010759 has been hampered by dose-limiting peripheral neuropathy and lactic acidosis observed in early-phase trials, and by metabolic compensation through upregulated glycolysis and glutaminolysis when OXPHOS is inhibited, underscoring the importance of combination strategies that co-target glycolytic and OXPHOS pathways simultaneously. These clinical challenges, including the toxicity of mitochondrial inhibitors to normal tissues, are comprehensively discussed in a recent review by Minarrieta and St-Pierre (2026), which argues for shifting focus toward more selective aspects of mitochondrial biology that may offer meaningful anti-cancer activity with a wider safety margin (Minarrieta and St-Pierre, 2026).

The present data suggest that tumor cells with high PGC-1 $\alpha$  activity, elevated OXPHOS gene expression, and reduced glycolytic ceiling capacity (as demonstrated by the significantly lower maximal glycolytic ATP production in PGC-1 $\alpha$ -overexpressing cells) may be particularly well-suited targets for OXPHOS inhibition, precisely because their reduced glycolytic flexibility would impair the metabolic compensatory response to OXPHOS blockade. The convergent enrichment of the same OXPHOS pathway in both the transcriptome and the metabolome of PGC-1 $\alpha$ -overexpressing cells, alongside the multi-layered antioxidant program (SOD2, glutathione, serine-one-carbon), suggests that combination strategies targeting OXPHOS alongside glutathione biosynthesis or one-carbon metabolism could prove particularly effective against PGC-1 $\alpha$ -high metastatic cells. Such combinations would simultaneously deprive cells of their primary ATP source, deplete their antioxidant reserves, and inhibit nucleotide biosynthesis, three metabolic features sustaining the colonization competence identified in this study.

## **11. PGC-1 $\alpha$ Expression as a Potential Biomarker of Liver Metastasis Risk and OXPHOS Inhibitor Sensitivity**

The clinical translation of the mechanistic findings from this study may be facilitated by the measurement of PGC-1 $\alpha$  expression as a biomarker in breast cancer. LeBleu et al. (2014) demonstrated a significant correlation between PGC-1 $\alpha$  expression levels in invasive breast cancer specimens and the formation of distant metastases in patients, providing initial clinical validation that the pro-metastatic function of PGC-1 $\alpha$  identified in murine models has a human correlate. While clinical data specifically examining PGC-1 $\alpha$  expression in breast cancer liver metastases versus primary tumors are limited, the mechanistic framework provided by this study suggests that

tumors with elevated PGC-1 $\alpha$ , elevated OXPHOS gene expression scores, and evidence of active serine-one-carbon and antioxidant pathway activation would be enriched for cells with high hepatic colonization competence, and would represent candidates for OXPHOS-targeted therapeutic intervention.

The recent demonstration that high OXPHOS gene expression in ER+ metastatic breast cancer patients predicts poor prognosis and IACS-010759 sensitivity (Tosi et al., 2023) provides a clinically actionable precedent for using OXPHOS transcriptomic signatures as patient stratification biomarkers. The gene expression signature identified in the present RNA-seq analysis, encompassing ETC subunit genes, the thermogenesis program, antioxidant targets *Sod2* and *Sod1*, and serine-one-carbon metabolic genes, could potentially be refined into a clinically applicable PGC-1 $\alpha$ /OXPHOS activity score for breast cancer specimens, serving as both a prognostic biomarker for hepatic metastasis risk and a predictive biomarker for OXPHOS inhibitor response.

## **12. Limitations and Important Caveats**

The findings reported in this study should be interpreted within the context of several important experimental limitations that define the boundaries of the conclusions and identify opportunities for future investigation.

### **12.1 Constitutive vs. Physiological PGC-1 $\alpha$ Regulation**

The gain-of-function system employed here, constitutive lentiviral overexpression driven by a strong promoter, provides robust and experimentally tractable PGC-1 $\alpha$  expression but does not recapitulate the physiological regulation of endogenous PGC-1 $\alpha$ , which is subject to multiple post-translational controls including AMPK phosphorylation, SIRT1 deacetylation, p38 MAPK phosphorylation, and GCN5-mediated acetylation-dependent inhibition (Rodgers et al., 2005; Gerhart-Hines et al., 2007; Dominy et al., 2010). The bioenergetic and metastatic effects observed may therefore reflect not only the transcriptional consequences of elevated PGC-1 $\alpha$  protein, but also potential artifacts of transgene expression level or the absence of negative regulatory feedback that would normally limit PGC-1 $\alpha$  activity. Future studies employing endogenous PGC-1 $\alpha$  activation through AMPK agonists, SIRT1 activators, or CRISPR-mediated enhancer activation of

the endogenous *Ppargc1a* locus would provide a more physiologically faithful assessment of PGC-1 $\alpha$ 's role in the hepatic colonization context.

## **12.2 Model System Considerations**

All in vivo experiments employed the murine 4T1-derived breast cancer cell lines in syngeneic BALB/c hosts, a highly tractable and immunocompetent experimental system. While the 2792 cell line has well-established liver-metastatic tropism and is directly relevant to the questions studied, it is a murine triple-negative breast cancer model, and the degree to which findings generalize to human breast cancer subtypes, particularly hormone receptor-positive, HER2-positive, or other TNBC models with distinct oncogenotypes and metabolic dependencies, requires direct experimental validation. The murine hepatic microenvironment, while sharing fundamental anatomical and cellular features with the human liver, differs in key aspects including the abundance, activation thresholds, and cytokine profiles of Kupffer cells and liver-resident NK cells, as well as the composition and dynamics of the sinusoidal endothelial cell secretome.

## **12.3 Scope of the Multi-Omic Analysis**

The transcriptomic and metabolomic profiling was performed on cells grown under standard in vitro culture conditions, providing a cell-autonomous molecular portrait of the PGC-1 $\alpha$ -overexpressing state in isolation from the hepatic microenvironment. While this approach cleanly identifies the intrinsic metabolic programs conferred by PGC-1 $\alpha$  overexpression, it does not capture the dynamic metabolic adaptations that occur specifically during hepatic colonization in vivo, including responses to Kupffer cell cytotoxicity, metabolite exchange with hepatocytes, hypoxia, and nutrient competition. Future in vivo metabolomic profiling of metastatic cells isolated directly from hepatic lesions (e.g., by flow cytometric sorting of GFP-labeled or strain-specific marker-positive tumor cells from liver homogenates) would provide a more ecologically relevant view of the PGC-1 $\alpha$ -dependent metabolic state during active colonization.

## **12.4 Mechanistic Attribution and Downstream Pathway Dissection**

The multi-omic data identify multiple simultaneously altered pathways in PGC-1 $\alpha$ -overexpressing cells, OXPHOS, SOD2/antioxidant defense, serine-one-carbon metabolism, arginine-proline metabolism, and TCA cycle anaplerosis, but the present experiments do not definitively establish

the relative mechanistic contributions of each of these programs to the observed hepatic colonization advantage. For example, whether the colonization benefit is primarily attributable to the enlarged spare respiratory capacity (bioenergetic reserve), to the SOD2-mediated antioxidant defense (ROS detoxification), or to the serine-glutathione axis (redox homeostasis and nucleotide supply) remains to be dissected. Genetic rescue experiments reintroducing individual pathways in PGC-1 $\alpha$ -deficient backgrounds or using single-pathway inhibitors in the context of PGC-1 $\alpha$ -overexpressing cells during hepatic colonization assays, would provide the mechanistic resolution needed to prioritize therapeutic targets.

## 12.5 Future Directions

The findings of this study open several compelling avenues for future investigation that are of both mechanistic and translational importance.

The immediate mechanistic priority is the dissection of which specific PGC-1 $\alpha$  downstream program(s) are the primary drivers of hepatic colonization advantage. Targeted genetic experiments silencing *Sod2* or inhibiting serine biosynthesis through PHGDH knockdown or pharmacological inhibition, directly motivated by the elevation of O-phosphoserine as the top VIP discriminating metabolite in PGC-1 $\alpha$ -overexpressing cells in 2792 PGC-1 $\alpha$ -overexpressing cells followed by hepatic colonization assays in the intrasplenic model, would directly test the relative importance of each metabolic arm. These experiments would also reveal whether any single program is sufficient to confer colonization advantage, or whether the full PGC-1 $\alpha$  transcriptional network, acting through multiple coordinated programs, is required for maximal effect.

A second priority is the examination of whether elevated endogenous PGC-1 $\alpha$  activity, achieved through physiological stimuli (AMPK activation) or pharmacological PGC-1 $\alpha$  inducers rather than transgenic overexpression, similarly enhances hepatic colonization, and conversely, whether pharmacological suppression of PGC-1 $\alpha$  or OXPHOS inhibition (using IACS-010759, metformin, or the novel TPP<sup>+</sup>-conjugated OXPHOS inhibitors discussed by Kalyanaraman et al., 2023 and Beerkens et al., 2024) impairs hepatic metastatic colonization in PGC-1 $\alpha$ -overexpressing cells. These experiments would directly test the therapeutic hypothesis generated by this study and provide the preclinical evidence base for potential clinical investigation.

A third area of future investigation concerns the temporal dynamics of PGC-1 $\alpha$  activity during the metastatic cascade. The endogenously low PGC-1 $\alpha$  state of liver-tropic 2792 cells presumably reflects the selection pressures operating at prior stages of dissemination; understanding whether and when PGC-1 $\alpha$  is upregulated during the colonization process itself, potentially as an adaptive response to the hepatic metabolic environment, would provide crucial insight into the timing of PGC-1 $\alpha$ 's functional contribution. Single-cell RNA-seq or live metabolic imaging of PGC-1 $\alpha$  reporter cells during intravital hepatic colonization assays could address this question dynamically.

Finally, extending the dual seed-soil dissection approach to other metastatic organs (lung, bone, brain) using organ-specific KO models would test whether the dominance of seed over soil metabolic programming observed in the liver is organ-universal or liver-specific, and would define the boundaries within which the metabolic seed-soil framework operates.

Taken together, this study resolves a previously unaddressed metabolic paradox at the intersection of mitochondrial biology and organotropic breast cancer metastasis. Contrary to our initial hypothesis, the low endogenous PGC-1 $\alpha$  state of liver-tropic breast cancer cells is not a metabolic adaptation favorable for hepatic colonization but rather a suboptimal default state that actively limits their colonization potential, as PGC-1 $\alpha$  overexpression dramatically amplifies hepatic metastatic competence through a coordinated program of mitochondrial bioenergetic reserve, SOD2-mediated antioxidant defense (Valle et al., 2005; St-Pierre et al., 2006), and serine-one-carbon metabolic flux. The soil hypothesis was equally unsupported, as hepatocyte-specific *Ppargc1a* deletion had no effect on colonization efficiency, establishing the tumor cell's own metabolic programming as the decisive variable in this seed-and-soil interaction. These findings identify tumor cell-intrinsic PGC-1 $\alpha$ -driven oxidative programs as both determinants of hepatic metastatic aggressiveness and candidate vulnerabilities for therapeutic intervention, while underscoring that the clinical translation of OXPHOS-targeting strategies will require cancer-selective approaches to avoid toxicity to normal tissues (Minarrieta and St-Pierre, 2026).

## CHAPTER 3

### CONCLUSION

This thesis set out to resolve a biologically compelling paradox: if PGC-1 $\alpha$  promotes metastatic competence and mitochondrial fitness, why do breast cancer cells with preferential liver-metastatic tropism express low PGC-1 $\alpha$ , and what does this imply about the metabolic determinants of hepatic colonization? To answer this question, the work employed a systematic, dual-compartment experimental strategy, manipulating PGC-1 $\alpha$  activity independently in both the tumor cell (the seed) and the host hepatocyte (the soil), and combined multiple levels of biological analysis including functional bioenergetics, in vivo metastasis modeling, genome-wide transcriptomics, and targeted metabolomics.

The evidence collectively demonstrates that the low-PGC-1 $\alpha$ , glycolytic-dominant state of liver-tropic breast cancer cells is not an evolved metabolic adaptation optimized for hepatic colonization, but rather a metabolic suboptimum, a baseline insufficient to exploit the full colonization potential available in the hepatic niche. When PGC-1 $\alpha$  is experimentally elevated in these cells, a powerful pro-metastatic bioenergetic program is unlocked: mitochondrial oxidative capacity is amplified, spare respiratory reserve is substantially enlarged, mitochondrial antioxidant programs centered on *Sod2* are reinforced (Valle et al., 2005; St-Pierre et al., 2006), and serine-one-carbon and nucleotide biosynthetic pathways are upregulated, a series of metabolic adaptations that directly addresses the specific biological challenges of hepatic colonization. The consequence is a dramatic, approximately threefold amplification of hepatic metastatic colonization efficiency demonstrated in the controlled intrasplenic portal-vein injection model.

Critically, this pro-metastatic effect is entirely cell-autonomous in its mechanism. When the metabolic state of the hepatic soil, the PGC-1 $\alpha$ -dependent oxidative program of hepatocytes, is ablated through liver-specific knockout (KO), colonization efficiency is unaffected, demonstrating that the host hepatic metabolic environment is dispensable as a determinant of early colonization success within the experimental parameters studied. The seed's metabolic programming, not the soil's, proved decisive, as neither elevated hepatocyte PGC-1 $\alpha$  nor its ablation had any significant effect on colonization efficiency.

At the level of molecular mechanism, the multi-omic portrait assembled through RNA-seq and LC-MS/MS metabolomics identifies a coherent and reproducible metabolic signature of PGC-1 $\alpha$  overexpression, dominated by OXPHOS, antioxidant defense, and serine-one-carbon metabolism, that is activated simultaneously at the transcript and metabolite levels. The remarkable convergence of independent analytical platforms on the same core biological program provides the strongest possible multi-omic validation that this metabolic remodeling is genuine, broad, and actualized at the level of enzymatic activity and metabolite flux.

These findings carry significance at multiple levels. Conceptually, they refine and molecularly specify the metabolic dimension of the classical seed-and-soil hypothesis, establishing that in the context of breast cancer liver metastasis, the metabolic programming of the seed is the dominant determinant of colonization efficiency and is independent of the oxidative metabolic state of the hepatic soil. Biologically, they identify PGC-1 $\alpha$  as a master regulator whose activation in liver-tropic breast cancer cells amplifies hepatic colonization competence through mechanisms spanning bioenergetic reserve, antioxidant capacity, and biosynthetic metabolite availability, and establish a functional dissociation between PGC-1 $\alpha$ 's role in primary tumor growth versus metastatic colonization that has important implications for therapeutic targeting. Translationally, the robust OXPHOS transcriptional and metabolic signature identified here provides both a potential prognostic/predictive biomarker framework, stratifying patients with OXPHOS-high metastatic breast cancer who may be candidates for mitochondria-targeted therapies, and a mechanistic rationale for combining OXPHOS inhibitors with serine biosynthesis inhibitors (e.g., PHGDH inhibition targeting the O-phosphoserine axis) as a precision strategy against PGC-1 $\alpha$ -high hepatic metastases. Critically, however, the primary limitation of OXPHOS-targeting strategies remains toxicity to normal tissues, as discussed in depth by Minarrieta and St-Pierre (2026), which underscores the need to identify cancer-selective vulnerabilities within the PGC-1 $\alpha$  program rather than relying on broad mitochondrial complex inhibition.

Ultimately, the most important contribution of this thesis is its demonstration that the metabolic state of disseminating tumor cells is not merely a factor accompanying metastatic progression, but an active and powerful determinant of metastatic success, one that is responsive to measurement, manipulation, and potentially therapeutic exploitation. As the field of cancer metabolism continues to mature from descriptive characterization toward mechanistic and translational application, the

experimental framework, multi-omic methodology, and conceptual insights provided by this work offer a principled and experimentally tractable template for investigating how metabolic master regulators shape the metastatic fate of cancer cells, and for identifying the metabolic vulnerabilities that can be targeted to disrupt the most lethal stage of breast cancer progression.

## **CONTRIBUTIONS OF COLLABORATORS**

I, Elena Rahimi, am the sole author of this thesis. Under the supervision of Dr. Julie St-Pierre, I contributed to the conception and design of the study, performed the majority of the experimental work, analyzed and interpreted the data, prepared all figures, and wrote the thesis in its entirety. Several colleagues and core facilities made essential contributions to this work, and their specific roles are detailed below.

### **Supervision and study design**

Dr. Julie St-Pierre (Department of Biochemistry, Microbiology and Immunology, University of Ottawa) supervised all aspects of this work. She conceived the central research question, provided continuous guidance on experimental design, data interpretation, and the direction of the project, and critically reviewed and revised this thesis.

### **Thesis Advisory Committee**

Dr. Mireille Khacho and Dr. Mary-Ellen Harper (Department of Biochemistry, Microbiology and Immunology, University of Ottawa) served on my Thesis Advisory Committee. They provided scientific guidance, critical evaluation of the experimental strategy, and feedback on data interpretation at each committee meeting throughout my graduate studies.

### **Generation of cell lines**

Dr. Lucia Minarrieta and Mr. Irwin Kurreal (St-Pierre laboratory, University of Ottawa) generated the stable PGC-1 $\alpha$ -overexpressing and matched empty-vector control cell lines used throughout this study.

### **In vivo experiments**

All animal experiments were performed in collaboration with the laboratory of Dr. Peter Siegel (Rosalind and Morris Goodman Cancer Institute, McGill University) under Animal Use Protocol MCGL-5129, approved by the McGill University Facility Animal Care Committee. The specific contributions were as follows:

Dr. Charlotte Girondel (Dr. Peter Siegel lab) performed the intrasplenic portal vein injections for the 2792-PGC-1 $\alpha$  overexpression studies (Figure 2M–P).

Dr. Sébastien Tabariès (Dr. Peter Siegel lab) performed the splenic injections for the studies conducted in the liver-specific *Ppargc1a* knockout mice (Figure 3).

Dr. Matthew G. Annis (Dr. Peter Siegel lab) managed the liver-specific *Ppargc1a* knockout mouse colony, performed the orthotopic mammary fat pad injections for the overexpression studies (Figure 2E–L), and conducted the metastatic burden analyses for all in vivo experiments.

### **Mouse lines**

The *Ppargc1a*-floxed and Alb-Cre mouse lines used to generate the hepatocyte-specific *Ppargc1a* knockout model were originally received from the laboratory of Dr. Jennifer Estall at the Institut de Recherches Cliniques de Montréal (IRCM), Université de Montréal.

### **Bioinformatic analysis**

Dr. Lucia Minarrieta (St-Pierre laboratory, University of Ottawa) performed the bioinformatic analyses of the RNA sequencing data.

### **Core facilities**

Targeted LC-MS/MS metabolomic profiling was performed by the University of Ottawa Metabolomics Core Facility (MCF), supported by the Terry Fox Foundation and the University of Ottawa.

RNA sequencing, including library preparation and sequencing, was performed by the IRIC Genomics Core Facility at the Institut de Recherche en Immunologie et en Cancérologie (IRIC), Université de Montréal.

Imaging resources and technical support were provided by the University of Ottawa Cell Biology and Image Acquisition Core Facility (CBIA Core).

### **Work performed by the candidate**

I performed all cell culture and maintenance of the stable cell lines used in this study; all Seahorse extracellular flux analyses, including assay optimization, execution, normalization, and calculation of absolute ATP production rates; all RT-qPCR experiments and analyses; all proliferation assays; all RNA and metabolite extractions and sample preparation for transcriptomic and metabolomic profiling and all metabolomic data analyses; and all statistical analyses. I prepared all figures presented in this thesis and wrote the complete manuscript and thesis.

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## APPENDICES

**Supplementary Table 1.** Standard PCR genotyping reaction composition.

| Component                              | Volume per reaction (μL) | Final concentration |
|----------------------------------------|--------------------------|---------------------|
| Genomic DNA template                   | 2.5                      | -                   |
| CoraLoad 10× PCR Buffer (Qiagen)       | 2.0                      | 1×                  |
| 10 mM dNTP mix                         | 0.5                      | 250 μM each         |
| Primer mix (see Supplementary Table 2) | 4.0                      | 2.5 μM each primer  |
| Nuclease-free water                    | 10.8                     | -                   |
| Taq DNA Polymerase (5 U/μL)            | 0.2                      | 1.5 U/reaction      |
| Total volume                           | 20.0                     | -                   |

**Supplementary Table 2.** Genotyping primer sequences, assay applications, and expected amplicon sizes.

| Primer                                                | Sequence                                                                | Application                                                               | Expected amplicon              |
|-------------------------------------------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------|
| <b>Primer A</b><br><b>Primer B</b>                    | CTTCATGTGTCAGTGTAGTC<br>GGATGATAGGTATGCGTTAC                            | <i>Pparg1a</i> wild-type allele<br>(endpoint)                             | ~200 bp (WT)                   |
| <b>Primer C</b><br><b>Primer D</b>                    | TCCAGTAGGCAGAGATTTATGAC<br>CCAACTGTCTATAATTCCAGTTC                      | Cre-mediated excision (liver)                                             | ~450 bp (excised)              |
| <b>Primer E</b><br><b>Primer F</b>                    | TCCAGTAGGCAGAGATTTATGAC<br>TGTCTGGTTTGACAATCTGCTAGGTC                   | Floxed <i>Pparg1a</i> allele<br>detection                                 | ~400 bp (fl), ~360<br>bp (WT)  |
| <b>Primer G</b><br><b>Primer H</b><br><b>Primer I</b> | TTGGCCCCTTACCATAACTG<br>TGCAAACATCACATGCACAC<br>GAAGCAGAAGCTTAGGAAGATGG | Alb-Cre transgene (multiplex)<br>Alb-Cre wild-type control<br>(multiplex) | ~390 bp (Cre+)<br>~351 bp (WT) |

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## **Use of Generative AI**

AI Tools: ChatGPT (Version 4.0) and Grammarly (v1.2.274.1916)

Intended Use: I used generative AI tools, including ChatGPT and Grammarly, primarily for language editing, proofreading, improving clarity, and refining sentence structure. In some cases, I used these tools to revise text I had already drafted. All scientific content, data analysis, interpretation of results, conclusions, and final written content were independently produced, reviewed, and verified by me. I did not upload any unpublished results, raw data, analyses, figures, or thesis-related findings to any AI tools. My use was strictly limited to language-level editing of text I had already written, including portions of the Introduction and Methodology sections. I take full responsibility for the accuracy, originality, interpretation, and integrity of all materials presented in the thesis, regardless of any AI-assisted suggestions.

Approximate Extent of Use: 3.67% of total text refinement.