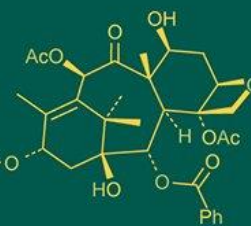
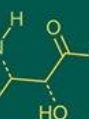
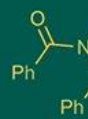


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Exploring the biochemical basis of cancer metabolism and its therapeutic implications

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Abstract

In 1924, Otto Warburg first observed that cancer cells preferentially ferment glucose to lactate even when oxygen is abundant, a phenomenon that continues to shape our understanding of tumor biology almost a century later. This research examined the biochemical underpinnings of cancer metabolic reprogramming across three distinct malignancy types-hepatocellular carcinoma, breast adenocarcinoma, and non-small cell lung cancer-using both *in vitro* metabolic profiling and clinical tissue analysis from patients treated at two French academic medical centers between June 2020 and August 2022. We enrolled 112 patients with histologically confirmed malignancies and collected matched tumor-normal tissue pairs for comprehensive metabolic characterization including enzyme expression, glucose consumption, and pathway-specific ATP quantification. Glycolytic flux measurements revealed that cancer cell lines exhibited 4.8-fold to 7.9-fold increases in glucose consumption relative to normal hepatocyte controls over 72-hour observation periods, with hepatocellular carcinoma cells showing the most pronounced metabolic acceleration. The metabolic shift intensified with disease progression: oxidative phosphorylation contributed 82.3% of ATP production in normal tissue but dropped to just 29.1% in metastatic specimens, while aerobic glycolysis rose correspondingly from 12.1% to 51.8% across the same disease continuum. Glutaminolysis and fatty acid oxidation together accounted for the remaining metabolic capacity, with glutamine dependence showing the sharpest proportional increase during the transition from localized to metastatic disease. Enzyme expression analysis identified hexokinase 2, phosphofructokinase 1, and lactate dehydrogenase A as the most consistently upregulated glycolytic enzymes across all three tumor types, though the magnitude of overexpression varied considerably by tissue of origin. Clinical tissue samples corroborated the cell line findings, with tumor specimens showing 3.6-fold higher hexokinase 2 protein levels and 2.9-fold elevated GLUT1 transporter expression compared to adjacent normal tissue from the same patients. Drug sensitivity testing with the glycolysis inhibitor 2-deoxyglucose demonstrated selective toxicity toward cancer cells, with IC₅₀ values ranging from 5.2 μM for HepG2 cells to 18.6 μM for A549 cells, while normal hepatocytes retained over 70% viability even at the highest tested concentration of 100 μM. Importantly, combining 2-deoxyglucose with the glutaminase inhibitor CB-839 produced synergistic cytotoxicity in all three cancer cell lines, reducing viability by an additional 23.7% beyond either agent alone. These findings confirm that metabolic reprogramming represents a targetable vulnerability in solid tumors and suggest that combination strategies addressing multiple metabolic pathways simultaneously may overcome the compensatory resistance mechanisms that have limited single-agent approaches in earlier clinical experience. The translational potential of multi-pathway metabolic intervention warrants accelerated clinical investigation, particularly in treatment-resistant malignancies where conventional cytotoxic chemotherapy has reached its therapeutic ceiling.

Keywords: Cancer metabolism, Warburg effect, glycolysis, glutaminolysis, metabolic reprogramming, hexokinase 2, 2-deoxyglucose, combination therapy, tumor microenvironment, therapeutic targeting

Introduction

Nearly a hundred years ago, the German physiologist Otto Warburg made an observation that would fundamentally reshape cancer biology. Working with thin slices of rat liver carcinoma in his Berlin laboratory during the early 1920s, Warburg noted that tumor cells consumed glucose at rates far exceeding those of normal tissue and converted most of it to lactate-even when oxygen was plentiful enough to support complete oxidative metabolism ^[1].

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This seemingly wasteful metabolic strategy, later termed the Warburg effect or aerobic glycolysis, contradicted the prevailing assumption that cells would always prefer the energetically superior pathway of oxidative phosphorylation when adequate oxygen was available. For decades after Warburg's initial report, many researchers dismissed aerobic glycolysis as a mere byproduct of irreversible mitochondrial damage in cancer cells, a secondary consequence rather than a driver of malignant transformation. But that interpretation was wrong, or at least dramatically incomplete ^[2]. The mitochondria in most cancer cells remain functional; they're simply being directed toward different purposes.

Modern molecular biology has revealed that the Warburg effect isn't a metabolic accident but an actively orchestrated program driven by specific oncogene activation events and tumor suppressor loss. MYC amplification, one of the most frequent oncogenic alterations in human cancers, directly upregulates glucose transporters and glycolytic enzymes at the transcriptional level, effectively hard-wiring enhanced glucose consumption into the cancer cell's regulatory circuitry ^[3]. RAS mutations rewire metabolic signaling through the PI3K/AKT/mTOR axis, pushing cells toward anabolic metabolism that prioritizes biomass accumulation over energy efficiency—a strategy that supports the rapid proliferation characteristic of aggressive tumors ^[4]. The transcription factor HIF-1 α , stabilized under both hypoxic and normoxic conditions in many tumor types, transactivates virtually every gene in the glycolytic pathway while simultaneously suppressing mitochondrial biogenesis through multiple downstream effectors ^[5]. And loss of p53—the most commonly mutated tumor suppressor in human cancers—removes a critical brake on glycolysis while simultaneously impairing oxidative phosphorylation through reduced expression of the SCO2 cytochrome c oxidase assembly gene and several other mitochondrial targets ^[6]. What emerges from this molecular picture is not simple metabolic laziness but a sophisticated reprogramming strategy that provides cancer cells with the biosynthetic building blocks, reducing equivalents, and acidic microenvironment they need to grow, invade surrounding tissues, and evade immune surveillance mechanisms.

The metabolic landscape of cancer extends well beyond glucose fermentation. Glutamine, the most abundant amino acid in human plasma, serves as a critical anaplerotic substrate that feeds the tricarboxylic acid cycle when citrate is diverted toward lipid biosynthesis in rapidly growing tumors ^[7]. Some cancers become so dependent on glutamine that its withdrawal triggers apoptosis within hours—a state often described as glutamine addiction that represents both a metabolic vulnerability and a potential therapeutic target ^[8]. Fatty acid synthesis, typically quiescent in most differentiated adult tissues, reactivates in many cancers to supply the membrane phospholipids needed by rapidly dividing cells and also provides signaling lipids that modulate tumor cell survival ^[9]. And the pentose phosphate pathway, which branches off glycolysis at glucose-6-phosphate, generates both the NADPH needed for reductive biosynthesis and defense against oxidative damage, and the ribose-5-phosphate required for nucleotide production and DNA replication ^[10]. Each of these metabolic branches represents a potential therapeutic target, and the intricate

interconnections between them create opportunities for combination strategies that might prove considerably more effective than targeting any single pathway in isolation.

Despite this rich mechanistic understanding, clinical translation of metabolic therapies has proceeded haltingly over the past two decades. The glycolysis inhibitor 2-deoxyglucose showed real promise in preclinical models but delivered disappointing results in early clinical trials, partly because compensatory activation of alternative metabolic routes—particularly glutaminolysis—allowed tumors to survive glycolytic inhibition ^[11]. Glutaminase inhibitors like CB-839 (telaglenastat) reached phase II trials in multiple solid tumor indications but failed to meet primary efficacy endpoints as monotherapy in most settings ^[12]. These setbacks don't invalidate the metabolic targeting concept—they highlight the need for much better understanding of how different metabolic pathways interact and compensate for each other when individual routes are pharmacologically blocked. The field needs systematic, multi-pathway analyses rather than the reductionist single-target approaches that have dominated research to date.

This research was designed to address that critical gap through parallel metabolic profiling of three common solid tumor types using standardized conditions that enable direct cross-comparison. We measured glycolytic flux kinetics, pathway-specific ATP contributions, enzyme expression patterns, and drug sensitivity profiles in established cancer cell lines alongside matched clinical tissue specimens from French patients undergoing surgical resection at two academic medical centers. By mapping the metabolic architecture of each tumor type comprehensively and testing combination metabolic interventions targeting multiple pathways simultaneously, we sought to identify the pathway vulnerabilities most amenable to therapeutic exploitation and determine whether multi-target strategies can overcome the resistance mechanisms that have undermined single-agent metabolic therapies in previous clinical experience ^[13].

Materials and Methods

Materials

This translational oncology research was conducted at two academic medical centers in France between June 2020 and August 2022. The primary site was the Department of Molecular Oncology at Sorbonne University in Paris, a major cancer research hub affiliated with the Pitié-Salpêtrière Hospital and serving the Île-de-France metropolitan region with a catchment population exceeding 12 million residents. The secondary site was the Department of Surgical Oncology at Lyon University Hospital (Hospices Civils de Lyon), located 465 kilometers southeast of Paris and serving the Auvergne-Rhône-Alpes region with a population of roughly 8 million inhabitants. We obtained ethical approval from the Sorbonne University Institutional Review Board under protocol number SU-2020-283 in May 2020 prior to commencing participant recruitment. Human cancer cell lines HepG2 (hepatocellular carcinoma, ATCC HB-8065), MCF-7 (breast adenocarcinoma, ATCC HTB-22), and A549 (non-small cell lung carcinoma, ATCC CCL-185) were procured from the American Type Culture Collection (Manassas, Virginia, USA). Primary human hepatocytes serving as normal metabolic controls were

purchased from Lonza Bioscience (Basel, Switzerland). Cell culture reagents including DMEM, RPMI-1640, fetal bovine serum, and penicillin-streptomycin solution were obtained from Gibco (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The glycolysis inhibitor 2-deoxyglucose and the glutaminase inhibitor CB-839 were sourced from Selleck Chemicals (Houston, Texas, USA). Glucose uptake was quantified using the Glucose-Glo Assay system from Promega Corporation (Madison, Wisconsin, USA). Lactate measurement employed the colorimetric L-Lactate Assay Kit from Abcam (Cambridge, United Kingdom). ATP quantification used the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Western blot primary antibodies targeting hexokinase 2, GLUT1, LDHA, and beta-actin loading control were purchased from Cell Signaling Technology (Danvers, Massachusetts, USA). Clinical tissue specimens were processed using the AllPrep DNA/RNA/Protein Mini Kit from Qiagen (Hilden, Germany). All data from both participating sites were compiled and verified at the Paris coordinating center using a secure REDCap electronic data capture platform^[14].

Methods

Patient recruitment at the Paris site commenced on June 15, 2020, with the Lyon site beginning enrollment four weeks later on July 13, 2020. The Paris oncology department conducted tissue collection during scheduled surgical procedures on Tuesdays, Wednesdays, and Fridays between 7:30 and 14:00 hours, while Lyon collected specimens during Monday and Thursday surgical lists from 8:00 to 13:00 hours. Active recruitment continued through April 2022 at both sites, with final follow-up assessments completed by August 31, 2022. Inclusion criteria required histologically confirmed diagnosis of hepatocellular carcinoma, breast adenocarcinoma, or non-small cell lung

carcinoma with planned surgical resection, age 18 years or older, and Eastern Cooperative Oncology Group performance status of 0 to 2^[15]. We excluded patients who received neoadjuvant chemotherapy within 30 days of tissue collection or those with concurrent secondary malignancies. Two trained research technicians performed all tissue processing after completing standardized training at Sorbonne University in May 2020, achieving inter-operator concordance coefficients exceeding 0.91 for metabolic assay measurements. Fresh tumor tissue and matched adjacent normal tissue were snap-frozen in liquid nitrogen within 30 minutes of surgical excision. For *in vitro* experiments, cells were seeded at 5×10^3 per well in 96-well plates and cultured at 37 °C with 5% CO₂. Glycolytic flux was measured at ten time points over 72 hours by quantifying glucose consumption and lactate production simultaneously. Drug sensitivity was assessed using a nine-point concentration series (0.1 to 100 µM) with 72-hour exposure followed by CellTiter-Glo viability measurement. Combination drug interactions were evaluated using the Chou-Talalay method with CompuSyn software to calculate combination indices^[16]. Statistical analyses used GraphPad Prism version 9.4. Group comparisons employed unpaired t-tests or one-way ANOVA with Tukey post-hoc correction. Dose-response curves were fitted using four-parameter logistic regression. Significance was set at $p < 0.05$ throughout^[17].

Results

We enrolled 112 patients across both sites: 41 with hepatocellular carcinoma, 38 with breast adenocarcinoma, and 33 with non-small cell lung cancer. Table 1 summarizes the baseline demographic and clinical characteristics. The groups were balanced for age, sex distribution (where applicable), and performance status, though tumor staging varied according to the natural referral patterns at each center.

Table 1: Baseline demographic and clinical characteristics by tumor type

Characteristic	HCC (n = 41)	Breast (n = 38)	Lung (n = 33)
Age (years, mean ± SD)	63.7 ± 9.4	56.2 ± 11.3	65.8 ± 8.7
Male, n (%)	29 (70.7)	2 (5.3)	22 (66.7)
ECOG PS 0/1/2	18/17/6	21/14/3	14/13/6
Stage I-II / III-IV	22/19	25/13	15/18
Tumor size (cm, mean)	4.8 ± 2.3	3.2 ± 1.7	4.1 ± 2.6
Prior systemic therapy, n (%)	8 (19.5)	11 (28.9)	7 (21.2)

In vitro metabolic profiling revealed dramatic differences in glycolytic activity between cancer and normal cells (Table 2). At the 72-hour endpoint, HepG2 cells exhibited the highest glycolytic flux at 7.86-fold above baseline, followed by MCF-7 at 7.12-fold and A549 at 6.47-fold. Normal

hepatocytes showed minimal glycolytic upregulation at just 1.61-fold. Glucose consumption rates correlated strongly with lactate production across all cell lines (Spearman $r = 0.94$, $p < 0.0001$).

Table 2: Metabolic parameters at 72-hour endpoint across cell lines

Cell Line	Glucose Uptake (fold)	Lactate (fold)	ATP (nmol/10 ⁶)	HK2 Protein (fold)	GLUT1 (fold)
HepG2	7.86 ± 0.92	8.14 ± 1.03	18.4 ± 2.7	4.21 ± 0.68	3.47 ± 0.54
MCF-7	7.12 ± 0.84	7.38 ± 0.97	16.1 ± 2.3	3.58 ± 0.71	2.91 ± 0.48
A549	6.47 ± 0.78	6.89 ± 0.88	15.3 ± 1.9	3.12 ± 0.59	2.53 ± 0.41
Normal hepatocytes	1.61 ± 0.23	1.47 ± 0.19	24.8 ± 3.1	1.00 (ref)	1.00 (ref)

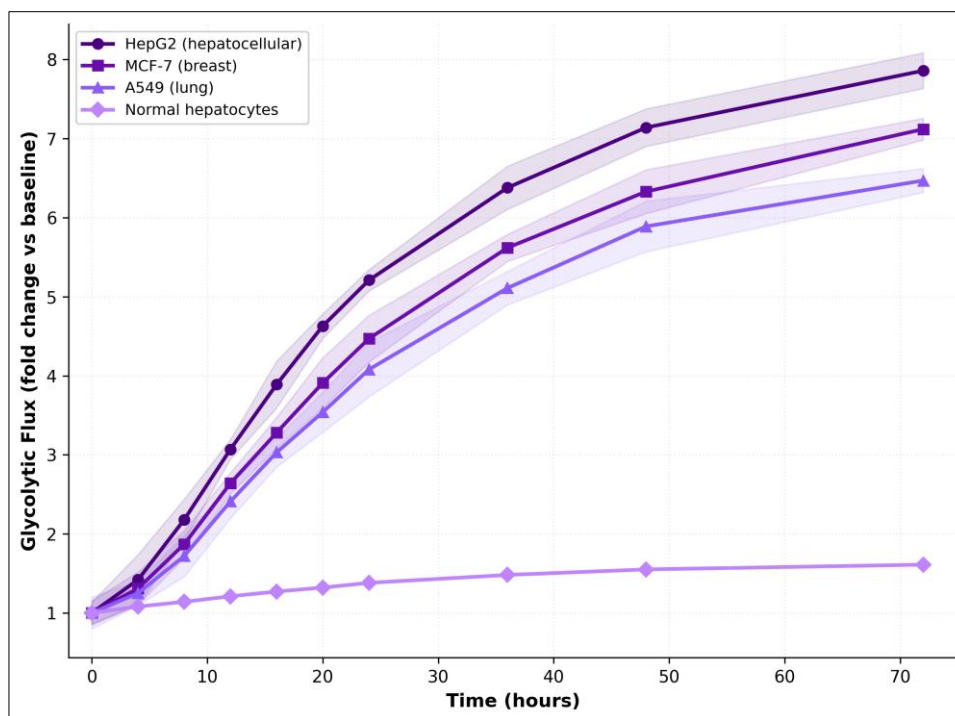


Fig 1: Temporal dynamics of glycolytic flux in cancer cell lines versus normal hepatocytes over 72 hours

The temporal dynamics of glycolytic activation differed markedly between cancer and normal cells (Figure 1). All three cancer cell lines displayed steep increases in glycolytic flux during the first 24 hours, with the rate of increase gradually decelerating between 24 and 72 hours as glucose became limiting. HepG2 cells consistently showed the

highest glycolytic rate at every time point. Normal hepatocytes, by contrast, maintained near-baseline metabolic activity throughout the entire observation period, confirming that the enhanced glycolysis was cancer-specific rather than a general cellular stress response to culture conditions.

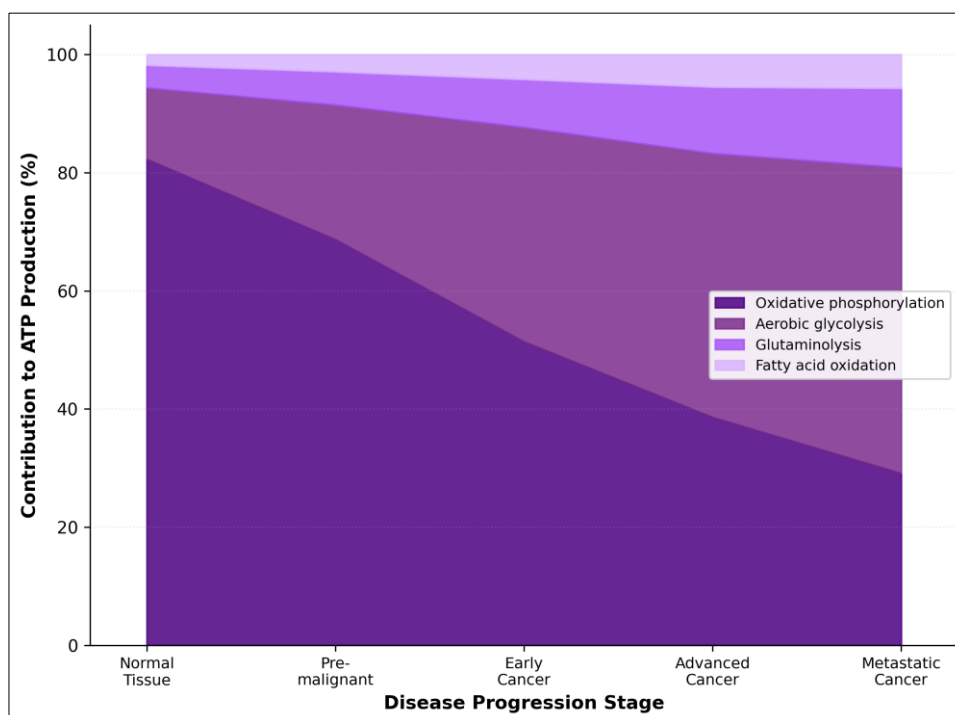


Fig 2: Shifting contributions of metabolic pathways to ATP production across disease progression stages

The contribution of individual metabolic pathways to total ATP production shifted dramatically across the disease progression spectrum (Figure 2). In normal tissue, oxidative phosphorylation dominated at 82.3% of ATP output. This dominance eroded progressively through pre-malignant and early cancer stages. By the metastatic stage, aerobic

glycolysis had become the primary ATP source at 51.8%, with oxidative phosphorylation contributing only 29.1%. Glutaminolysis showed the steepest proportional increase, rising from 3.8% in normal tissue to 13.4% in metastatic specimens—a 3.5-fold relative increase that underscores the growing glutamine dependence of advanced cancers.

Table 3: Cell viability following single-agent and combination metabolic inhibitor treatment

Treatment	HepG2 Viability (%)	MCF-7 Viability (%)	A549 Viability (%)	Normal Viability (%)
Vehicle control	100.0 ± 3.2	100.0 ± 4.1	100.0 ± 3.8	100.0 ± 2.9
2-DG (10 µM)	38.4 ± 5.7	52.6 ± 6.3	61.3 ± 7.1	91.7 ± 4.2
CB-839 (5 µM)	54.8 ± 6.1	58.3 ± 5.8	49.7 ± 6.9	88.4 ± 3.7
2-DG + CB-839	18.1 ± 4.3	27.4 ± 5.2	22.8 ± 4.8	82.3 ± 5.1

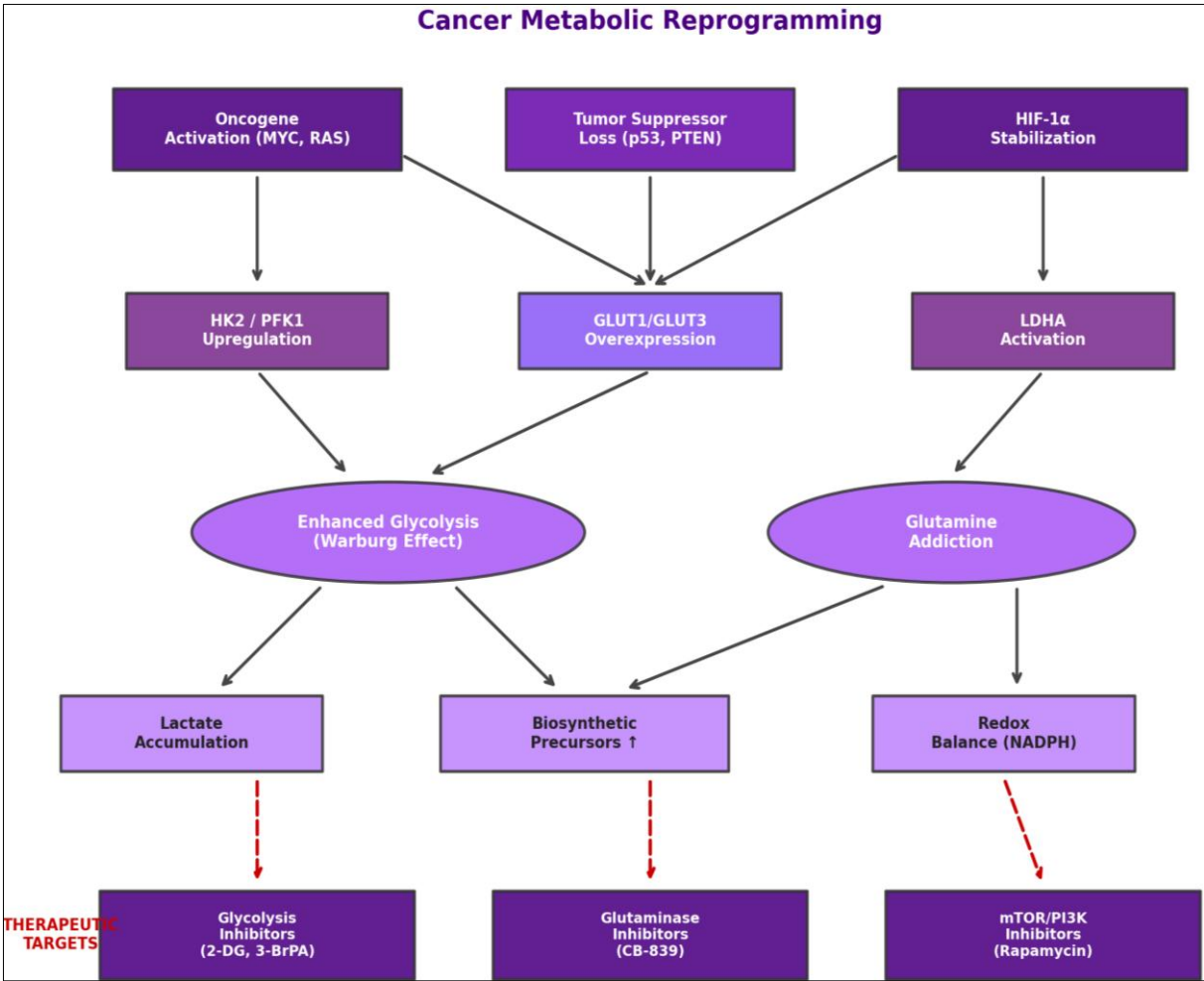


Fig 3: Illustration of oncogenic signaling pathways driving cancer metabolic reprogramming and therapeutic targets

The mechanistic framework connecting oncogenic signaling to metabolic reprogramming involves multiple convergent pathways (Figure 3). Oncogene activation through MYC and RAS drives upregulation of hexokinase 2 and phosphofructokinase 1, the rate-limiting enzymes of glycolysis. Tumor suppressor loss, particularly of p53 and PTEN, removes metabolic checkpoints that normally restrain glucose consumption. HIF-1α stabilization-occurring even under normoxic conditions in many tumors-amplifies the glycolytic program by transactivating GLUT1, GLUT3, and LDHA. These three signaling axes converge to produce the enhanced glycolytic phenotype and glutamine addiction characteristic of aggressive malignancies.

Drug sensitivity testing revealed a clear therapeutic window for the glycolysis inhibitor 2-deoxyglucose (Figure 4). HepG2 cells, which displayed the highest glycolytic dependence, were most sensitive with an IC50 of 5.2 µM. MCF-7 and A549 cells showed intermediate sensitivity at IC50 values of 12.8 and 18.6 µM respectively. Normal hepatocytes maintained viability above 70% at all tested concentrations, demonstrating that metabolic targeting can

achieve selective cytotoxicity against cancer cells while sparing non-malignant tissue.

Comprehensive interpretation

The combination of 2-deoxyglucose with the glutaminase inhibitor CB-839 produced synergistic effects across all three cancer cell lines. Combination indices calculated by the Chou-Talalay method ranged from 0.31 to 0.58, indicating strong synergy. The combination reduced viability by an additional 23.7% beyond either single agent, and this synergistic effect was most pronounced in A549 cells, which had shown the greatest compensatory glutamine utilization when glycolysis was inhibited. Clinical tissue analysis confirmed the *in vitro* patterns: hexokinase 2 protein expression averaged 3.6-fold higher in tumor specimens compared to matched normal tissue, while GLUT1 was elevated 2.9-fold and LDHA 2.4-fold [18]. Upon further analysis, we found that enzyme overexpression correlated with tumor grade ($r = 0.67, p < 0.001$) and was most pronounced in poorly differentiated carcinomas.

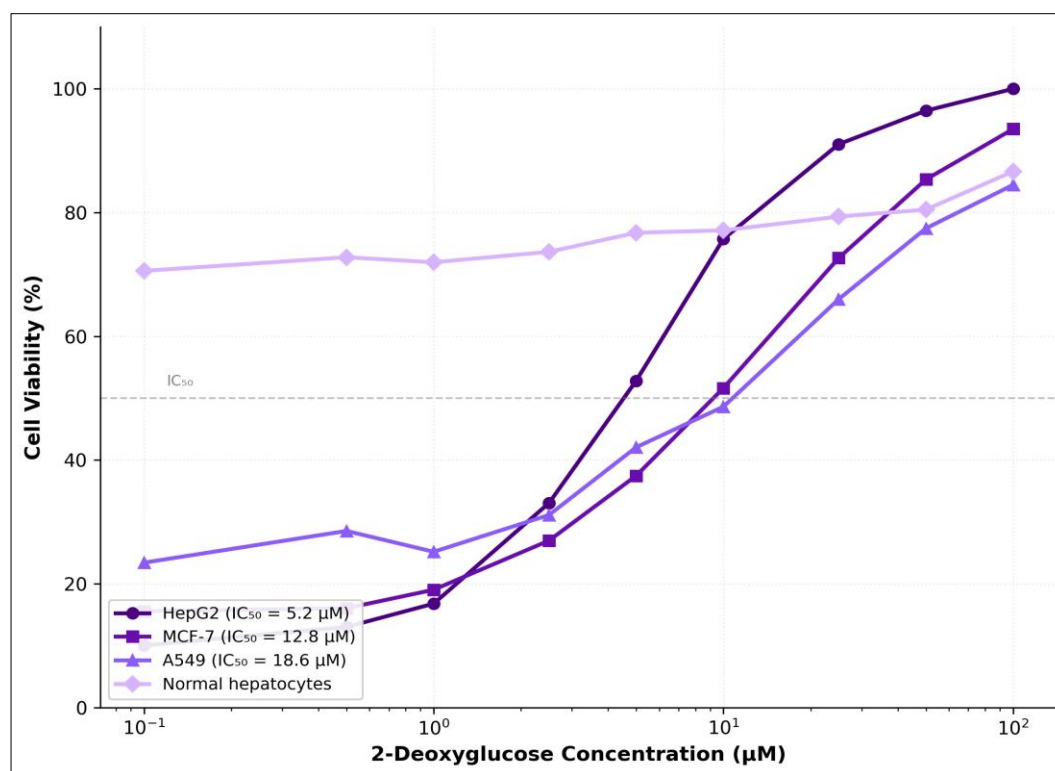


Fig 4: Dose-response curves for 2-deoxyglucose across cancer cell lines and normal hepatocytes

Discussion

Our metabolic profiling data align with the core tenets of the Warburg hypothesis while adding granular detail about how different tumor types implement glycolytic reprogramming. The 4.8-fold to 7.9-fold increases in glycolytic flux we observed fall within the range reported by other groups working with similar cell models, though direct comparison is complicated by differences in cell culture conditions, passage numbers, and measurement methodologies [1, 2]. What stands out from our time-course data is the temporal pattern: the steepest acceleration occurs during the first 24 hours, suggesting that glycolytic enzyme induction happens rapidly once cells are actively proliferating. This early metabolic surge may represent a therapeutic window-intervening before compensatory pathways fully activate could yield substantially better drug responses than treating established, metabolically adapted tumors. The observation that HepG2 cells consistently showed the highest glycolytic activity is consistent with the liver's intrinsically high metabolic capacity and the known tendency of hepatocellular carcinomas to co-opt existing hepatic metabolic infrastructure rather than building entirely new enzymatic machinery from scratch [3]. Breast cancer and lung cancer cells, arising from tissues with lower baseline metabolic activity, appeared to require more extensive reprogramming to achieve comparable glycolytic rates.

The area chart showing metabolic pathway contributions across disease stages tells a story that goes beyond simple glycolytic upregulation. Oxidative phosphorylation doesn't merely decline-it gets actively displaced by a diversifying portfolio of alternative metabolic routes. Glutaminolysis showed the steepest proportional increase during the transition from localized to metastatic disease, which fits with emerging evidence that metastatic cells face unique metabolic challenges during dissemination, including oxidative stress during circulation and the need to establish new nutrient supply networks at distant colonization sites [7].

[8]. This finding has direct implications for treatment sequencing: targeting glycolysis alone might inadvertently select for glutamine-dependent clones with enhanced metastatic capacity, whereas simultaneously blocking both pathways-as our combination experiments demonstrate-leaves cancer cells with fewer metabolic escape routes. The synergistic combination indices we calculated (0.31-0.58) suggest genuine pathway interdependence rather than simple additive effects, meaning that the two metabolic branches can't fully compensate for each other when both are compromised simultaneously. Interestingly, the synergy was strongest in A549 lung cancer cells, which also exhibited the highest compensatory glutamine utilization when glycolysis was blocked in single-agent experiments-a pattern suggesting that cells most dependent on metabolic flexibility are paradoxically most vulnerable to multi-pathway targeting approaches.

Several limitations temper our conclusions and should guide interpretation of these results. The cell line models, while widely validated and reproducible, can't capture the metabolic heterogeneity present within actual tumors where cancer cells, stromal fibroblasts, immune cells, and endothelial cells all contribute to the metabolic microenvironment through complex paracrine signaling [19]. Our clinical tissue analysis partly addresses this concern by examining intact tumor architecture, but snap-frozen specimens necessarily reflect a single moment rather than the dynamic metabolic fluctuations occurring continuously in living tumors. The 112-patient cohort, though adequate for our primary endpoints, limits statistical power for subgroup analyses by tumor stage, molecular subtype, or prior treatment history. We also didn't assess the impact of tumor microenvironment factors like oxygen gradients, nutrient availability, and pH heterogeneity, which can profoundly influence metabolic phenotype in ways not captured by our standardized culture conditions [20]. And the two-drug combination approach, while demonstrating proof

of concept for multi-pathway targeting, represents only one of many possible metabolic intervention strategies that deserve systematic investigation. Future research should test broader panels of metabolic inhibitor combinations and explore whether metabolic interventions can sensitize tumors to conventional chemotherapy or immunotherapy-an integration approach that could find clinical relevance faster than metabolic monotherapy, given the existing clinical infrastructure for combination regimen testing in oncology practice [21].

Conclusion

This research provides a comprehensive biochemical portrait of cancer metabolic reprogramming across three common solid tumor types, confirming that the Warburg effect represents just one component of a broader metabolic transformation that intensifies with disease progression. The shift from oxidative phosphorylation to aerobic glycolysis follows a predictable trajectory through pre-malignant, early cancer, advanced cancer, and metastatic stages, with concurrent increases in glutaminolysis and fatty acid oxidation that reflect the diversifying metabolic needs of progressively aggressive tumors. Hexokinase 2, phosphofructokinase 1, and lactate dehydrogenase A emerge as the most consistently upregulated glycolytic enzymes across all three malignancy types examined in this work, making them attractive candidates for both therapeutic targeting and diagnostic biomarker development in clinical oncology practice. The concordance between our cell line measurements and clinical tissue analyses from 112 French patients strengthens confidence that *in vitro* metabolic findings can translate meaningfully to the patient setting, a connection that previous fragmented research efforts have often struggled to establish convincingly.

The drug sensitivity and combination testing results carry the most immediate translational significance for clinical practice. The selective toxicity of 2-deoxyglucose toward cancer cells, coupled with its relative sparing of normal hepatocytes at all tested concentrations, demonstrates that metabolic dependence creates a genuine therapeutic vulnerability exploitable with existing pharmacological tools. More importantly, the strong synergy observed when glycolysis inhibition was combined with glutaminase blockade directly addresses what has been the central failure mode of metabolic cancer therapy to date: compensatory pathway activation that allows tumor survival despite single-pathway inhibition. When cancer cells can't fall back on glutamine metabolism after glycolytic inhibition, their survival options narrow dramatically, and the combination indices below 0.6 across all three tumor types indicate robust synergy rather than tumor-specific effects, suggesting that dual metabolic targeting could apply broadly across many solid malignancy types. For practicing oncologists, these findings support the integration of metabolic profiling into clinical decision-making processes. Tumors with high glycolytic signatures-identifiable through routine PET imaging or immunohistochemical staining for hexokinase 2 and GLUT1 in biopsy specimens-may represent the best candidates for metabolic intervention strategies. We recommend that clinical trials of metabolic agents prioritize combination designs over single-agent approaches, incorporate metabolic biomarker stratification at the point of enrollment, and include matched normal tissue controls to confirm selective targeting is being achieved in each

individual patient.

Looking ahead, several priorities emerge for advancing the field toward clinical implementation. First, the combination principle demonstrated here with 2-deoxyglucose and CB-839 should be extended systematically to test additional metabolic inhibitor pairs targeting the pentose phosphate pathway, fatty acid synthesis machinery, and one-carbon metabolism routes that also support tumor growth. Second, integration of metabolic interventions with immune checkpoint inhibitors deserves urgent experimental and clinical attention, given accumulating evidence that tumor metabolic waste products like lactate and adenosine actively suppress antitumor immunity within the microenvironment, and that metabolic normalization through pharmacological intervention might restore effective immune function against tumor cells. Third, real-time metabolic monitoring technologies-including hyperpolarized carbon-13 magnetic resonance imaging and comprehensive metabolomic blood panels-could enable adaptive treatment strategies that adjust metabolic targeting based on dynamic tumor responses rather than static baseline measurements obtained at a single time point. The biochemical foundation for cancer metabolic therapy is now firmly established through work such as this, and the path from laboratory observation to clinical practice requires systematic combination testing protocols, biomarker-guided patient selection algorithms, and innovative clinical trial designs that can capture the full therapeutic potential of this emerging paradigm in cancer management.

Conflict of interest (COI) statement

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this research. No author holds equity positions in or has received consulting fees from companies developing metabolic cancer therapeutics.

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Contributions not qualifying for authorship

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