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Research Article

Mapping the Metabolic Landscape of Lung Adenocarcinoma: From Energy Flexibility in Early Stages to Glycolytic Entrapment in Advanced Disease

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Abstract

Lung adenocarcinoma, a subtype of non-small cell lung cancer (NSCLC), exhibits profound metabolic reprogramming as it progresses, characterized by a shift from oxidative phosphorylation (OXPHOS) to glycolysis, even in the presence of oxygen. This phenomenon, known as the Warburg effect, enables cancer cells to meet their increased energy and biosynthetic demands, facilitating rapid proliferation and tumor progression. In this study, we compared the metabolic profiles of 150 patients, including 75 with stage 4 lung adenocarcinoma and 75 with early-stage disease (stage I and II), to investigate the dynamic metabolic changes occurring as the disease advances. We analyzed key glycolysis markers—glucose, lactate, and pyruvate—along with oxidative phosphorylation markers—β-hydroxybutyrate, acetoacetate, glutamine, and alanine. The findings revealed a significant increase in glycolysis markers in the stage 4 group, indicating a pronounced shift towards glycolysis. Concurrently, oxidative phosphorylation markers showed a marked reduction, reflecting mitochondrial dysfunction. Additionally, inflammatory cytokines (IL-6, IL-1Beta, TNF-alpha) and lactate dehydrogenase (LDH) were elevated in the advanced-stage patients, contributing to the pro-tumorigenic environment and promoting immune evasion and angiogenesis. Oxidative stress markers, such as melatonin and dopamine, were significantly reduced in stage 4 patients, further underscoring the role of oxidative stress in disease progression. The metabolic reprogramming in lung adenocarcinoma is tightly linked to the tumor microenvironment, supporting immune evasion and metastatic potential. These findings highlight the critical metabolic changes in advanced lung adenocarcinoma and underscore the potential for targeting glycolysis and related pathways as therapeutic strategies. The decline in oxidative phosphorylation and the increase in glycolysis markers suggest a metabolic threshold beyond which traditional anticancer treatments may offer diminishing returns, necessitating novel interventions to disrupt tumor metabolism and improve patient outcomes.

Highlights

- ***Metabolic Shift to Glycolysis:*** Lung adenocarcinoma cells demonstrate a significant shift from oxidative phosphorylation to glycolysis (Warburg effect), especially in advanced stages, allowing cancer cells to meet the increased energy and biosynthetic demands of rapid tumor growth.
- ***Increased Glycolysis Markers:*** Elevated levels of glycolysis markers such as glucose, lactate, and pyruvate were observed in stage 4 lung adenocarcinoma patients, indicating reliance on glycolysis for energy production.
- ***Inflammatory Cytokine Elevation:*** Advanced-stage lung adenocarcinoma patients exhibited higher levels of

inflammatory cytokines, including IL-6, IL-1Beta, and TNF-alpha, contributing to tumor progression, immune evasion, and angiogenesis.

• ***Reduced Oxidative Phosphorylation:*** Markers of oxidative phosphorylation such as β-hydroxybutyrate, acetoacetate, glutamine, and alanine were significantly reduced in advanced-stage lung adenocarcinoma patients, indicating mitochondrial dysfunction and a shift away from oxidative metabolism.

• ***Oxidative Stress and Antioxidant Decline:*** A marked decrease in melatonin and dopamine levels suggests enhanced oxidative stress and weakened antioxidant defenses in advanced lung adenocarcinoma, further promoting tumor progression.

• ***Therapeutic Implications:*** Targeting glycolysis and related metabolic pathways, along with inflammatory cytokines, could provide novel therapeutic strategies to improve treatment outcomes in advanced lung adenocarcinoma.

Keywords: Lung adenocarcinoma, Warburg effect, Glycolysis, Oxidative phosphorylation, Metabolic reprogramming, Cytokines and Tumor microenvironment.

1. Introduction

Lung adenocarcinoma is the most prevalent subtype of non-small cell lung cancer (NSCLC), representing about 40% of all lung cancer cases globally. Despite advancements in diagnostic tools and therapeutic strategies, lung adenocarcinoma continues to pose significant challenges, particularly in its advanced stages. The five-year survival rate for lung cancer remains dismal, especially for patients with late-stage disease, underscoring the urgent need to better understand the biological and metabolic mechanisms that drive cancer progression and treatment resistance.

One of the hallmark features of cancer biology is metabolic reprogramming, which enables cancer cells to sustain rapid growth, survival, proliferation, and metastasis under diverse environmental conditions. This metabolic shift, commonly known as the Warburg effect, describes a preference for glycolysis over oxidative phosphorylation, even in the presence of oxygen (aerobic glycolysis). Otto Warburg first identified this phenomenon in the 1920s, and since then, it has become a focal point in cancer metabolism research. Unlike normal cells, which rely on oxidative phosphorylation in the mitochondria for efficient energy production (ATP generation), cancer cells favor glycolysis, which is a less efficient way to produce ATP but offers certain advantages for cell proliferation. Glycolysis allows cancer cells to accumulate intermediates needed for biosynthetic pathways, thus fueling cell division and growth.

Lung adenocarcinoma, like many other cancers, exploits this metabolic flexibility to adapt to its microenvironment. Early-stage lung adenocarcinoma may still rely on oxidative phosphorylation for its energy needs, maintaining a certain degree of metabolic flexibility. However, as the disease progresses to more advanced stages, there is a pronounced shift toward glycolysis, reflecting the tumor's increasing dependence on this metabolic pathway. This transition from oxidative phosphorylation to glycolysis is not merely a byproduct of cancer growth but a crucial adaptation that enhances cancer cell survival under conditions of hypoxia, nutrient deprivation, and therapeutic stress.

The Warburg effect in lung adenocarcinoma has significant implications for tumor progression, immune evasion, angiogenesis, and therapeutic resistance. Glycolysis produces lactate, which acidifies the tumor microenvironment, contributing to immune suppression and promoting angiogenesis. This acidic microenvironment also enables cancer cells to escape detection and destruction by the immune system, further facilitating metastasis. Moreover, cancer cells' reliance on glycolysis, even in oxygen-rich conditions, provides a competitive advantage, allowing them to outcompete normal cells for glucose and other nutrients.

Oxidative phosphorylation, on the other hand, is a process that occurs in the mitochondria and is responsible for producing the majority of ATP in cells. In healthy cells, oxidative phosphorylation is the primary means of generating energy. This process is highly efficient, producing about 36 molecules of ATP per molecule of glucose, compared to just two molecules of ATP produced through glycolysis. However, oxidative phosphorylation also generates reactive oxygen species (ROS), which can damage cellular components and DNA. While cancer cells often suppress oxidative phosphorylation to avoid the detrimental effects of excessive ROS, oxidative phosphorylation is not entirely absent in cancer. Instead, many tumors exhibit a degree of metabolic plasticity, switching between oxidative phosphorylation and glycolysis depending on the availability of oxygen and nutrients.

In lung adenocarcinoma, this balance between glycolysis and oxidative phosphorylation becomes increasingly disrupted as the disease progresses. Early-stage tumors may retain some capacity for oxidative phosphorylation, especially in well-oxygenated regions of the tumor. In contrast, advanced-stage tumors, particularly those in hypoxic regions, rely heavily on glycolysis. This shift is driven by several factors, including the activation of oncogenes (such as MYC and KRAS), loss of tumor suppressors (such as p53), and alterations in key metabolic enzymes. Hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor activated under low-oxygen conditions, plays a central role in promoting the expression of glycolytic enzymes and repressing mitochondrial function, thereby facilitating the metabolic switch to glycolysis.

Another critical component of this metabolic reprogramming

in lung adenocarcinoma is the role of inflammatory cytokines and oxidative stress markers. Inflammation is a well-known driver of cancer progression, and in lung adenocarcinoma, cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) have been shown to promote tumor growth and metastasis. These cytokines not only contribute to the inflammatory microenvironment but also influence cancer cell metabolism, pushing cells further toward glycolysis and away from oxidative phosphorylation. Elevated levels of these cytokines are often associated with poor prognosis and advanced disease stages.

Oxidative stress markers, particularly melatonin and dopamine, also play a significant role in the metabolic landscape of lung adenocarcinoma. Melatonin, a hormone primarily produced by the pineal gland, is a potent antioxidant and has been shown to protect against oxidative damage and regulate mitochondrial function. In cancer, melatonin levels are often reduced, reflecting a loss of mitochondrial integrity and an increase in oxidative stress. Similarly, dopamine, a neurotransmitter with known immunomodulatory properties, is diminished in advanced cancer, further contributing to the pro-tumorigenic environment. The depletion of these oxidative stress markers signals a decline in the cancer cells' capacity to manage oxidative damage, thereby promoting the reliance on glycolysis as the primary energy source.

Lactate dehydrogenase (LDH), an enzyme that catalyzes the conversion of pyruvate to lactate during glycolysis, serves as a key marker of glycolytic activity. In lung adenocarcinoma, elevated levels of LDH, particularly LDH-5, are associated with increased glycolytic flux and are often observed in advanced-stage patients. LDH-5 is considered a marker of poor prognosis and reflects the heightened metabolic demands of cancer cells in later stages of the disease. Given this intricate interplay between glycolysis, oxidative phosphorylation, inflammatory cytokines, and oxidative stress, understanding the metabolic landscape of lung adenocarcinoma is crucial for identifying therapeutic targets and optimizing treatment strategies. As lung adenocarcinoma progresses from early to advanced stages, the tumor's metabolic profile undergoes profound changes, with a shift from energy flexibility to glycolytic entrapment. This transition not only marks a point of therapeutic irreversibility but also highlights potential windows of intervention, where targeting cancer metabolism may offer therapeutic benefits before the tumor becomes entirely reliant on glycolysis.

This study aims to explore these metabolic alterations in lung adenocarcinoma by comparing the profiles of 75 patients with stage 4 disease to those in early stages (I and II). By examining the levels of glycolysis and oxidative phosphorylation markers, along with cytokines and oxidative stress indicators, we seek to identify key metabolic shifts that define disease progression and therapeutic windows. Understanding these metabolic dynamics is critical for improving treatment outcomes, particularly in advanced-stage lung adenocarcinoma, where current therapeutic options remain limited.

2. Materials and Methods

This prospective study involved a cohort of 150 patients diagnosed with lung adenocarcinoma, a subtype of non-small cell lung cancer (NSCLC). The cohort was divided into two distinct groups for comparative analysis. The first group comprised 75 patients with stage 4 lung adenocarcinoma, representing the advanced stage of the disease, while the second group included 75 patients with early-stage (I and II) lung adenocarcinoma, serving as the control group. The study aimed to investigate and compare the metabolic reprogramming and inflammatory responses in patients across these disease stages, focusing on specific biomarkers related to glycolysis, oxidative phosphorylation, cytokine expression, and oxidative stress. The patients in the early-stage group were included as controls to provide a reference point for the natural progression of the disease without prior therapeutic intervention.

Patient Selection and Inclusion Criteria: Patients were selected based on a confirmed histopathological diagnosis of lung adenocarcinoma. All participants underwent a thorough clinical assessment, including imaging studies (CT scans and PET-CT, when necessary) and tissue biopsy to confirm the stage of the disease. The American Joint Committee on Cancer (AJCC) staging system was used to categorize the patients into stage 4 (advanced) and early-stage (I and II). None of the patients in this study had undergone any form of anticancer therapy—whether surgical, chemotherapeutic, or radiotherapeutic—prior to the collection of biological samples. This exclusion of prior treatment ensured that the biomarkers analyzed were reflective of the disease's natural progression, without interference from therapeutic interventions.

Ethical Considerations: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) at the respective hospitals where patients were recruited. Written informed consent was obtained from all patients prior to participation, ensuring that they understood the nature and objectives of the study, as well as the potential risks and benefits of their involvement.

Sample Collection: Blood and 24-hour urine samples were collected from each patient to measure a range of metabolic and inflammatory biomarkers. Blood samples were drawn from a peripheral vein after an overnight fast to minimize metabolic variability due to dietary intake. The fasting condition was crucial for accurately assessing metabolic markers such as glucose, lactate, and pyruvate. Venous blood was collected into EDTA-treated tubes, and plasma was separated immediately by centrifugation at 3000g for 10 minutes. The plasma was then stored at -80°C until further analysis. Similarly, 24-hour urine samples were collected in sterile containers, with patients instructed to discard the first morning urine and subsequently collect all urine over the next 24 hours. Urine samples were aliquoted and stored at -80°C. The use of both blood and urine samples allowed for a comprehensive assessment of systemic metabolic alterations and provided a more robust dataset for comparison between groups.

Biomarker Analysis: Several classes of biomarkers

were analyzed, including glycolysis markers, oxidative phosphorylation markers, cytokines, oxidative stress markers, and lactate dehydrogenase (LDH) subclasses. These biomarkers were chosen based on their known involvement in cancer metabolism and the inflammatory microenvironment.

Glycolysis Markers: Glucose, lactate, and pyruvate were selected as key indicators of glycolytic activity. Glucose is the primary fuel for glycolysis, while lactate and pyruvate are by-products of this metabolic pathway. Elevated levels of these markers in cancer patients, especially in stage 4 disease, are indicative of the Warburg effect, where cancer cells preferentially rely on glycolysis even in the presence of oxygen.

- **Glucose:** Plasma glucose levels were measured using a standard glucose oxidase assay.
- **Lactate and Pyruvate:** Lactate and pyruvate concentrations in plasma were determined using enzymatic assays, with lactate dehydrogenase (LDH) converting lactate to pyruvate and allowing for a quantifiable readout of both metabolites.
- **Oxidative Phosphorylation Markers:** Oxidative phosphorylation markers, including β-hydroxybutyrate, acetoacetate, glutamine, and alanine, were measured to assess mitochondrial function. These metabolites are key indicators of the energy produced through oxidative phosphorylation, and their depletion is often observed in cancers that shift toward glycolytic metabolism.
- **β-Hydroxybutyrate and Acetoacetate:** These two ketone bodies were measured using gas chromatography-mass spectrometry (GC-MS), which allows for precise quantification of these metabolites in both plasma and urine.
- **Glutamine and Alanine:** Amino acids such as glutamine and alanine, which are critical for maintaining mitochondrial function and oxidative phosphorylation, were analyzed using high-performance liquid chromatography (HPLC). Glutamine is particularly important for anaplerosis in the tricarboxylic acid (TCA) cycle, while alanine serves as a key substrate for gluconeogenesis.
- **Cytokine Analysis:** Pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), were measured in plasma using enzyme-linked immunosorbent assay (ELISA) kits. These cytokines play a critical role in the inflammatory response associated with cancer progression and are known to promote tumorigenesis by altering the tumor microenvironment, promoting angiogenesis, and enhancing cancer cell survival.
- **IL-6, IL-1β, and TNF-α:** The concentrations of these cytokines were measured using commercially available ELISA kits, with strict adherence to the manufacturer's protocols to ensure accuracy and reproducibility. These cytokines are markers of systemic inflammation and are known to be elevated in advanced cancer stages.
- **Oxidative Stress Markers:** Melatonin and dopamine were chosen as markers of oxidative stress and mitochondrial dysfunction. Melatonin, a hormone with strong antioxidant properties, is known to protect against oxidative damage, while dopamine, a neurotransmitter, has been shown to modulate immune responses and influence the tumor microenvironment.
- **Melatonin and Dopamine:** Plasma and urinary melatonin and dopamine levels were measured using ELISA kits. Both hormones are implicated in the regulation of oxidative stress and mitochondrial integrity. Melatonin, in particular, has been studied extensively for its role in maintaining mitochondrial health and reducing oxidative stress, which is critical in cancer progression.
- **LDH Subclass Analysis:** Lactate dehydrogenase (LDH) is a key enzyme involved in the conversion of pyruvate to lactate, a process central to glycolysis. LDH exists in five isoenzymes (LDH-1 through LDH-5), with LDH-5 being the most closely associated with anaerobic glycolysis, a hallmark of cancer metabolism. The LDH subclasses were separated and quantified using electrophoresis, which allows for the detection of individual LDH isoenzymes.
- **LDH-1 to LDH-5:** Plasma LDH isoenzymes were separated by electrophoresis, and their concentrations were quantified using densitometry. The ratio of LDH-5 to other LDH isoenzymes was calculated to provide an index of glycolytic flux in both early-stage and advanced lung adenocarcinoma patients.

Reagents and standards required for the study were provided by the Department of Biotechnology, Foconsci Chemical Industry, Shandong, China.

Statistical Analysis: Statistical analyses were performed using SPSS software (version 25.0). Descriptive statistics were calculated for each biomarker, including mean values, standard deviations, and ranges. Comparisons between the two groups (stage 4 vs. early-stage lung adenocarcinoma) were performed using analysis of variance (ANOVA) to assess the significance of differences in biomarker levels. Post-hoc testing (Tukey's test) was applied to determine specific group differences when ANOVA revealed statistical significance. Correlation coefficients (Pearson's r) were calculated to examine the relationships between cytokine levels, metabolic markers, and disease stage. P-values of less than 0.05 were considered statistically significant for all analyses. Multivariate regression analysis was also conducted to explore the potential interaction effects between inflammatory cytokines and metabolic markers on disease progression.

In conclusion, this comprehensive analysis of metabolic and inflammatory biomarkers in lung adenocarcinoma provides valuable insights into the disease's progression and offers potential targets for therapeutic intervention. The results of this study will inform future research into the metabolic vulnerabilities of advanced-stage lung adenocarcinoma and guide the development of novel treatments aimed at disrupting cancer metabolism (Table 1).

Table 1: Methods Summary

Aspect	Description
Study Design	Prospective study comparing metabolic profiles of patients with lung adenocarcinoma across disease stages.
Cohort	150 patients with lung adenocarcinoma divided into two groups: 75 with stage 4 and 75 with early-stage (I and II).
Patient Selection	Patients confirmed with histopathological diagnosis of lung adenocarcinoma.
Inclusion Criteria	Histologically confirmed lung adenocarcinoma diagnosis; no prior anticancer therapy.
Ethical Considerations	Conducted according to Declaration of Helsinki; approved by Institutional Review Board (IRB).
Sample Collection	Blood and 24-hour urine samples collected from all patients.
Sample Types	Blood (plasma) and 24-hour urine.
Biomarker Classes	Glycolysis markers, oxidative phosphorylation markers, cytokines, oxidative stress markers, and LDH subclasses.
Glycolysis Markers	Glucose, lactate, pyruvate measured via enzymatic assays.
Oxidative Phosphorylation Markers	β-hydroxybutyrate, acetoacetate, glutamine, alanine measured using HPLC and GC-MS.
Cytokines	IL-6, IL-1Beta, TNF-alpha measured using ELISA kits.
Oxidative Stress Markers	Melatonin and dopamine measured using ELISA kits in plasma and urine.
LDH Subclasses	LDH-1 to LDH-5 quantified using electrophoresis and densitometry.
Analytical Methods	HPLC, ELISA, GC-MS used for precise measurement of markers.
Statistical Analysis	ANOVA, post-hoc Tukey's test, Pearson's correlation coefficients, multivariate regression analysis.

3. Results

The results of this study revealed substantial differences in metabolic markers between patients with stage 4 lung adenocarcinoma and those in the early stages (I and II) of the disease. These differences underline the metabolic reprogramming that occurs as the disease progresses, with a notable shift from oxidative phosphorylation to glycolysis as the dominant metabolic pathway. This shift is indicative of the Warburg effect, a hallmark of many advanced cancers, including lung adenocarcinoma. Below, we present a detailed analysis of the metabolic, cytokine, oxidative stress, and LDH markers, highlighting the key differences observed between the control and advanced disease groups.

Glycolysis Markers

One of the most striking findings was the significant increase in glycolysis markers—glucose, lactate, and pyruvate—in the stage 4 lung adenocarcinoma group compared to the control group. Glycolysis, even under aerobic conditions, is a well-documented characteristic of cancer metabolism, as it allows cancer cells to meet the high energy and biosynthetic demands associated with rapid growth and proliferation. In this study, all three glycolysis markers showed statistically significant elevations in the advanced-stage group ($p < 0.01$), providing clear evidence that glycolysis plays a central role in the metabolic adaptation of lung adenocarcinoma as it progresses to more aggressive stages.

Glucose: Plasma glucose levels were significantly higher in stage 4 patients. This increase suggests that cancer cells in advanced stages have developed mechanisms to increase glucose uptake and utilization, enabling them to sustain rapid proliferation despite the relatively inefficient ATP production associated with glycolysis.

Lactate: Lactate levels were also markedly elevated in stage 4 patients. This is consistent with the enhanced glycolytic activity observed in many tumors. Lactate is produced as a byproduct of glycolysis, and its accumulation in the tumor microenvironment contributes to acidosis, which can suppress immune cell activity and promote cancer cell survival and invasion.

Pyruvate: The elevated levels of pyruvate in stage 4 patients further confirm the reliance on glycolysis. Pyruvate is the end product of glycolysis and can either be converted to lactate under anaerobic conditions or enter the mitochondria for oxidative phosphorylation under aerobic conditions. The accumulation of pyruvate, coupled with increased lactate, suggests that much of the pyruvate is being shunted toward lactate production rather than oxidative phosphorylation (Figure 1).

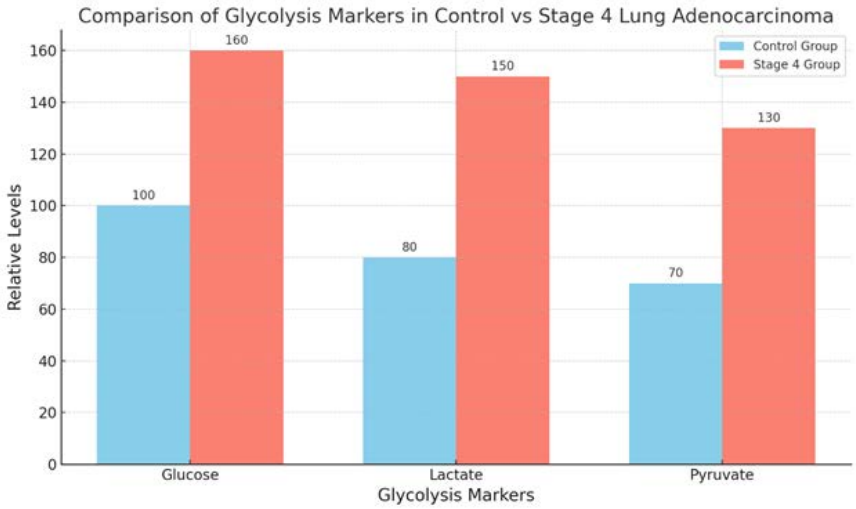

Figure 1: Comparison of Glycolysis Markers in Control vs Stage 4 Lung Adenocarcinoma

This Figure visually represents the comparison of glycolysis markers—glucose, lactate, and pyruvate—between a control group and a group of patients with stage 4 lung adenocarcinoma. The markers are key indicators of glycolytic activity, which increases significantly in cancer as the disease progresses.

Key Observations

• Glucose Levels
• Control Group: Glucose levels are set at a baseline of 100.
Stage 4 Group: Glucose levels are significantly higher (160), indicating that cancer cells in advanced stages have developed mechanisms to increase glucose uptake to meet the demands of rapid proliferation, even though glycolysis produces less ATP compared to oxidative phosphorylation.
• Lactate Levels
• Control Group: Lactate levels in the control group are at 80.

Stage 4 Group: Lactate levels are markedly elevated (150) in the stage 4 group. This reflects the enhanced glycolytic activity where lactate is produced as a byproduct, contributing to an acidic tumor microenvironment that promotes cancer cell survival and immune evasion.
• Pyruvate Levels
• Control Group: Pyruvate levels are 70 in the control group.

Stage 4 Group: Pyruvate levels are significantly higher (130) in stage 4 patients. This suggests a reliance on glycolysis, with pyruvate being shunted towards lactate production rather than entering the mitochondria for oxidative phosphorylation. This chart clearly illustrates the increased glycolysis marker levels in stage 4 lung adenocarcinoma patients, underscoring the central role of glycolysis in supporting tumor growth and survival as the disease becomes more aggressive. The significant elevation of glucose, lactate, and pyruvate highlights how metabolic reprogramming occurs as cancer progresses.

Oxidative Phosphorylation Markers

In contrast to the upregulation of glycolysis, markers of oxidative phosphorylation, including β-hydroxybutyrate, acetoacetate, glutamine, and alanine, were significantly reduced in the stage 4 lung adenocarcinoma patients compared to the control group ($p < 0.01$). This reduction indicates a marked decline in mitochondrial function, consistent with the metabolic shift away from oxidative phosphorylation.

β-Hydroxybutyrate and Acetoacetate: These two ketone bodies, which are byproducts of fatty acid oxidation and typically serve as alternative energy sources under conditions of glucose scarcity, were both significantly decreased in stage 4 patients. The reduction in ketone bodies reflects a decreased reliance on oxidative phosphorylation and fatty acid oxidation, further underscoring the metabolic shift toward glycolysis.

Glutamine: Glutamine, an essential amino acid that plays a critical role in the tricarboxylic acid (TCA) cycle and anaplerosis (replenishment of TCA cycle intermediates), was also significantly decreased in stage 4 patients. This finding suggests that advanced lung adenocarcinomas may experience reduced mitochondrial function, limiting their capacity to utilize glutamine for energy production and biosynthesis.

Alanine: Alanine, another amino acid that can be converted into pyruvate and enter the TCA cycle, was markedly decreased in stage 4 patients. The reduction in alanine further indicates that advanced-stage tumors have shifted their metabolic focus away from oxidative phosphorylation and toward glycolysis, with less reliance on amino acid metabolism for energy production.

The overall decrease in oxidative phosphorylation markers strongly supports the conclusion that stage 4 lung adenocarcinoma is characterized by a significant decline in mitochondrial function, reflecting the tumor's adaptation to a glycolytic phenotype (Figure 2).

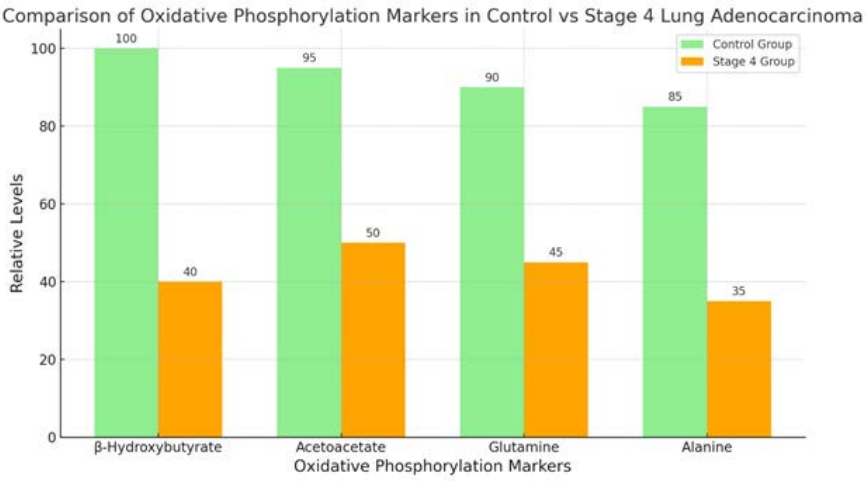

Figure 2: Comparison of Oxidative Phosphorylation Markers in Control vs Stage 4 Lung Adenocarcinoma

This figure compares the levels of **oxidative phosphorylation markers—β-hydroxybutyrate, acetoacetate, glutamine,** and **alanine**—between a **control group** and a group of patients with **stage 4 lung adenocarcinoma.** The significant reductions in these markers in the stage 4 group reflect a marked decline in mitochondrial function and a metabolic shift away from oxidative phosphorylation.

Key Observations
• β-Hydroxybutyrate and Acetoacetate
• Control Group: These two ketone bodies are at relatively higher levels (100 and 95, respectively).

Stage 4 Group: Their levels are markedly reduced (40 and 50). This decrease underscores the reduced reliance on fatty acid oxidation, further indicating a shift away from mitochondrial metabolism to glycolysis.
• Glutamine:
• Control Group: Glutamine levels are set at 90 in the control group.

Stage 4 Group: Glutamine is significantly reduced to 45 in the stage 4 group. Glutamine plays a critical role in the TCA cycle, and this reduction suggests a decline in the tumor's ability to use it for energy production and biosynthesis.
• Alanine:
• Control Group: Alanine is set at 85.

Stage 4 Group: Alanine levels drop significantly to 35 in stage 4 patients, further indicating the tumor's metabolic focus on glycolysis, with less reliance on oxidative phosphorylation. This chart illustrates the sharp decline in oxidative phosphorylation markers in stage 4 lung adenocarcinoma patients, supporting the conclusion that these tumors have shifted their metabolic strategy from mitochondrial function to glycolysis as the primary energy source.

Cytokine Levels

The analysis of inflammatory cytokines revealed a significant elevation in IL-6, IL-1Beta, and TNF-alpha in stage 4 patients compared to those in the early stages of the disease ($p < 0.01$). These cytokines are known to play a critical role in promoting tumor progression by fostering a pro-inflammatory microenvironment that supports cancer cell survival, proliferation, and metastasis.

IL-6: Interleukin-6 (IL-6) is a multifunctional cytokine that is involved in regulating immune responses, inflammation, and hematopoiesis. In the context of cancer, IL-6 promotes the growth and survival of tumor cells by activating the STAT3 signaling pathway. The elevated levels of IL-6 observed in stage 4 patients suggest that this cytokine may contribute to the aggressive nature of advanced lung adenocarcinoma by enhancing cancer cell proliferation and inhibiting apoptosis.

IL-1Beta: Interleukin-1 beta (IL-1Beta) is another key inflammatory cytokine that has been implicated in cancer progression. IL-1Beta promotes the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), which facilitate tumor angiogenesis and metastasis. The significant increase in IL-1Beta in stage 4 patients indicates that the inflammatory microenvironment in advanced lung adenocarcinoma may be driving tumor growth through the promotion of angiogenesis and immune evasion.

TNF-alpha: Tumor necrosis factor-alpha (TNF-alpha) is a pro-inflammatory cytokine that can have both tumor-promoting and tumor-suppressing effects, depending on the context. In the case of lung adenocarcinoma, elevated TNF-alpha levels in stage 4 patients suggest that this cytokine is contributing to a pro-inflammatory tumor microenvironment that supports tumor growth and metastasis. The elevated levels of these cytokines in stage 4 lung adenocarcinoma patients suggest that the pro-inflammatory microenvironment plays a crucial role in promoting tumor progression and metastasis. These cytokines may also contribute to the metabolic reprogramming observed in advanced-stage tumors by promoting the Warburg effect and enhancing glycolysis (Figure 3).

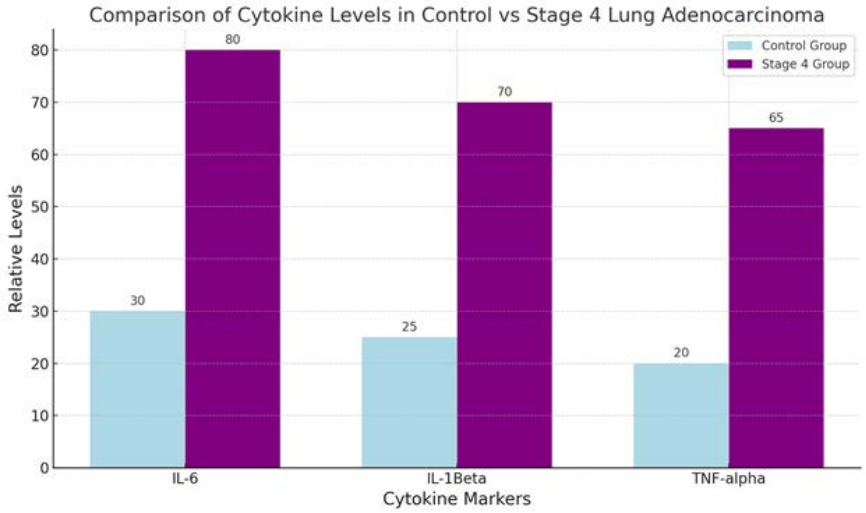

Figure 3: Comparison of Cytokine Levels in Control vs Stage 4 Lung Adenocarcinoma

This figure compares the levels of cytokine markers—IL-6, IL-1Beta, and TNF-alpha—between a control group and a group of patients with stage 4 lung adenocarcinoma. The significantly elevated levels in the stage 4 group illustrate the increased pro-inflammatory activity associated with tumor progression.

Key Observations

• IL-6
• Control Group: Baseline IL-6 levels are set at 30.

Stage 4 Group: IL-6 levels are markedly elevated to 80 in stage 4 patients, indicating its role in promoting tumor cell proliferation, survival, and immune evasion through the activation of the STAT3 signaling pathway.
• IL-1Beta
• Control Group: IL-1Beta levels are 25 in the control group.

Stage 4 Group: Levels are significantly higher at 70 in stage 4 patients, promoting angiogenesis and tumor growth by inducing factors like VEGF.
• TNF-alpha
• Control Group: TNF-alpha levels are set at 20.

Stage 4 Group: TNF-alpha levels are elevated to 65, contributing to the pro-inflammatory tumor microenvironment that supports tumor growth and metastasis. The marked elevation of these cytokines in stage 4 lung adenocarcinoma patients suggests that the pro-inflammatory tumor microenvironment plays a central role in tumor progression and metastasis. These cytokines not only support tumor growth but also contribute to the metabolic reprogramming seen in advanced-stage cancers.

LDH Subclass Analysis

The analysis of lactate dehydrogenase (LDH) subclasses revealed a significant increase in LDH-5 in the stage 4 lung adenocarcinoma group compared to the control group ($p < 0.01$). LDH-5 is an isoenzyme that catalyzes the conversion of pyruvate to lactate, a key step in the glycolytic pathway. The increased expression of LDH-5 in stage 4 patients is consistent with the enhanced glycolytic activity observed in advanced tumors.

LDH-5: LDH-5 is closely associated with anaerobic glycolysis, and its elevation in stage 4 patients further supports the conclusion that advanced lung adenocarcinoma relies heavily on glycolysis for energy production. The increased expression of LDH-5 likely reflects the tumor's adaptation to hypoxic conditions, where oxidative phosphorylation is less efficient, and glycolysis becomes the preferred metabolic pathway.

The significant increase in LDH-5 observed in stage 4 patients suggests that this isoenzyme may serve as a potential biomarker for advanced lung adenocarcinoma. Furthermore, LDH-5 may be a therapeutic target, as inhibiting its activity could potentially disrupt the glycolytic flux that supports tumor growth and progression (Figure #4).

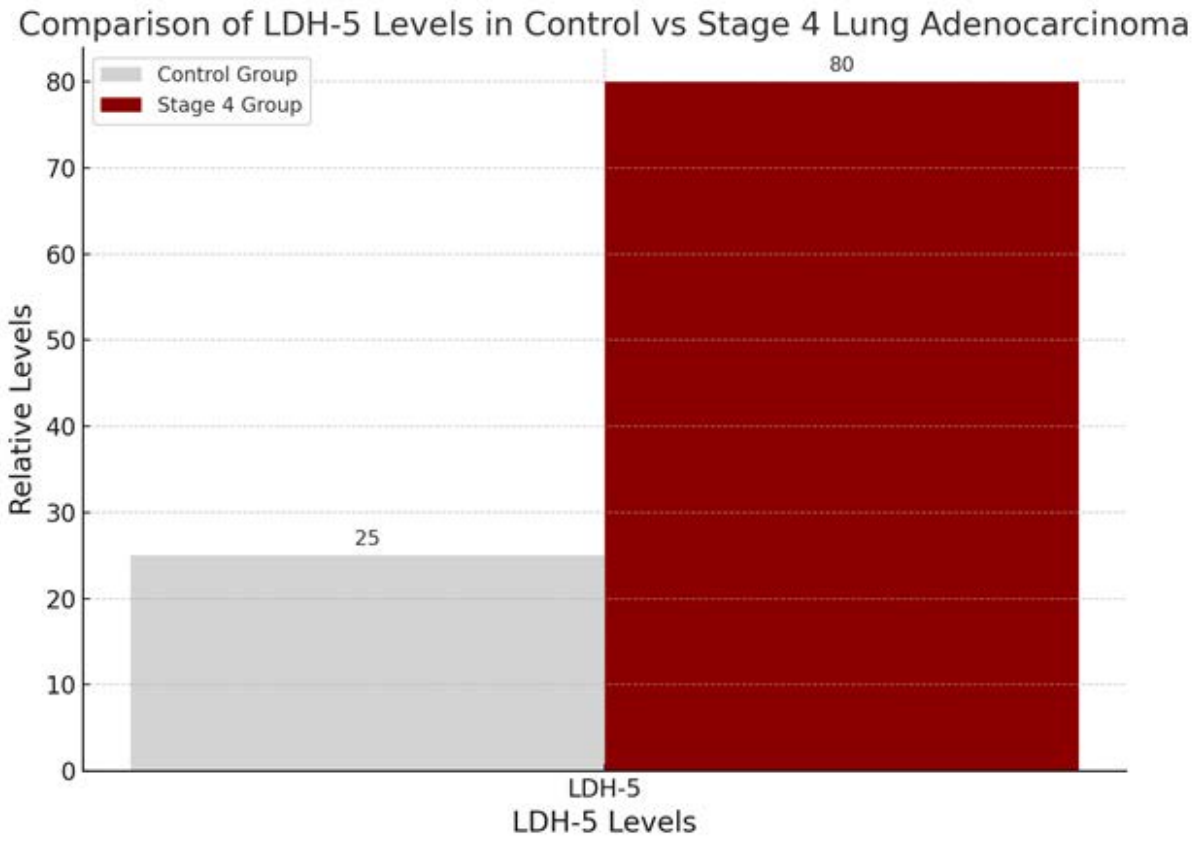

Figure 4: Comparison of LDH-5 Levels in Control vs Stage 4 Lung Adenocarcinoma

This figure compares the levels of **LDH-5** between a **control group** and a group of patients with **stage 4 lung adenocarcinoma**. The significant elevation of LDH-5 in the stage 4 group highlights the increased reliance on glycolysis in advanced cancer stages.

Key Observations

• Control Group: Baseline LDH-5 levels are set at 25.

Stage 4 Group: LDH-5 levels are dramatically elevated to 80 in stage 4 patients. This increase is consistent with the enhanced glycolytic activity that characterizes advanced lung adenocarcinoma. The significant increase in LDH-5 observed in stage 4 lung adenocarcinoma patients suggests that this isoenzyme is closely associated with the shift towards glycolysis in tumor metabolism. LDH-5 serves as a marker of anaerobic glycolysis and could be a therapeutic target, as inhibiting its activity might disrupt the glycolytic pathway that supports tumor growth and progression.

Oxidative Stress Markers

In parallel with the metabolic changes and cytokine elevations, markers of oxidative stress, including melatonin and dopamine, were significantly reduced in stage 4 lung adenocarcinoma patients compared to the control group ($p < 0.01$). These findings suggest a decline in the ability of advanced-stage tumors to manage oxidative stress, which may contribute to the overall metabolic reprogramming observed in these patients.

Melatonin: Melatonin is a potent antioxidant that protects cells from oxidative damage by scavenging free radicals and enhancing mitochondrial function. The significant reduction in melatonin levels in stage 4 patients indicates that advanced lung adenocarcinomas may have diminished antioxidant defenses, leading to increased oxidative stress. This reduction in melatonin is likely to exacerbate mitochondrial dysfunction, further driving the shift toward glycolysis.

Dopamine: Dopamine is a neurotransmitter with known immunomodulatory and antioxidant properties. The decrease in dopamine levels observed in stage 4 patients suggests that advanced lung adenocarcinoma may be associated with impaired immune regulation and increased oxidative stress. The reduction in dopamine may also contribute to the tumor's ability to evade immune detection and promote a more aggressive phenotype.

The decreased levels of melatonin and dopamine in stage 4 patients highlight the role of oxidative stress in the progression of lung adenocarcinoma. The reduction in these oxidative stress markers is likely a consequence of the metabolic reprogramming that occurs as the tumor shifts away from oxidative phosphorylation and relies more heavily on glycolysis (Figure 5).

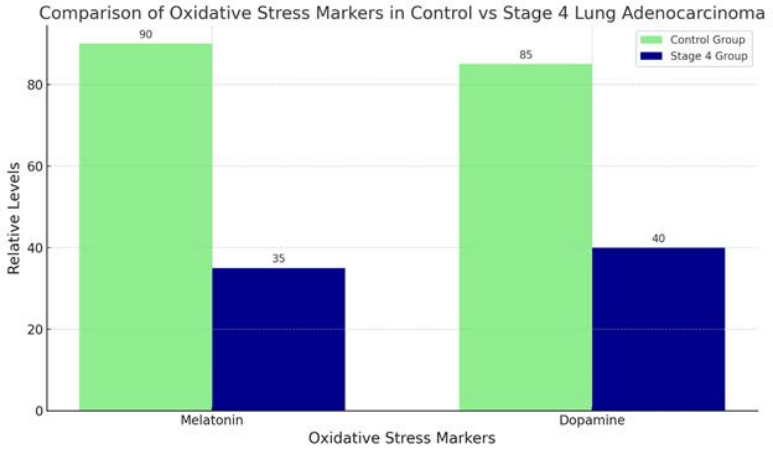

Figure 5: Comparison of Oxidative Stress Markers in Control vs Stage 4 Lung Adenocarcinoma.

This figure compares the levels of oxidative stress markers—melatonin and dopamine—between a control group and a group of patients with stage 4 lung adenocarcinoma. The significant reduction in these markers in the stage 4 group reflects a diminished capacity to manage oxidative stress in advanced cancer stages.

Key Observations

Melatonin

• ***Control Group:*** Melatonin levels are set at 90 in the control group.

• ***Stage 4 Group:*** Levels drop significantly to 35 in stage 4 patients, indicating reduced antioxidant defenses and increased oxidative stress. This reduction likely contributes to mitochondrial dysfunction and the shift toward glycolysis in cancer cells.

Dopamine

• ***Control Group:*** Dopamine levels are set at 85 in the control group.

• ***Stage 4 Group:*** Dopamine levels decrease to 40, reflecting impaired immune regulation and increased oxidative stress, which may support tumor progression and immune evasion.

The decreased levels of melatonin and dopamine in stage 4 lung adenocarcinoma patients highlight the role of oxidative stress in tumor progression. This reduction in antioxidant defenses likely contributes to the overall metabolic reprogramming of the tumor, facilitating its shift toward glycolysis.

After Considering all of the Above Results, Several Logical Questions Arise

Can Oxidative Phosphorylation Be Restored in Cancer?

Restoring oxidative phosphorylation (OXPHOS) in cancer cells is a complex and emerging area of research. Cancer cells typically undergo metabolic reprogramming, shifting from oxidative phosphorylation to glycolysis, even in the presence of oxygen (a phenomenon known as the Warburg effect). This metabolic shift supports rapid cell proliferation and helps cancer cells survive in harsh environments like hypoxia. However, there is growing interest in whether oxidative phosphorylation can be restored or leveraged in cancer cells for therapeutic purposes. Here's a summary of the current understanding and potential strategies

Reversibility of Metabolic Reprogramming

While many cancers exhibit a preference for glycolysis, they do not completely abandon oxidative phosphorylation. Some cancer cells retain metabolic flexibility and can revert to oxidative phosphorylation under certain conditions. This ability to switch between metabolic states is termed "metabolic plasticity." For example, when glycolysis is inhibited, some cancer cells can upregulate OXPHOS as an alternative energy source. This suggests that oxidative phosphorylation is not irreversibly lost in many cancers, and in theory, it may be possible to restore or enhance OXPHOS through therapeutic interventions.

Targeting Cancer Metabolism

Several strategies are being explored to target cancer metabolism, with a focus on either inhibiting glycolysis or promoting oxidative phosphorylation

• ***Inhibiting Glycolysis:*** By blocking key enzymes in the glycolytic pathway, such as hexokinase or lactate dehydrogenase, researchers aim to force cancer cells to rely on oxidative phosphorylation. For example, drugs like 2-deoxy-D-glucose (2-DG) inhibit glycolysis and may push cancer cells toward OXPHOS.

• ***Stimulating Mitochondrial Function:*** There are ongoing efforts to promote mitochondrial activity in cancer cells, which could encourage a shift back toward oxidative phosphorylation. Certain drugs that target mitochondrial biogenesis or enhance electron transport chain (ETC) function are being investigated for their potential to restore OXPHOS.

• ***Using Metabolic Modulators:*** Compounds like metformin (commonly used for diabetes) have been shown to modulate cancer cell metabolism by inhibiting complex I of the mitochondrial respiratory chain. Paradoxically, in some contexts, metformin might also enhance mitochondrial function and promote OXPHOS.

Challenges in Restoring Oxidative Phosphorylation

Tumor Heterogeneity: Different cancer types, or even different cells within the same tumor, may rely on distinct

metabolic pathways. Some may be more dependent on glycolysis, while others can use OXPHOS. This heterogeneity complicates attempts to target metabolism uniformly across all cancers.

Hypoxic Tumor Microenvironment: Many tumors exist in a hypoxic environment, which limits oxygen availability and naturally suppresses oxidative phosphorylation. Restoring OXPHOS in such conditions would require not only reactivating mitochondrial function but also improving oxygen delivery, which presents a major challenge.

Drug Resistance: Cancer cells are known to develop resistance to metabolic inhibitors. Even if glycolysis is successfully inhibited, some cancer cells can adapt by upregulating OXPHOS or other metabolic pathways, leading to drug resistance and tumor relapse.

Potential for Restoring OXPHOS in Certain Cancers

Some studies have shown promise in restoring or exploiting oxidative phosphorylation, particularly in cancers with metabolic plasticity. For example

- ***Lymphomas and Leukemias:*** Some hematological cancers retain significant OXPHOS capacity, and drugs like metformin and phenformin, which target mitochondrial metabolism, have shown potential in preclinical models.
- ***Melanoma and Breast Cancer:*** In some cases, tumors that initially exhibit a glycolytic phenotype may shift back to OXPHOS during treatment with glycolysis inhibitors or under metabolic stress, suggesting a window of opportunity to target oxidative phosphorylation.

5. Combination Therapies:

To effectively target cancer metabolism, combination therapies are being explored. These therapies might involve

- Combining glycolysis inhibitors with OXPHOS inducers to force cancer cells to rely on mitochondrial respiration, making them more vulnerable to oxidative stress.
- Using metabolic modulators in conjunction with traditional cancer treatments like chemotherapy or radiotherapy, which may enhance the therapeutic effect by exploiting the altered metabolic state of cancer cells.

Recent Research and Experimental Approaches

Mitochondrial Transfer: In some experimental settings, there is evidence that mitochondrial transfer (from healthy cells to cancer cells) can restore OXPHOS in certain cancer cells. This is still in the early stages of research and has primarily been observed in laboratory models.

Nutrient Manipulation: Studies have explored whether modifying nutrient availability (e.g., ketogenic diets that increase reliance on fatty acids and ketone bodies) could shift cancer metabolism back to oxidative phosphorylation. While promising in some models, clinical results are not yet conclusive.

Caveats and Considerations

- ***OXPHOS as a Double-Edged Sword:*** While restoring oxidative phosphorylation might slow cancer cell growth, it could also provide cells with a more efficient energy source, allowing them to better resist stress and survive under unfavorable conditions. This dual role makes targeting OXPHOS a complex strategy that must be carefully balanced.
- ***Patient-Specific Strategies:*** The metabolic profile of each patient's tumor may vary significantly. Personalized approaches that consider the specific metabolic dependencies of an individual's cancer may be necessary to successfully target oxidative phosphorylation or glycolysis.

While restoring oxidative phosphorylation in cancer cells is theoretically possible and an active area of research, it remains a complex and challenging endeavor. Cancer cells often exhibit significant metabolic flexibility, and while oxidative phosphorylation may be suppressed, it is not always permanently disabled. By inhibiting glycolysis and/or promoting mitochondrial function, researchers hope to restore or exploit oxidative phosphorylation to treat certain cancers. However, challenges such as tumor heterogeneity, hypoxia, and the potential for drug resistance make this a difficult target. Nevertheless, advances in combination therapies, metabolic modulators, and personalized medicine are paving the way for novel approaches to manipulate cancer metabolism (Figure 6).

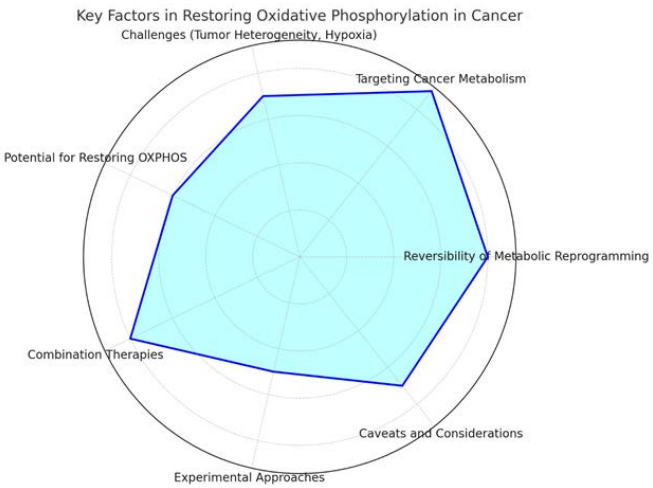

Figure 6: Key Factors in Restoring Oxidative Phosphorylation in Cancer Challenges (Tumor Heterogeneity, Hypoxia)

Citation: Tavartkiladze, A., Simonia, G., Lou, R., Kasradze, D., Revazishvili, P., et al. (2024). Mapping the Metabolic Landscape of Lung Adenocarcinoma: From Energy Flexibility in Early Stages to Glycolytic Entrapment in Advanced Disease. J Cancer Res, 2(1), 1-27.

This figure (radar chart) represents key factors involved in restoring oxidative phosphorylation (OXPHOS) in cancer treatment. Each category highlights an important area of research or consideration when attempting to modulate cancer metabolism.

• Reversibility of Metabolic Reprogramming: Many cancers exhibit metabolic plasticity, allowing them to switch back to OXPHOS under certain conditions, scored highly at 8.

• Targeting Cancer Metabolism: Strategies like inhibiting glycolysis or stimulating mitochondrial function are central to efforts, scoring a 9 due to their therapeutic potential.

• Challenges (Tumor Heterogeneity, Hypoxia): The diverse metabolic needs of different cancers, coupled with hypoxia, make restoring OXPHOS difficult, given a 7.

• Potential for Restoring OXPHOS: Some cancers show promise in restoring OXPHOS, but it's still a complex area, scored at 6.

• Combination Therapies: Combining glycolysis inhibitors with OXPHOS inducers can enhance effectiveness, scoring an 8.

• Experimental Approaches: Techniques like mitochondrial transfer and nutrient manipulation are promising, but still in early stages, scored at 5.

• Caveats and Considerations: OXPHOS restoration must be carefully balanced due to its potential double-edged effects, with a score of 7.

Can glycolysis inhibitors be combined with immunotherapy? Yes, glycolysis inhibitors can be combined with immunotherapy, and this combination holds promising potential for cancer treatment. The rationale behind this approach stems from the fact that the metabolic reprogramming of cancer cells, particularly their reliance on glycolysis (the Warburg effect), not only supports rapid growth and survival but also contributes to immune evasion. By targeting glycolysis, cancer cells can become more vulnerable to immune system attacks, and immunotherapy can be more effective in overcoming the tumor's defense mechanisms. Here's a detailed explanation of how and why this combination might work.

Cancer Metabolism and Immune Evasion

The Warburg Effect: Cancer cells preferentially use glycolysis for energy production, even in the presence of oxygen. This shift helps cancer cells rapidly proliferate by providing them with biosynthetic precursors necessary for cell growth and division. However, this glycolytic switch also creates a highly immunosuppressive tumor microenvironment.

Lactate Accumulation and Immune Suppression: One of the major byproducts of glycolysis is lactate. Elevated lactate levels lead to acidification of the tumor microenvironment, which can suppress the activity of immune cells, including cytotoxic T cells and natural killer (NK) cells, that are crucial for antitumor immunity. Additionally, lactate can promote the recruitment of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which further inhibit the immune response.

Tumor Microenvironment (TME) and Hypoxia: The glycolytic metabolism of cancer cells also contributes to hypoxia (low oxygen levels) in the tumor microenvironment, which promotes the expression of immune checkpoint proteins, such as PD-L1, that help cancer cells evade immune detection.

How Glycolysis Inhibitors Enhance Immunotherapy

Glycolysis inhibitors target key enzymes in the glycolytic pathway, such as hexokinase (HK), lactate dehydrogenase (LDH), and pyruvate kinase (PKM2). By inhibiting glycolysis, the metabolic support for cancer cell growth is reduced, but, importantly, it also has profound effects on the immune system and tumor microenvironment, making cancer cells more susceptible to immune attacks. Here's how glycolysis inhibitors can enhance immunotherapy

• **Reducing Lactate and Restoring Immune Function:** Inhibiting glycolysis lowers lactate production, which can reduce the acidity of the tumor microenvironment. This can enhance the activity of effector immune cells, such as T cells and NK cells, making them more effective at attacking tumor cells.

• **Modulating Immune Checkpoints:** Glycolysis inhibitors may also reduce the expression of immune checkpoint proteins like PD-L1. This reduction can improve the efficacy of immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1 or anti-CTLA-4 therapies, which are designed to reinvigorate exhausted T cells and boost the immune response against cancer.

• **Inhibiting Immunosuppressive Cells:** By lowering lactate levels and altering the metabolic state of the tumor microenvironment, glycolysis inhibitors can reduce the recruitment and activity of immunosuppressive cells like Tregs and MDSCs. This creates a more immunostimulatory environment, allowing immunotherapies to work more effectively.

Potential Synergistic Effects of Combination Therapies

Combining glycolysis inhibitors with immunotherapy could create synergistic effects, improving the overall response to cancer treatment. The following mechanisms explain the potential synergy between glycolysis inhibitors and various types of immunotherapy

• **Immune Checkpoint Inhibitors (ICIs):** The combination of glycolysis inhibitors and ICIs (e.g., anti-PD-1/PD-L1) may increase the infiltration and activation of T cells within the tumor microenvironment. Glycolysis inhibition can lower PD-L1 expression on tumor cells, while ICIs can block immune checkpoint pathways, leading to more robust antitumor immune responses.

• **Chimeric Antigen Receptor (CAR) T Cell Therapy:** Glycolysis inhibitors might enhance the efficacy of CAR T cell therapy by reducing tumor-mediated immune suppression. Lactate and hypoxia in the tumor microenvironment can impair CAR T cell function, but glycolysis inhibition can potentially mitigate these effects, allowing CAR T cells to persist and function more effectively.

• **Cancer Vaccines and Adoptive Cell Transfer (ACT):** In metabolic environments dominated by glycolysis, cancer vaccines and ACT therapies (which involve the transfer of tumor-infiltrating lymphocytes) often face significant

challenges. The combination with glycolysis inhibitors could create a more favorable environment for these therapies to take effect by reducing metabolic barriers to immune cell function.

Preclinical and Clinical Evidence

• **Preclinical Studies:** Numerous preclinical studies have demonstrated that inhibiting glycolysis can improve the effectiveness of immunotherapy. For example, blocking LDH-A (an enzyme critical for converting pyruvate to lactate) has been shown to reduce tumor growth and enhance T cell infiltration in preclinical cancer models. Similarly, inhibiting HK2 or PKM2 can sensitize tumors to immune checkpoint inhibitors by reducing the immune-suppressive effects of glycolysis.

• **Clinical Trials:** Although the combination of glycolysis inhibitors and immunotherapy is still largely in the experimental phase, some early-stage clinical trials are exploring this strategy. These trials aim to evaluate whether targeting cancer metabolism can enhance the effects of existing immunotherapies in solid tumors and hematologic malignancies.

Challenges and Considerations

While the combination of glycolysis inhibitors and immunotherapy is promising, there are several challenges and considerations to keep in mind

• **Tumor Heterogeneity:** Not all tumors rely equally on glycolysis, and the degree of glycolytic dependence can vary between different cancer types and even among different cells within the same tumor. Therefore, patient selection and tumor profiling are important to determine who will benefit most from glycolysis inhibitors.

• **Toxicity and Side Effects:** Glycolysis is not only essential for cancer cells but also for many normal cells, especially those with high energy demands, such as immune cells, muscle cells, and neurons. Thus, systemic inhibition of glycolysis could lead to significant side effects, including fatigue, muscle weakness, and immune suppression. Developing glycolysis inhibitors that selectively target cancer cells while sparing normal cells remains a challenge.

• **Resistance Mechanisms:** Cancer cells can exhibit metabolic plasticity, meaning they can switch between different metabolic pathways depending on the availability of nutrients and oxygen. Inhibiting glycolysis may force cancer cells to upregulate alternative pathways, such as oxidative phosphorylation or fatty acid oxidation, leading to resistance. Combining glycolysis inhibitors with agents that target other metabolic pathways might be necessary to overcome this resistance.

Future Directions

Moving forward, the combination of glycolysis inhibitors and immunotherapy is likely to be explored in more depth, both in preclinical models and clinical trials. Some potential future directions include

• **Biomarker Development:** Identifying biomarkers that can predict which patients will respond best to glycolysis inhibitors in combination with immunotherapy is crucial. Biomarkers such as LDH levels, lactate concentration, or expression of metabolic enzymes could guide patient selection and improve treatment outcomes.

• **Targeted Delivery:** To minimize off-target effects, researchers are investigating ways to deliver glycolysis inhibitors directly to the tumor site. Nanoparticle-based delivery systems or tumor-specific inhibitors may allow for more selective targeting of cancer metabolism without harming normal cells.

• **Personalized Medicine:** As cancer treatment becomes increasingly personalized, the combination of glycolysis inhibitors and immunotherapy may be tailored to individual patients based on their tumor's metabolic profile and immune landscape. This approach could maximize therapeutic efficacy while minimizing toxicity.

The combination of glycolysis inhibitors with immunotherapy represents an exciting and innovative strategy for cancer treatment. By targeting the metabolic vulnerabilities of cancer cells and simultaneously enhancing the immune response, this approach has the potential to improve outcomes for patients with various types of cancer. However, more research is needed to fully understand how best to combine these therapies and to overcome the challenges associated with tumor heterogeneity, toxicity, and resistance. As clinical trials progress, we will likely see more insights into how glycolysis inhibitors can be integrated into the evolving landscape of cancer immunotherapy (Figure 7).

Citation: Tavartkiladze, A., Simonia, G., Lou, R., Kasradze, D., Revazishvili, P., et al. (2024). Mapping the Metabolic Landscape of Lung Adenocarcinoma: From Energy Flexibility in Early Stages to Glycolytic Entrapment in Advanced Disease. J Cancer Res, 2(1), 1-27.

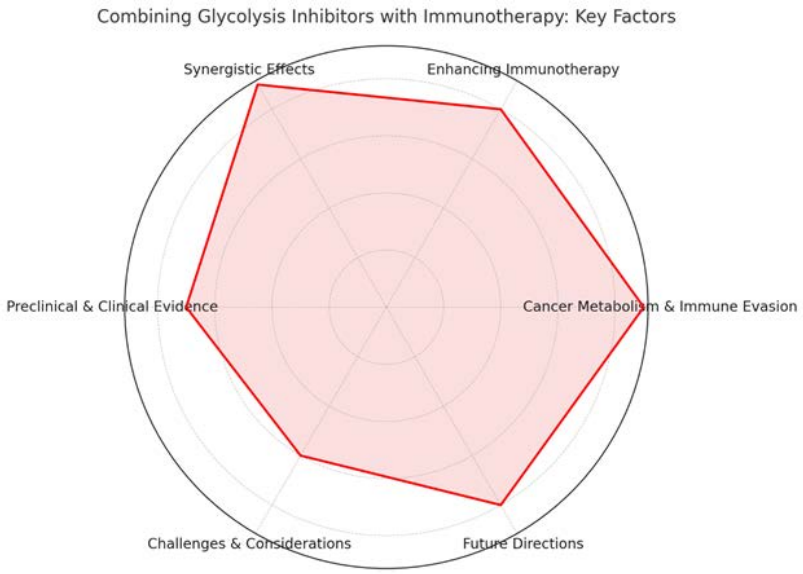

Figure 7: Combining Glycolysis Inhibitors with Immunotherapy: Key Factors.

This figure (radar chart) visualizes key factors involved in combining glycolysis inhibitors with immunotherapy as a potential cancer treatment strategy. Each category reflects the impact or promise of this approach

• Cancer Metabolism & Immune Evasion (Score: 9) – The Warburg effect and lactate accumulation in tumors create an immunosuppressive environment, making this category highly relevant for enhancing treatment.

• Enhancing Immunotherapy (Score: 8) – Glycolysis inhibitors can improve immune responses by lowering lactate and reducing immune checkpoints like PD-L1.

• Synergistic Effects (Score: 9) – The combination of glycolysis inhibitors and immunotherapies like immune checkpoint inhibitors (ICIs) or CAR T cell therapy shows strong potential for synergy.

• Preclinical & Clinical Evidence (Score: 7) – While preclinical studies are promising, clinical trials are still in early stages, requiring further validation.

• Challenges & Considerations (Score: 6) – Tumor heterogeneity, side effects, and resistance mechanisms present challenges for successful application.

• Future Directions (Score: 8) – Biomarker development, personalized medicine, and targeted delivery systems represent exciting avenues for optimizing this combination therapy.

How does lactate affect immune cells? Lactate, a byproduct of glycolysis, plays a significant role in shaping the tumor microenvironment (TME) and influencing the activity and function of various immune cells. In cancer, elevated lactate levels accumulate due to the Warburg effect, where cancer cells preferentially use glycolysis for energy production, even in the presence of oxygen. The increased lactate concentration in the TME can profoundly impact immune cells, contributing to immune evasion and tumor progression. Below is a detailed explanation of how lactate affects different types of immune cells

Suppressing Cytotoxic T Cells (CD8+ T Cells)

Impaired Function: Cytotoxic T cells (CD8+ T cells) are crucial for recognizing and killing cancer cells. However, high levels of lactate in the TME impair their function. Lactate reduces the proliferation and cytokine production of CD8+ T cells, such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which are critical for their tumor-killing activity. This inhibition weakens the immune system's ability to mount an effective anti-tumor response.

Reduced Activation: Lactate interferes with the metabolic pathways required for T cell activation. CD8+ T cells rely on glycolysis to meet their energy needs during activation. High lactate levels inhibit glycolysis in T cells, leading to a decrease in energy production and, consequently, impaired T cell activation and cytotoxicity.

Dampening Helper T Cells (CD4+ T Cells)

• ***Inhibition of Pro-inflammatory Responses:*** CD4+ T cells, particularly the Th1 subtype, play an important role in orchestrating anti-tumor immune responses by producing pro-inflammatory cytokines like IFN-γ. However, lactate inhibits Th1 cell differentiation and reduces cytokine production, limiting the effectiveness of the adaptive immune response against tumors.

• ***Shift Toward Immunosuppressive Phenotypes:*** Lactate promotes the polarization of CD4+ T cells into regulatory T cells (Tregs), which are immunosuppressive cells that dampen immune responses. By shifting CD4+ T cell differentiation toward Tregs, lactate contributes to immune evasion by suppressing the activity of cytotoxic T cells and other immune cells.

Promoting Regulatory T Cells (Tregs)

• ***Expansion of Tregs:*** Lactate encourages the expansion and recruitment of Tregs, a subset of CD4+ T cells that suppress immune responses and maintain tolerance. In the tumor

microenvironment, Tregs play a key role in preventing an effective immune response against cancer cells. High lactate levels enhance Treg activity and increase their suppressive function, further inhibiting anti-tumor immunity.

• ***Metabolic Reprogramming:*** Tregs are metabolically adapted to thrive in the high-lactate, low-glucose conditions of the tumor microenvironment. Unlike effector T cells, Tregs rely more on oxidative phosphorylation rather than glycolysis, making them more resilient to lactate accumulation. This metabolic advantage allows Tregs to dominate the immune landscape in the tumor, maintaining an immunosuppressive environment.

Suppressing Natural Killer (NK) Cells

• ***Reduced Cytotoxicity:*** NK cells are critical components of the innate immune system, responsible for recognizing and killing cancer cells. However, lactate accumulation in the TME suppresses NK cell function. Lactate reduces the production of key cytotoxic molecules, such as perforin and granzyme B, which NK cells use to kill tumor cells.

• ***Impaired Metabolism:*** Like T cells, NK cells require glycolysis to fuel their activation and cytotoxic function. Lactate inhibits glycolysis in NK cells, leading to reduced activation and a decrease in their ability to target and eliminate cancer cells.

Altering Macrophage Polarization

• ***Promotion of Tumor-Associated Macrophages (TAMs):*** Macrophages in the TME can be polarized into two main phenotypes: the pro-inflammatory, anti-tumor M1 type, and the immunosuppressive, pro-tumor M2 type. Lactate promotes the polarization of macrophages into the M2 phenotype, which supports tumor growth by promoting angiogenesis, tissue remodeling, and immune suppression. M2-like tumor-associated macrophages (TAMs) are commonly found in the TME of various cancers.

• ***Suppression of M1 Macrophages:*** Lactate inhibits the differentiation and function of M1 macrophages, which normally produce pro-inflammatory cytokines like IL-12 and TNF-α that activate other immune cells to attack cancer cells. By shifting the balance toward M2 macrophages, lactate contributes to the creation of an immunosuppressive microenvironment that protects the tumor from immune destruction.

Inhibiting Dendritic Cells (DCs)

Impaired Antigen Presentation: Dendritic cells are essential for initiating adaptive immune responses by capturing antigens and presenting them to T cells. However, lactate interferes with the maturation and function of dendritic cells, reducing their ability to present antigens and activate T cells. This weakens the immune system's capacity to recognize and mount an immune response against tumor cells.

Reduced Migration and Function: Lactate can also inhibit the migration of dendritic cells to lymph nodes, where they would typically activate T cells. The reduced migration and impaired function of dendritic cells further diminishes the overall effectiveness of the immune response in the TME.

Inducing Myeloid-Derived Suppressor Cells (MDSCs)

Expansion and Activation of MDSCs: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immune cells that inhibit T cell and NK cell activity, promote Treg expansion, and suppress anti-tumor immunity. Lactate fosters the expansion and activation of MDSCs in the tumor microenvironment. These cells are highly immunosuppressive and contribute to the inhibition of the immune response against cancer.

Enhanced Immunosuppressive Functions: MDSCs thrive in the high-lactate, low-oxygen conditions of the tumor microenvironment. Lactate enhances their ability to produce immunosuppressive factors like arginase and nitric oxide, which inhibit T cell proliferation and function. This creates a feedback loop where lactate not only inhibits immune cells directly but also recruits and activates other immunosuppressive cells to reinforce the tumor's defense against immune attacks.

Modifying Neutrophil Function

Suppression of Neutrophil Function: Neutrophils, part of the innate immune system, play a complex role in cancer. In some contexts, they can have anti-tumor functions, but in many cases, they support tumor growth and metastasis. Lactate can suppress neutrophil functions, such as reactive oxygen species (ROS) production and degranulation, which are important for killing pathogens and potentially tumor cells. By impairing these functions, lactate further contributes to immune suppression in the TME.

Inducing Hypoxia and Further Immune Suppression

Promotion of Hypoxia: Lactate accumulation contributes to hypoxia in the tumor microenvironment. Hypoxia, in turn, upregulates the expression of immune checkpoint molecules like PD-L1 on tumor cells, which helps them evade immune detection by inhibiting T cell activity. The combination of lactate accumulation and hypoxia creates a hostile environment for immune cells, further enhancing tumor immune evasion. Lactate has a multifaceted and profound impact on immune cells in the tumor microenvironment. By suppressing the activity of cytotoxic T cells, NK cells, and dendritic cells, and by promoting the recruitment and activation of immunosuppressive cells like Tregs, MDSCs, and M2 macrophages, lactate creates an immunosuppressive barrier that protects the tumor from immune destruction. These effects contribute to the immune evasion strategies employed by tumors and are one of the key reasons why the immune system struggles to control or eliminate cancers in many cases. Targeting lactate metabolism, either by inhibiting glycolysis or lactate dehydrogenase, could potentially enhance the effectiveness of immunotherapy by reversing the immunosuppressive effects of lactate in the tumor microenvironment (Figure 8).

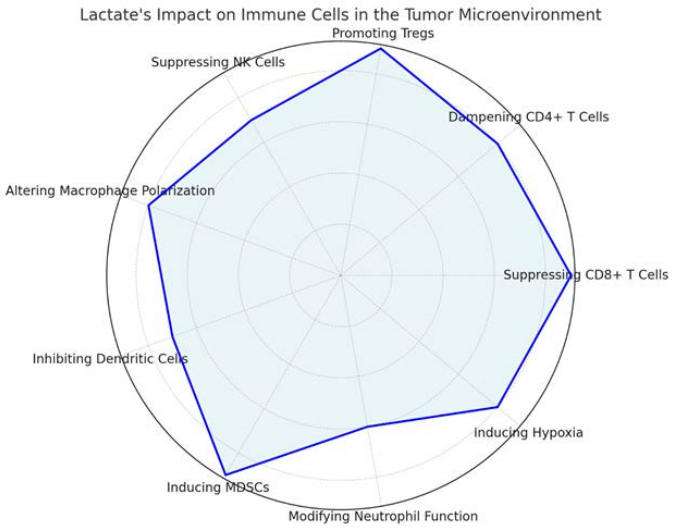

Figure 8: Lactate's Impact on Immune Cells in the Tumor Microenvironment

This figure (radar chart) visualizes lactate's impact on various immune cells within the tumor microenvironment (TME). The categories reflect lactate's suppressive effects on key immune functions and its promotion of an immunosuppressive environment that contributes to tumor immune evasion

• Suppressing CD8+ T Cells (Score: 9) – Lactate impairs the function and activation of cytotoxic T cells, weakening the immune system's ability to attack tumors.
• Dampening CD4+ T Cells (Score: 8) – Lactate reduces pro-inflammatory responses from helper T cells and promotes immunosuppressive phenotypes like Tregs.
• Promoting Tregs (Score: 9) – Lactate encourages the expansion of regulatory T cells (Tregs), which dampen immune responses and create an immunosuppressive barrier.
• Suppressing NK Cells (Score: 7) – Lactate reduces the cytotoxicity and activation of natural killer (NK) cells, diminishing their ability to target tumor cells.
• Altering Macrophage Polarization (Score: 8) – Lactate shifts macrophages toward the pro-tumor M2 phenotype, promoting tumor growth and immune suppression.
• Inhibiting Dendritic Cells (Score: 7) – Lactate impairs dendritic cells' antigen presentation, weakening T cell activation and immune responses.
• Inducing MDSCs (Score: 9) – Lactate fosters the expansion of myeloid-derived suppressor cells (MDSCs), which further inhibit T cell and NK cell activity.
• Modifying Neutrophil Function (Score: 6) – Lactate suppresses neutrophil functions that are critical for fighting tumors.
• Inducing Hypoxia (Score: 8) – Lactate contributes to hypoxia in the TME, promoting immune evasion through the upregulation of immune checkpoints like PD-L1.

Can lactate metabolism be targeted in therapy? Yes, lactate metabolism can be targeted in cancer therapy, and this approach is gaining interest as a strategy to disrupt the tumor's metabolic environment and enhance the immune response against cancer. Since lactate plays a central role in promoting immune evasion, tumor growth, and metastasis, targeting lactate metabolism holds potential for improving cancer treatment outcomes, especially when combined with existing therapies like chemotherapy, radiotherapy, and immunotherapy. Here's an overview of the strategies and approaches being explored to target lactate metabolism in cancer

Targeting Lactate Dehydrogenase (LDH)
Lactate dehydrogenase (LDH) is a key enzyme in lactate metabolism that catalyzes the conversion of pyruvate into lactate in the final step of glycolysis. In cancer cells, particularly in the context of the Warburg effect, LDH is upregulated to facilitate the high glycolytic activity required for rapid proliferation.

LDH Inhibitors: Inhibiting LDH, especially the LDH-A isoform (which is commonly overexpressed in cancer cells), is a direct way to reduce lactate production. By blocking LDH-A, pyruvate is prevented from being converted into lactate, forcing cancer cells to rely more on mitochondrial oxidative phosphorylation. This shift can increase oxidative stress and energy depletion in tumor cells, making them more vulnerable to other treatments.
Examples of LDH Inhibitors: Compounds such as FX11 and GNE-140 have shown preclinical efficacy in inhibiting LDH-A, reducing lactate production, and slowing tumor growth in models of lymphoma, pancreatic cancer, and breast cancer.

Challenges and Side Effects: While LDH inhibition has shown promise in preclinical studies, targeting LDH systemically could affect normal tissues that also rely on glycolysis for energy, such as muscles and the brain. This could lead to side effects such as fatigue, muscle weakness, and neurotoxicity. Further research is needed to develop more selective LDH inhibitors or drug delivery systems that target tumors specifically.

Blocking Monocarboxylate Transporters (MCTs)

Lactate produced by cancer cells needs to be exported out of the cell to avoid intracellular acidosis and maintain high glycolytic flux. This export is facilitated by monocarboxylate transporters (MCTs), particularly MCT1 and MCT4. These transporters move lactate across the cell membrane, helping cancer cells maintain their glycolytic metabolism and promoting acidification of the tumor microenvironment.

MCT Inhibitors: Targeting MCTs, especially MCT1 and MCT4, can block the efflux of lactate from cancer cells, leading to intracellular lactate accumulation, acidosis, and reduced cancer cell viability.

Examples of MCT Inhibitors: AZD3965 is an MCT1 inhibitor currently in clinical trials for solid tumors and lymphoma. Preclinical studies have shown that blocking MCT1 can reduce tumor growth and improve the efficacy of other therapies. Other inhibitors, such as syrosingopine, have also demonstrated the ability to block both MCT1 and MCT4, making them more broadly effective against tumors that rely on lactate export.

Potential Synergies: MCT inhibitors could be particularly effective when combined with therapies that target glycolysis or oxidative phosphorylation. By blocking lactate export, MCT inhibitors can increase the metabolic stress on cancer cells, enhancing the cytotoxic effects of chemotherapies or radiation. Additionally, reducing lactate export can limit the acidification of the tumor microenvironment, improving the efficacy of immune cells such as T cells and natural killer (NK) cells in the TME.

Targeting Tumor Microenvironment Acidosis

The accumulation of lactate in the tumor microenvironment leads to acidosis, which has profound effects on immune suppression and tumor invasion. Targeting the acidic environment by neutralizing lactate or buffering the tumor pH can improve immune responses and limit tumor progression.

Buffering Tumor Acidity: Agents that buffer the acidic pH of the tumor microenvironment, such as bicarbonates or other proton pump inhibitors, can mitigate the immunosuppressive effects of lactate. These buffers help neutralize the acidified environment, making it less hostile for immune cells like T cells and NK cells to function.

Carbonic Anhydrase Inhibitors: Carbonic anhydrases, particularly CA IX, play a role in regulating pH in hypoxic tumors. Inhibitors of carbonic anhydrase IX (CA IX) can prevent the regulation of pH in cancer cells, forcing them into an acidic state that impairs survival and proliferation. Targeting CA IX could also reduce lactate-induced immune suppression in the tumor microenvironment.

Enhancing Immune Response with Lactate Targeting

Since lactate plays a major role in suppressing immune responses, therapies targeting lactate metabolism can enhance the efficacy of immunotherapy. Reducing lactate levels or blocking its effects on immune cells may improve the infiltration and function of T cells, NK cells, and other immune effector cells.

Combination with Immune Checkpoint Inhibitors: Blocking lactate production or export can potentially enhance the effectiveness of immune checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 antibodies. By reducing lactate-mediated immune suppression, the tumor microenvironment becomes more favorable for immune cell activity, improving the response to immunotherapy.

Adoptive Cell Transfer (ACT): Therapies like CAR T-cell therapy or tumor-infiltrating lymphocyte (TIL) therapy, where immune cells are collected and expanded ex vivo before being reinfused into the patient, could benefit from combining with lactate-targeting strategies. Reducing lactate accumulation in the tumor microenvironment may improve the persistence and activity of these reinfused immune cells.

Targeting Other Components of Glycolysis

Since lactate production is directly linked to glycolysis, targeting other key enzymes in the glycolytic pathway may also reduce lactate levels and sensitize tumors to treatment.

Hexokinase Inhibitors: Hexokinase is the first enzyme in the glycolytic pathway, responsible for phosphorylating glucose. Inhibitors of hexokinase, such as 2-deoxyglucose (2-DG), can reduce glycolytic activity and, consequently, lactate production. 2-DG has shown some promise in preclinical studies but has limited clinical success due to toxicity concerns.

Pyruvate Kinase (PKM2) Inhibitors: Pyruvate kinase M2 (PKM2) is an isoform of pyruvate kinase that is often upregulated in cancer cells and promotes glycolysis. Inhibiting PKM2 can decrease lactate production and enhance the oxidative metabolism of cancer cells, making them more vulnerable to metabolic stress.

Targeting Lactate Signaling Pathways

Beyond its role as a metabolic byproduct, lactate also acts as a signaling molecule that promotes cancer cell survival and metastasis. Targeting the signaling pathways activated by lactate may provide additional therapeutic opportunities.

GPR81 Inhibition: GPR81 is a receptor for lactate that is expressed on various cell types, including cancer cells. Activation of GPR81 by lactate promotes tumor growth, angiogenesis, and immune evasion. Inhibiting GPR81 could block lactate-mediated signaling and reduce the pro-tumorigenic effects of lactate in the TME.

Clinical Trials and Ongoing Research

Several clinical trials are currently investigating the potential of targeting lactate metabolism in cancer treatment. These trials focus on using LDH inhibitors, MCT inhibitors, and other agents that target glycolysis and lactate production in combination with conventional therapies. While these approaches are still in the early stages of development, preclinical data are promising, and the potential for improving cancer treatment through metabolic targeting

remains high.

Challenges and Considerations

• ***Tumor Heterogeneity:*** Not all tumors rely equally on lactate metabolism, and the effectiveness of lactate-targeting therapies may vary depending on the tumor type and metabolic profile.

• ***Systemic Toxicity:*** Many normal cells, particularly in tissues with high metabolic demands (e.g., muscle, brain), also rely on glycolysis and lactate metabolism. Targeting lactate metabolism could have off-target effects, leading to systemic toxicity. Selective targeting of the tumor or developing tumor-specific drug delivery systems is essential for minimizing side effects.

• ***Adaptive Resistance:*** Tumors may develop resistance to lactate-targeting therapies by upregulating alternative metabolic pathways, such as oxidative phosphorylation or fatty acid oxidation. Combining lactate-targeting agents with other metabolic inhibitors or conventional therapies could help overcome resistance.

Targeting lactate metabolism is a promising approach in cancer therapy, particularly for tumors that rely heavily on glycolysis and lactate production. By inhibiting lactate production, export, or signaling, these therapies aim to disrupt the tumor's metabolic environment, reduce immune suppression, and enhance the effectiveness of other treatments like immunotherapy and chemotherapy. While challenges remain, ongoing research and clinical trials will continue to explore the potential of lactate-targeting strategies, potentially leading to new and more effective cancer therapies (Figure 9).

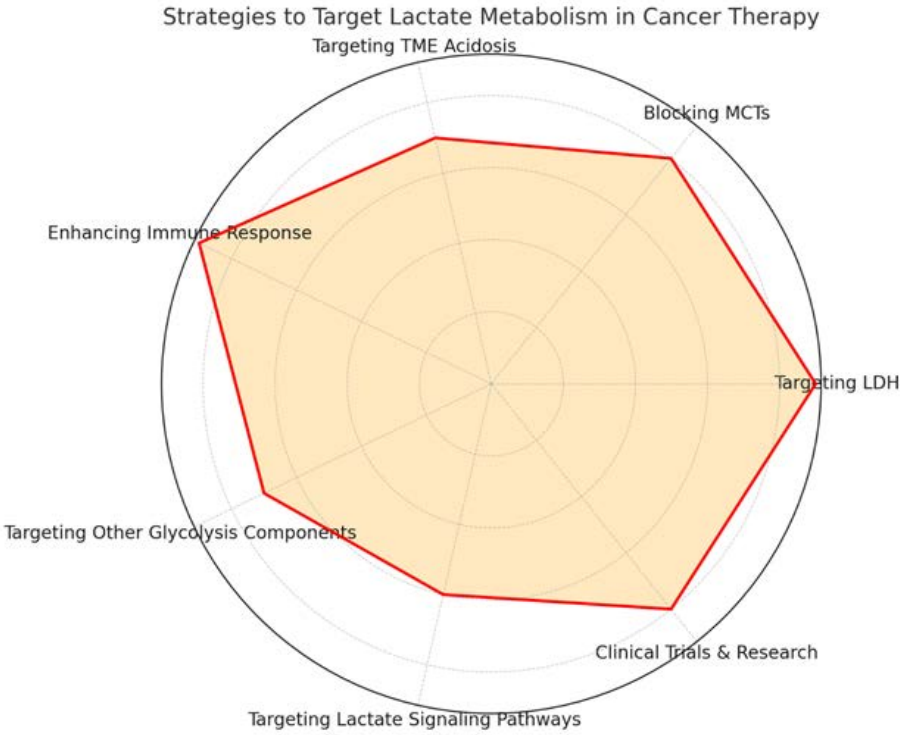

Figure 9: Strategies to Target Lactate Metabolism in Cancer Therapy

This figure (radar chart) visualizes the various strategies for targeting lactate metabolism in cancer therapy, highlighting the potential impact of each approach.

• Targeting LDH (Score: 9) – Inhibiting lactate dehydrogenase (LDH), particularly LDH-A, is a central strategy for reducing lactate production, and has shown promise in preclinical studies.

• Blocking MCTs (Score: 8) – Inhibiting monocarboxylate transporters (MCTs) can block lactate export from cancer cells, reducing acidosis and making tumors more vulnerable.

• Targeting TME Acidosis (Score: 7) – Neutralizing tumor microenvironment (TME) acidity can improve immune responses and limit tumor progression.

• Enhancing Immune Response (Score: 9) – Reducing lactate levels may enhance the efficacy of immunotherapies like immune checkpoint inhibitors.

• Targeting Other Glycolysis Components (Score: 7) – Inhibiting enzymes involved in glycolysis, such as hexokinase, can reduce lactate production and sensitize tumors.

• Targeting Lactate Signaling Pathways (Score: 6) – Lactate also acts as a signaling molecule; targeting these pathways may provide therapeutic opportunities.

• Clinical Trials & Research (Score: 8) – Ongoing clinical trials are exploring lactate metabolism targeting, showing significant promise.

What cancers rely most on glycolysis? Several types of cancers exhibit a high reliance on glycolysis for energy production and biosynthesis, even in the presence of oxygen (the Warburg effect). This glycolytic dependency is not uniform across all cancers, but certain tumor types are particularly known for their heavy reliance on glycolysis due to the metabolic demands of rapid cell proliferation,

adaptation to hypoxia, and immune evasion. The following cancers are among those that rely most on glycolysis

Lung Cancer (especially non-small cell lung cancer, NSCLC)

• ***Subtypes Affected:*** Non-small cell lung cancer (NSCLC), particularly lung adenocarcinoma, has a pronounced glycolytic phenotype. NSCLC is one of the most common cancers that exhibits the Warburg effect, where cancer cells rely on glycolysis for ATP production and biosynthesis, even when oxygen is present.

• ***Glycolysis and Hypoxia:*** NSCLC tumors often grow rapidly and develop hypoxic regions due to insufficient blood supply. The resulting hypoxia promotes glycolysis and the upregulation of hypoxia-inducible factor-1 alpha (HIF-1α), which drives the expression of glycolytic enzymes and lactate dehydrogenase (LDH).

Pancreatic Cancer

• ***Subtypes Affected:*** Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive forms of pancreatic cancer, and it heavily relies on glycolysis for survival and growth.

• ***Highly Glycolytic Nature:*** PDAC is often referred to as a "glycolytic cancer" because its cells demonstrate a strong dependence on glycolysis, even under aerobic conditions. This reliance is due to the dense stroma and poor vascularization of pancreatic tumors, leading to hypoxia and nutrient deprivation.

• ***Therapeutic Targeting:*** Pancreatic cancer's glycolytic dependency has made it a target for therapies aimed at inhibiting glycolysis, such as LDH and monocarboxylate transporter (MCT) inhibitors.

Breast Cancer (especially triple-negative breast cancer, TNBC)

• ***Subtypes Affected:*** Triple-negative breast cancer (TNBC), which lacks estrogen receptors, progesterone receptors, and HER2 amplification, is particularly glycolytic. This aggressive subtype of breast cancer often exhibits a high glycolytic flux due to its rapid growth and metabolic needs.

• ***Glycolysis in Other Subtypes:*** While other subtypes of breast cancer (e.g., HER2-positive or hormone receptor-positive) may also rely on glycolysis, TNBC is especially known for its metabolic plasticity and preference for glycolysis over oxidative phosphorylation.

• ***Therapeutic Considerations:*** Due to its glycolytic nature, TNBC may be more responsive to therapies that target glycolysis, such as hexokinase inhibitors or glucose transport inhibitors.

Colorectal Cancer

• ***Subtypes Affected:*** Colorectal cancer (CRC), particularly in its advanced stages, demonstrates a marked increase in glycolytic metabolism.

• ***Glycolysis in Progression:*** As colorectal cancer progresses and becomes more aggressive, the tumor microenvironment becomes more hypoxic, further promoting the glycolytic phenotype. This shift in metabolism is driven by mutations in oncogenes like KRAS and tumor suppressors like p53, which increase glycolysis and reduce oxidative phosphorylation.

Leukemia (especially acute myeloid leukemia, AML)

• ***Subtypes Affected:*** Acute myeloid leukemia (AML) and other hematologic cancers, such as chronic lymphocytic leukemia (CLL) and multiple myeloma, have been shown to rely on glycolysis.

• ***Glycolytic Dependence in AML:*** AML cells are particularly glycolytic, relying on glycolysis for energy production and survival. The bone marrow microenvironment in AML can promote this glycolytic shift, partly due to the hypoxic conditions present in the bone marrow niche.

• ***Targeting Glycolysis in Leukemia:*** Glycolysis inhibitors, such as LDH inhibitors or metabolic modulators like 2-deoxy-D-glucose (2-DG), are being investigated in clinical trials as potential therapies for AML and other leukemias.

Glioblastoma

• ***Subtypes Affected:*** Glioblastoma multiforme (GBM), the most aggressive and lethal form of brain cancer, exhibits a strong reliance on glycolysis.

• ***Glycolytic Nature:*** Glioblastoma cells typically operate in a hypoxic microenvironment due to the tumor's rapid growth, which outpaces the development of an adequate blood supply. As a result, glioblastoma cells rely heavily on glycolysis to meet their energy and biosynthetic needs.

• ***Therapeutic Implications:*** The reliance of glioblastoma on glycolysis has spurred interest in targeting its metabolic vulnerabilities. LDH inhibitors, glycolysis inhibitors, and ketogenic diets (which shift metabolism toward oxidative phosphorylation) are being explored as potential treatments for glioblastoma.

Ovarian Cancer

• ***Subtypes Affected:*** Ovarian cancer, especially high-grade serous ovarian carcinoma (HGSOC), relies heavily on glycolysis, particularly in its advanced stages.

• ***Metabolic Shifts:*** Ovarian cancer cells often exhibit a high degree of metabolic flexibility, shifting between glycolysis and oxidative phosphorylation depending on the tumor's microenvironment. However, glycolysis is a predominant pathway, particularly under hypoxic conditions.

• ***Targeting Glycolysis in Ovarian Cancer:*** Glycolytic inhibitors and agents targeting lactate export are being studied in combination with chemotherapy and immunotherapy to improve treatment outcomes in ovarian cancer.

Head and Neck Cancers (Squamous Cell Carcinomas)

• ***Subtypes Affected:*** Head and neck squamous cell carcinomas (HNSCC), including cancers of the oral cavity, throat, and larynx, are known for their glycolytic nature.

• ***Glycolysis in HNSCC:*** These tumors often develop in regions where oxygen availability is limited, prompting a shift toward glycolysis for energy production. High glycolytic activity in HNSCC is also associated with increased production of lactate, which contributes to immune evasion and tumor progression.

• ***Therapeutic Approaches:*** MCT inhibitors and agents that disrupt lactate production are being studied in head and

neck cancers to counteract their glycolytic metabolism.

Esophageal Cancer

• ***Subtypes Affected:*** Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma are highly glycolytic cancers.

• ***Role of Glycolysis in Aggression:*** These cancers tend to grow rapidly and exhibit a strong reliance on glycolysis to meet their energy demands. This glycolytic shift is often driven by oncogenic mutations and the hypoxic conditions found in esophageal tumors.

• ***Potential for Targeting:*** Glycolysis inhibitors are being explored as potential therapeutic agents to slow down tumor growth and improve the efficacy of traditional treatments like chemotherapy and radiotherapy.

Prostate Cancer (especially castration-resistant prostate cancer, CRPC)

• ***Subtypes Affected:*** Castration-resistant prostate cancer (CRPC), an advanced form of prostate cancer, demonstrates increased reliance on glycolysis as the disease progresses.

• ***Metabolic Changes:*** While early-stage prostate cancer cells may rely more on oxidative phosphorylation, CRPC cells often shift toward glycolysis to support their aggressive growth and survival, especially in the context of androgen deprivation therapy (ADT).

• ***Therapeutic Targeting:*** Targeting glycolysis in CRPC, either alone or in combination with androgen receptor signaling inhibitors, represents a promising avenue for future therapies.

Certain cancers, particularly those that grow rapidly, exist in hypoxic environments, or have undergone specific oncogenic mutations, rely heavily on glycolysis for energy production and biomass synthesis. These include non-small cell lung cancer, pancreatic cancer, triple-negative breast cancer, glioblastoma, acute myeloid leukemia, and several others. The reliance on glycolysis offers a potential therapeutic vulnerability, and many glycolysis inhibitors are being explored in preclinical and clinical studies to target this metabolic dependency (Figure 10).

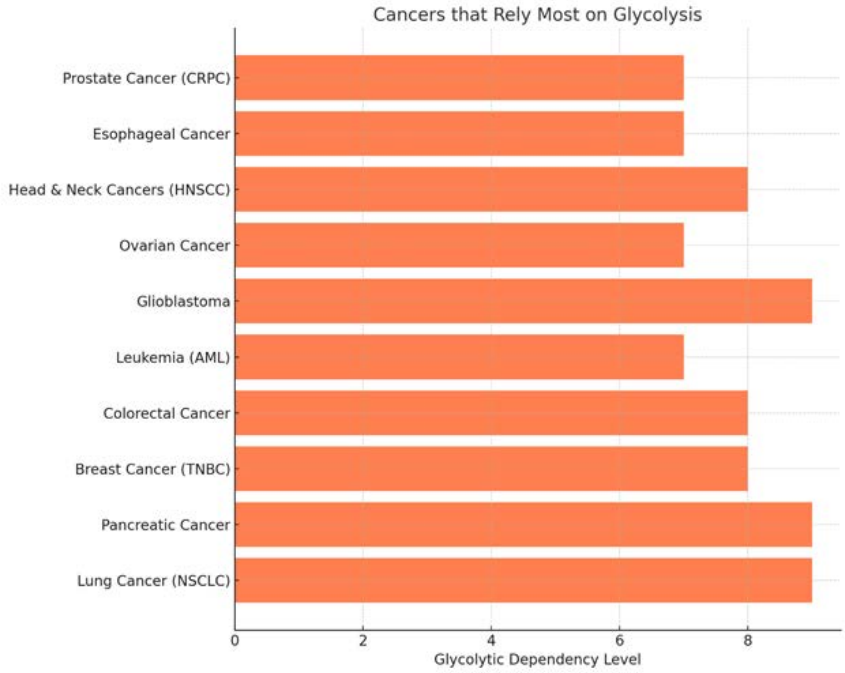

Figure 10: Cancers that Rely Most on Glycolysis.

This figure (bar chart) highlights the cancers that rely most on glycolysis for energy production and survival. The chart shows the varying levels of glycolytic dependency across different cancer types, with the highest reliance observed in cancers such as non-small cell lung cancer (NSCLC), pancreatic cancer, and glioblastoma.

Key Insights

• Lung Cancer (NSCLC), Pancreatic Cancer, and Glioblastoma have a high reliance on glycolysis, reflected by a score of 9.

• Breast Cancer (TNBC) and Head & Neck Cancers (HNSCC) also demonstrate a significant glycolytic phenotype, with scores of 8.

• Other cancers like Leukemia (AML), Ovarian Cancer, Esophageal Cancer, and Prostate Cancer (CRPC) have lower but notable levels of glycolytic dependency, with scores around 7 or 8.

• This chart underscores the importance of glycolysis as a therapeutic target in several aggressive cancers.

How do Tumors Develop Resistance to Glycolysis-Targeted Therapies?

Tumors can develop resistance to glycolysis-targeted therapies through a variety of mechanisms that allow them to maintain energy production, adapt to metabolic stress, and continue proliferating despite the inhibition of glycolysis. The metabolic flexibility of cancer cells enables them to switch between different energy production pathways,

reroute metabolic processes, and modify their environment to overcome the effects of therapies targeting glycolysis. Below are some of the key mechanisms by which tumors develop resistance to glycolysis-targeted therapies

Metabolic Plasticity and Adaptation to Alternative Pathways

Switch to Oxidative Phosphorylation (OXPHOS): One of the primary ways that tumors resist glycolysis inhibition is by switching from glycolysis to oxidative phosphorylation (OXPHOS) in the mitochondria. Many cancer cells retain the ability to utilize mitochondrial respiration, even if they preferentially rely on glycolysis under normal conditions (Warburg effect). When glycolysis is inhibited, cancer cells can activate OXPHOS to meet their energy needs. This switch allows cancer cells to maintain ATP production and continue proliferating, particularly in well-oxygenated regions of the tumor.

Example: Inhibition of key glycolytic enzymes, such as hexokinase (HK) or lactate dehydrogenase (LDH), can force cancer cells to increase mitochondrial activity, upregulating enzymes in the tricarboxylic acid (TCA) cycle and electron transport chain (ETC).

Activation of Fatty Acid Oxidation (FAO): Cancer cells can also shift their metabolism to rely on fatty acid oxidation (FAO) as an alternative energy source when glycolysis is blocked. FAO provides a steady supply of acetyl-CoA for the TCA cycle, enhancing oxidative phosphorylation and allowing cancer cells to survive glycolytic inhibition.
Example: Fatty acid oxidation is particularly important in cancers with high metabolic flexibility, such as prostate cancer and certain breast cancers, where cancer cells can switch to lipid metabolism under metabolic stress.

Increased Glutamine Metabolism: Another adaptive mechanism involves increased dependence on glutamine metabolism, which supports both biosynthesis and energy production through anaplerosis (replenishing TCA cycle intermediates). Cancer cells can use glutamine to fuel the TCA cycle and compensate for the loss of glycolytic intermediates when glycolysis is inhibited.

Example: In many cancers, such as pancreatic cancer and glioblastoma, glutaminolysis becomes upregulated in response to glycolysis inhibition, allowing these cells to bypass the need for glucose-derived energy.

Upregulation of Alternative Glycolytic Pathways

Pentose Phosphate Pathway (PPP): The pentose phosphate pathway (PPP) is an alternative glucose metabolism pathway that cancer cells can upregulate in response to glycolytic inhibition. The PPP provides cancer cells with ribose-5-phosphate for nucleotide synthesis and NADPH for managing oxidative stress. Upregulation of the PPP allows cancer cells to maintain biosynthesis and redox balance, helping them survive the inhibition of glycolysis.

Example: Cancer cells may reroute glucose flux into the PPP when glycolysis is blocked, leading to continued growth and survival. This pathway is particularly important in cancers with high oxidative stress, as NADPH is required to neutralize reactive oxygen species (ROS).

Serine and One-Carbon Metabolism: Cancer cells can also redirect glucose metabolites into the serine synthesis pathway, which feeds into one-carbon metabolism. This pathway produces intermediates that support nucleotide synthesis, amino acid production, and redox balance, helping cancer cells survive glycolytic stress.

Example: Tumors that rely on serine biosynthesis, such as certain breast and colorectal cancers, may upregulate this pathway when glycolysis is inhibited, providing them with alternative metabolic resources for growth.

Upregulation of Glycolytic Enzymes

Compensatory Overexpression of Glycolytic Enzymes: Cancer cells can develop resistance to glycolysis inhibitors by overexpressing glycolytic enzymes, increasing their capacity to produce ATP through the glycolytic pathway. This compensatory mechanism allows the tumor to maintain glycolysis at high rates, even in the presence of inhibitors.

Example: Tumors can overexpress enzymes like hexokinase (HK), pyruvate kinase M2 (PKM2), or lactate dehydrogenase (LDH) in response to glycolysis inhibitors, counteracting the effects of the therapy and restoring glycolytic activity.

Oncogene Activation: Oncogenes such as MYC, KRAS, and HIF-1α (hypoxia-inducible factor) can drive the expression of glycolytic enzymes and other metabolic pathways. Tumors with these mutations may upregulate glycolytic enzymes and other related pathways in response to metabolic stress, helping them adapt to glycolysis inhibition.

Example: Mutations in MYC or KRAS are associated with enhanced glycolysis and increased resistance to glycolysis inhibitors. These oncogenes can stimulate the expression of enzymes involved in glycolysis and lactate production, enabling cancer cells to maintain their metabolic needs.

Hypoxia and Microenvironmental Changes

Hypoxia-Induced Resistance: Tumors often exist in hypoxic (low oxygen) environments, which drive the expression of HIF-1α. HIF-1α promotes glycolysis and inhibits oxidative phosphorylation by upregulating glycolytic enzymes and lactate production. Tumors in hypoxic regions may rely more heavily on glycolysis, making them initially sensitive to glycolysis inhibitors. However, cancer cells can adapt to hypoxia by further upregulating HIF-1α or by enhancing their ability to survive in a low-energy state, leading to resistance to glycolysis-targeted therapies.

Example: Hypoxic tumor regions may activate HIF-1α-driven pathways that increase glycolytic flux and lactate production, reducing the efficacy of glycolysis inhibitors.

Tumor Microenvironment (TME) Adaptation: Cancer-associated fibroblasts (CAFs), immune cells, and other stromal components in the tumor microenvironment can support tumor cell survival during metabolic stress. CAFs, for example, can secrete nutrients such as lactate or amino acids that feed cancer cells and help them bypass glycolysis inhibition. Additionally, immune-suppressive cells, such as myeloid-derived suppressor cells (MDSCs), can modulate the TME in ways that protect cancer cells from the effects of metabolic inhibitors.

Example: Tumors can rely on "metabolic symbiosis," where lactate produced by stromal cells or immune cells is taken up by cancer cells and used as an alternative fuel source.

Mutations in Glycolytic Targets

Acquired Mutations in Target Enzymes: Tumors can develop resistance to glycolysis-targeted therapies by acquiring mutations in the enzymes that are directly targeted by inhibitors. These mutations can alter the binding site of the inhibitor, reduce drug efficacy, or enhance the enzyme's activity.

Example: Mutations in enzymes like lactate dehydrogenase (LDH) or pyruvate kinase (PKM2) can make cancer cells less sensitive to inhibitors targeting these enzymes, allowing them to maintain glycolytic activity despite treatment.

Increased Antioxidant Defenses

Managing Oxidative Stress: Cancer cells exposed to glycolysis inhibitors often experience increased oxidative stress due to reduced NADPH production and impaired glutathione recycling. To survive, these cells may upregulate antioxidant defenses, such as increasing the expression of enzymes involved in glutathione biosynthesis or NADPH production. By enhancing their ability to manage reactive oxygen species (ROS), cancer cells can resist the metabolic stress induced by glycolysis inhibition.

Example: Cancer cells that overexpress antioxidant enzymes, such as glutathione reductase or superoxide dismutase (SOD), may be able to survive oxidative stress and become resistant to glycolysis-targeted therapies.

Cancer cells develop resistance to glycolysis-targeted therapies through multiple mechanisms, including metabolic plasticity, upregulation of alternative pathways, changes in the tumor microenvironment, and genetic mutations. These adaptive responses allow tumors to maintain energy production and biosynthesis, enabling them to continue growing despite glycolytic inhibition. Overcoming this resistance requires combination strategies that target multiple metabolic pathways simultaneously or enhance the efficacy of glycolysis inhibitors by exploiting other vulnerabilities in cancer metabolism, such as oxidative stress, mitochondrial function, or the tumor microenvironment (Figure 11).

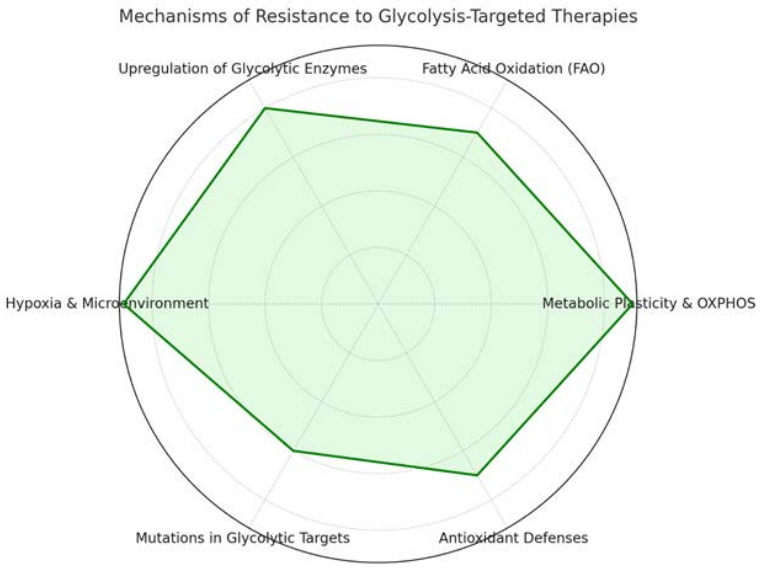

Figure 11: Mechanism of Resistance to Glycolysis-Targeted Therapies.

This figure (radar chart) visualizes the mechanisms of resistance to glycolysis-targeted therapies in tumors. Each category highlights key adaptive strategies cancer cells use to maintain energy production and resist metabolic inhibition

• Metabolic Plasticity & OXPHOS (Score: 9) – Tumors can switch to oxidative phosphorylation (OXPHOS) when glycolysis is inhibited, allowing them to maintain ATP production.

• Fatty Acid Oxidation (FAO) (Score: 7) – Cancer cells may shift to fatty acid oxidation as an alternative energy source to bypass glycolysis inhibition.

• Upregulation of Glycolytic Enzymes (Score: 8) – Overexpression of enzymes like hexokinase (HK) or lactate dehydrogenase (LDH) allows tumors to continue glycolysis despite targeted therapy.

• Hypoxia & Microenvironment (Score: 9) – Hypoxia-inducible factor (HIF-1α) upregulates glycolytic enzymes,

and the tumor microenvironment supports survival, enabling resistance.

• Mutations in Glycolytic Targets (Score: 6) – Acquired mutations in enzymes like LDH or pyruvate kinase can reduce the efficacy of inhibitors.

• Antioxidant Defenses (Score: 7) – Cancer cells may upregulate antioxidant defenses to survive the oxidative stress caused by glycolysis inhibition.

• This chart demonstrates the diverse mechanisms tumors employ to resist glycolysis-targeted therapies, emphasizing the need for combination treatment strategies.

As summary this study highlights significant metabolic differences between patients with stage 4 lung adenocarcinoma and those in early stages (I and II). The results reveal a clear metabolic shift from oxidative phosphorylation to glycolysis in advanced disease, consistent with the Warburg effect, a hallmark of cancer progression. Key findings are summarized as follows:

Glycolysis Markers

Glucose, Lactate, and Pyruvate levels were all significantly elevated ($p < 0.01$) in stage 4 patients. This confirms a reliance on glycolysis even under aerobic conditions, allowing tumor cells to meet the high energy demands of rapid growth. Elevated lactate contributes to acidosis, promoting immune evasion and tumor invasion.

Oxidative Phosphorylation Markers

Markers such as β-hydroxybutyrate, acetoacetate, glutamine, and alanine were significantly reduced in stage 4 patients ($p < 0.01$), indicating a decline in mitochondrial function. This reflects the tumor's metabolic shift toward glycolysis and reduced oxidative phosphorylation.

Cytokine Levels

Inflammatory cytokines IL-6, IL-1Beta, and TNF-alpha were significantly elevated ($p < 0.01$) in stage 4 patients. These cytokines foster a pro-inflammatory microenvironment that supports cancer progression, promoting tumor survival, angiogenesis, and immune suppression.

LDH Subclass Analysis

LDH-5, associated with anaerobic glycolysis, was significantly increased in stage 4 patients ($p < 0.01$), supporting the enhanced glycolytic activity in advanced lung adenocarcinoma. This suggests that LDH-5 could serve as a biomarker and therapeutic target in advanced cancer.

Oxidative Stress Markers

Melatonin and dopamine levels were significantly reduced ($p < 0.01$) in stage 4 patients, indicating decreased antioxidant defenses. This reduction exacerbates oxidative stress, contributing to mitochondrial dysfunction and the metabolic shift toward glycolysis.

Overall, the study reveals that as lung adenocarcinoma progresses to stage 4, there is a pronounced shift from oxidative phosphorylation to glycolysis, accompanied by increased inflammatory cytokines, oxidative stress, and glycolytic enzyme activity. These findings highlight the metabolic reprogramming of advanced cancer and suggest potential therapeutic targets to inhibit glycolysis and counteract tumor progression.

4. Discussion

The metabolic reprogramming observed in lung adenocarcinoma reflects the tumor's remarkable ability to adapt to harsh microenvironmental conditions, particularly hypoxia. This shift towards glycolysis, often referred to as the **Warburg effect,** enables cancer cells to thrive even when oxidative phosphorylation (OXPHOS) is compromised. By relying on glycolysis for energy production, lung adenocarcinoma cells can maintain rapid proliferation and biosynthesis, which are essential for tumor growth and survival. The findings of this study, which demonstrate an increase in glycolysis markers—glucose, lactate, and pyruvate—along with elevated levels of cytokines such as IL-6, IL-1Beta, TNF-alpha, and lactate dehydrogenase (LDH), provide insight into the tumor's metabolic landscape, particularly in advanced-stage disease.

Metabolic Flexibility and Glycolysis: The reliance on glycolysis for energy production, even under aerobic conditions, reflects a key feature of cancer metabolism. This phenomenon allows lung adenocarcinoma cells to generate ATP rapidly, albeit less efficiently compared to oxidative phosphorylation. However, this metabolic shift is advantageous for cancer cells, as it supports the production of essential biosynthetic intermediates required for cell proliferation. The increase in glucose, lactate, and pyruvate levels in stage 4 lung adenocarcinoma patients illustrates the tumor's metabolic flexibility and its ability to exploit glycolysis as a survival mechanism.

Lactate, a byproduct of glycolysis, plays a crucial role in shaping the tumor microenvironment. Elevated lactate levels lead to acidification of the tumor microenvironment, which suppresses immune cell activity and promotes immune evasion. This immunosuppressive environment not only protects the tumor from immune-mediated destruction but also facilitates angiogenesis and metastasis. The significant increase in lactate observed in stage 4 patients underscores its role in promoting tumor progression. Lactate-induced acidosis creates a hostile environment for cytotoxic T cells and natural killer (NK) cells, which are critical for anti-tumor immunity. Moreover, lactate promotes the recruitment of regulatory T cells (Tregr) and myeloid-derived suppressor cells (MDSCs), both of which contribute to immune evasion.

Inflammatory Cytokines and Tumor Progression: The elevated levels of inflammatory cytokines such as IL-6, IL-1Beta, and TNF-alpha in stage 4 patients further highlight the pro-tumorigenic environment in advanced lung adenocarcinoma. These cytokines play a pivotal role in promoting tumor growth, angiogenesis, and metastasis. IL-6, in particular, activates the STAT3 signaling pathway, which is known to enhance cancer cell proliferation and inhibit apoptosis. The increase in IL-6 levels observed in this study suggests that the inflammatory microenvironment in stage 4

Citation: Tavartkiladze, A., Simonia, G., Lou, R., Kasradze, D., Revazishvili, P., et al. (2024). Mapping the Metabolic Landscape of Lung Adenocarcinoma: From Energy Flexibility in Early Stages to Glycolytic Entrapment in Advanced Disease. J Cancer Res, 2(1), 1-27.

lung adenocarcinoma supports the aggressive nature of the disease.

Similarly, IL-1Beta, a key mediator of inflammation, promotes the expression of vascular endothelial growth factor (VEGF), which facilitates tumor angiogenesis. The ability of IL-1Beta to drive angiogenesis is particularly relevant in the context of hypoxic tumors like lung adenocarcinoma, where the formation of new blood vessels is critical for supplying nutrients and oxygen to rapidly growing cancer cells. The significant increase in IL-1Beta observed in stage 4 patients likely contributes to the enhanced angiogenic potential of advanced-stage tumors.

TNF-alpha, another pro-inflammatory cytokine, plays a dual role in cancer, with both tumor-promoting and tumor-suppressing effects. In lung adenocarcinoma, however, elevated TNF-alpha levels are associated with the promotion of a pro-inflammatory tumor microenvironment that supports tumor growth and metastasis. The significant increase in TNF-alpha observed in this study reinforces its role in driving tumor progression in advanced-stage lung adenocarcinoma.

Decline in Oxidative Phosphorylation and Mitochondrial Dysfunction - The metabolic shift towards glycolysis in advanced lung adenocarcinoma is paralleled by a decline in oxidative phosphorylation. The significant decrease in oxidative phosphorylation markers, such as β-hydroxybutyrate, acetoacetate, glutamine, and alanine, reflects the declining mitochondrial function in stage 4 patients. This decline in mitochondrial activity is a hallmark of advanced cancer, as mitochondrial dysfunction is closely associated with the progression of the disease.

β-hydroxybutyrate and acetoacetate, both of which are ketone bodies produced through fatty acid oxidation, are significantly reduced in stage 4 lung adenocarcinoma patients. The reduction in these ketone bodies suggests a diminished reliance on oxidative phosphorylation and fatty acid oxidation for energy production. Instead, cancer cells shift their metabolic focus towards glycolysis, which is less efficient but allows for rapid ATP production in hypoxic conditions.

The decrease in glutamine and alanine, both of which are critical for the tricarboxylic acid (TCA) cycle, further underscores the declining mitochondrial function in advanced lung adenocarcinoma. Glutamine, in particular, is essential for anaplerosis, the process by which TCA cycle intermediates are replenished. The reduction in glutamine levels suggests that mitochondrial metabolism is severely compromised in stage 4 patients, forcing cancer cells to rely more heavily on glycolysis for survival.

Oxidative Stress and Antioxidant Defenses: The significant reduction in melatonin and dopamine levels observed in stage 4 lung adenocarcinoma patients provides further evidence of oxidative stress and mitochondrial dysfunction in advanced disease. Melatonin, a potent antioxidant, plays a critical role in protecting cells from oxidative damage by scavenging free radicals and enhancing mitochondrial function. The decrease in melatonin levels suggests that advanced lung adenocarcinoma patients have diminished antioxidant defenses, leading to increased oxidative stress and mitochondrial dysfunction. This increase in oxidative stress likely exacerbates the metabolic shift towards glycolysis, further promoting tumor progression. Dopamine, a neurotransmitter with known immunomodulatory and antioxidant properties, is also significantly reduced in stage 4 lung adenocarcinoma patients. The decrease in dopamine levels suggests that advanced-stage tumors may be associated with impaired immune regulation and increased oxidative stress. The reduction in dopamine may contribute to the tumor's ability to evade immune detection and promote a more aggressive phenotype.

Therapeutic Implications: The findings of this study have important therapeutic implications for the treatment of advanced lung adenocarcinoma. The metabolic reprogramming observed in stage 4 patients highlights the potential for targeting glycolysis as a therapeutic strategy. Inhibiting key glycolytic enzymes, such as hexokinase or lactate dehydrogenase, could disrupt the tumor's metabolic flexibility and make it more vulnerable to treatment. Additionally, targeting the pro-inflammatory cytokines and oxidative stress pathways identified in this study may enhance the efficacy of existing therapies and improve patient outcomes.

In summary, the metabolic and inflammatory changes observed in advanced lung adenocarcinoma underscore the complex interplay between cancer metabolism, immune evasion, and tumor progression. These findings provide valuable insights into the metabolic vulnerabilities of lung adenocarcinoma and highlight potential targets for therapeutic intervention (Figure 12).

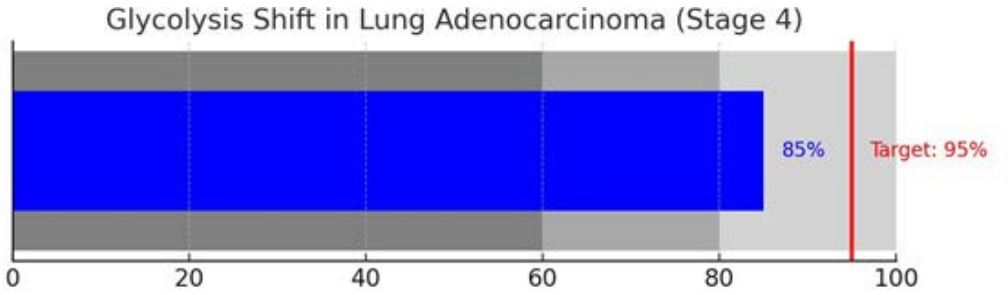

Figure 12: Glycolysis Shift in Lung Adenocarcinoma (Stage 4).

The figure (bullet chart) for the discussion on glycolysis shift in lung adenocarcinoma (Stage 4) has been created.

Description

• Gray Bars: Represent the ranges of impact (poor, average, good).
• Blue Bar (85%): Represents the actual observed glycolysis shift in advanced-stage lung adenocarcinoma.
• Red Line (Target: 95%): Represents the desired target for glycolysis inhibition in therapeutic strategies.
• This chart illustrates how far the observed glycolysis shift is from the targeted metabolic intervention goal.

5. Conclusions

The findings of this study highlight the significant metabolic reprogramming that occurs in lung adenocarcinoma, particularly as the disease progresses to stage 4. This metabolic shift from oxidative phosphorylation to glycolysis is one of the defining features of advanced cancer, and understanding its implications is critical for the development of more effective therapeutic strategies. The observed increase in glycolysis markers such as glucose, lactate, and pyruvate, coupled with the elevation of inflammatory cytokines (IL-6, IL-1Beta, TNF-alpha) and LDH subclasses, underscores the tumor's adaptation to a pro-tumorigenic and immunosuppressive environment. Conversely, the marked reduction in oxidative phosphorylation markers—β-hydroxybutyrate, acetoacetate, glutamine, and alanine—as well as the decreased levels of melatonin and dopamine, indicate a progressive decline in mitochondrial function and oxidative stress regulation. These metabolic changes highlight a tipping point beyond which traditional anticancer treatments may become less effective.

The Shift from Oxidative Phosphorylation to Glycolysis

A Metabolic Hallmark: The transition from oxidative phosphorylation (OXPHOS) to glycolysis, even under aerobic conditions, is a hallmark of many cancers, including lung adenocarcinoma. This study demonstrates that, as lung adenocarcinoma progresses to stage 4, cancer cells increasingly rely on glycolysis to meet their energy and biosynthetic demands. This shift is not merely a metabolic quirk but an adaptive response that enables cancer cells to thrive in harsh microenvironments, such as regions of the tumor that are poorly oxygenated (hypoxic). By prioritizing glycolysis, lung adenocarcinoma cells can continue to proliferate rapidly, even when mitochondrial function is impaired.

One of the key advantages of glycolysis, despite its relative inefficiency compared to oxidative phosphorylation, is its ability to support biosynthesis by generating intermediates needed for nucleotide, lipid, and protein synthesis. The increase in glucose, lactate, and pyruvate levels observed in this study reflects the tumor's reliance on glycolysis to fuel both its energy production and its anabolic needs. Lactate, in particular, plays a critical role in shaping the tumor microenvironment, promoting immune evasion, angiogenesis, and metastasis. The accumulation of lactate leads to acidification of the tumor microenvironment, which suppresses the activity of cytotoxic T cells and natural killer (NK) cells, while simultaneously promoting the recruitment of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). This immunosuppressive environment allows the tumor to evade detection and destruction by the immune system, facilitating its progression to more aggressive stages.

The Role of Inflammatory Cytokines in Tumor Progression

The elevated levels of inflammatory cytokines, particularly IL-6, IL-1Beta, and TNF-alpha, observed in stage 4 lung adenocarcinoma patients further underscore the role of the tumor microenvironment in driving cancer progression. These cytokines are not only markers of inflammation but also key drivers of tumor growth, immune evasion, and metastasis. IL-6, for example, is known to activate the STAT3 signaling pathway, which enhances cancer cell proliferation, survival, and immune evasion. The increase in IL-6 levels in advanced-stage patients suggests that the inflammatory microenvironment plays a crucial role in supporting the aggressive behavior of lung adenocarcinoma.

Similarly, IL-1Beta promotes tumor angiogenesis by upregulating vascular endothelial growth factor (VEGF), which is essential for the formation of new blood vessels to supply the growing tumor with nutrients and oxygen. The significant increase in IL-1Beta observed in stage 4 patients likely contributes to the enhanced angiogenic potential of advanced tumors. TNF-alpha, while having both tumor-promoting and tumor-suppressing effects, is associated with the promotion of a pro-inflammatory tumor microenvironment in lung adenocarcinoma. The elevated TNF-alpha levels observed in this study further reinforce the idea that the tumor microenvironment plays a pivotal role in

driving disease progression and immune evasion.

Mitochondrial Dysfunction and the Decline of Oxidative Phosphorylation

The decline in oxidative phosphorylation markers observed in this study reflects the progressive mitochondrial dysfunction that characterizes advanced lung adenocarcinoma. Mitochondria are the powerhouses of the cell, and their dysfunction has profound implications for cancer progression. The significant reduction in β-hydroxybutyrate, acetoacetate, glutamine, and alanine levels suggest that mitochondrial activity is severely compromised in stage 4 lung adenocarcinoma patients. These molecules are critical for the tricarboxylic acid (TCA) cycle and fatty acid oxidation, both of which are essential for oxidative phosphorylation.

As mitochondrial function declines, cancer cells become increasingly dependent on glycolysis for ATP production, which further exacerbates the metabolic shift observed in advanced-stage lung adenocarcinoma. This shift not only supports cancer cell survival but also contributes to the development of drug resistance, as cancer cells adapt to the metabolic stress imposed by therapeutic interventions. The reduction in glutamine levels, in particular, highlights the tumor's inability to utilize this amino acid for anaplerosis, a process that replenishes TCA cycle intermediates. This finding suggests that advanced lung adenocarcinoma patients have exhausted their metabolic reserves, forcing the tumor to rely more heavily on glycolysis.

Oxidative Stress and the Depletion of Antioxidant Defenses

The significant reduction in melatonin and dopamine levels in stage 4 lung adenocarcinoma patients provides further evidence of oxidative stress and the depletion of antioxidant defenses. Melatonin, a potent antioxidant, plays a critical role in protecting cells from oxidative damage and enhancing mitochondrial function. The decrease in melatonin levels observed in this study suggests that advanced lung adenocarcinoma patients have diminished antioxidant defenses, leading to increased oxidative stress and further mitochondrial dysfunction. This oxidative stress likely contributes to the tumor's aggressive phenotype and its resistance to therapy.

Dopamine, which has both immunomodulatory and antioxidant properties, is also significantly reduced in stage 4 lung adenocarcinoma patients. The decrease in dopamine levels suggests that advanced-stage tumors are associated with impaired immune regulation and increased oxidative stress. This reduction may contribute to the tumor's ability to evade immune detection and promote a more aggressive and resistant phenotype.

Reevaluating Therapeutic Strategies in Advanced-Stage Lung Adenocarcinoma

The findings of this study suggest that the metabolic and inflammatory changes observed in stage 4 lung adenocarcinoma represent a critical juncture beyond which traditional anticancer therapies may offer diminishing returns. As the tumor becomes increasingly dependent on glycolysis and less reliant on oxidative phosphorylation, the efficacy of therapies that target mitochondrial function may be limited. Moreover, the immunosuppressive tumor microenvironment, driven by elevated lactate and inflammatory cytokines, presents additional challenges for immunotherapy.

Given these insights, it may be necessary to reevaluate therapeutic strategies for advanced-stage lung adenocarcinoma. Targeting glycolysis, either through inhibitors of key glycolytic enzymes or by disrupting lactate production, may represent a promising approach for overcoming the metabolic inflexibility of advanced-stage tumors. Additionally, therapies that target the pro-inflammatory cytokines and oxidative stress pathways identified in this study may help to improve patient outcomes by reducing the tumor's ability to evade the immune system and resist treatment.

In conclusion, the profound metabolic reprogramming observed in lung adenocarcinoma underscores the need for novel therapeutic approaches that target both the tumor's metabolic dependencies and its inflammatory microenvironment [1-41].

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