Targeting Glucose Metabolism in Cancer Immunotherapy: A Promising Avenue for Enhanced Treatment Strategies

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Abstract. Cancer immunotherapy has emerged as an innovative strategy for treating multiple types of cancer. Recent breakthroughs have emphasized the significance of altering glucose metabolism in malignant cells; specifically, the heightened dependency of cancer cells on glucose metabolism ("Warburg effect"), where cancer cells alter their metabolic pathway so that they utilize glucose as an energy source, even when oxygen is present. This adaptation allows for rapid tumor cell proliferation, enabling them to grow and divide at an accelerated pace. Current research concentrates on the development of cancer treatments aimed at inhibiting glucose metabolism in cancer cells. This approach aims to improve the effectiveness of existing immunotherapies by utilizing the metabolic pathways of malignant cells through direct and indirect inhibition of glycolysis. This review discusses the interplay between glucose metabolism and immune response, explores current efforts in manipulating glucose metabolism for therapeutic benefits, and provides insights into future directions for optimizing this strategy.

Keywords: cancer immunotherapy; glucose metabolism; glycolysis.

1. Introduction

It has been known that cancer cells and normal cells take on different metabolic pathways. Specifically, cancer cells exhibit altered metabolic pathways with a heightened preference for glycolysis, even when sufficient oxygen is available (Warburg effect) [1]. Emerging studies show that the Warburg effect significantly contributes to tumor progression by enabling quick proliferation; enhanced survival, invasion, metastasis; and resistance to existing treatments [2]. Given that cancer cells rely on glycolysis for energy, targeting the glycolytic pathways could be a viable approach for cancer research aimed at creating new targeted cancer therapies [1]. However, in order to get these new cancer therapies, such as 2-DG and ritonavir, in the market, they must pass clinical trials, but there are limitations that prevent that.

This review will present glucose metabolism and immune cell interplay, explore the current efforts in manipulating glucose metabolism for therapeutic benefits, and provide insights into future directions for optimizing this strategy.

2. Glucose Metabolism and Immune Regulation

2.1. Glucose Regulation of Immune System

2.1.1 Aerobic Glycolysis in Malignant Cells

When there is an adequate amount of oxygen available, normal cells initiate glycolysis, followed by mitochondrial oxidative phosphorylation (OXPHOS) to obtain energy. Under conditions of oxygen deficiency, cells utilize anaerobic glycolysis instead of OXPHOS, which consumes oxygen. However, cancer cells undergo a different metabolic pathway [2]. The tendency for malignant cells to rely on aerobic glycolysis regardless of the oxygen levels present within the cell was discovered by Otto Warburg, which is accordingly termed the “Warburg effect.” [1].
2.1.2 Glycolysis in Immune Cells

Fig. 1 Different metabolic pathways of normal and cancer cells: oxidative phosphorylation, anaerobic glycolysis, and aerobic glycolysis. Normal cells undergo both oxidative phosphorylation and anaerobic glycolysis as their metabolic pathways. Through the aid of oxygen, OXPHOS breaks down glucose and other molecules to produce energy that is crucial for numerous cellular functions.

In situations of oxygen deprivation, anaerobic glycolysis plays a vital function in energy production. This pathway occurs in the cytoplasm of cells and begins with glucose breakdown into two pyruvate molecules, producing small amounts of ATP. Pyruvate then undergoes fermentation to produce lactate, which gets released into the bloodstream. This process helps maintain the energy supply to the cells but at a lower rate than oxidative phosphorylation. In contrast, tumor cells undergo aerobic glycolysis regardless of oxygen availability, leading to high levels of lactate production, which is exported from the cell to the extracellular microenvironment. Compared to OXPHOS, the aerobic glycolysis pathway is relatively inefficient, but it provides biosynthetic precursors and substrates that support the cellular growth and proliferation of tumors. Abbreviation: OXPHOS, Oxidative phosphorylation. Figure credit: original.

As shown in Figure 1, OXPHOS is the most energy-efficient production route, generating approximately 36 ATPs per glucose molecule, whereas anaerobic glycolysis produces about two ATP molecules per glucose molecule and aerobic glycolysis (Warburg effect) four. Because OXPHOS is the most energy efficient, naïve T cells usually utilize OXPHOS. On the other hand, the Warburg effect causes T cells to shift from OXPHOS to aerobic glycolysis when triggered. Said T cells exhibited heightened glucose absorption, glycolysis, and lactate secretion [3]. Furthermore, elevated expression of glycolysis-associated proteins, specifically glucose transporter 1 (GLUT1) and hexokinase 2 (HK2), were detected in these cells [3]. By upregulating these glycolysis-associated proteins, more lactic acid is generated and then secreted into the cell microenvironment. Due to the decreased pH and increase in lactic acid concentration in the TME, lactic acid secretion from T cells is hindered, leading to a significant decline in cytokine production and proliferation [2]. This reduction of cytokines means that the signals the immune system receives to come to the site of the cancer cell are reduced, meaning that this process promotes cancer metastasis. Glycolysis is also very important in the metabolic reconfiguration of T cells, emphasized by the inhibition of effector T cell (Teffs) infiltration, resulting in cell death of regulatory T cells (Tregs).

Aside from its ability to hinder T cells’ cytolytic functions, lactate is recognized for promoting the progression of myeloid-derived suppressor cells (MDSCs). In particular, MDSCs are amplified by
lactate and innate immune effector activity is limited by inhibiting natural killer (NK) cell activity. This demonstrates lactate’s role in establishing this immunosuppressive microenvironment that is commonly found in developing tumors [2].

2.2. Impact on Immunosuppression

2.2.1 Impact of Glucose Availability on Immune Cell Function

Cancer cells upregulate glucose and glutamine uptake to keep cell proliferation. These nutrients are then broken down, functioning as building blocks for macromolecule production, while also serving as reducing powers for macromolecule production and ATP generation. The metabolic processes in tumor and immune cells, namely cytotoxic T lymphocytes (CTL) and Teffs, are fairly similar, and that causes competition for the scarcity of glucose and glutamine [4]. The lack of glucose and glutamine in the microenvironment could lead to nutrient stress on both cancer and normal cells. Because the Warburg effect facilitates tumor growth and escalates the need for macromolecules, the tumor cells have an external benefit. This includes acceleration in glucose consumption for tumor cells and restrictions related to glucose consumption for T cells that have invaded the tumor, leading to starvation among immune cells and a reduction in antitumor immune activity [5]. For example, in a low-glucose environment, CD4+ and CD8+ cell function diminished, resulting in decreased interferon-γ (IFN-γ), granzyme B, and intermediate-17 (IL-17) production [5]. In brief, glucose metabolism can directly control T cell activation, and the limited glucose and glutamine in the TME have the potential to compromise immune cell function, suppressing anti-tumor activity.

2.2.2 Immunosuppressive Effects of Lactate and Acidosis

Furthermore, glycolysis leads to acidification of the microenvironment. Since lactate and carbon dioxide (CO₂) are produced as a product of glycolysis, the microenvironment becomes acidic, promoting the progression of highly aggressive cancer cells [6]. Metabolic stresses, such as acidosis, have immunosuppressive qualities, and T cells exhibit a low tolerance towards these stresses as a result of Treg cell development in a TME with limited glucose [6]. Various researchers have also indicated that the deficiency of nutrients can hinder Teff cytokine production, macrophage phagocytosis, and superoxide generation [5]. Thus, the metabolic conditions in the TME provide malignant cells with an advantage because they can tolerate metabolic stress and proliferate under this condition.

3. Targeting Glucose Metabolism in Cancer Immunotherapy

3.1. Direct Inhibition of Glycolysis

Because of the Warburg effect, there’s a lack of glucose and a surplus of lactate in the cell environment, hindering immune cell activity. Therefore, inhibiting glycolysis-related enzymes could help impede tumor growth while improving immune cell function. Before producing CAR T cells, T cells were isolated and cultured for 3 days. During this period, cytokines, particularly interleukin-2 (IL-2), are included to enhance T cell expansion. Current research suggests that T cell metabolism is vital in controlling their growth and differentiation. Furthermore, it has been found that these two processes can be separated from each other by altering metabolic pathways [3].

As seen in Table 1, the glucose inhibitor 2-deoxyglucose (2-DG) inhibits glycolysis by targeting hexokinase. After being transported to the cytoplasm via glucose transporters, hexokinase phosphorylates 2-DG, leading to the formation of 2-DG-6-phosphate. The inability to metabolize the phosphorylated form, 2-DG-6-phosphate, causes inhibition of hexokinase. Sukumar et al. conducted a study that uncovered that while 2-DG was capable of inhibiting tumor cell growth as well as enhancing memory CD8+ T cell generation, it had a detrimental effect on the Teff function [7]. When 2-DG was introduced during in vitro expansion, it hindered T cell glycolysis and contributed to memory T cell production, indicating that an elevated glycolytic metabolism has an impact on memory T cell formation [7].
When phosphofructokinase-1 is inhibited by dichloroacetate (DCA), another glucose inhibitor, the cancer cells go from using glycolysis to using OXPHOS, suppressing tumor cell growth [8]. However, the problem with 2-DG and DCA is that they affect non-malignant cells, such as T cells besides tumor cells. Thus, the drugs end up inhibiting T cell function, which promotes immunosuppression. Thus, it is essential to ensure the precise targeting of tumor cells while using glycolysis inhibitors.

3.2. Indirect Inhibition of Glycolysis

As mentioned before, the PI3K-AKT pathway can be suppressed by glycolysis inhibitors, which helps to preserve the undifferentiated state of T cells. Additionally, the PI3K-AKT pathway is believed to impact various metabolic pathways that aren’t associated with glucose metabolism. Further exploration is needed to understand how T cell differentiation is affected by PI3K and AKT inhibitors.

Another approach to indirect inhibition of glycolysis is through utilizing the mitochondria. T cells are related to mitochondrial plasticity, as evidenced in studies that indicate the association between T cell differentiation and mitochondrial membrane potential ($\Delta \Psi_m$), dynamics, and mitophagy [9]. Tetramethylrhodamine methyl ester absorption is utilized in CD8+ T cell differentiation with different levels of $\Delta \Psi_m$; T cells exhibiting a low $\Delta \Psi_m$ possess better metabolic adaptability as well as metabolic features akin to memory CD8+ T cells [11]. A new and emerging method in promoting prolonged survival and antitumor defense mechanisms includes separating T cells by $\Delta \Psi_m$.

In addition, various studies have linked the mitochondria to cell metabolism and mitochondrial fission and fusion to immunotherapy [11]. Naïve T cells display comparatively smaller, fragmented mitochondria supported by OXPHOS, as indicated by various studies [11]. Because of mitochondrial fission, mitochondria in Teff cells display a more round, punctiform shape, and an increase in aerobic glycolysis [12]. Memory CD8+ T cells exhibit an augmentation in both mitochondrial fusion and mass, evident in their slender mitochondrial network [12]. Studies have shown that suppressing mitochondrial fission or stimulating fusion may result in elevated levels of OXPHOS and enhance the T cells’ killing ability. For example, Buck et al. found that Mdivi-1, an inhibitor of mitochondrial fission, Drp1 inhibition, and OPA1 overexpression, encourage the development of CD8+ T cell memory [12].

Another important regulatory mechanism in the mitochondria is mitophagy, which clears out flawed or inefficient mitochondria to preserve their functionality [1]. It is believed that mitophagy contributes towards the development of effector memory in CD8+ T cells. Nonetheless, there is a need for further investigation in future research to confirm the mechanisms of mitophagy during the development of central memory in T cells.

In summary, promoting mitochondrial function will enhance the differentiation in CAR T cells and enhance their ability to eliminate malignant cells.

4. Combination Therapies

Combinatorial approaches that merge glucose metabolism-targeting agents with existing immunotherapies hold promise for synergistically enhancing treatment outcomes.

Despite the rapid development of various glycolytic inhibitors, there are still constraints on clinical application. However, when combined with other immunotherapies, the tumor-suppressive effects are enhanced. In a study conducted by Mohammed and colleagues, a mouse model with ductal carcinoma showed that the co-administration of PKM2 activators and lactate dehydrogenase A (LDHA) inhibitors resulted in a substantial reduction in metastatic occurrences, showing potential in combining glycolysis-targeting compounds with multiple targets [2].

An emerging body of evidence suggests that in addition to fueling cancer growth, aerobic glycolysis also plays a role in chemotherapy resistance. Hence, medications aimed at inhibiting aerobic glycolysis could potentially enhance the effectiveness of chemotherapy by increasing its cytotoxicity. To treat liver cancer cells, Korga et al. employed a combination of 2-DG and the
chemotherapy drug adriamycin [13]. The findings demonstrated that the combined therapy outperformed solo adriamycin treatment by effectively suppressing liver cancer cell activity and enhancing apoptosis [13]. Additional research has uncovered that 2-DG's impact extends to inhibiting protein N-glycosylation, rendering it effective in sensitizing cells and reversing resistance to various chemotherapy treatments. This encompasses 5-FU for prostate cancer cells, trastuzumab for breast cancer cells, as well as Bcl-2 inhibitors for leukemia cells [13]. Zhang et al. found that in mice with transplanted osteosarcoma and non-small cell lung cancer, the addition of 2-DG was discovered to mitigate resistance to paclitaxel and adriamycin when compared to chemotherapy alone [12]. This implies that the fusion of chemotherapeutic drugs with glycolysis inhibitors represents a promising therapeutic approach for cancer.

Studies have also indicated the potential of combining immune checkpoint inhibitors with glycolytic inhibitors as an encouraging therapeutic approach. Studies have shown that the co-administration of a PD-1 inhibitor alongside FX11, an LDH inhibitor, led to heightened infiltration of CD8+ and NK cells, supporting the idea that the concurrent use of immune checkpoint and glycolytic inhibitors can augment their anticancer effects [14]. Renner et al. found that diclofenac, previously shown to be an MCT1/4 inhibitor, demonstrated a reduction in lactate secretion and an enhancement in the cytotoxicity of infiltrating T cells [15]. As mentioned before, within the TME, there’s a metabolic conflict between infiltrating T cells and cancer cells. This competition can be influenced by lowering glucose levels within the cell microenvironment, which could potentially result in the inhibition of infiltrating T cell responses to tumor cells [15]. Furthermore, PD-1 can be expressed on the cell membrane of T cells, whereas cancer cells exhibit PD-L1 on their cell membrane, providing them with a means to escape anti-tumor immune cells. Fang et al. conducted a study, in which they observed that combining MCT inhibitors and PD-1 monoclonal antibodies led to a reduction in tumor development and enhanced infiltration of CD8+ T cells in mice models with hepatocellular carcinoma [15]. Combining immune checkpoint inhibitors with glycolytic inhibitors carries a promising future.

5. Challenges and Future Perspectives

5.1. Limitations in Glucose Metabolism Inhibitors

5.1.1 Selectivity and Toxicity Issues of Glucose Metabolism Inhibitors

For decades, researchers have recognized that tumor cells heavily depend on aerobic glycolysis for their nutritional requirements. Despite this knowledge, the implementation of targeting this pathway for clinical therapy is still in progress. Among the significant limitations of glucose metabolism inhibitors is their low selectivity. Given the widespread presence of enzymes involved in glucose metabolism, low selectivity poses a prevalent problem. Although several targeted drugs have demonstrated efficacy and some have progressed to clinical trials, inadequate targeting can result in toxic side effects, posing challenges to fulfilling the requirements of cancer treatment [16]. The rise of selective targeting coupled with precise delivery techniques has introduced an alternative approach to mitigate the issue of systemic toxicity. This approach has witnessed a resurgence of interest in recent years. Given the extensive research conducted on the genetic sequences and protein configurations related to glycolytic enzymes, it's feasible to utilize computer-assisted drug design to create inhibitors by leveraging the structure of glycolytic enzymes. Critical aspects in designing small-molecule drugs capable of enhancing drug potency while circumventing systemic toxicity include scrutinizing the binding sites of glycolytic enzymes, analyzing their interactions with substrates, and understanding the properties of these binding sites, along with identifying pivotal binding sites [16]. Nevertheless, these methods prove to be successful solely in the treatment of localized cancers; treatment for widely metastatic cancers requires more research.
5.1.2 Metabolic Heterogeneity in Cancer Cells

Another significant limitation of using glucose metabolism inhibitors is the metabolic heterogeneity across cancer cells. Cancer cells near blood vessels predominantly utilize OXPHOS and rely on monocarboxylate transporter 1 (MCT1) to take up lactate. They utilize this lactate as fuel for the tricarboxylic acid cycle, which supplies them with energy. Contrarily, cancer cells located further away from blood vessels rely on glucose for energy production through glycolysis, resulting in the release of lactate [16]. The latter demonstrates metabolic symbiosis, giving them an adaptive advantage over the TME [16]. Accordingly, cancer cells with metabolic heterogeneity cannot be eradicated by a single glycolytic targeting drug. Rather, the alteration of tumor cells’ metabolic programming is stimulated by the metabolic stress caused by these glycolytic-targeted drugs. For example, increased dependency on glutamine metabolism results in resistance to glycolytic-targeted drugs. To address this issue, one strategy is to first treat the diverse metabolic patterns within the TME to promote a more homogeneous metabolic profile among the cells. Then, the focus can be on targeting this relatively uniform metabolic population to disrupt it effectively. Chaube et al. employed a two-step approach to treat melanoma. Initially, they used metformin to hinder mitochondrial respiration in the cells, shifting their reliance toward glycolysis. Following this, LDH inhibitors were introduced [17]. The metformin caused the cancer cells to become relatively homogenous, which led to better therapeutic results.

5.1.3 Immune Response to Glucose Metabolism Inhibitors

Furthermore, the immune system's reaction to the drug can present a constraint in glycolysis-targeted therapies. Immune cells with anticancer properties, such as cytotoxic T lymphocytes which require glucose for interferon-gamma synthesis; DCs which rely on glycolysis to facilitate the production of interleukin 12 (IL-12) and stimulate the proliferation of T-cells; NK cells which rely on glycolysis for activation; Th1 and Th17 cells which depend on glucose metabolism for differentiation; or macrophages which rely on glycolysis for tumor necrosis factor (TNF) secretion, rely on glucose metabolism to function [2]. Because so many anticancer cells rely on glycolysis, inhibiting glycolytic enzymes with targeted therapies can impede not only tumor cell proliferation but also dampen the anticancer immune response.

5.2. Clinical Translation

As of now, the clinical translation of glucose metabolism inhibitors remains limited (Table 1). However, various glucose metabolism enzymes show therapeutic promise.

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Table 1. Primary targets and drugs impacting cancer cell glucose metabolism

Abbreviations: GLUT, glucose transporter; HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase.

Glucose transporters manage glucose entry — GLUT-1 in particular. WZB117 and STF-31, both GLUT-1 inhibitors, have been tested in preclinical trials [16]. STF-31 proved to be efficient in blocking GLUT-1 intake, triggering apoptosis, and impeding cancer cell proliferation. Nonetheless, due to its molecular limitations, STF-31 shows limited therapeutic promise [16]. WZB117 was
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effective in inhibiting GLUT-1 uptake and tumor development, but there is still uncertainty about its effectiveness [16].

Current research suggests inhibition of lactate transport as a possible strategy. However, LDHA inhibitors face limitations of high toxicity and an absence of LDHA reliance on inhibitors for human tumors [17]. Nonetheless, galloflavin, a natural compound formed by the oxidation of gallic acid, acts as a blocker for certain human LDH enzymes [16].

FX11, an LDHA inhibitor, has been proven effective in increasing oxidative stress levels, resulting in cancer cell inhibition in vitro and in vivo [17].

Furthermore, novel drugs have been developed to achieve dual inhibition, for example: targeting both the adaptability and survival mechanisms related to glucose metabolism in cancer cells. Anticancer effects have been observed with the inhibition of glucose, glutamine, and lactate metabolism, effectively hindering the proliferation of cancer-related endothelial cells [18]. Glutor, a GLUT inhibitor, has shown success in reducing glycolytic flux, which suppresses the growth of various cancer cells [19]. A combination of Glutor and CB-839, a glutaminase inhibitor, has been observed to have an enhanced effect in inhibiting the proliferation of colon cancer cells [19].

Recent studies have shown that by combining treatment with vitamin C, the functioning of both glucose metabolism and the TCA cycle can be impeded, causing a decrease in ATP and NADPH production. This disruption triggers oxidative stress, resulting in amplified cancer cell death and further suppression of cancer cell survival and invasion [20]. Based on preclinical studies, there is evidence that a vitamin C concentration under 5 mM can inhibit the tumor cell proliferation, likely due to its antioxidant properties [21].

The combination of metformin, a drug commonly used for diabetes, and ritonavir targets GLUT-4 and has the potential to successfully inhibit the phosphorylation of the AKT mTORC1 pathway and MCL1 [21]. This is crucial because AKT and mTORC1 are both proteins that enhance cell growth and proliferation. Excessive activation of these proteins has been linked to tumor development and progression, so restraint of their activity is a desirable therapeutic effect. Similarly, MCL1 is a protein linked to tumor cell viability, so a reduction in MCL1 activity could potentially result in a lowered survival rate for malignant cells.

Over the last 20 years, the potential of aspirin to treat various cancer types and tumor cell lines has been extensively researched because of its capacity to function as both a common anti-inflammatory drug and pain reliever, as well as an inhibitor of MCL1 activity [22]. It was found that after administering aspirin to mice with tumors, there was a notable change in the expression of pH regulators MCT-1 and V-ATPase in T cell lymphoma cells, in addition to changes in regulatory molecules responsible for cell survival, including GLUT-1 [23]. Aspirin has the capability of reducing the expression or activity of the GLUT-1 protein, and by inhibiting its function, aspirin can modulate glucose uptake by targeting signaling pathways such as NF-κB or NF κB/HIF-1α. As a result, cancer cell proliferation can be inhibited [1].

6. Conclusion

Targeting glucose metabolism in cancer immunotherapy has emerged as a compelling avenue for enhancing the efficacy of cancer immunotherapies. However, immunosuppressive factors in the TME, such as glucose starvation and acidosis continue to impede the success of cancer immunotherapies. Through the manipulation of key glycolytic enzymes and pathways involved in glucose production, this approach holds the potential to impede abnormal cancer cell growth and enhance the anticancer effects of other immunotherapies. Novel inhibitors of glucose metabolism include 2-DG and londamine. While substantial advancements have been achieved in comprehending the mechanisms governing cancer cell glucose metabolism and in developing potent inhibitors, further research is essential to optimize their specificity and minimize potential side effects. The ongoing exploration of glucose metabolism inhibitors holds the promise of novel therapeutic strategies that could...
significantly impact the lives of individuals affected by refractory malignancies, paving the way for improved management and treatment approaches in the future.

References


