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# Selective Metabolic Targets and Their Inhibitors in Oncological Diseases: Potential and Prospects

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**Abstract**

Reprogramming of glucose and lipid metabolism is one of the key properties intrinsic in aggressive cancer cells, due to which they acquire advantages for their survival, growth and avoidance of the antitumor immune response. The aim of this review is to search for selective oncotargets underlying the reprogramming of metabolic oncopathways, as well as to identify their most effective inhibitors. The main inhibitors capable of effectively suppressing cancer aerobic glycolysis and triggering cancer cell death were analyzed to test this assumption. Attention was also focused on the ability of cancer cells to collectively suppress the local immune response, in particular, due to the synthesis and secretion of cancer lipid granules. A metabolic model explaining the origin of this effect in the cancer microenvironment was also developed.

**Keywords:** metabolic targets, Warburg effect; cell dedifferentiation; tumor microenvironment; intracellular pH regulation; aerobic glycolysis

## INTRODUCTION

Aggressive forms of cancer have been known for a long time. They consist predominantly of poorly differentiated malignant cells capable of metastasis and invasion, and are resistant to various therapeutic effects [1, 2]. These features can be realized through a change in metabolism, as a result of which malignant cells prefer aerobic glycolysis to oxidative phosphorylation even under conditions of sufficient oxygen supply. This phenomenon was described by Otto Warburg and is often referred to as the Warburg effect [3]. It is assumed that such a metabolic transformation can be a trigger for the emergence of immature malignant cells that acquire resistance to damage and the ability to survive and grow under unfavorable conditions [4]. However, increased aerobic glycolysis is not only a distinctive feature of undifferentiated cancer cells; it can also be induced in normal mature cells under conditions of chronic stress (under nutritional deficiency and hypoxia). Adapting to these unfavorable conditions, mature cells have to switch from energy-consuming to energy-saving metabolism (glycolysis), which is accompanied by a partial loss of their specific properties, i.e. dedifferentiation [5]. It has been hypothesized that malignant cells use

this mechanism to protect themselves from lethal damage and to survive in unfavorable conditions. Such protection is achieved by switching to Warburg metabolism, which reduces the maturation of cancer cells and increases their survival [4, 5]. Therefore, the search for effective inhibitors of the Warburg effect and stimulators of cancer cell differentiation may, in the long term, increase the effectiveness of the treatment of aggressive forms of cancer.

## HOW DOES THE WARBURG EFFECT INFLUENCE THE DIFFERENTIATION OF MALIGNANT CELLS?

Aerobic glycolysis is a hallmark of malignancy and is often found in aggressive tumors [6]. However, this metabolic pathway is not bioenergetically efficient, producing only 2 ATP molecules per glucose as compared to 36 ATP with complete oxidation of glucose. The question of why so many malignant forms of cancer prefer aerobic glycolysis remains open. Warburg suggested this to be due to mitochondrial dysfunction [3]. However, a large number of studies have not supported his "respiratory injury" theory, having experimentally established that tumor development is not accompanied by the dysfunction of cancer mitochondria when aerobic glycolysis is activated. Another theory suggests that glycolysis enhances tumor cell proliferation by generating anabolic building blocks necessary for the synthesis of macromolecules. However, most of the carbon derived from glucose is exported from the cell as lactate [7]. Russell et al. [8] hypothesize the existence of acid-mediated invasion. The hypothesis is based on the observation that maintenance of chronic pH imbalance inside and outside cancer cells can provide increased fermentation of glucose to lactate. It has been established that the accumulation of lactate in the cancer microenvironment contributes to the maintenance of an undifferentiated state in aggressive cancer cells, such as melanoma cells. The authors have found that chronic adaptation of cancer cells to acidosis stimulates expression of stem markers, and also increases the ability of cancer cells to form new clones of aggressive cancer cells *in vitro* and *in vivo*. However, the undifferentiated phenotype of melanoma cells caused by acidosis can be reversible and to be associated with epithelial-mesenchymal transition (EMT) [9].

EMT is known as a cellular process in which cells lose their epithelial markers (E-cadherin) and acquire mesenchymal markers (N-cadherin, vimentin). EMT is triggered by external and internal signals, inducing the transformation of epithelial cells into a phenotype similar to mesenchymal cells. In this case, epithelial cells lose cell–cell contacts and acquire the capability of invasion and migration, which increases the risk of the occurrence and development of metastases, since adult human tissues [10] do not synthesize embryonic factors required for controlled reversal of EMT [11]. What is more, lactate produced through the Warburg effect is also necessary for the transcriptional activation of proteins that support the EMT process [12]. In

other words, under conditions of extracellular acidosis, EMT transformation allows malignant cells to acquire additional advantages in migration, survival, growth, and evasion of the immune response [13]. It is important to note that normal, benign or differentiated tumor cells do not possess such adaptive capabilities [14], whereas cells in solid tumors often experience hypoxia, which can induce the loss of dedifferentiation and the acquisition by cancer cells of a phenotype characteristic of immature stem cells [15-16].

Earlier studies have shown that cancer cells using aerobic glycolysis require an active uptake of glucose. To be realized, this mechanism requires an increase in the number of glucose transporters on the surface of malignant cells. This fact is used to determine the level of differentiation of cancer cells by positron emission tomography, which allows measuring the level of fluorodeoxyglucose (FDG) uptake by tumor cells. The degree of cell differentiation was found to be closely related to glucose uptake: a well-differentiated tumor had a low FDG uptake, while a poorly differentiated aggressive tumor had a higher level of glucose uptake usually characteristic of immature stem cells [17]. These results support the hypothesis that cancer evolution is characterized by progression towards an embryonic-like, poorly differentiated phenotype, which also occurs in the metabolic development program. In particular, embryonic tail bud cells exhibit a high Warburg metabolism responsible for elevated intracellular pH ($pH_i$) and low extracellular pH ($pH_e$) [18]. This enables the authors to suggest that the "primary cause" of cancer underlying the induction of the Warburg effect is poorly controlled intracellular alkalinization of cells observed in various malignancies and leukemias [19-20]. This effect can be induced by a wide range of mediating factors [21-22]. However, transient intracellular alkalinization can also be observed in physiological conditions, for example, in amniotic embryos [18]. Probably, cytoplasmic pH serves as a secondary messenger capable of controlling glucose intake into cells [21]. Specifically, glucose increases $pH_i$ in pancreatic β-cells, whereas glucose starvation results in a marked decrease in $pH_i$ that is restored by re-addition of glucose to starved cells. In other words, a transient increase in $pH_i$ in normal cells is a reversible response that must be carefully controlled by extracellular nutrients, whereas aggressive cancer cells become tolerant to glucose and oxygen deprivation. This condition may be termed the cancer "$pH_i$–$pH_e$ paradox"; it is characterized by a cancer-specific increase in a combination of intracellular alkalosis and secondary extracellular acidosis that may influence the differentiated state in various cells. What are the critical targets and factors that may control this paradox?

## CANCER $pH_i$–$pH_e$ PARADOX: SEARCH FOR POTENTIAL TARGETS AND THEIR INHIBITORS

It is known that the removal of $H^+$ from cells occurs via membrane-associated pumps, ion channels, and proton transporters. In cancer cells of various origins, they maintain $pH_i$ within the normal to elevated range even under unfavorable conditions of the cancer microenvironment, such as hypoxia, nutrient deficiency, and extracellular acidosis. Invasive cancer cells demonstrate higher $pH_i$, higher rates of hydrogen ion production and export into the cancer microenvironment than noninvasive cells. It is suggested that modulation of tumor $pH_i$ may affect various cell functions, such as proliferation, apoptosis, and chemotherapy resistance [22, 23].

Which hormones can modulate $pH_i$ and influence the development of these effects? Insulin is an important hormone that can stimulate the functioning of $Na^+$-$H^+$ exchangers, increasing $pH_i$ and enhancing glycolysis, which ultimately stimulates tumor growth [24-26]. Elevated levels of insulin accelerate the development of various human malignancies, whereas its inhibition, on the contrary, delays their growth [27]. High glucose loads (with or without insulin) can also stimulate oncogene expression and accelerate the cell cycle [28]. Therefore, antidiabetic drugs that lower glucose levels, such as metformin or phenformin, have a negative effect on cancer development, in particular, *metformin* – by reducing intracellular pH in aggressive cancer cells [29]. This effect can be enhanced by combinations of drugs consisting of mitochondrial complex I inhibitors and $H^+$-ionophores, which cause specific death of cancer cells due to a decrease in $pH_i$ [30]. Other combinations of drugs are also effective; in particular, a combination of phenformin with oxamate, a lactate dehydrogenase-A inhibitor, increases synergistic anticancer efficacy [30-31]. It should be noted that the resistance of cancer cells to anticancer drugs such as doxorubicin or cisplatin depends on the level of expression and activity of P-glycoprotein, which is responsible for multidrug resistance of cancer cells and controls $pH_i$. It has been experimentally shown that such cellular resistance to doxorubicin increases more than 1000-fold at $pH_i$ 7.4 compared to pHi 6.9 [32]. A detailed review of observations on the role and influence of $pH_i$ in response to various anticancer chemotherapeutic agents is presented in [23]. It is assumed that the use of $H^+$-ionophores is promising for increasing the effectiveness of treatment of cancer patients.

Particular attention should be paid to *melatonin*. This is a well-known compound produced endogenously and controlling circadian rhythms, apoptosis and the host immune response. Melatonin has also been shown to suppress aerobic glycolysis and metastasis of malignant cells. This effect correlates with the activation of mitochondrial metabolism and suppression of Warburg metabolism, which increases the activity of the pyruvate dehydrogenase complex, deacetylase and sirtuin 3 that control the differentiation of cancer cells [33]. An example of this effect of melatonin are embryonic gastric cancer cells, which, after their *in vitro* treatment with melatonin, acquire a

more mature, differentiated phenotype, compared to untreated cells. Melatonin also triggers differentiation in gastric adenocarcinoma cells, which confirms its anticancer potential [33]. Another promising compound that inhibits the $Na^+/H^+$ antiporter and acidifies the cell cytoplasm is *amiloride*. It inhibits vascular growth and is able to completely abolish the metastasis process after transplantation of mammary gland cancer cells in rats [23]. *Dichloroacetate* is also able to inhibit aerobic metabolism by shifting $pH_i$ to the acidic side, inducing apoptosis and suppressing the proliferation and metastasis of cancer cells. Dichloroacetate was especially effective against highly metastatic tumors [34]. $H^+$ ionophores such as monensin and salinomycin are often used in veterinary practice. However, *salinomycin* is also able to act on cancer stem cells, in particular, melanoma, suppressing the formation of metastases in the liver [35].

The mechanisms by which $pH_i$ controls cell behavior remain an open question. In this regard, the ability of $pH_i$ to influence the expression of eukaryotic genes via epigenetic mechanisms attracts particular attention. A correlation was previously found between the alkaline $pH_i$ inherent in malignant cells and lower cellular levels of histone acetylation. This observation suggested that the use of histone deacetylase inhibitors that disrupt the buffering capacity of $pH_i$ in cancer cells could be promising for clinical testing to improve the treatment outcomes in cancer patients [36]. It has been established that *butyrate*, one of the most widely studied short-chain fatty acids, acts as a histone deacetylase inhibitor, negatively affecting undifferentiated cancer cells, including stem cells [37-39]. This effect can be explained by the fact that healthy colonocytes primarily break down butyrate by β-oxidation and use it as an energy source, whereas cancer cells or undifferentiated colonocytes in which the Warburg effect is activated preferentially use glucose rather than butyrate for their energy needs. As a result, butyrate accumulates and induces epigenetic changes in the expression of genes that stimulate cell differentiation [40]. The opposite effect is exerted by lactate, which suppresses differentiation in malignant cells at chronic activation of Warburg metabolism and its key enzyme, lactate dehydrogenase [41]; as a result, lactate accumulates in the microenvironment of cancer cells.

## DUAL DEFENSE OF MALIGNANT CELLS AND ITS KEY TARGETS

How can the apparent $pH_i$–$pH_e$ cancer paradox be explained? One possible explanation is that cancer cells are able to protect themselves by building two “lines of defense” both within the cells and by creating a protective microenvironment in which the antitumor immune response is suppressed. Indeed, aggressive cancer cells differ from mature normal cells or differentiated tumors in many important characteristics: increased survival, invasiveness, and low differentiation. Malignant cancer cells also demonstrate resistance to oxidative stress and metabolic flexibility necessary for survival in an environment with limited nutrients and oxygen

[42–43]. Moreover, hypoxia supports additional survival of cancer cells to many therapeutic interventions. The metabolic features of normal and benign tumor cells do not allow them to develop under hypoxic conditions [14], whereas cancer cells, on the contrary, have acquired the ability to survive and evade the immune response under hypoxic conditions. How do cancer cells gain a selective advantage for survival in these conditions, and could the Warburg effect be involved in this process?

Cancer cells are more dependent than normal cells on the efficiency of their antioxidants, in particular, glutathione, thioredoxin and their regulators [43-44]. Glutathione acts as a redox buffer, which is present mainly in the reduced form inside the cells. Nicotinamide adenine dinucleotide phosphate (NADPH) is an important cofactor of glutathione reductase and thioredoxin reductase. These enzymes are able to regenerate reduced glutathione or thioredoxin from its oxidized forms. Activated glycolysis and the pentose phosphate pathway are the main sources of cytoplasmic NADPH in cancer cells, the level of which is associated with the redox state of the cells. Decreased NADPH levels cause oxidative stress due to increased free radical production, while increased NADPH can lead to resistance to oxidative stress [45]. One of the main properties of cancer metabolism is the increased production of NADPH, which is involved in enhancing the activity of key antioxidants. As a result, cancer cells are more resistant to free radical damage than normal cells [46]. Thus, the determination of the level of endogenous NADPH in single living cancer cells can be used as an indicator of their resistance to oxidative damage. Experimentally, this can be determined *in vitro* by measuring the level of endogenous NADPH fluorescence in single cancer cells, as well as by measuring their ability to partially restore the intensity of NADPH fluorescence after oxidative damage. This method can help to detect individual aggressive cancer cells that have a higher resistance to oxidative stress than other cancer cells [45]. It can also be useful for the search for new antitumor drugs that are able to more effectively destroy aggressive cancer cells by reducing their NADPH levels. Similar effects are demonstrated by drugs such as sulfasalazine (a glutathione inhibitor) and chaetocin (a thioredoxin inhibitor) [46]. Interestingly, the antidiabetic drug metformin exerts its anticancer effects, in particular, by reducing the NAD/NADH ratio in the cytoplasm. A combined use of metformin and resveratrol has been shown to be more effective in the treatment of aggressive forms of breast cancer [47]. Many prooxidant small molecules have also been tested in clinical settings in the study of various forms of cancer. It has been found that imexon, which binds to thiols, is able to suppress the antioxidant effect of glutathione, which increases the level of free radicals and leads to the death of cancer cells. This compound has been successfully tested against non-Hodgkin's lymphoma [48].

Current studies have established a close relationship between glucose and lipid metabolism inherent in cancer cells. In particular, an increase in NADPH in the cytoplasm of cancer cells, which is necessary to maintain lipid synthesis. Unlike normal cells, which use exogenous fatty acids for their needs, most cancer cells can actively synthesize fatty acids themselves [49-50]. Moreover, lipid synthesis pathways in aggressive cancer cells are usually chronically activated, and lipid breakdown processes are inhibited [51]. Synthesized lipids are often stored in special reservoirs – lipid granules (LG)/lipid droplets (LD), which can subsequently be used as an important source of energy necessary for the survival of cancer cells in unfavorable conditions. LG are also able to act as an intracellular lipid buffer, preventing the toxic effect of free fatty acids by isolating them in the form of neutral triglycerides [50-52]. Accumulation of LG in cancer cells can be enhanced by hypoxia/acidosis in the cancer microenvironment, which is observed during the steady-state growth phase of ascites cancers *in vivo* [53-54]. However, our study has shown that intracellular LG and their orderly packed clusters can appear already at the mitotic stage, in particular, in dividing Seidel ascites hepatoma cells. Importantly, these aggressive cancer cells are also a source of extracellular LG, which enter the ascites fluid from their surface LG-containing vesicles [52]. As a result, the cancer microenvironment is enriched with lipids that can reprogram the metabolism of various immune cells, suppressing their antitumor response. This assumption was confirmed in the study of dendritic cells, natural killer cells, tumor myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), and T-regulatory lymphocytes. For example, when dendritic cells accumulate lipids, they acquire a tolerant phenotype, losing the ability to cross-present the antigen necessary for recognition of immune cells, which ultimately increases the survival of cancer cells [55]. When accumulating lipids from the cancer microenvironment, MDSC cells also support cancer development by blocking the antitumor response of T lymphocytes [54]. Under similar conditions, TAM macrophages and natural killer cells also increase the expression of receptors responsible for lipid transport. This leads to intracellular accumulation of lipids, which suppresses the ability of these cells to recognize and destroy cancer cells [56-59]. The use of specific inhibitors of C75 fatty acid synthetase, which reduce the entry and accumulation of fatty acids into LG, as well as LG degradation by tumor macrophages, made it possible to normalize the antitumor activity of TAM [60].

Recent studies have established the ability of LG to both accumulate various cytokines that suppress the immune response and express cyclooxygenase-2 [59], which maintains a tolerant immune response. In other words, similar pathways that reprogram lipid metabolism can be activated in different immune cells. These pathways can act synergistically to suppress the anti-cancer immune response when cancer cells are in an acidic, lipid-rich microenvironment. Figure 1 illustrates how cancer cells are able to protect themselves by building two “lines of defense”

both inside the cells and creating a protective microenvironment around themselves in which the anti-tumor immune response is suppressed. As a result, malignant cells rich in lipids gain an advantage both for their survival and for evading immune surveillance. Since lipogenesis is not characteristic of normal cells, the search for anticancer compounds among inhibitors of lipogenic enzymes and their signaling cascades is promising for the development of new anticancer drugs.

## DOES THE TISSUE "TUMOR FIELD" MIMIC THE ACTION OF A NON-HEALING WOUND?

Common epigenetic and stromal changes have previously been identified in both peritumoral tissue and in wound healing regions [61, 62]. Virchow was the first to establish a link between tissue injury and cancer progression [63]. This idea was subsequently formulated as the concept that "cancer acts like a wound that does not heal". It has been proposed that tumor cells can induce the emergence of a stroma required for further cancer cell growth that is very similar to the stroma in a non-healing wound. This concept is based on the identification of similar cellular and molecular changes found in non-healing wounds caused by both injury and the cancer microenvironment that develops in response to chronic cycles of tissue damage and repair [62]. Many authors have suggested that cancer may arise from chronic tissue injury if homeostatic signals for stem cell renewal are continually reactivated during adult tissue repair. Under these conditions, tumor cells can originate from tissue stem cells or dedifferentiated somatic cells, both of which acquire embryonic-like markers [63-64]. It has been proposed [65-67] to consider cancers as a group of different diseases resulting from a "disruption of the embryonic cell development program". The program can be disrupted at different stages of fetal development. However, fetal and adult tissues use two different types of strategies for repair and healing of wound injuries. Wound healing in the fetus occurs rapidly by regeneration without inflammation and scar formation, while in adults it is accompanied by inflammation and scar formation. During pregnancy, fetal and maternal (adult) tissues are not in direct contact, whereas invasive cancers are in close proximity to surrounding tissues that heal poorly after surgical trauma [68-70]. Both trauma and cancer development may be associated with a process called epithelial-mesenchymal transition, in which intercellular adherent epithelial cells acquire mesenchymal-like characteristics, including high migratory and invasive capacity and lower cellular–extracellular adhesion. Because of this, invasive cancer and its tissue microenvironment can usually be repaired by recruiting adjacent wound fibroblasts into the tumor stroma. The unexpected appearance of these fibroblasts supports the idea that "a tumor is a wound that does not heal" [62, 67].

A large number of cellular and molecular studies have found that invasive cancers, particularly carcinomas, activate a latent host wound healing program, but in a chronic manner. It

has been shown that malignant progression positively correlates with the emergence of an EMT state in aggressive cancer cells [68]. The EMT process is often associated with the development of metastasis and resistance in cancer cells, and metabolic targets that support its development are therefore therapeutic targets in cancer treatment. A study showed that in resistant prostate cancer cell lines, ethyl pyruvate, as an inhibitor of lactate dehydrogenase, effectively blocks the EMT phenotype, as evidenced by decreased vimentin expression and increased E-cadherin protein levels. Ethyl pyruvate treatment also inhibits the ability to form tumor spheres *in vitro* [69]. Another study found ethyl pyruvate to have a therapeutic effect on leukemia cells while being safe for normal blood cells [70].

## ON THE NEGATIVE INFLUENCE OF THE "TUMOR FIELD" ON THE DIFFERENTIATION OF TUMOR CELLS

A metabolic feature of undifferentiated and aggressive cancer cells is their ability to obtain essential nutrients from an environment often depleted of nutrients and to use them subsequently both to maintain their survival and to build new tumor cells. However, the population of cancer cells is heterogeneous, it contains both rapidly dividing cancer cells and slowly proliferating stem cells, as well as a population consisting of their immature descendants that do not exhibit the ability to differentiate under "tumor field" conditions.

Which metabolic regulators from the "tumor field" are able to maintain the undifferentiated state of cancer cells? What factors can induce the maturation of immature, poorly differentiated cancer cells? Previous studies have shown that hypoxia stimulates the transition of highly differentiated neuroblastoma cells to a more immature, poorly differentiated state. The emergence of such immature cells is considered a sign of cancer aggressiveness with a poor clinical prognosis. Using a model of neuroblastoma cell differentiation, it has been shown that hypoxia suppresses differentiation by decreasing acetyl-CoA levels, which in turn suppresses histone acetylation, accessibility to chromatin, in parallel with an increase in glycolysis and disruption of oxidative phosphorylation [71, 72]. Moreover, under hypoxic and stress conditions, neuroblastoma cells also express β3-adrenergic receptors (β3-AR), which can participate both in the activation of proliferation and in the regulation of the transition between the stem state of cells and differentiation, using cross-interaction with sphingosine kinase 2 receptors. Under experimental conditions both *in vivo* and *in vitro*, a specific β3-AR antagonist is able to inhibit neuroblastoma cell growth and tumor progression by switching these cells from an immature stem state to cell differentiation [73].

Acidosis, like hypoxia, is a distinctive feature of the "tumor field". Important sources of increased extracellular $H^+$ levels in the tumor microenvironment may be both activation of

Warburg metabolism, accompanied by lactate secretion, and activation of hydration processes of $CO_2$ molecules formed during decarboxylation of intermediate metabolic products, which also leads to the production of $H^+$ [7]. It has been established that extracellular acidosis supports the further development of cancer cells, which is achieved both by increasing the invasive potential of the cancer cells themselves and by their evasion of the host's immune surveillance. Russell et al. suggest that lactate accumulation in the "tumor field" is capable of maintaining an undifferentiated state in malignant cells, in particular, in melanoma cells [8]. This hypothesis is based on experimental evidence that chronic adaptation to acidosis stimulates the expression of markers characteristic of immature stem cells, which increases the survival of cancer cells and their ability to grow and trans-differentiate *in vitro* and *in vivo*. However, this acidosis-induced stem cell phenotype of melanoma cells was reversible and was associated with epithelial-mesenchymal transition. Moreover, chronic acidosis also induces the differentiation of monocytes into macrophages, which acquire pro-tumor and inflammatory characteristics. These macrophages produce tumor growth factors, inflammatory cytokines and chemokines. However, this effect is reversible and is probably associated with the stabilization of HIF1α [7]. Lactate has also been reported to affect the process of stem cell maturation in the bone marrow [74].

Hussain and co-authors [75] have shown that addition of lactate to the culture medium before induction of bone marrow stem cell differentiation results in a significant increase in the production of myeloid-derived suppressor cells (MDSC) as compared to the controls. MDSC have predominantly immunosuppressive effects and play an important role in cancer development. It can be assumed that the use of agents that control extracellular acidosis in the cancer microenvironment may be of great importance in clinical practice for the treatment of aggressive forms of cancer. For this reason, numerous groups are working on the development of therapeutic approaches to inhibit or target tumor acidity. These approaches can be divided into two groups: (1) direct approaches using oral buffers, diets, or targeted agents to increase tumor pH; (2) development or reuse of acid-activated proton pump inhibitors [76-78]. Therefore, the search for other potential inhibitors of the Warburg effect may be considered as a promising pharmacological target for the treatment of cancer, one example of which is the use of cyclic AMP (cAMP), which suppresses the Warburg effect by switching aggressive, poorly differentiated glioblastoma cells to more mature astroglial cells. Importantly, cAMP activators also caused inhibition of tumor growth and differentiation in patient-derived glioblastoma samples [79].

## WARBURG EFFECT: AN EPIGENETIC REGULATOR OF CELL DIFFERENTIATION

Presumably, eukaryotic cells evolved the ability to differentiate after they acquired mitochondria, the descendants of ancient aerobic bacteria. Disruption of mitochondrial respiration and increased glycolysis in eukaryotic cells often leads to the development of cancer and is the essence of the Warburg theory of cancer origination. However, the question remains: why do different eukaryotic/somatic cells become dedifferentiated as soon as their mitochondrial energy supply is replaced by glycolytic energy? Warburg associated this effect with irreversible disruptions in mitochondria and considered them as a common cause of the development of different forms of cancer [3]. Many studies have found no irreversible injury in cancer mitochondria. However, a number of mitochondrial metabolites have been found to be substrates for nuclear enzymes that regulate the expression of nuclear genes through epigenetic mechanisms. In other words, changes in the functional state of mitochondria can affect which nuclear genes are active and which are not. This effect proved to be potentially reversible and was capable of controlling cell differentiation [4, 79]. Such critical substrates include: (1) acetyl coenzyme A [acetyl-CoA], a substrate for histone acetyltransferases; activation of this enzyme stimulates transcription of genes responsible for stress response; (2) α-ketoglutarate (KG), a critical substrate of α-KG-dependent dioxygenases, an enzyme that regulates DNA demethylation; (3) succinate and fumarate, the metabolites that inhibit the activity of α-KG-dependent demethylase; (4) $NAD^+$, it regulates the activity of $NAD^+$-dependent deacetylase; (5) 2-hydroglutarate, it inhibits α-ketoglutarate-dependent demethylase (data taken from [77, 79]). Which of these metabolic pathways support cell dedifferentiation in the Warburg effect and how can it be restored?

It is known that the Warburg effect suppresses cell differentiation, in particular by inhibiting the conversion of pyruvate to acetyl-CoA, the reduction of which reduces histone acetylation and suppresses the expression of genes responsible for differentiation. Acetate supplementation restores chromatin acetylation and increases tumor cell differentiation under hypoxia [79, 80]. Butyrate (the end product of intestinal bacterial fermentation) also triggers differentiation in HT-29 adenocarcinoma cells, in particular by increasing histone acetylation [72] and/or inducing differentiation in cancer stem cells [80, 81]. Another pathway by which the Warburg effect may act is to induce cell dedifferentiation, in particular by disrupting DNA methylation. In this case, the expression of lactate dehydrogenase and M2 isoform of pyruvate kinase, the key enzymes of glycolysis, increases [82]. In other words, the Warburg effect can induce structural changes in the DNA epigenetics of mature cells, which causes their transformation into immature embryonic-like cells that are already capable of maintaining Warburg metabolism [83]. Many of the above effects are associated with disturbances in the functioning of mitochondria. What compounds are potentially capable of restoring the functioning of cancer mitochondria? Such compounds, first of all, include retinoic acid (RA), a derivative of

vitamin A with antitumor properties. RA has been found to be capable of inducing cell differentiation, stimulating the biogenesis of mitochondria and their functional activity [82]. RA has proven to be very effective in the treatment of neuroblastoma and acute promyelocytic leukemia [78,84]. Cyclic AMP (cAMP) also has the ability to stimulate differentiation in human glioma cells, since it suppresses the Warburg effect, enhancing mitochondrial biogenesis [78]. It has been shown that cAMP triggers neuronal differentiation and suppresses the self-renewal capacity of glioma stem cells. Importantly, cAMP induction is mediated through melatonin receptors, the expression of which has been found in many cell lines [78, 84]. Melatonin can also significantly enhance mitochondrial energy metabolism and suppress the Warburg effect, stimulating tumor cell differentiation [33]. Thus, it is possible to change gene readout patterns in tumor cells. Such epigenetic "switchovers" may be reversible, but the question remains as to how stable they are to be used in clinical practice.

**Conclusion**

Currently, most scientists agree that the initiation of a malignant tumor depends on two groups of factors: on the one hand, on hereditary predisposition or immunity of the body; on the other hand, on external influences that change the body's resistance to cancer during life. The survival rate of cancer cells depends on the degree of their differentiation, which largely determines the severity of the disease and the effectiveness of treatment. One of the ways to control the survival of cancer cells is to influence their reprogrammed metabolism. The Warburg metabolism has been found to disrupt cell maturation, inducing dedifferentiation in them by epigenetic mechanisms [4, 78]. For this reason, research into the mechanism of dedifferentiation can determine new directions in the diagnosis and therapy of oncological diseases. It is known that undifferentiated cancer cells are characterized by high resistance and aggressiveness; they are also capable of creating a protective "tumor field" around themselves, in which antitumor immunity does not work. This adaptive response may arise due to multiple cell-metabolism regulation changes responsible for the increased survival of cancer cells and their growth under unfavorable environmental conditions. As a result, such cancer cells acquire resistance to radio- and chemotherapy. This review analyzes metabolic pathways, their key targets, and inhibitors capable of suppressing the aggressive behavior of cancer cells, which can increase treatment efficacy and be further utilized in developing combination therapies. To achieve this goal, however, it is first necessary to investigate how metabolic inhibitors act in combination with chemotherapy and immunotherapy. Despite the ongoing intensive research in this field [85–90], as well as the experimental and preclinical trials of Warburg effect inhibitors [91–101] presented in Table 1,

research into these compounds is still underway. Knowledge of metabolic oncotargets and their inhibitors can also be successfully applied to prevent the development of various forms of cancer.

**List of abbreviations**.

2-deoxy-D-glucose (2-DG); 3-bromopyruvate (3-BP); acetyl coenzyme A (acetyl-CoA); α-ketoglutarate (KG); AMP-activated protein kinase (AMPK); adenosine triphosphate (ATP); β3-adrenergic receptors (β3-AR); cyclic AMP (cAMP); dichloroacetate (DCA); epithelial–mesenchymal transition (EMT); extracellular pH (pHe); fluorodeoxyglucose (FDG); glyceraldehyde-3-phosphate dehydrogenase (GAPDH); glucose transporters (GLUT1/GLUT4); hexokinase (HK/HK2); hypoxia-inducible factor (HIF); intracellular pH (pHi); lactate dehydrogenase A (LDHA); lipid droplets (LD); lipid granules (LG); mechanistic target of rapamycin (mTOR); monocarboxylate transporters (MCT1/MCT4); myeloid-derived suppressor cells (MDSCs); nicotinamide adenine dinucleotide ($NAD^+$); reduced nicotinamide adenine dinucleotide (NADH); nicotinamide adenine dinucleotide phosphate (NADPH); pyruvate dehydrogenase (PDH); pyruvate dehydrogenase kinase (PDK); pyruvate kinase M2 (PKM2); retinoic acid (RA); tumor-associated macrophages (TAM).

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## CONFLICTS OF INTEREST

As the author of this work, I declare that I have no conflict of interest.


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**Table 1. The list of drugs used to interfere with the Warburg effect and the several other effects due to the increment of glycolysis.**

| Drug / Compound | Target / Mechanism of Action | Stage of Development / Notes |
|---|---|---|
| **2-Deoxy-D-glucose (2-DG)** | Inhibits Hexokinase (HK); competitive inhibitor of glucose | Clinical trials / Research; disrupts both glycolysis and N-linked glycosylation [91] |
| **3-Bromo-pyruvate (3-BP)** | Inhibits Hexokinase II (HK2) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). | Preclinical / Research; powerful alkylating agent that depletes ATP [92] |
| **Dichloroacetate (DCA)** | Inhibits Pyruvate Dehydrogenase Kinase (PDK). | Clinical trials; reactivates PDH, shifting metabolism from glycolysis back to oxidative phosphorylation [93] |
| **Lonidamine** | Inhibits Hexokinase II (HK2) and mitochondrial respiration. | Approved in some countries / Clinical trials; alters mitochondrial membrane potential [94] |
| **FX11** | Selective inhibitor of Lactate Dehydrogenase A (LDHA). | Preclinical; reduces lactate production and inhibits tumor growth [95] |
| **Phloretin / Ritonavir** | Inhibitors of Glucose Transporters (GLUT1/GLUT4). | Research / Repurposed drugs; block glucose uptake into cancer cells [96] |
| **WZB117** | Small-molecule inhibitor of Glucose Transporter 1 (GLUT1). | Preclinical; downregulates glycolysis and intracellular ATP levels [97] |

| | | |
|---|---|---|
| **Shikonin** | Specific inhibitor of Pyruvate Kinase M2 (PKM2). | Preclinical / Natural compound; induces necroptosis by targeting glycolytic flux [98] |
| **AZD3965** | Inhibits Monocarboxylate Transporter 1 (MCT1). | Clinical trials; blocks lactate efflux, leading to intracellular acidification [99] |
| **Syrosingopine** | Dual inhibitor of Monocarboxylate Transporters (MCT1 and MCT4). | Preclinical research; disrupts lactate export, especially when combined with Metformin [100] |
| **Metformin** | Inhibits Mitochondrial Complex I; indirectly suppresses mTOR signaling. | Widely used clinical drug / Investigated in oncology; alters cellular energy sensing (AMPK activation) [101] |

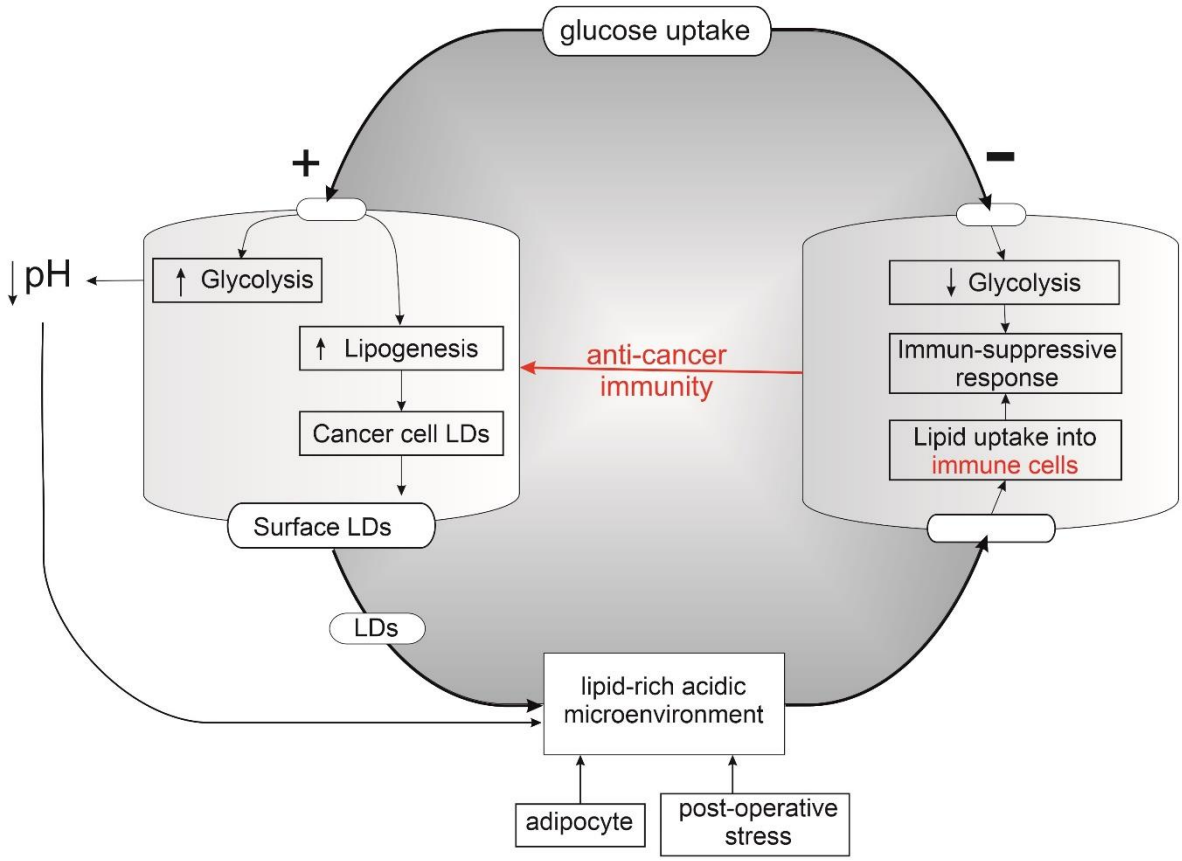


**Figure 1:** A working model illustrating the central role of inverse metabolic dysregulation between cancer and immune cells, namely, in their glucose-lipid pathways. Unlike immune cells, cancer cells are capable of efficiently taking up glucose and upregulating fatty acid synthesis, accumulating the resulting lipids in lipid droplets (LDs). Together with adipocyte-derived LDs, cancer-derived LDs can be involved in a creation of lipid-rich acidic microenvironment, in which different immune cells loss the ability to recognize and kill cancer cells. As a result, malignant cells gain an advantage in survival, growth, and evading the immune response by residing in a lipid-rich, acidic microenvironment.