Historical perspective of tumor glycolysis: a century with Otto Warburg

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Abstract

Tumors have long been known to rewire their metabolism to endorse their proliferation, growth, survival, and invasiveness. One of the common characteristics of these alterations is the enhanced glucose uptake and its subsequent transformation into lactic acid by means of glycolysis, regardless the availability of oxygen or the mitochondria effectiveness. This phenomenon is called the "Warburg effect", which has turned into a century of age now, since its first disclosure by German physiologist Otto Heinrich Warburg. Since then, this peculiar metabolic switch in tumors has been addressed by extensive studies covering several areas of research. In this historical perspective we aim at illustrating the evolution of these studies over time and their implication in various fields of science.

Keywords: aerobic glycolysis, Warburg effect, Reverse Warburg effect, tumor metabolism, historical perspective

Introduction

Cancer has classically been considered only as a genetic disease provoked by mutations in genes that control cell growth and division. Nonetheless, more recently cancer was alternatively reconsidered as a metabolic disease involving alterations in energy production.[1,2] Cancer cells are characterized by an unrestrained proliferation, thus requiring a large amount of nutrients and energy, which consequently causes changes and adaptations in their metabolic profile. This metabolic reprogramming is essential to support the rapid growth of the tumor even in adverse conditions, such as limited oxygen and nutrient availabilities.[3–5] Dysregulation in carbohydrate, aminoacid and lipid metabolism are the main characteristics of cancer cell alterations.[6,7] In contrast to healthy cells, most cancer cells produce energy *via* glycolysis even under normoxic conditions and with fully functioning mitochondria: the metabolic switch from the oxidative to the glycolytic pathway is known as “aerobic glycolysis” or the “Warburg effect”. Enhanced aerobic glycolysis is one of the most common phenotypes found in tumors and it is a well-established hallmark of cancer.[8]

The present perspective has the aim to retrace the main historical stages of the understanding of tumor glycolysis in cancer biology starting from the first findings of Otto Warburg in the early 1920s until the present day. Moreover, a general overview about the development of anti-cancer therapies exploiting tumor glycolysis is also shortly introduced.

Otto Warburg, the father of tumor glycolysis

Glycolysis is a biochemical process that converts one molecule of glucose into two molecules of pyruvate to generate energy (ATP). Glucose is internalized by glucose transporters (GLUTs) into the cytosol and its demolition starts with sequential phosphorylation/isomerization reactions operated by hexokinase (HK), glucose-6-phosphate isomerase (GPI) and phosphofructokinase (PFK), thus forming fructose-1,6-bisphosphate (Figure 1). Then, aldolase (ALD) demolishes the 6-carbon skeleton of fructose-1,6-bisphosphate into two 3-carbon units: glyceraldehyde-3-phosphate and dihydroxyacetonephosphate (Figure 1). These molecules quickly interconvert into each other thanks to triosephosphate isomerase (TPI). Glyceraldehyde-3-phosphate undergoes a sequence of reactions catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase (ENO) and finally pyruvate kinase (PK) leading to the generation of two molecules of pyruvate (Figure 1), thus reaching the “bifurcation point” of the glycolytic pathway.

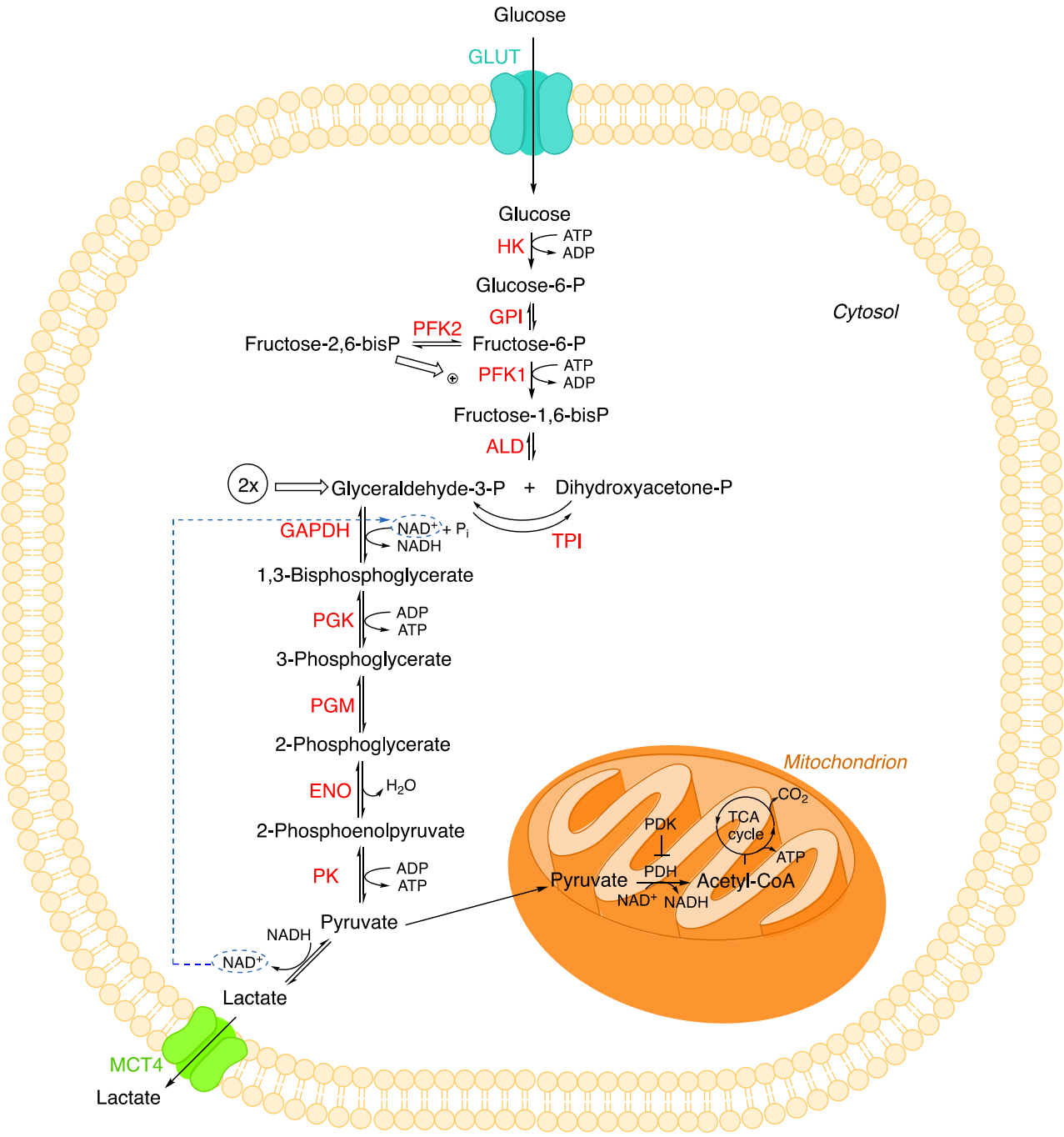

Figure 1. The glycolytic pathway.

This metabolic pathway does not require the presence of oxygen and in normal conditions it is usually coupled to mitochondrial oxidative phosphorylation (OXPHOS). Therefore, pyruvate is oxidized in mitochondria by pyruvate dehydrogenase (PDH) to acetyl-CoA, which enters the tricarboxylic acid cycle (TCA) and is converted to CO_2 and ATP. In the case of low oxygen concentrations (hypoxia), OXPHOS cannot occur, thus pyruvate is transformed to lactate by lactate dehydrogenase (LDH) and

regenerates the oxidized cofactor NAD^+, which is crucial for the progression of glycolysis even under oxygen deprivation conditions. Finally, lactate is extruded by monocarboxylate transporter 4 (MCT4) into the extracellular space to maintain a stable intracellular pH.

Oppositely to healthy cells, the metabolism of cancer cells mainly relies on glycolysis, uncoupled to OXPHOS, even under normal oxygen concentrations and fully functioning mitochondria. This metabolic reprogramming leads to high levels of lactate in the tumor microenvironment that becomes more acidic. Tumor acidosis plays an important role in cancer proliferation because it favors cancer cell adaptation to hypoxic conditions and stimulates tumor growth and invasiveness. This effect is known as the "Warburg effect" after Otto Warburg, the biochemist who first proved this metabolic alteration in cancer.

Otto Warburg started to investigate cancer cell metabolism at the beginning of 1920s aiming to better understand the origin of tumors and the cause of their rapid growth.[9,10] One of the first studies concerning this topic dates back to 1925, where he expected to find a high increase in respiration rate of carcinoma cells compared to normal epithelium.[11] Curiously, the tissue selected by Warburg (the Flexner rat carcinoma) showed an unexpected lower respiration rate than normal kidney and liver tissues. However, the Flexner rat carcinoma exhibited an enhanced lactate production that made markedly acidic the medium containing the slices of tumor tissue. Warburg ascribed the augmented levels of lactate to a high "glycolytic capacity" (*glykolytische Fähigkeit* in German) of this type of carcinoma cells.[9,12]

In the same year, pharmacological studies conducted by the spouses Carl and Gerty Cori confirmed that Warburg's *in vitro* findings also occurred *in vivo*; indeed, large amounts of glucose were converted into lactic acid in tumor tissues of living animals.[12–14]

Further *in vitro* and *in vivo* investigations on the metabolism of tumors conducted in Warburg's laboratory, such as the chance of killing tumor cells by lack of oxygen, highlighted that the presence of oxygen could not inhibit glycolysis, in disagreement with the Pasteur effect that, instead, describes

an inhibiting effect of oxygen on the production of lactate (fermentation process) in living cells. Thus, Warburg hypothesized that an impairment in respiration could lead to carcinogenesis.[15,16]

Two years later, the English biochemist Herbert Crabtree, explored the heterogeneity of glycolysis in several strains of mouse tumors.[17] Warburg's findings were corroborated, but he also found out that the magnitude of respiration in tumors was highly variable. Crabtree concluded that cancer cells exhibited high aerobic glycolysis to produce energy for their unbridled proliferation; moreover, he supposed that there was variability in the intervention of fermentation that was caused by environmental or genetic causes.[17,18]

In 1956, Warburg formally postulated his metabolic theory on the origin of cancer, assessing that dysfunctions in mitochondria constitute the basis of tumor aerobic glycolysis.[19] In particular, the he emphatically affirmed that cancer cells originated from healthy body cells in two phases: *i)* the first phase consisted in the irreversible damage of respiration, *ii)* the second phase was the replacement of the permanently loss of respiration energy with fermentation energy (conversion of glucose to lactate). Later, researcher Efraim Racker will be the first to coin the term "Warburg effect" in 1972 to indicate the augmented glycolytic capacity of tumors.[20]

Despite the Warburg theory about the origin of cancer cells convinced many researchers of that time, some of them raised reservations over this hypothesis. In the 1950s, Britton Chance, Professor of Biophysics and Physical Biochemistry at the University of Pennsylvania, rejected Warburg's hypothesis, because he found out that rates of respiration for ascites cells were similar to those of muscle and yeast cells. Therefore, he stated that the enhanced secretion of lactic acid was not due to respiration injury as Warburg previously affirmed.[21,22] One of the major critics of Warburg's hypothesis was Sidney Weinhouse, a renowned cancer researcher and member of the American Association of Cancer Research, who published an article standing up against Warburg right after his *"On the origin of cancer cells"* publication in Science in 1956.[23] The confutation of Warburg's theory was principally based on copious findings demonstrating that many cancer cells exhibited high oxygen consumption and CO_2 production. In addition, Weinhouse discredited Warburg proposal

about abnormalities of structure and function of mitochondria because of the absence of significant evidence indicating a permanent respiratory impairment in tumors; indeed, rat hepatoma cells showed fully functioning mitochondria and an active respiratory capacity.[24,25] Only two years later, in 1978, Pedersen extensively described in a detailed review that abundant alterations in the structure and function of mitochondria occurred in cancer cells.[26]

Warburg himself in 1962 admitted that the statement he made about insufficient, rather than damaged, respiration had led to "fruitless controversy".[27,28]

At the time the "anti-Warburg sentiment"[26] was very common, and another prominent researcher working at the Harvard Medical School, Alan C. Aisenberg, published a monograph where he criticized the concept of respiratory defect as the basis of cancer origin.[29] Nevertheless, emerging data continued to strongly supported the high aerobic glycolysis rate of tumors, as later noted by Sidney Colowick and Peter Pedersen.[26,30]

The hypothesis about the central role of mitochondrial dysfunction in the onset and progression of cancer still has remained controversial and only in the 2000s the Warburg effect will become a well-established hallmark of cancer.[8,31]

Recent evolution of the role of tumor glycolysis in cancer biology

Nowadays, glucose metabolism reprogramming is widely considered as a characteristic phenotype of cancer cells that mainly relies on two biochemical events: *i)* aerobic glycolysis and *ii)* augmented glucose uptake.[18] Noteworthy, an important advancement in the field of cancer diagnosis deriving from these considerations was achieved in 1980, when the fluorinated glucose analogue 2-^{18}fluoro-2-deoxy-D-glucose (FDG) was introduced in positron emission tomography (PET) for the imaging of glycolytic cancer cells/tissues.[32] The radiotracer, characterized by the radionuclide ^{18}F bound at the C2 position of 2-deoxyglucose (DG), exploits the Warburg effect, since it selectively accumulates in tumor regions due to the increased glucose uptake consequent to an augmented glycolytic pathway

occurring in cancer cells.[33] Since then, PET-FDG has now become a routine clinical practice in the diagnostic procedure of cancer patients.

Tumor glycolysis raised wide interest in the scientific community and in the late 1980s its regulation by oncogenes was brought to light thanks to investigators who figured out that aerobic glycolysis was a process directly controlled by growth factor signaling. In 1985, Boerner and colleagues showed that preparations of transforming growth factor β (TGF-β) from human platelets stimulated glycolysis in normal rat kidney cells (NRK-49F) after 24 hours of incubation.[34] Another study of the same year by Inman *et al.* corroborated these observations and reported that glucose uptake into mouse 3T3 cells was stimulated by epidermal growth factor (EGF) and TGF-β.[35] Two years later, the accelerated rate of glucose transport characterizing the metabolic transformation of cancer cells demonstrated to be activated by *ras* and *src* oncogenes. Indeed, the resulting increased rate of glucose uptake was the direct consequence of the augmented expression of the structural gene encoding the glucose transport protein.[36,37] Additional investigations confirmed that the glucose transport system was regulated at a transcriptional level by serum growth factors.[38]

It was then that the Warburg hypothesis found a new implication in cancer biology and targeting tumor glycolysis became an even more appealing strategy to develop new potential anti-cancer agents.[39]

At the end of the 1990s, new evidence supported the correlation between cellular metabolism and cell death, cell proliferation and tissue homeostasis.[40–42] A relevant step forward in this period was achieved in 1996 by Liu and collaborators who demonstrated that mitochondria regulated apoptosis by releasing cytochrome *c* in the cytosol.[40] This finding confirmed that the energy production machinery played an important role in the regulation of cell death. At that time, further pharmacological and genetic studies highlighted that the Warburg effect was needed for tumor growth.[18] For example, the regulation of LDH-A expression at transcriptional level by *c-Myc* oncogene and the closely coupling of LDH-A to glucose metabolism demonstrated that tumor glycolysis could be potentially exploited for the development of cancer therapeutics.[41,42]

Starting from the new millennium, the benefits and the evolutionary advantages of aerobic glycolysis in tumors were elucidated.[39] It is well-known that glycolysis is much less efficient than OXPHOS in providing energy, since only two molecules of ATP are generated by each glucose molecule; on the other hand, the TCA cycle in mitochondria normally produces about 36 molecules of ATP for each glucose molecule. Despite this, glycolysis generates ATP very quickly with an ATP production rate of 100 times faster than OXPHOS.[43] This feature confers a selective advantage to glycolytic cancer cells and favors their rapid and uncontrolled growth.[44,45] In fact, it was proved that increased glucose consumption of glycolytic cancer cells has the major function to maintain high levels of glycolytic intermediates to support the biosynthesis of macromolecules that determines cancer cell proliferation.[46,47] Moreover, intracellular ATP levels proved to be a fundamental cause of chemoresistance in colon cancer cells.[48,49]

Another benefit of the Warburg effect for cancer progression is the acidification of the tumor microenvironment because of augmented lactate secretion (lactagenesis). Tumor acidosis allows cancer cells to survive in adverse conditions (such as nutrient-limiting conditions) and to adapt to hypoxia; in addition, it stimulates tumor mass growth and invasiveness.[50] The environmental acidosis also leads to cellular toxicity and protects the cancer cells against attack from the immune system.[45,51,52] Tumor cells are able to adapt to low oxygen concentration conditions by means of the hypoxia-inducible factor 1 (HIF-1). In 2000s, Semenza and collaborators found out that this transcription factor also regulated at transcriptional levels genes encoding for several glycolytic enzymes. Moreover, it also increased the expression of glucose transporters and many other genes correlated to aerobic glycolysis.[53,54] Therefore, tumor environmental acidosis also demonstrated to induce expression of several glycolytic enzymes, thus resulting in high rate of glycolysis.[55]

Importantly, in 2008 Sonveaux and colleagues, found out that lactate, the end-product of glycolysis, was not just a waste product of this biochemical process, but it also acted as an important substrate that fueled the oxidative metabolism of oxygenated cancer cells. In this context, monocarboxylate

transporter 1 (MCT1) was identified as the facilitator of lactate uptake by oxidative tumor cells.[56] Therefore, lactate became a key player in all processes involved in tumorigenesis.[57]

Although it is still unresolved if the Warburg effect is the cause or the effect of carcinogenesis, some proposals about its role were suggested.[12,18] Besides its role in the rapid ATP synthesis, the production of building blocks for cancer growth and the acidification of tumor microenvironment to favor cancer cell survival, the Warburg effect was also believed to implement direct signaling functions to tumor cells.[18,58] Therefore, cellular metabolism and cellular signaling are tightly linked.[59] In particular, tumor glycolysis seemed to be connected to cellular signaling by the generation and the modulation of reactive oxygen species (ROS) and the regulation of chromatin state.[18,60,61] Noteworthy, in 2008 McFate and colleagues highlighted the correlation between pyruvate dehydrogenase complex (PDC) inhibition and the Warburg effect[62] thus giving rise to a new hypothesis that the Warburg effect coincides with the beginning of carcinogenesis.[63]

Very recently, the role of tumor glycolysis in several types of tumors emerged.[64] In 2019 Yang *et al.* demonstrated that pancreatic ductal adenocarcinoma (PDAC), one of the most aggressive malignancies, was characterized by an enhanced glycolysis to ensure its survival in nutrient-limiting and hypoxic conditions caused by its hypovascularization.[65] Moreover, the high-rate glycolysis showed to be strongly associated to pancreatic cancer metastasis.[66] Aerobic glycolysis also plays a central role in breast cancer (BC);[67] indeed, the expression of several glycolytic genes promoting the Warburg effect were directly increased by the transcription factor sine oculis homeobox 1 (SIX1).[64] In 2022, a cohort study conducted by Janniskens and colleagues on male subjects aged 55-69 years old demonstrated that the expression of proteins associated with the Warburg effect, such as GLUT1 and LDH-A, was directly involved in the correlation between adolescent body mass index (BMI) and colorectal cancer (CRC) risk.[68]

It was also shown that the enhancement of tumor glycolysis, thanks to the activation of AKT signaling pathway by the chemokine (C-C motif) ligand 2 (CCL2), promoted chemoresistance in glioma cells.[69]

Reverse Warburg effect and mixed scenarios

An important turning point regarding a modern perspective of the Warburg effect dates back to 2009.[70] In that year the research group of Professor Michael Lisanti observed the occurrence of aerobic glycolysis in the nearby stromal cells or cancer-associated fibroblasts (CAFs), rather than in cancer cells themselves.[71] Therefore, CAFs undergo myo-fibroblastic differentiation and secrete lactate. Epithelial cancer cells can then use these energy-rich substrates in the mitochondrial TCA cycle, thus efficiently generating energy and resulting in a higher proliferative capacity. This revisited two-compartment model was termed the "Reverse Warburg effect". Hence, the Warburg effect was reconsidered as a stromal phenomenon. Moreover, tumors with an augmented percentage of stroma showed to have a worse prognosis, because of their increased lactate production/secretion according to the "Reverse Warburg effect".[70] This vectorial transport of energy-rich metabolites from the fibroblastic tumor stroma to anabolic cancer cells demonstrated to involve monocarboxylate transporter 4 (MCT4), which is normally responsible of lactate efflux from glycolytic muscle fibers and astrocytes in the brain. In 2011, a study conducted by the same research group proved the existence of a stromal-epithelial lactate shuttle in human tumors, thus corroborating the "Reverse Warburg effect".[72]

These findings about the "Reverse Warburg effect" had relevant new implications in cancer research field considering that lactate transport inhibitors could be an innovative and promising therapeutic strategy to tackle cancer disease.[70,72,73]

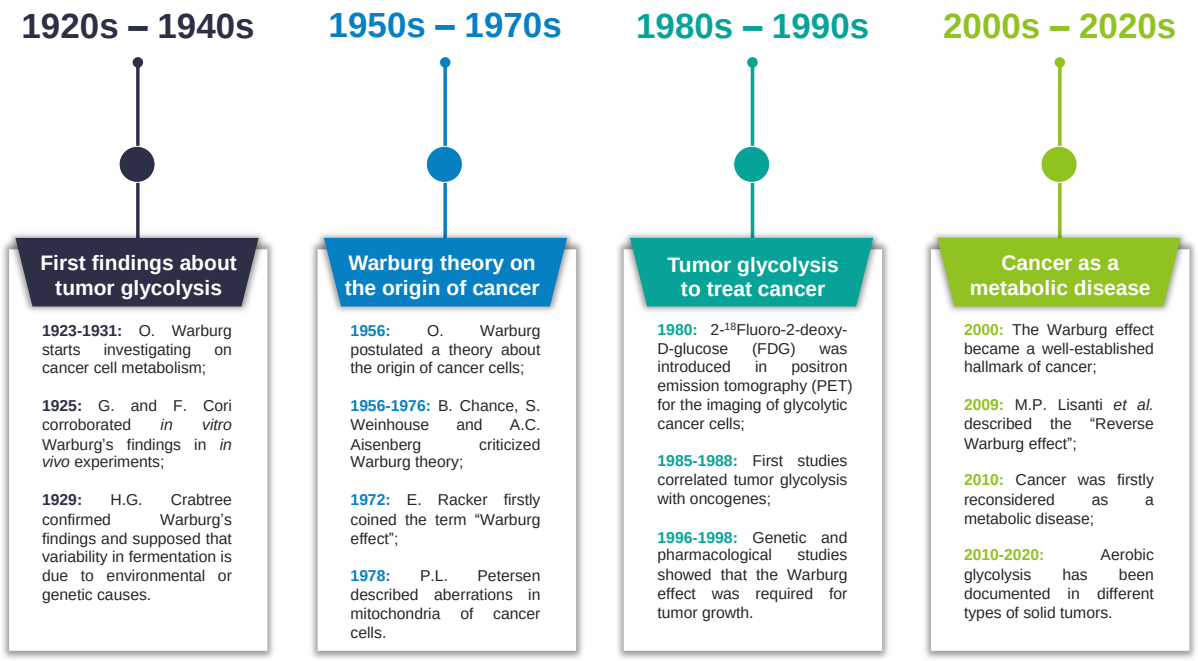

Figure 2. Main historical stages concerning tumor glycolysis from 1920s until today.

As a matter of fact, it was later demonstrated that in real tumors the situation is often more heterogeneous, where both tumor and stroma cells may be characterized by either a Warburg-type or by a Reverse Warburg-type behavior. A representative example of this situation was described in 2013 by a Korean research group, where tissues from 132 triple-negative breast cancer (TNBC) patients were evaluated to classify the subtypes of cancer and stroma cells. The classification was defined as "Warburg-type" (cancer: glycolytic, stroma: non-glycolytic), "Reverse Warburg-type" (cancer: non-glycolytic, stroma: glycolytic), "mixed metabolic-type" (both cancer and stroma: glycolytic), and "metabolic null-type" (both cancer and stroma: non-glycolytic). The results indicated that all these classes were populated to a certain extent, although noticeable differences were found among them. The most abundant class was the Warburg-type (around 60%), followed by the mixed metabolic-type (18%), then by the metabolic null-type (17%) and, finally, by the Reverse Warburg-type, which was displayed only by a 5% of the samples.[74]

Evolutionary advantages of the Warburg phenotype in cancer: a matter of Applied Mathematics and Thermodynamics

Rapidly growing cancers represent an appropriate competitive scenario for the application of mathematical models, such as the "Game Theory", that predict benefits deriving from certain choices. One of these models based on the "Prisoner's dilemma" was developed by Irina Kareva in 2011 to elucidate the competitive benefit that glycolytic cells obtain over their aerobic counterparts, in spite of the lower energetic efficiency of glycolysis.[75] Briefly, when the number of glycolytic cancer cells reach a critical mass, they produce an environment that favors invasion (abundant production of lactate). In tumors there are several trigger events that may contribute to reach this critical mass and overcome the disadvantages of glycolytic metabolism: abundant nutrient availability (glucose concentration), decreased oxygen content (tumor hypoxia), and increased cell turnover (which, unfortunately, may be promoted by some types of chemotherapy regimens too).

An interesting connection between cancer, their bioenergetic profiles, and entropy was described in 2022 by Bartolomé Sabater,[76] who acknowledge that cancer tissues are less organized than healthy tissues and, as such, are characterized by a higher entropy level. Nevertheless, the relationship between tumors and thermodynamics is not straight and clear. In fact, cancer cells displaying the Warburg effect convert glucose to lactate (lower entropy) at a higher rate than that of healthy cells, where most glucose is eventually transformed into carbonic anhydride (higher entropy). This apparent contradiction in terms of entropy stemming out of the different bioenergetic profiles of cancer vs. normal cells was proposed to be in agreement with the Prigogine theorem. In fact, the association of the Warburg effect and cancer is understandable from a thermodynamic point of view in a model where the total rate of entropy production tends to a minimum, as predicted by Prigogine in the case of "dissipative structures", among which Warburg-type cancer cells may now be enumerated.

Implication of the Warburg Effect in infective pathologies: Covid-19

Over the past two years, Warburg effect was found to be correlated with Coronavirus Disease 2019 (Covid-19). In particular, aerobic glycolysis seemed to favor SARS-CoV2 replication in nasal epithelial and pneumocyte cells that express angiotensin-converting enzyme 2 (ACE2).[77]Moreover, the Warburg effect was induced by hypoxia in endothelial cells where, in the presence of atherosclerosis, it supported micro thrombosis and vasoconstriction.[77] Finally, aerobic glycolysis also promoted the activation of neutrophiles and M1 macrophages, thus exerting pro-inflammatory effects.[77] An interesting study showed that the exogenously administration of melatonin, an agent that could be potentially used in the treatment of SARS-CoV2 infection, reversed aerobic glycolysis in immune cells, hence inhibiting the "cytokine storm" that causes the typical massive tissue damage of Covid-19 disease.[78]

Recently, A. Cossarizza and co-workers found a Warburg-type bioenergetic profile in in neutrophils obtained from patients affected by severe Covid-19 pneumonia. These neutrophils display an increased glycolysis associated to a significant overexpression of glucose transporter 1 (GLUT1) and lactate dehydrogenase A (LDHA). Apparently, the metabolic remodeling in these immune cells is responsible for the neutrophil inflammatory response and for the formation of neutrophil extracellular traps (NET).[79]

Development of anti-cancer agents targeting tumor glycolysis

Taking into account the multiple evolutionary advantages that aerobic glycolysis provides to cancer cells (*e.g.*, promoting tumor growth and resistance to chemotherapy) and its role in different types of malignancies, nowadays tumor glycolysis is considered as a feasible target for the development of anti-cancer therapies.[39]

The road to the development of metabolic agents targeting tumor glycolysis has been long and tortuous, and it was only in the 2000s that the pharmaceutical industries started to invest resources in this scientific field.[12,80]

Some of the most relevant examples of anti-cancer agents counteracting tumor glycolysis that entered clinical phase studies are discussed below.

In the past, the only drugs used for several years in cancer chemotherapy were DNA damaging agents, such as alkylating agents and antimetabolites that inhibit nucleic acid synthesis or function. Along with the elucidation of the differences in the genomics, the proteomics and the metabolism of cancer cells, during the past 50 years, new therapeutic approaches began to emerge. In this framework, enzymes and transporters involved in glucose metabolism, especially in the glycolytic process, have started to be increasingly considered as promising targets for the development of new anti-cancer therapies.[81,82]

As said before, the increased glycolytic pathway of the cancer cells reflects a higher glucose uptake. This mechanism was exploited in tumor diagnosis by using ^{18}FDG as imaging agent in PET.[32] Therefore, exploiting the uptake of glucose was considered as one of the first strategies to counteract the metabolic peculiarity of cancer. For example, overexpression of GLUTs by cancer cells was exploited to improve the uptake of anticancer agents by conjugating sugar moieties to small organic molecules[83] or metal complexes with therapeutic or diagnostic purposes.[84]

Two representative examples that reached clinical trials deriving from this approach are SNAP and glucophosphamide (Figure 3).[85]

The same phenomenon of enhanced glucose uptake deriving from overexpression of transmembrane transporter GLUTs represented another direct strategy to tackle tumors. However, very few GLUT inhibitors have reached advanced clinical trials so far, despite their high potential as anticancer agents, probably due to the difficulty to specifically inhibit this protein only in tumors, without affecting normal cells. Some examples of GLUT inhibitors are Silybin, a natural flavonoid with broad biological activities that underwent a phase I clinical trial in patient with prostate cancer (2007),[86] and Fasentin and STF31 that show promising *in vivo* activity (Figure 3).[87,88]

Once the glucose enters the cell, the first enzyme involved in the glycolytic process is hexokinase (HK) that catalyzes the rate-limiting phosphorylation of glucose to give glucose 6-phosphate (Figure

1), in order to trap the molecule inside the cell and then activate it for the glycolytic or the pentose phosphate pathway (PPP). This enzyme was studied in the 2000s as a suitable target for anticancer agents, and a large variety of molecules have been identified as hexokinase inhibitors. Among them, the glucose analog 2-deoxy-D-glucose (2-DG, Figure 3) is one of the most famous competitive HK inhibitors known since the 1970s.[89] 2-DG was used as a starting point in the late 2000s to generate more potent HK inhibitors., such as the non-radioactive FDG (Figure 3),[90] and it recently underwent a phase II clinical trial, terminated due to slow accrual.[91] Nevertheless, it is still studied as a suitable therapeutic agent in combination with radiotherapy or other antineoplastic drugs, such as docetaxel or metformin.[92,93] Another well-known HK direct inhibitor showing synergism with other anticancer agents is Lonidamine (Figure 3), that was also selected for a phase III clinical trial in 2007, unfortunately interrupted because of adverse hepatic effects.[94]

Another well-studied enzyme involved in the glycolytic pathway is GADPH. This enzyme catalyzes the addition of a phosphate group to glyceraldeyde-3-phosphate to give 1,3-diphosphoglycerate with the simultaneous reduction of NAD^+ to NADH (Figure 1).[95] Furthermore, it was demonstrated that GADPH is over-expressed in several malignant cancer types and it also exhibits non-glycolytic effects enhancing cell survival.[96] In 2019, a novel GADPH inhibitor, named GP-2250 (Figure 3), entered a phase I/II study in combination with the chemotherapeutic agent gemcitabine in subjects affected by advanced pancreatic cancer, highlighting its feasible employment in cancer therapy.[97]

Recently, thanks to the increasingly awareness of lactate role in processes involved in carcinogenesis, targeting LDH enzyme and MCTs transporters became a widely explored therapeutic approach to tackle cancer considering their overexpression in cancer cells. As said before, LDH catalyzes the reduction of pyruvate to lactate regenerating cofactor NAD^+ (Figure 1), that is fundamental for the progression of glycolysis. This step enables the cell to sustain itself on glycolysis decoupled from oxidative metabolism in mitochondria, so it represents a crucial point for proliferation in hypoxic environment.[98] At present, only few LDH inhibitors have reached clinical trials. One of them is a natural polyphenolic aldehyde derivative, gossypol (Figure 3), involved in a large number of clinical

trials in combination with other anticancer agents.[99] Noteworthy, it also modulates other targets, such as GADPH, and it has many other biological activities, such as antioxidant properties.[100]

In the past few years, the crucial role of lactate for cancer cells energy production, proliferation, invasion, immune escape, chemo- and radio-resistance has been largely confirmed.[101] The ability of cancer to transport lactate molecules between hypoxic and normoxic region of the tumor ("lactate shuttle") is promoted by monocarboxylate transporters (MCTs). Consequently, MCTs are gaining a great interest as suitable anticancer targets, as demonstrated by AZD3965 (Figure 3), a MCT1 inhibitor belonging to a class of molecule originally produced as immunosuppressors, which is currently in an ongoing phase I clinical trial for the treatment of advanced solid tumors, such as prostate cancer and non-Hodgkin lymphoma.[102]

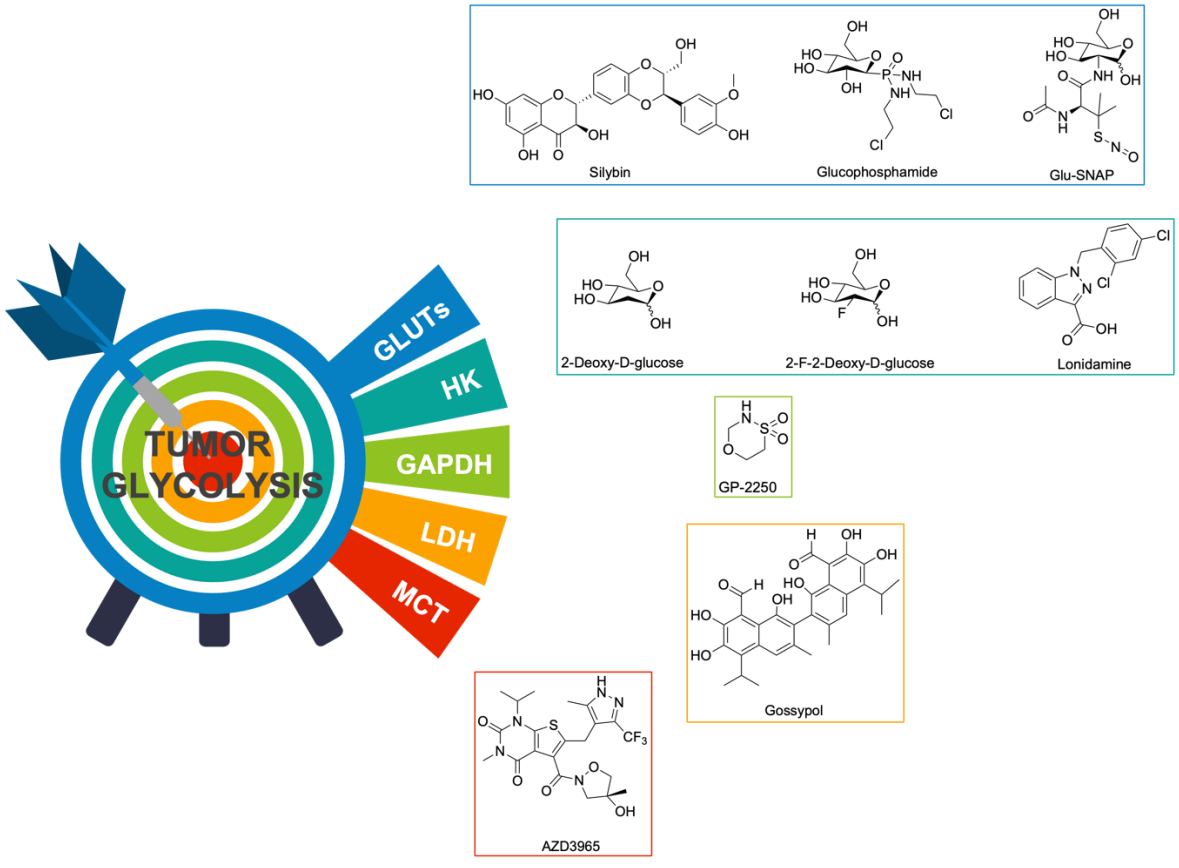

Figure 3. Some examples of anti-cancer agents targeting tumor glycolysis in clinical trials.

Conclusion

Over the past century, the scientific community has demonstrated an increasing interest in the study of the metabolic rewiring occurring in tumors, as described by the Warburg effect. A remarkable number of articles reported to have established that this effect is either a cause or an effect of cancer, and this dispute is still ongoing. For sure, this peculiarity of cancer cells definitely needs to be exploited more and more for both diagnostic and therapeutic purposes, since our capacity to spot tumors earlier and treat them more successfully will strongly depend on our increasing knowledge about this phenomenon. A better understanding of tumor biology is fundamental for producing progresses in treating and preventing cancer by using dietary and pharmacological interventions in metabolism. Unfortunately, as of yet it is not clear which glycolytic targets are more suitable than others in this endeavor, but this research field is incrementally growing, as highlighted by the increasing number of patent applications and ongoing clinical trials involving glycolytic inhibitors. Looking at the growing efforts internationally placed in this research field, we believe that in the near future novel therapeutic agents targeting glycolytic metabolism will be successfully approved for clinical uses as effective and selective anti-cancer drugs.

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