Journal Pre-proof

The Dichotomous Role of the Glycolytic Metabolism Pathway in Cancer Metastasis: Interplay with the Complex Tumor Microenvironment and Novel Therapeutic Strategies

Btissame El Hassouni, Carlotta Granchi, Andrea Vallés-Martí, I Gede Putu Supadmanaba, Giulia Bononi, Tiziano Tuccinardi, Niccola Funel, Connie R. Jimenez, Godefridus J. Peters, Elisa Giovannetti, Filippo Minutolo

PII: S1044-579X(19)30180-4

DOI: https://doi.org/10.1016/j.semcancer.2019.08.025

Reference: YSCBI 1654

To appear in: *Seminars in Cancer Biology*

Received Date: 24 June 2019

Revised Date: 17 August 2019

Accepted Date: 20 August 2019

Please cite this article as: El Hassouni B, Granchi C, Vallés-Martí A, Supadmanaba IGP, Bononi G, Tuccinardi T, Funel N, Jimenez CR, Peters GJ, Giovannetti E, Minutolo F, The Dichotomous Role of the Glycolytic Metabolism Pathway in Cancer Metastasis: Interplay with the Complex Tumor Microenvironment and Novel Therapeutic Strategies, *Seminars in Cancer Biology* (2019), doi: https://doi.org/10.1016/j.semcancer.2019.08.025

The Dichotomous Role of the Glycolytic Metabolism Pathway in Cancer Metastasis: Interplay with the Complex Tumor Microenvironment and Novel Therapeutic Strategies

Btissame El Hassouni[a*], Carlotta Granchi[b*], Andrea Vallés-Martí[a], I Gede Putu Supadmanaba[a], Giulia Bononi[b], Tiziano Tuccinardi[b], Niccola Funel[c], Connie R. Jimenez[a], Godefridus J. Peters[a,d], Elisa Giovannetti[a,c,e**], Filippo Minutolo[b**]

[a]Department of Medical Oncology, Amsterdam UMC, VU University Medical Center, Amsterdam, The Netherlands;

[b]Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6 and 33, 56126 Pisa, Italy;

[c]Cancer Pharmacology Lab, AIRC Start Up Unit, Pisa, Italy;

[d]Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland

[e]Fondazione Pisana per la Scienza, Pisa, Italy

*Both authors contributed equally to this paper, ** Both senior authors contributed equally

Corresponding author:

Btissame El Hassouni, Department of Medical Oncology, Amsterdam UMC, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. Telephone: +31-204442267, Fax: +31-204443844, E-mail: b.elhassouni@amsterdamumc.nl

Elisa Giovannetti, MD, PhD, Department of Medical Oncology, Amsterdam UMC, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. Telephone: +31-204442633 , Fax: +31-204443844 , E-mail: e.giovannetti@amsterdamumc.nl

Abstract

Cancer metastasis to distant organs is initiated by tumor cells that disseminate from primary heterogeneous tumors. The subsequent growth and survival of tumor metastases depend on different metabolic changes, which constitute one of the enigmatic properties of tumor cells. Aerobic glycolysis, 'the Warburg effect', contributes to tumor energy supply, by oxidizing glucose in a faster manner compared to oxidative phosphorylation, leading to an increased lactate production by lactate dehydrogenase A (LDH-A), which in turn affects the immune response. Surrounding stromal cells contribute to feedback mechanisms further prompting the acquisition of pro-invasive metabolic features. Hence, therapeutic strategies targeting the glycolytic pathway are intensively investigated, with a special interest on their anti-metastatic properties. Various small molecules, such as LDH-A inhibitors, have shown pre-clinical activity against different cancer types, and blocking LDH-A could also help in designing future complimentary therapies. Modulation of specific targets in cells with an altered glycolytic metabolism should indeed result in a milder and distinct toxicity profile, compared to conventional cytotoxic therapy, while a combination treatment with vitamin C leading to increasing reactive oxygen species levels, should further inhibit cancer cell survival and invasion. In this review we describe the impact of metabolic reprogramming in cancer metastasis, the contribution of lactate in this aberrant process and its effect on oncogenic processes. Furthermore, we discuss experimental compounds that target glycolytic metabolism, such as LDH-A inhibitors, and their potential to improve current and experimental therapeutics against metastatic tumors.

Keywords: glycolysis, metastasis, LDH, tumor microenvironment, vitamin C

Introduction

Cancer development is a multi-step process that results from genomic instability. Genetic alterations enabling the extensively described "cancers' hallmarks", include proliferative signaling sustenance, angiogenesis induction, cell death resistance, growth suppressors circumvention and replicative immortality, immune evasion and metabolic reprogramming have recently emerged as core traits of tumors [1].

More specifically, the altered glycolytic metabolism pathway is implicated in various cancer types, where cells switch from oxidative phosphorylation (OXPHOS) in the mitochondria to aerobic glycolysis. The latter is also known as the "Warburg effect", which is described as a high rate glycolysis followed by lactic acid fermentation despite the presence of available oxygen [2]. In addition, several cancers are much more hypoxic than normal tissues and therefore, altered lactate production may also result from an adaption to tumor environmental cues [3]. These metabolic changes confer selective advantages to cancer cells, by providing nutrients and essential components to build biomass, such as amino acids, lipids, and nucleotides. Altered metabolism also contributes to the modulation of apoptosis, angiogenesis, anti-anoikis and anchorage-independent expansion, conferring a metastatic phenotype to the cells [4].

The renewed interest in tumor metabolism during the last decade has generated hope that a new class of effective anti-cancer treatment strategies might finally arise. Enzymes playing an essential role in alterations in glycolysis and glutamine metabolism in tumors proved to be promising targets in preclinical studies, warranting further studies on cell type specific interactions which might limit their clinical activity. Remarkably, an improved knowledge of the influence of tumor metabolism in supporting the metastatic behavior is also essential to combat the most aggressive tumors. To date, the biological complexity coupled with invasion and metastatic cascades have indeed limited the research and investment in potentially valuable novel therapeutic agents [5]. However, the growth and survival of tumor metastases depend on

particular metabolic pathways that facilitate reciprocal interactions between cancer cells and host homeostatic mechanisms in the metastatic niches [6]. Targeting these interactions, in addition to the key signaling pathways driving tumorigenesis, should produce synergistic therapeutic effects. Thus, in order to enhance efficacy of current treatments as well as prompt the development of more effective therapies, it is crucial to get more insight into metabolic signaling alterations that lead to metastatic phenotypes.

Several studies revealed that a peculiar glycolytic metabolism is associated with cancer stemness, invasive behavior and poor prognosis [7]. Over the past few years, many molecules have been investigated for targeting the glycolytic pathway by inhibiting lactate dehydrogenase A (LDH-A) [8–10], a key enzyme in the conversion of pyruvate into lactate. Remarkably, LDH-A overexpression is correlated with a poor survival in patients with solid tumors and has also been linked to chemo- and radiotherapy resistance [11]. The investigated experimental molecules vary from penicillic acid and oxamate, the more historical inhibitors, to bi-functional inhibitors and *N*-hydroxyindole based compounds that show selectivity for LDH-A compared to other isoforms. A promising compound is glucose-conjugated lactate dehydrogenase inhibitor (NHI-Glc-2), which showed a higher intracellular concentration due to its uptake by cancer cell overexpressing glucose transporter 1 (GLUT-1) and activity in preclinical models of hypoxic tumors, such as peritoneal mesothelioma and pancreatic cancer [9].

In this review we describe the impact of metabolic reprogramming in cancer metastasis, the contribution of lactate in this aberrant process and its effect on oncogenic signaling pathways. Furthermore, experimental compounds that target metabolism, with a special focus on LDH inhibitors, and their potential novel applications in cancer treatment will be discussed.

Cancer cell glycolytic metabolism

Pioneer Otto Warburg, described the metabolism of carcinoma cells almost a century ago [2]. This ingenious study was initiated by the observation of sea urchin eggs that increased in size during fertilization, leading to the hypothesis that an acceleration of respiration might apply to carcinomas as well. Warburg and collaborators found a lower respiration in the tumor of a seminal vesicle in a rat, prompting investigation of nutritional molecules such as amino acids, glycose and fatty acids. Unexpectedly, amino acids did not affect the respiration whereas lactate production from glucose stopped respiration, which led to the investigation of glycolysis in presence or absence of oxygen in carcinoma tissues. Cancer cells were found to prefer aerobic glycolysis, despite the presence of oxygen and the energetically more efficient OXPHOS. These findings are described as the "Warburg effect" and in the past decade, a renewed interest on this phenomenon has prompted more research on cancer cell metabolism, including multi-scale computational studies on both Warburg effect, glutamine addiction and the "reverse Warburg effect", in which tumor cells force non-cancerous cells in the tumor's microenvironment to consume glucose [12,13]. More specifically, epithelial tumor cells can induce aerobic glycolysis in neighboring stromal fibroblasts, which in turn causes their differentiation into myo-fibroblasts/cancer-associated fibroblasts (CAFs) [13]. In these myo-fibroblasts, the produced lactate and pyruvate are transported via the monocarboxylate transporter (MCT) allowing the uptake of lactate and pyruvate by epithelial cells. Subsequently, pyruvate and lactate are then used for ATP production via the energetically favorable tricarboxylic acid (TCA) cycle in the epithelial cells, enabling a higher cell proliferation capacity. This phenomenon is also reported by Vincent and collaborators [14], where human keloid fibroblasts (KFs) generated the required ATP via glycolysis. Unraveling the causes underlying these molecular mechanisms could pave the way to innovative therapeutic strategies targeting cancer metabolism.

Glycolysis initiation requires glucose uptake, which is performed by the glucose transporters via facilitated diffusion (GLUTs) or by the sodium-glucose linked transporters (SGLTs) (**Figure 1**) [15]. Then, hexokinase (HK) catalyzes the first critical and irreversible step in glycolysis: conversion of glucose to glucose-6-phosphate (G6P) [16–18]. The conversion ensures that the glucose will not diffuse back out of the cells. There are 4 types of HK that have been intensively studied, namely HK1, HK2, HK3, and HK4 [19]. HK4 is the only low-affinity HK known, while the rest of these hexokinases are considered as high-affinity enzymes. In cancer cells, due to accelerated glucose metabolism, high affinity HKs are often over-expressed, particularly HK2 [20]. HK2 has been studied extensively as a prognostic biomarker in several solid tumors and has been linked to a poor clinical outcome in hepatocellular carcinoma and colorectal carcinoma [21]. Notably, it has also been linked with larger tumor size and higher tendency of nodal involvement.

Glucose-6-phosphate is further metabolized into fructose-6-phosphate (F6-P), fructose-1,6-biphosphate (F1,6BP) and glyceraldehyde-3-phosphate (G3P), by glucose-6-phosphate isomerase, phosphofructokinase and aldolase, respectively [18,22]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) converts G3P to glycerate-1,3-diphoshate (G1,3DP) and subsequently to 3-phosphoglycerate (3PG). Next, 3-phosphoglycerate is converted into pyruvate via 2–phosphoglycerate (2PG) and phosphoenolpyruvate (PEP), mediated by enolase and pyruvate kinases, respectively. Pyruvate is taken up into mitochondria, converted into acetyl coenzyme A (Acetyl-CoA) and can then enter into the TCA cycle. Pyruvate can also be reduced to lactate by LDH-A, which is a target gene of c-Myc and hypoxia-inducible factor (HIF-1α) [23]. Simultaneously, LDH-A regenerates nicotinamide adenine dinucleotide (NAD^+) molecules to meet the high energy demand of a cancer cell [24]. The LDH family of enzymes consists of five isoforms, which are tetrameric structures with distinct catalytic activities [25]. LDH-A has been proven to be associated with cell proliferation, survival, angiogenesis,

invasion and metastasis, as well as immune escape [25–28]. Its clinical importance is reflected by its high expression in many types of solid cancers [29] and its overexpression has been established as a prognostic factor in renal [30], pancreatic [31], liver [32], lung [33] and breast cancer [34]. The reverse function, conversion of lactate to pyruvate, is mainly led by LDH-B isoform, which converts lactic acid to pyruvate and regenerates nicotinamide adenine dinucleotide (NADH) molecules. In cancer, due to the high levels of extracellular lactic acid, LDH-B is mostly expressed in surrounding tissue and tumor microenvironment [35]. Most importantly, disruption of both isoforms has been shown to enhance OXPHOS signaling [35], which eventually can be blocked by electron transport chain (ETC) inhibitors, leading to cancer cell death [36]. These results underline the need of new drugs specifically targeting different enzymes of the cancer glycolytic metabolism, as discussed in the following paragraphs.

[Position figure 1]

Cancer metastasis: role of glycolytic metabolism

Over a century ago, Stephan Paget described cancer metastasis as the seed and soil phenomenon, where cancer cells represent the seeds that find a new soil (other organ microenvironments) for further growth [37,38]. Paget emphasized that metastasis is dependent on both the characteristics of the cancer cell and the responses of the soil. The circulating cancer cell should survive the multiple steps of metastasis and find an organ that can host (‘function as the soil’) this cancer cell. Importantly, not all organs can serve as hosts, and most frequently metastasis are found in the brain, lungs, liver and bone. Interestingly, an increasing extent of evidences supports the hypothesis that metabolic flexibility is a key determinant for the success of the metastatic process as well as in the interaction with the metastatic microenvironments of specific organs [39].

Cancer metastasis to distant organs is initiated by tumor cells that disseminate from primary heterogeneous tumors, invade surrounding tissue, and enter the circulation. The circulating tumor cells establish pre-metastatic niches in distant organs usually in a dormant state, and require activation stimuli from the primary tumor to produce micro-metastases [6]. Cancer cells that shed from a primary tumor need to overcome the energy deficit to survive and form metastases, and it has been suggested that most of them might acquire a glycolytic phenotype [40]. The oxidation of glucose into lactate indeed offers a crucial growth advantage. Though with glycolysis the cancer cells produce less ATP/mol glucose compared to cells using the citric acid cycle and the respiratory chain, this production is about 100 times faster due to the much shorter reaction pathway [41]. In addition, because of the glycolytic switch and increase of the "waste" product lactate, the cancer cells can influence the microenvironment by stimulating angiogenesis, via activation of HIF1α and upregulation of the vascular endothelial growth factor (VEGF) and VEGF receptors [42] as well as increased extracellular acidification, which contributes to evading surveillance by the immune system [43]. For instance, breast cancer cells metastasizing to the liver had a dominant aerobic glycolytic metabolic phenotype, compared to lung and bone metastases, which were more dependent on OXPHOS [44].

However, a number of studies have reported differing metabolic changes underlying metastasis, showing for instance that the KISS1 metastasis suppressor protein reverses the Warburg effect by shifting from glycolysis to mitochondrial beta-oxidation, but also that enhanced tumor invasiveness is associated with elevated ATP production, pyruvate uptake, and oxygen consumption [45]. These controversial findings can be explained by the fact that even within a hypoxic tumor mass where most cancer cells rely only on glycolysis, a small percentage (5-10%) of glucose is still used for the OXPHOS via the mitochondria and the respiratory chain [46]. Accordingly, preclinical models of breast cancer and melanoma preclinical models showed that some metastatic cancer cells have a high oxidative metabolism, which increases in

parallel to the progressive acquisition of metastatic traits [47,48]. In particular, Porporato and collaborators [48] described a different "metabolic switch" affecting mitochondria, which involves overload of the ETC or partial ETC inhibition associated with enhanced superoxide production. These phenotypes result in superoxide-dependent cancer cell migration, invasion, and metastasis.

Another pivotal process enabling metastasis is the cell-biological program named epithelial-mesenchymal transition (EMT), by which epithelial cells lose their polarity and cell-cell adhesion, while gaining migratory and invasive properties to become more mesenchymal. Notably, analysis of the metabolic gene expression signature in 20 different tumor types showed that metastasis correlated with downregulation of mitochondrial genes, which was also associated with EMT [49]. Similar results were reported in a metabolite profiling approach in pancreatic cancer cells, which showed that the subpopulation enriched for glycolytic-related metabolites is characterized by a mesenchymal phenotype. Furthermore, pancreatic cancer cells exposed to EMT inducers, such as transforming growth factor β (TGF-β) and tumor necrosis factor-α (TNF-α) increased glucose uptake and lactate secretion, without affecting OXPHOS metabolism [50]. However, other studies described a metabolic signature characterized by reduced glycolysis and increased consumption of glucose through the TCA cycle for ATP and glutamate production in lung cancer mesenchymal cells [51].

Recently, Zhu and collaborators [52] reported the inhibition of cancer cell apoptosis and metastasis xenografts by Kudingcha, a Chinese traditional medicine, in triple negative breast cancer cell lines (MDA-MB-231 and HCC1806) and in immune-competent and -deficient mice bearing HCC1806 xenografts. Kudingcha treatment increased protein expression of apoptosis markers cleaved PARP, cleaved caspase-3 and decreased Bcl-2 levels, which was prevented by apoptosis inhibitor Z-VAD-FMK. Moreover, it inhibited cell migration and down-regulated the protein expression of beta-catenin, snail and vimentin, which are commonly overexpressed in

metastasis. To explain these results Zhu and collaborators propose that ROS production upon treatment modulates cell death and inhibits EMT. Remarkably, Kudingcha induced metabolic changes, where key components of glycolysis HK2, PKM2, phopshofructosekinase and transporters GLUT1 and GLUT2 protein expression was decreased after treatment, while lactate production was diminished.

Surrounding stromal cells contribute to feedback mechanisms prompting the acquisition of pro-invasive metabolic features. In particular, the conversion of glucose leads to an increase of lactate that is secreted mainly by hypoxic cells and taken up by neighboring cells via bidirectional MCTs, where it can fuel anabolic pathways. Cancer-associated fibroblasts can also serve as a source of lactate, and play a role in the activation of the NF-kB signaling pathway and epigenetic reprogramming [53]. Interestingly, in an immunodeficient murine system, lactate promoted breast cancer stem cell (CSC)-like properties that could be reverted by vitamin C, a LDH-A lowering agent [54], supporting further studies to unravel new mechanisms underlying the still controversial anti-cancer property of vitamin C.

Of note, glycolytic metabolism has also been associated with a CSC phenotype, which is involved in both tumor relapse and invasion in several cancer types [26]. However, different studies in CSC showed a glycolytic or OXPHOS addicted metabolic phenotype in various tumor types and even within different cell lines of the same tumor type. For instance, in breast cancer cells MDA-MB-231 and lung cancer H1299 cells, CSC related RNA binding protein LIN28B promoted aerobic glycolysis and increased lactate secretion [55]. LIN28B knockdown by shRNA led to inhibition of glycolysis and conversely an increase in OXPHOS. Moreover, shLIN28B-MDA-MB-231 cells injected in mice formed smaller tumors with low levels of lactic acid, and produced less metastatic sites in the lung compared to wild type cells. Breast cancer stem cells, identified by a high aldehyde dehydrogenase 1 (ALDH1) expression and grown as spheroids, showed a higher glycolytic capacity compared to non-spheroid cells and lactate

increased the number of MDA-MB-231 cells with high expression of ALDH or CD44+CD24- MCG-7, promoting migration. Briefly, in this study lactate or low pH contributed to cancer-associated stemness in breast cancer cells and LIN28B increased glycolysis via the MYC/miR-34a-5p axis. Conversely, the same MDA-MB-231 cells, as well as SUM159PT, MCF-7, and T47D breast cancer cell lines propagated as mammospheres. Therefore models enriched in CSCs produced less lactate and are much more dependent on OXPHOS than their differentiated progeny [56]. Similarly, KRAS ablation-resistant lung and pancreatic cancer cells displaying CSC traits were less dependent on glycolysis than on OXPHOS [57]. However, a resistant subpopulation of pancreatic cancer CSC clones showed reduced sensitivity to mitochondrial targeting with metformin because of a peculiar intermediate glycolytic/respiratory phenotype [58]. These results underline the metabolic plasticity of cancer cells, and support further studies on combinations of metabolic inhibitors.

Moreover, co-culturing of renal cancer cells (786-O) with human vascular endothelial (HUVEC) cells showed an enhanced proliferation, migration and invasion compared to single cell type culturing [59], supporting the notion of cancer cell promoting interactions with TME. In this study the MCT inhibitor 7AAC1 reduced the increased viability of the co-cultured cells, their migratory ability and invasion.

Similarly, patient-derived CAFs co-cultured with primary lymphoma cells increased cell viability and supported tumor cell survival, whereas mono-cultured primary lymphoma cells with a higher yH2A.X and caspase-3 expression could not survive [60]. Moreover, less ROS production was measured in the co-culture.

Anti-cancer agents targeting the Warburg Effect

Many small inhibitory molecules that target glycolysis have been discovered, but most of these molecules are still in pre-clinical phase (**Table 1**). So far, the main targets include glucose uptake (mediated mostly by GLUT-1), glucose retention (through HK), and lactate production (catalyzed by LDH-A). Therefore, we describe in the following paragraphs the most important studies on GLUT-1, HK and LDH-A inhibitors, reporting, when available, the findings on modulation of cancer cells invasion/migration and metastasis.

[Position figure 2]

GLUT-1 inhibitors

Due to high proliferative and glycolytic rate, cancer cells have high glucose consumption rate [16]. In order to compensate this consumption, enzymes and transport proteins involved in glucose uptake and glucose metabolism are upregulated in cancer cells. In particular, the glucose transporter GLUT-1, also known as solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1), is overexpressed in various tumor types [61,62]. Normally present in red blood cells, GLUT-1 is a rate limiting glucose transporter that enables cancer cells to accumulate glucose to fulfill their metabolic requirements [63].

A large number of studies have demonstrated the pivotal role of GLUT-1 in carcinogenesis in a wide array of tumors. Most importantly, increased expression of GLUT-1 has also been linked to invasive behavior and increased metastatic features [62,64,65]. For instance, it has been directly linked to vessel density and a higher degree of vascular invasion in esophageal carcinoma [64]. The clinical implication of GLUT-1 emerged in several clinical studies. GLUT-1 over-expression was significantly associated with poor prognosis in breast cancer, salivary gland tumor, ovarian cancer, bladder cancer, colorectal cancer, esophageal cancer and oral squamous cell carcinoma [64]. However, it should be noted that other studies have failed to find a similar correlation [62]. Nevertheless, due to the abundant evidence of the Warburg effect

in cancer, it can be concluded that there is high chance that GLUT-1 plays a critical role in cancer aggressive behavior.

Only a few GLUT-1 inhibitory agents have been tested, including STF-31 and WZB117 (**Figure 2**) [66,67]. STF-31 was effective in reducing glucose uptake, inhibiting tumor progression and inducing apoptosis in von Hippel-Lindau (VHL) deficient renal cell carcinoma. However, this study used cells lacking VHL while cancer cells with functional VHL survived the treatment. Therefore, STF-31 has a narrow therapeutic potential due to molecular restriction [66]. WZB117 has been tested against human ductal breast carcinoma and non-small cell lung cancer cells with promising results [67]. WZB117 effectively inhibits glucose uptake, proliferation, and tumor progression both in vitro and in vivo. However, despite its efficacy, up-regulation of several glycolytic enzymes and autophagy should be taken into consideration as these may reflect the compensative mechanism of the tumor cells towards a decreased supply of glucose.

More recently, new oxime-based inhibitors, originally designed as estrogen receptor (ER) ligands, proved to be able to efficiently hinder glucose uptake and cell growth in H1299 lung cancer cells [68]. Of note, one of these compounds was able to restore the repression of aerobic glycolysis induced by inhibitors of the PI3K/mTOR pathway in primary pancreatic cancer resistant cells, supporting their synergistic interaction with cytotoxic compounds [69]. These results should prompt additional studies to unravel the role of cell metabolism in resistance to new anti-signaling therapies and to identify innovative metabolic promising inhibitors that might be exploited by combination therapies.

Hexokinase Inhibitors

The aforementioned HK is considered one of the most promising potential targets for anti-glycolytic therapies and one compound developed against HK already managed to enter clinical

trials. This compound, 2-deoxy-D-glucose (2-DG, Figure 2), acts as competitive inhibitor for glucose due to its structural similarity[70–73]. However, instead of being processed via the glycolytic pathway, 2-DG is trapped in its phosphorylated form and accumulates in the cytoplasm and inhibits HK, leading to depletion of glucose supply and ATP [71,74,75].

The results of preclinical studies of 2-DG were convincing and a phase I dose-escalation trial of combination of 2-DG and docetaxel in 34 patients with metastatic or advanced solid tumors reported that the clinically tolerated dose for 2-DG was 63 mg/Kg [71]. Eleven of the thirty-four (11/34) patients had stable disease that remained until the 4^{th} cycle while 22/34 patients progressed. Only 1 out of 34 patients experienced partial response (PR), but this patient had previously received intensive treatments. Hyperglycemia was the most common side effect, whereas gastrointestinal bleeding and QT prolongation (measured by electrocardiogram) were less frequent [71]. Another phase I dose-escalating study evaluated the effective dose and efficacy of 2-DG in castration resistant prostate cancer by monitoring the level of p62 protein to determine the tumor burden [72]. This study recommended a lower clinical dose (i.e., 45 mg/Kg) due to prominent QT prolongation in patients who received 60 mg/Kg 2-DG. The level of p62 protein was decreased in all subjects but no other examination was reported [72].

2-DG also has been studied in glioma multiforme in combination with radiotherapy [73]. In this study, the patients were treated with four weekly fractions of oral 2-DG (200 mg/kg body weight) followed by whole brain irradiation (5 Gy). Nausea and vomiting were observed during the days of combined therapy in 50% of the patients. However, no significant brain tissue damage was reported, and all the twenty patients completed the treatment without interruption, with seven patients surviving more than 18 months.

Overall, the application of 2-DG needs to be further evaluated due to difference in clinically recommended dose as well as side effects such as hypoglycemia [71–73]. The clinical efficacy of this agent is also still questionable due to lack of response [71,72]. However, a recent report

from Cheong and collaborators support the continuation of 2-DG application in clinical setting. These researchers indeed reported that the combination of 2-DG with metformin increased AMPK activation, which enhanced apoptosis induction in gastric cancer, esophageal cancer, osteosarcoma, and breast cancer, both *in vitro* and *in vivo* [76–78]. However, no clinical evidence is yet available to justify this combination in the clinical setting. Of note, a preclinical study in glioma cells suggested that 2-DG treatment activated the anti-apoptotic protein kinase B (Akt) signaling that might, in turn, reduce its clinical efficacy [79]. In this context, simultaneous inhibition of the NADPH oxidase 4/Akt signaling enhanced 2-DG-induced suppression of glycolysis. In addition, this combination reduced migration, and invasion of glioma cells *in vitro* and induced anti-angiogenic effects *in vivo*, suggesting a role of this therapeutic strategy in reducing cancer metastasis.

Historical overview of LDH inhibitors

LDH is the primary glycolytic enzyme that catalyzes the synthesis of lactate from pyruvate and vice versa. This enzyme plays a key role in the interaction between tumor and stroma. For instance, CAF-induced lactate production increases activity of sirtuin 1 (SIRT1) with consequent deacetylation/activation of peroxisome proliferator-activated receptor gamma coactivator (PPARG) 1 alpha, a master regulator of mitochondrial biogenesis, involved in EMT and invasion of prostate cancer cells [80]. A seminal study showed that silencing of LDH impaired tumor initiation and progression [81]. Thus, several different classes of LDH inhibitors have been described or synthesized, and here we present an overview of these compounds (**Figure 2**), with special emphasis on the potential role of small molecule inhibitors in targeting LDH activity and modulating cancer metastatic behavior.

To develop LDH inhibitors, the chemists have used different approaches including fragment-based lead generation coupled with X-ray crystallography, molecular dynamics and

simulations, receptor-based pharmacophore modeling and structure-based virtual screening, as recently reviewed [82].

One of the first inhibitors of LDH is penicillic acid, a mycotoxin produced by different species of Penicillum. This fungal toxin showed antibiotic activity and toxicity against animals, and had a carcinogenic effect in rats and mice [83,84]. I*n vitro* studies on rabbit-muscle LDH highlighted that penicillic acid is a competitive inhibitor of LDH-A [85].

Oxamic acid (Oxamate) is a pyruvate competing inhibitor of LDH-A (K_i = 136 μM) [86] and LDH-B (K_i = 94.4 μM) [87]. Nevertheless, oxamate showed better affinity with *Plasmodium falciparum pf*LDH (IC_{50} = 94 μM) than human LDHs, thus leading to the development of new oxamic acid analogues as potential anti-malaria agents [88]. In addition, oxamic acid also exerts an inhibitory activity on aspartate aminotransferase (AAT), an enzyme involved in the malate-aspartate shuttle, showing better inhibition of AAT than LDH-A. Interestingly oxamate suppressed tumor metastasis in an orthotopic renal xenograft model [89]. However, this compound is characterized by non-enzyme specific inhibition, weak potency, and poor cellular uptake due to its high polarity, which requires the use of high oxamate concentrations in order to obtain the inhibition of aerobic glycolysis and tumor cell proliferation *in vitro* [90–93], limiting its further development.

Gossypol (reported as (*R*)-(−)-Gossypol in the **Figure 2**) is a natural structure extracted from the cotton seeds [94,95]. Gossypol exists as two enantiomers and some preliminary studies highlight that the bioactivity of gossypol is influenced by its chirality. (*R*)-(−)-gossypol induces dose-dependent cytotoxic effects on a series of cancer cell lines including melanoma, breast, lung, leukemia and cervix with a mean IC_{50} value of 20 μM, and it is more potent than the (*S*)-(+)-gossypol [96]. Despite the importance of the chirality on gossypol activity, most of the biological assays reported in literature have evaluated both enantiomers. Gossypol mainly acts on dehydrogenase enzymes such as LDH-A but can also inhibit GAPDH [97]. Gossypol non-

selectively inhibits LDH-A and LDH-B by competing with NADH, exhibiting K_i values of 1.9 and 1.4 μM, respectively [98]. Moreover, gossypol can chelate metal ions [99], resulting in non-specific toxicity that leads to side effects such as cardiac arrhythmias, hypokalemia, renal failure, muscle weakness, fatigue and occasionally paralysis [100]. The first dose escalation clinical trials were conducted with patients with metastatic adrenal cancer and malignant glioma [101,102]. Flack and collaborators reported that gossypol was generally well tolerated by metastatic adrenal cancer patients with minor side effects, but the response rate was considerably low [101]. The trial in malignant glioma conducted by Bushunow and collaborators reported similar findings of a well-tolerated drug with minor side effects, but low response rate (only 2 of 15 subjects showed partial response and 4 subjects stable disease) [102]. Another Phase I/II clinical trial conducted in metastatic breast cancer patients, refractory to anthracyclin and taxane, reported similar findings. However, this study also found that gossypol modulates retinoblastoma (Rb) and cyclin D1 expression [103]. Of note, Rb has a key role in the control of cell adhesion and how aberrations in these adhesive features of cancer cells may drive metastasis [104].

In these clinical studies, oral doses ranged from 30 to 70 mg/day, without correlation between dose and adverse effects and it has therefore been difficult to develop promising anticancer agents with gossypol as lead compound.

Among 2,3-dihydroxy-1-naphtoic acid derivatives structurally related to gossypol, FX11 (**Figure 2**) was previously developed as antimalarial agent and further selected as potential anticancer agents, because it preferentially inhibits LDH-A [105]. Further studies on purified human liver LDH-A showed that FX-11 efficiently inhibits LDHA with a K_i value of 8 μM in competition with NADH. FX-11 also negatively affects cellular energy supply, decreases cellular production of lactate, induces oxidative stress and finally provokes cell death. FX-11 reduces cancer cell growth in lymphoma and pancreatic cancer cell and xenograft models

[23,106]. Remarkably, it has also been tested against more aggressive pancreatic xenograft LZ10.7 *in vivo* and proved to be effective even as single agent [23]. Moreover, inhibition of LDHA activities by FX11 inhibited migration and invasion of different cancer cells, such as PC-3 and DU145 cells [107]. However, to date, no clinical trial is yet conducted to evaluate the clinical activity of FX-11.

NADH-competing agent quinoline-3-sulfonamide (**Figure 2**) has great selectivity against LDH-A over LDH-B with an effective concentration of 2-3 nM [108]. *In vitro* experiments of this agent in the hepatocellular carcinoma cell line Snu398 showed a significant decrease in lactate production, whereas oxygen consumption, glycolytic rate and TCA metabolites were elevated. Unfortunately, due to the low clearance rate of quinoline-3-sulfonamide, *in vivo* experiments were not performed [108,109].

Galloflavin (**Figure 2**) is a product of oxidation of gallic acid, identified as an inhibitor of both human isoforms LDH-A and LDH-B. I*n vitro* studies on hepatocellular carcinoma PLC/PFR/5 cells showed inhibition of aerobic glycolysis, decreased lactate production, ATP levels and cell growth. In addition, the anti-tumor effect of galloflavin has been shown in various breast cancer cell lines [110], representing the different pathological subtypes of this tumor: MCF-7 (well differentiated form), MDA-MB-231 (aggressive triple negative tumor) and MCF-Tam (tamoxifene resistant sub-line of MCF). Although MDA-MB-231 and MCF-Tam showed higher LDH levels and glucose uptake and had a lower capacity to consume oxygen, galloflavin exerted similar growth inhibition effects, suggesting its potential in inhibiting more aggressive cancer cells. Moreover, this study showed that blocking the oxidative stress function drove growth inhibition of MCF-Tam and MDA-MB-231 cells. These important results should encourage the further development of galloflavin as a promising antitumor agent. However, it is important to consider that galloflavin has a more complex mechanism of action [111]. It primarily binds to free LDH-A and, thus, blocks glycolysis but also interferes with LDH-

A/ssDNA in a lesser extent and inhibits RNA synthesis in vitro. Further testing is needed to confirm its efficacy, including anti-metastatic activity and possible side effects *in vivo*.

Novel LDH inhibitors

A new class of *N*-hydroxyindole based compounds were reported as selective LDHA inhibitors with inhibition values in the low micromolar range by competing with both the substrate pyruvate and the cofactor NADH, as demonstrated by modeling studies [112]. The most representative *N*-hydroxyindole based compounds are reported in **Figure 2**. The carboxylic acid derivative NHI-1 inhibited LDH-A with a K_i value of 8.9 μM with respect to the cofactor NADH and 4.7 μM with respect to the substrate pyruvate, [113]. Interestingly, its methyl ester analogue NHI-2 was more effective in cell-based assays due to its better cellular permeability and efficient lactate production reduction in cancer cells [114]. However, both compounds NHI-1 and NHI-2 were effective in reducing highly glycolytic CSC in glioblastoma multiforme, triggering apoptosis and cell differentiation [115], though the anti-proliferative effect exerted by the two compounds was transient. These two compounds were also particularly effective in hypoxic models of pancreatic cancer, where they induced apoptosis, affected invasiveness, spheroid-growth and reduced expression of metalloproteinases [9]. Of note, their synergistic interaction with gemcitabine was attributed to modulation of gemcitabine metabolism, overcoming the reduced synthesis of phosphorylated metabolites in hypoxia.

A recent development of this class of derivatives includes the glucose-conjugated methyl ester NHI-Glc-2, which is a weaker inhibitor (K_i value of 37.8 μM) than the *N*-OH methyl ester NHI-2 (K_i value of 5.1 μM) on the isolated enzyme [116]. However, NHI-Glc-2 exploits the GLUT-1 overexpression, leading to an increased cellular uptake and proved to reduce lactate production and decreased cancer cell proliferation. In a recent study, NHI-Glc-2 was combined with deoxynyboquinone (DNQ) creating the simultaneous generation of superoxide and hydrogen peroxide which caused synergistic cancer cell death [117]. An increased OXPHOS

was the result of LDH-A inhibition by NHI-Glc-2 leading to the generation of hydrogen peroxide, while DNQ was reduced by NAD(P)H quinone oxidoreductase 1 (NQO1), simultaneously forming superoxide. These results suggest that simultaneous exposure to reactive oxygen species (ROS)-inducing agents can be a powerful and selective anticancer strategy for preventing metastatic progression [118]. Moreover, it should prompt further studies on the role of OXPHOS in modulating LDH-A inhibition, as described in the following chapter.

[Position Table 1]

LDH-A inhibition, oxidative stress and the potential role of vitamin C

In pre-clinical studies, LDH-A inhibitors have shown to successfully impair tumor growth mostly by enhancing oxidative stress [23,119]. However, resistance has been linked to OXPHOS and off-target effects are not yet described [24]. Le and collaborators showed that LDH-A inhibition increased oxidative and bioenergetics stress by enhancing mitochondrial oxygen consumption and ROS levels in lymphoma and pancreatic cancer cell lines [23]. Interestingly, adding the antioxidant *N*-acetylcysteine (NAC), which diminished cell death, restored this oxidative stress.

It is also well-known that cancer cells switch from glycolysis to OXPHOS signalling to cope with high oxidative stress levels and produce enough energy to survive. Remarkable research in the field demonstrates that this metabolic plasticity is essential for tumor progression and metastasis development, where mitochondrial activity plays a key role [6,120,121]. Cancer cells also use this metabolic flexibility to sense changes occurring in the tumor microenvironment, such as hypoxia, as well as to interact and exchange metabolites, such as lactate, with stromal/epithelial cells and others [122,123]. In addition, accumulation of these onco-metabolites in the cytosol and in the mitochondrial matrix has a tremendous effect on ROS homeostasis and antioxidant response, angiogenesis and EMT induction (**Figure 1**) [39,120]. For instance, accumulation of succinate, fumarate and D-2-HG inhibits ten-eleven translocation (TET) enzyme function, inducing EMT gene expression and thus, promoting cell migration.

Due to the high metabolic heterogeneity of tumors, new combination strategies are needed to target not only the rapid proliferating and glycolytic cells, i.e. LDH-A/B inhibitors, but also the OXPHOS-dependent tumor cells, i.e. metformin, and NQO1 inhibitors [124]. A re-emerging anti-cancer compound, known to induce cancer cell death by boosting oxidative stress and interfering with cancer metabolism, is high-dose intravenous vitamin C (IVC) [125–129].

Clinical studies have shown that high IVC allows plasma peak concentrations of ~ 20 mM, needed for cancer cell killing [130,131]. Interestingly, preclinical studies show vitamin C concentrations below 5 mM are sufficient to successfully impair cancer cell growth in most cancer cells [132,133]. Moreover, recent studies show vitamin C to have a beneficial and epigenetic effect in some tumors by acting as a co-factor for TET dioxygenases, influencing cancer cell migration [134–136].

The exact mechanisms of vitamin C hydrogen peroxide (H_2O_2)-mediated cytotoxicity and cancer susceptibility have not yet been revealed. A great number of studies have shown redox balance, iron and energy metabolism, hypoxia, epigenetics and chromatin remodelling to play a key role in the response to high dose vitamin C. Several studies have explored the immediate vitamin C effects on cancer cell metabolism [137]. Researchers showed that vitamin C interfered with glycolysis and the TCA cycle, hindering the energy flux and thus inhibiting ATP and NADPH production [137] (**Figure 1**). It is known that high levels of ATP are crucial for circulating tumor cells, which use their antioxidant mechanisms, such as Nrf-2 signalling, and thus produce high levels of NAPDH [6]**.** Due to its pro-oxidant capacity, vitamin C might be able to impair survival of circulating tumor cells by reversing ROS scavenging and thus preventing metastatic niche formation **(Figure 1**). In addition, reduction in pyruvate and glutathione (GSH) has also been observed. By boosting ROS levels, vitamin C also reduces cancer cell motility and extracellular matrix remodelling mainly by increasing E-cadherin expression, by decreasing Snail expression and through the inhibition of matrix metalloproteinases (MMPs) [138,139].

In cancer cells, mitochondrial dysfunction can lead to increased invasiveness. H2O2 interferes with Fe-S proteins, which are involved in processes such as DNA replication and maintenance, TCA cycle, ATP synthesis, mitochondrial respiration (i.e. mitochondrial ETC complexes I-II) and fatty acid oxidation. Based on previous research, vitamin C can be hypothesized to also

disrupt OXPHOS signalling and thus impair cell invasion (**Figure 1**). If glycolysis is then blocked by simultaneous exposure to LDH inhibitors, cancer cells would no longer be able to meet the high energy demand, leading to cell death. Keeping with this hypothesis, increasing evidence supports tumor cell efficacy of the combination of glycolytic inhibitors and vitamin C in several cancer types [140], mainly due to a heightened oxidative stress and DNA fragmentation.

A number of studies also show reliable safety and tolerance [141,142] improved patient survival [127], selective cancer toxicity and potential efficacy of IVC [128,132,143]. For instance, clinical testing in combination with gemcitabine in a Phase I/IIa study showed potential synergism and reduced chemo-toxicity in pancreatic cancer patients. Currently, a total of 36 clinical trials -active, completed or terminated- are assessing IVC treatment outcome (https://clinicaltrials.gov/), and further studies on combination of IVC with LDH inhibitors are warranted.

Conclusions

Metabolic reprograming is a common mechanism of cancer cells to cope with the high energy demand. In particular, the glycolytic pathway is described in many cancer types to be upregulated and contributing to cancer cell development and metastasis.

Over the years, various small molecules are tested for the inhibition of the components of the glycolytic pathway, however only a small portion made it into the clinical trials, warranting novel preclinical and clinical development strategies, which should focus on the anti-metastatic properties of anti-glycolytic compounds.

In the preclinical setting, studies on both ortothopic patient-derived xenografts, reproducing clinical metastatic routes, as well as in immunocompetent animal models will help in better understanding the molecular interaction among tumor, tumor microenvironment and distant

metastasis, including analysis of immune modulation. These studies should guide rational drug development, which should also consider both the main features of metastatic cancers and tumor metabolic plasticity and heterogeneity.

Blocking LDH could also help in designing future complementary therapies. Monotherapy activity might indeed not be a realistic expectation for these agents, because cells driven by glycolytic metabolism represent only a subpopulation, though critically important, in most tumors. These agents should then have long half-lives to exploit fluctuating metabolism or for efficient bystander killing. However, inhibition of molecular targets in cells with glycolytic metabolism should offer a more benign and distinct toxicity profile, compared to the toxicity profile of conventional cytotoxic therapy, and therefore have greater opportunity for combination with current standards of care or with other agents, such as IVC, enhancing intratumoral ROS levels and thereby affecting cancer cell survival and invasion.

Funding Source

The CCA Foundation 2012 and 2015, Fondazione Pisana per la Scienza, KWF Dutch Cancer Society grants (KWF project#10401 and #11957, EG) and AIRC/Start-Up grants have funded this research. This work was also supported by the University of Pisa (Intramural Funds, CG, TT and FM).

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The CCA Foundation 2015, 2016 and 2018 grant (EG), KWF Dutch Cancer Society grants (KWF project#10401 and #11957, EG).(Italian Association for Cancer Research AIRC/Start-Up grant and National Science Center (Poland) project 2018/31/B/NZ7/02909 (GJP, EG) have funded this research. This work was also supported by the University of Pisa (Intramural Funds, CG, TT and FM).

References

[1] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: The next generation, Cell. 144 (2011) 646–674. doi:10.1016/j.cell.2011.02.013.

[2] O. Warburg, The metabolism of carcinoma cells, J. Cancer Res. (1925). doi:10.1158/jcr.1925.148.

[3] K.L. Eales, K.E.R. Hollinshead, D.A. Tennant, Hypoxia and metabolic adaptation of cancer cells, Oncogenesis. 5 (2016) e190–e190. doi:10.1038/oncsis.2015.50.

[4] G.F. Weber, Time and Circumstances: Cancer Cell Metabolism at Various Stages of Disease Progression, Front. Oncol. 6 (2016) 257. doi:10.3389/fonc.2016.00257.

[5] I.J. Fidler, M.L. Kripke, The challenge of targeting metastasis, Cancer Metastasis Rev. 34 (2015) 635–641. doi:10.1007/s10555-015-9586-9.

[6] I. Elia, G. Doglioni, S.M. Fendt, Metabolic Hallmarks of Metastasis Formation, Trends Cell Biol. 28 (2018) 673–684. doi:10.1016/j.tcb.2018.04.002.

[7] P. Danhier, P. Bański, V.L. Payen, D. Grasso, L. Ippolito, P. Sonveaux, P.E. Porporato, Cancer metabolism in space and time: Beyond the Warburg effect, Biochim. Biophys. Acta - Bioenerg. 1858 (2017) 556–572. doi:10.1016/j.bbabio.2017.02.001.

[8] C. Granchi, S. Roy, A. De Simone, I. Salvetti, T. Tuccinardi, A. Martinelli, M. MacChia, M. Lanza, L. Betti, G. Giannaccini, A. Lucacchini, E. Giovannetti, R. Sciarrillo, G.J. Peters, F. Minutolo, N-Hydroxyindole-based inhibitors of lactate dehydrogenase against cancer cell proliferation, Eur. J. Med. Chem. 46 (2011) 5398–5407. doi:10.1016/j.ejmech.2011.08.046.

[9] M. Maftouh, A. Avan, R. Sciarrillo, C. Granchi, L.G. Leon, R. Rani, N. Funel, K. Smid, R. Honeywell, U. Boggi, F. Minutolo, G.J. Peters, E. Giovannetti, Synergistic

interaction of novel lactate dehydrogenase inhibitors with gemcitabine against pancreatic cancer cells in hypoxia, Br. J. Cancer. 110 (2014) 172–182. doi:10.1038/bjc.2013.681.

[10] J.R. Doherty, J.L. Cleveland, Targeting lactate metabolism for cancer therapeutics Find the latest version : Review series Targeting lactate metabolism for cancer therapeutics, J. Clin. Invest. 123 (2013) 3685–3692. doi:10.1172/JCI69741.transcription.

[11] C. Grasso, G. Jansen, E. Giovannetti, Drug resistance in pancreatic cancer: Impact of altered energy metabolism, Crit. Rev. Oncol. Hematol. 114 (2017) 139–152. doi:10.1016/j.critrevonc.2017.03.026.

[12] M. Shan, D. Dai, A. Vudem, J.D. Varner, A.D. Stroock, Multi-scale computational study of the Warburg effect, reverse Warburg effect and glutamine addiction in solid tumors, PLoS Comput. Biol. 14 (2018) e1006584. doi:10.1371/journal.pcbi.1006584.

[13] S. Pavlides, D. Whitaker-Menezes, R. Castello-Cros, N. Flomenberg, A.K. Witkiewicz, P.G. Frank, M.C. Casimiro, C. Wang, P. Fortina, S. Addya, R.G. Pestell, U.E. Martinez-Outschoorn, F. Sotgia, M.P. Lisanti, The reverse Warburg effect: Aerobic glycolysis in cancer associated fibroblasts and the tumor stroma, Cell Cycle. 8 (2009) 3984–4001. doi:10.4161/cc.8.23.10238.

[14] A.S. Vincent, T.T. Phan, A. Mukhopadhyay, H.Y. Lim, B. Halliwell, K.P. Wong, Human skin keloid fibroblasts display bioenergetics of cancer cells, J. Invest. Dermatol. 128 (2008) 702–709. doi:10.1038/sj.jid.5701107.

[15] A.M. Navale, A.N. Paranjape, Glucose transporters: physiological and pathological roles, Biophys. Rev. 8 (2016) 5–9. doi:10.1007/s12551-015-0186-2.

[16] M. V. Liberti, J.W. Locasale, The Warburg Effect: How Does it Benefit Cancer Cells?,

Trends Biochem. Sci. 41 (2016) 211–218. doi:10.1016/j.tibs.2015.12.001.

[17] S.P. Mathupala, Y.H. Ko, P.L. Pedersen, Hexokinase-2 bound to mitochondria: Cancer's stygian link to the "Warburg effect" and a pivotal target for effective therapy, Semin. Cancer Biol. 19 (2009) 17–24. doi:10.1016/j.semcancer.2008.11.006.

[18] M. Akram, Mini-review on glycolysis and cancer, J. Cancer Educ. 28 (2013) 454–457. doi:10.1007/s13187-013-0486-9.

[19] S.P. Mathupala, Y.H. Ko, P.L. Pedersen, Hexokinase II: Cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria, Oncogene. 25 (2006) 4777–4786. doi:10.1038/sj.onc.1209603.

[20] P.L. Pedersen, S. Mathupala, A. Rempel, J.F. Geschwind, Y.H. Ko, Mitochondrial bound type II hexokinase: A key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention, Biochim. Biophys. Acta - Bioenerg. 1555 (2002) 14–20. doi:10.1016/S0005-2728(02)00248-7.

[21] J. Wu, L. Hu, F. Wu, L. Zou, T. He, Poor prognosis of hexokinase 2 overexpression in solid tumors of digestive system: a meta-analysis, Oncotarget. 8 (2017) 32332–32344. doi:10.18632/oncotarget.15974.

[22] T. Teslaa, M.A. Teitell, Techniques to monitor glycolysis, in: Methods Enzymol., 2014: pp. 91–114. doi:10.1016/B978-0-12-416618-9.00005-4.

[23] A. Le, C.R. Cooper, A.M. Gouw, R. Dinavahi, A. Maitra, L.M. Deck, R.E. Royer, D.L. Vander Jagt, G.L. Semenza, C. V. Dang, Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression, Proc. Natl. Acad. Sci. 107 (2010) 2037–2042. doi:10.1073/pnas.0914433107.

[24] M. Ždralević, M. Vučetić, B. Daher, I. Marchiq, S.K. Parks, J. Pouysségur, Disrupting

the ‘Warburg effect’ re-routes cancer cells to OXPHOS offering a vulnerability point via ‘ferroptosis’-induced cell death, Adv. Biol. Regul. 68 (2018) 55–63. doi:10.1016/j.jbior.2017.12.002.

[25] P. Miao, S. Sheng, X. Sun, J. Liu, G. Huang, Lactate dehydrogenase a in cancer: A promising target for diagnosis and therapy, IUBMB Life. 65 (2013) 904–910. doi:10.1002/iub.1216.

[26] J. Ding, J.E. Karp, A. Emadi, Elevated lactate dehydrogenase (LDH) can be a marker of immune suppression in cancer: Interplay between hematologic and solid neoplastic clones and their microenvironments, Cancer Biomarkers. 19 (2017) 353–363. doi:10.3233/CBM-160336.

[27] S.L. Sheng, J.J. Liu, Y.H. Dai, X.G. Sun, X.P. Xiong, G. Huang, Knockdown of lactate dehydrogenase A suppresses tumor growth and metastasis of human hepatocellular carcinoma, FEBS J. 279 (2012) 3898–3910. doi:10.1111/j.1742-4658.2012.08748.x.

[28] L. Faloppi, M. Bianconi, R. Giampieri, A. Sobrero, R. Labianca, D. Ferrari, S. Barni, E. Aitini, A. Zaniboni, C. Boni, F. Caprioni, S. Mosconi, S. Fanello, R. Berardi, A. Bittoni, K. Andrikou, M. Cinquini, V. Torri, M. Scartozzi, S. Cascinu, The value of lactate dehydrogenase serum levels as a prognostic and predictive factor for advanced pancreatic cancer patients receiving sorafenib, Oncotarget. 6 (2015) 35087–94. doi:10.18632/oncotarget.5197.

[29] M. Yu, S. Chen, W. Hong, Y. Gu, B. Huang, Y. Lin, Y. Zhou, H. Jin, Y. Deng, L. Tu, B. Hou, Z. Jian, Prognostic role of glycolysis for cancer outcome: evidence from 86 studies, J. Cancer Res. Clin. Oncol. 145 (2019) 967–999. doi:10.1007/s00432-019-02847-w.

[30] J. Shen, Z. Chen, Q. Zhuang, M. Fan, T. Ding, H. Lu, X. He, Prognostic value of serum

lactate dehydrogenase in renal cell carcinoma: A systematic review and meta-analysis, PLoS One. 11 (2016). doi:10.1371/journal.pone.0166482.

[31] G.H. Mohammad, S.W.M.O. Damink, M. Malago, D.K. Dhar, S.P. Pereira, Pyruvate kinase M2 and lactate dehydrogenase a are overexpressed in pancreatic cancer and correlate with poor outcome, PLoS One. 11 (2016). doi:10.1371/journal.pone.0151635.

[32] W. Kong, X. Zuo, H. Liang, J. Hu, H. Zhang, X. Wang, W. Chen, Prognostic Value of Lactate Dehydrogenase in Patients with Hepatocellular Carcinoma: A Meta-Analysis, Biomed Res. Int. 2018 (2018). doi:10.1155/2018/1723184.

[33] F. Petrelli, M. Cabiddu, A. Coinu, K. Borgonovo, M. Ghilardi, V. Lonati, S. Barni, Prognostic role of lactate dehydrogenase in solid tumors: A systematic review and meta-analysis of 76 studies, Acta Oncol. (Madr). 54 (2015) 961–970. doi:10.3109/0284186X.2015.1043026.

[34] D. Liu, D. Wang, C. Wu, L. Zhang, Q. Mei, G. Hu, G. Long, W. Sun, Prognostic significance of serum lactate dehydrogenase in patients with breast cancer: A meta-analysis, Cancer Manag. Res. 11 (2019) 3611–3619. doi:10.2147/CMAR.S199260.

[35] M. Ždralević, A. Brand, L. Di Ianni, K. Dettmer, J. Reinders, K. Singer, K. Peter, A. Schnell, C. Bruss, S.M. Decking, G. Koehl, B. Felipe-Abrio, J. Durivault, P. Bayer, M. Evangelista, T. O'Brien, P.J. Oefner, K. Renner, J. Pouysségur, M. Kreutz, Double genetic disruption of lactate dehydrogenases A and B is required to ablate the "Warburg effect" restricting tumor growth to oxidative metabolism, J. Biol. Chem. 293 (2018) 15947–15961. doi:10.1074/jbc.RA118.004180.

[36] T.M. Ashton, W. Gillies McKenna, L.A. Kunz-Schughart, G.S. Higgins, Oxidative phosphorylation as an emerging target in cancer therapy, Clin. Cancer Res. 24 (2018) 2482–2490. doi:10.1158/1078-0432.CCR-17-3070.

[37] I.J. Fidler, The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited., Nat. Rev. Cancer. 3 (2003) 453–8. doi:10.1038/nrc1098.

[38] S. Paget, the Distribution of Secondary Growths in Cancer of the Breast., Lancet. 133 (1889) 571–573. doi:10.1016/S0140-6736(00)49915-0.

[39] C. Lehúede, F. Dupuy, R. Rabinovitch, R.G. Jones, P.M. Siegel, Metabolic plasticity as a determinant of tumor growth and metastasis, Cancer Res. 76 (2016) 5201–5208. doi:10.1158/0008-5472.CAN-16-0266.

[40] V.L. Payen, P.E. Porporato, B. Baselet, P. Sonveaux, Metabolic changes associated with tumor metastasis, part 1: Tumor pH, glycolysis and the pentose phosphate pathway, Cell. Mol. Life Sci. 73 (2016) 1333–1348. doi:10.1007/s00018-015-2098-5.

[41] R.A. Cairns, I.S. Harris, T.W. Mak, Regulation of cancer cell metabolism, Nat. Rev. Cancer. 11 (2011) 85–95. doi:10.1038/nrc2981.

[42] N. Draoui, P. De Zeeuw, P. Carmeliet, Angiogenesis revisited from a metabolic perspective: Role and therapeutic implications of endothelial cell metabolism, Open Biol. 7 (2017). doi:10.1098/rsob.170219.

[43] A. Brand, K. Singer, G.E. Koehl, M. Kolitzus, G. Schoenhammer, A. Thiel, C. Matos, C. Bruss, S. Klobuch, K. Peter, M. Kastenberger, C. Bogdan, U. Schleicher, A. Mackensen, E. Ullrich, S. Fichtner-Feigl, R. Kesselring, M. Mack, U. Ritter, M. Schmid, C. Blank, K. Dettmer, P.J. Oefner, P. Hoffmann, S. Walenta, E.K. Geissler, J. Pouyssegur, A. Villunger, A. Steven, B. Seliger, S. Schreml, S. Haferkamp, E. Kohl, S. Karrer, M. Berneburg, W. Herr, W. Mueller-Klieser, K. Renner, M. Kreutz, LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells, Cell Metab. 24 (2016) 657–671. doi:10.1016/j.cmet.2016.08.011.

[44] F. Dupuy, S. Tabariès, S. Andrzejewski, Z. Dong, J. Blagih, M.G. Annis, A. Omeroglu, D. Gao, S. Leung, E. Amir, M. Clemons, A. Aguilar-Mahecha, M. Basik, E.E. Vincent, J. St.-Pierre, R.G. Jones, P.M. Siegel, PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer, Cell Metab. 22 (2015) 577–589. doi:10.1016/j.cmet.2015.08.007.

[45] S.J. Manley, W. Liu, D.R. Welch, The KISS1 metastasis suppressor appears to reverse the Warburg effect by shifting from glycolysis to mitochondrial beta-oxidation, J. Mol. Med. 95 (2017) 951–963. doi:10.1007/s00109-017-1552-2.

[46] C. Gründker, M. Lässche, J.W. Hellinger, G. Emons, Mechanisms of Metastasis and Cell Mobility - The Role of Metabolism, Geburtshilfe Frauenheilkd. 79 (2019) 184–188. doi:10.1055/a-0805-9113.

[47] V.S. Lebleu, J.T. O'Connell, K.N. Gonzalez Herrera, H. Wikman, K. Pantel, M.C. Haigis, F.M. De Carvalho, A. Damascena, L.T. Domingos Chinen, R.M. Rocha, J.M. Asara, R. Kalluri, PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis, Nat. Cell Biol. 16 (2014) 992–1003. doi:10.1038/ncb3039.

[48] P.E. Porporato, V.L. Payen, J. Pérez-Escuredo, C.J. De Saedeleer, P. Danhier, T. Copetti, S. Dhup, M. Tardy, T. Vazeille, C. Bouzin, O. Feron, C. Michiels, B. Gallez, P. Sonveaux, A mitochondrial switch promotes tumor metastasis, Cell Rep. 8 (2014) 754–766. doi:10.1016/j.celrep.2014.06.043.

[49] E. Gaude, C. Frezza, Tissue-specific and convergent metabolic transformation of cancer correlates with metastatic potential and patient survival, Nat. Commun. 7 (2016) 13041. doi:10.1038/ncomms13041.

[50] M. Liu, L.-E. Quek, G. Sultani, N. Turner, Epithelial-mesenchymal transition induction

is associated with augmented glucose uptake and lactate production in pancreatic ductal adenocarcinoma, Cancer Metab. 4 (2016) 19. doi:10.1186/s40170-016-0160-x.

[51] Y. Sun, A. Daemen, G. Hatzivassiliou, D. Arnott, C. Wilson, G. Zhuang, M. Gao, P. Liu, A. Boudreau, L. Johnson, J. Settleman, Metabolic and transcriptional profiling reveals pyruvate dehydrogenase kinase 4 as a mediator of epithelial-mesenchymal transition and drug resistance in tumor cells, Cancer Metab. 2 (2014) 20. doi:10.1186/2049-3002-2-20.

[52] S. Zhu, L. Wei, G. Lin, Y. Tong, J. Zhang, X. Jiang, Q. He, X. Lu, D.D. Zhu, Y.Q. Chen, Metabolic Alterations Induced by Kudingcha Lead to Cancer Cell Apoptosis and Metastasis Inhibition, Nutr. Cancer. (2019) 1–12. doi:10.1080/01635581.2019.1645865.

[53] L. Ippolito, A. Morandi, E. Giannoni, P. Chiarugi, Lactate: A Metabolic Driver in the Tumour Landscape, Trends Biochem. Sci. 44 (2019) 153–166. doi:10.1016/j.tibs.2018.10.011.

[54] B. Cui, Y. Luo, P. Tian, F. Peng, J. Lu, Y. Yang, Q. Su, B. Liu, J. Yu, X. Luo, L. Yin, W. Cheng, F. An, B. He, D. Liang, S. Wu, P. Chu, L. Song, X. Liu, H. Luo, J. Xu, Y. Pan, Y. Wang, D. Li, P. Huang, Q. Yang, L. Zhang, B.P. Zhou, S. Liu, G. Xu, E.W.F. Lam, K.W. Kelley, Q. Liu, Stress-induced epinephrine enhances lactate dehydrogenase A and promotes breast cancer stem-like cells, J. Clin. Invest. 129 (2019) 1030–1046. doi:10.1172/JCI121685.

[55] C. Chen, L. Bai, F. Cao, S. Wang, H. He, M. Song, H. Chen, Y. Liu, J. Guo, Q. Si, Y. Pan, R. Zhu, T.H. Chuang, R. Xiang, Y. Luo, Targeting LIN28B reprograms tumor glucose metabolism and acidic microenvironment to suppress cancer stemness and metastasis, Oncogene. 38 (2019) 4527–4539. doi:10.1038/s41388-019-0735-4.

[56] E. Vlashi, C. Lagadec, L. Vergnes, K. Reue, P. Frohnen, M. Chan, Y. Alhiyari, M.B. Dratver, F. Pajonk, Metabolic differences in breast cancer stem cells and differentiated progeny, Breast Cancer Res. Treat. 146 (2014) 525–534. doi:10.1007/s10549-014-3051-2.

[57] H. Xie, J.I. Hanai, J.G. Ren, L. Kats, K. Burgess, P. Bhargava, S. Signoretti, J. Billiard, K.J. Duffy, A. Grant, X. Wang, P.K. Lorkiewicz, S. Schatzman, M. Bousamra, A.N. Lane, R.M. Higashi, T.W.M. Fan, P.P. Pandolfi, V.P. Sukhatme, P. Seth, Targeting lactate dehydrogenase-A inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor-initiating cells, Cell Metab. 19 (2014) 795–809. doi:10.1016/j.cmet.2014.03.003.

[58] P. Sancho, E. Burgos-Ramos, A. Tavera, T. Bou Kheir, P. Jagust, M. Schoenhals, D. Barneda, K. Sellers, R. Campos-Olivas, O. Graña, C.R. Viera, M. Yuneva, B. Sainz, C. Heeschen, MYC/PGC-1α balance determines the metabolic phenotype and plasticity of pancreatic cancer stem cells, Cell Metab. 22 (2015) 590–605. doi:10.1016/j.cmet.2015.08.015.

[59] C. Guo, T. Huang, Q.-H. Wang, H. Li, A. Khanal, E.-H. Kang, W. Zhang, H.-T. Niu, Z. Dong, Y.-W. Cao, Monocarboxylate transporter 1 and monocarboxylate transporter 4 in cancer-endothelial co-culturing microenvironments promote proliferation, migration, and invasion of renal cancer cells, Cancer Cell Int. 19 (2019). doi:10.1186/s12935-019-0889-8.

[60] A. Sakamoto, S. Kunou, K. Shimada, M. Tsunoda, T. Aoki, C. Iriyama, A. Tomita, S. Nakamura, F. Hayakawa, H. Kiyoi, Pyruvate secreted from patient-derived cancer-associated fibroblasts supports survival of primary lymphoma cells, Cancer Sci. 110 (2019) 269–278. doi:10.1111/cas.13873.

[61] X. Chen, P. Lu, S. Zhou, L. Zhang, J. Zhao, J. Tang, Predictive value of glucose transporter-1 and glucose transporter-3 for survival of cancer patients: A meta-analysis, Oncotarget. 8 (2017) 13206–13213. doi:10.18632/oncotarget.14570.

[62] J. Wang, C. Ye, C. Chen, H. Xiong, B. Xie, J. Zhou, Y. Chen, S. Zheng, L. Wang, Glucose transporter GLUT1 expression and clinical outcome in solid tumors: a systematic review and meta-analysis, Oncotarget. 8 (2017) 16875–16886. doi:10.18632/oncotarget.15171.

[63] P. Gonzalez-Menendez, D. Hevia, J.C. Mayo, R.M. Sainz, The dark side of glucose transporters in prostate cancer: Are they a new feature to characterize carcinomas?, Int. J. Cancer. 142 (2018) 2414–2424. doi:10.1002/ijc.31165.

[64] M. Yu, H. Yongzhi, S. Chen, X. Luo, Y. Lin, Y. Zhou, H. Jin, B. Hou, Y. Deng, L. Tu, Z. Jian, The prognostic value of GLUT1 in cancers: a systematic review and meta-analysis, Oncotarget. 8 (2017) 43356–43367. doi:10.18632/oncotarget.17445.

[65] B. Zhang, Z. Xie, B. Li, The clinicopathologic impacts and prognostic significance of GLUT1 expression in patients with lung cancer: A meta-analysis, Gene. 689 (2019) 76–83. doi:10.1016/j.gene.2018.12.006.

[66] Y. Liu, Y. Cao, W. Zhang, S. Bergmeier, Y. Qian, H. Akbar, R. Colvin, J. Ding, L. Tong, S. Wu, J. Hines, X. Chen, A Small-Molecule Inhibitor of Glucose Transporter 1 Downregulates Glycolysis, Induces Cell-Cycle Arrest, and Inhibits Cancer Cell Growth In Vitro and In Vivo, Mol. Cancer Ther. 11 (2012) 1672–1682. doi:10.1158/1535-7163.MCT-12-0131.

[67] D.A. Chan, P.D. Sutphin, P. Nguyen, S. Turcotte, E.W. Lai, A. Banh, G.E. Reynolds, J.T. Chi, J. Wu, D.E. Solow-Cordero, M. Bonnet, J.U. Flanagan, D.M. Bouley, E.E. Graves, W.A. Denny, M.P. Hay, A.J. Giaccia, Targeting GLUT1 and the Warburg

effect in renal cell carcinoma by chemical synthetic lethality, Sci. Transl. Med. 3 (2011) 94ra70. doi:10.1126/scitranslmed.3002394.

[68] C. Granchi, Y. Qian, H.Y. Lee, I. Paterni, C. Pasero, J. Iegre, K.E. Carlson, T. Tuccinardi, X. Chen, J.A. Katzenellenbogen, P.J. Hergenrother, F. Minutolo, Salicylketoximes that target glucose transporter 1 restrict energy supply to lung cancer cells, ChemMedChem. 10 (2015) 1892–1900. doi:10.1002/cmdc.201500320.

[69] D. Massihnia, A. Avan, N. Funel, M. Maftouh, A. Van Krieken, C. Granchi, R. Raktoe, U. Boggi, B. Aicher, F. Minutolo, A. Russo, L.G. Leon, G.J. Peters, E. Giovannetti, Phospho-Akt overexpression is prognostic and can be used to tailor the synergistic interaction of Akt inhibitors with gemcitabine in pancreatic cancer, J. Hematol. Oncol. 10 (2017) 9. doi:10.1186/s13045-016-0371-1.

[70] Q. Tran, H. Lee, J. Park, S.H. Kim, J. Park, Targeting cancer metabolism - revisiting the Warburg effects, Toxicol. Res. 32 (2016) 177–193. doi:10.5487/TR.2016.32.3.177.

[71] L.E. Raez, K. Papadopoulos, A.D. Ricart, E.G. Chiorean, R.S. Dipaola, M.N. Stein, C.M. Rocha Lima, J.J. Schlesselman, K. Tolba, V.K. Langmuir, S. Kroll, D.T. Jung, M. Kurtoglu, J. Rosenblatt, T.J. Lampidis, A phase i dose-escalation trial of 2-deoxy-d-glucose alone or combined with docetaxel in patients with advanced solid tumors, Cancer Chemother. Pharmacol. 71 (2013) 523–530. doi:10.1007/s00280-012-2045-1.

[72] M. Stein, H. Lin, C. Jeyamohan, D. Dvorzhinski, M. Gounder, K. Bray, S. Eddy, S. Goodin, E. White, R.S. Dipaola, Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies, Prostate. 70 (2010) 1388–1394. doi:10.1002/pros.21172.

[73] B.K. Mohanti, G.K. Rath, N. Anantha, V. Kannan, B.S. Das, B.A.R. Chandramouli, A.K. Banerjee, S. Das, A. Jena, R. Ravichandran, U.P. Sahi, R. Kumar, N. Kapoor,

V.K. Kalia, B.S. Dwarakanath, V. Jain, Improving cancer radiotherapy with 2-deoxy-D-glucose: Phase I/II clinical trials on human cerebral gliomas, Int. J. Radiat. Oncol. Biol. Phys. 35 (1996) 103–111. doi:10.1016/S0360-3016(96)85017-6.

[74] M. Ralser, M.M. Wamelink, E.A. Struys, C. Joppich, S. Krobitsch, C. Jakobs, H. Lehrach, A catabolic block does not sufficiently explain how 2-deoxy-D-glucose inhibits cell growth, Proc. Natl. Acad. Sci. 105 (2008) 17807–17811. doi:10.1073/pnas.0803090105.

[75] K. URAKAMI, V. ZANGIACOMI, K. YAMAGUCHI, M. KUSUHARA, Impact of 2-deoxy-D-glucose on the target metabolome profile of a human endometrial cancer cell line, Biomed. Res. 34 (2013) 221–229. doi:10.2220/biomedres.34.221.

[76] J.-H. Cheong, E.S. Park, J. Liang, J.B. Dennison, D. Tsavachidou, C. Nguyen-Charles, K. Wa Cheng, H. Hall, D. Zhang, Y. Lu, M. Ravoori, V. Kundra, J. Ajani, J.-S. Lee, W. Ki Hong, G.B. Mills, Dual Inhibition of Tumor Energy Pathway by 2-Deoxyglucose and Metformin Is Effective against a Broad Spectrum of Preclinical Cancer Models, Mol. Cancer Ther. 10 (2011) 2350–2362. doi:10.1158/1535-7163.MCT-11-0497.

[77] M. Bizjak, P. Malavašič, K. Dolinar, J. Pohar, S. Pirkmajer, M. Pavlin, Combined treatment with Metformin and 2-deoxy glucose induces detachment of viable MDA-MB-231 breast cancer cells in vitro, Sci. Rep. 7 (2017) 1761. doi:10.1038/s41598-017-01801-5.

[78] J. Zhu, Y. Zheng, H. Zhang, H. Sun, Targeting cancer cell metabolism: The combination of metformin and 2-Deoxyglucose regulates apoptosis in ovarian cancer cells via p38 MAPK/JNK signaling pathway., Am. J. Transl. Res. 8 (2016) 4812–4821.

[79] P. Gupta, K. Jagavelu, D.P. Mishra, Inhibition of NADPH Oxidase-4 Potentiates 2-

Deoxy- d -Glucose-Induced Suppression of Glycolysis, Migration, and Invasion in Glioblastoma Cells: Role of the Akt/HIF1α/HK-2 Signaling Axis , Antioxid. Redox Signal. 23 (2015) 665–681. doi:10.1089/ars.2014.5973.

[80] L. Ippolito, A. Morandi, M.L. Taddei, M. Parri, G. Comito, A. Iscaro, M.R. Raspollini, F. Magherini, E. Rapizzi, J. Masquelier, G.G. Muccioli, P. Sonveaux, P. Chiarugi, E. Giannoni, Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mitochondrial transfer, Oncogene. (2019). doi:10.1038/s41388-019-0805-7.

[81] V.R. Fantin, J. St-Pierre, P. Leder, Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance, Cancer Cell. 9 (2006) 425–434. doi:10.1016/j.ccr.2006.04.023.

[82] D. Mishra, D. Banerjee, Lactate Dehydrogenases as Metabolic Links between Tumor and Stroma in the Tumor Microenvironment, Cancers (Basel). 11 (2019) 750. doi:10.3390/cancers11060750.

[83] F. Dickens, H.E. Jones, Carcinogenic activity of a series of reactive lactones and related substances, Br. J. Cancer. 15 (1961) 85–100. doi:10.1038/bjc.1961.10.

[84] F. Dickens, H.E.H. Jones, Further studies on the carcinogenic action of certain lactones and related substances in the rat and mouse, Br. J. Cancer. 19 (1965) 392–403. doi:10.1038/bjc.1965.47.

[85] S.H. Ashoor, F.S. Chu, Inhibition of alcohol and lactic dehydrogenases by patulin and penicillic acid in vitro, Food Cosmet. Toxicol. 11 (1973) 617–624. doi:10.1016/S0015-6264(73)80332-3.

[86] J.M. Thornburg, K.K. Nelson, B.F. Clem, A.N. Lane, S. Arumugam, A. Simmons, J.W.

Eaton, S. Telang, J. Chesney, Targeting aspartate aminotransferase in breast cancer, Breast Cancer Res. 10 (2008) R84. doi:10.1186/bcr2154.

[87] S.R. Choi, A.B. Beeler, A. Pradhan, E.B. Watkins, J.M. Rimoldi, B. Tekwani, M.A. Avery, Generation of oxamic acid libraries: Antimalarials and inhibitors of plasmodium falciparum lactate dehydrogenase, J. Comb. Chem. 9 (2007) 292–300. doi:10.1021/cc060110n.

[88] S.R. Choi, A. Pradhan, N.L. Hammond, A.G. Chittiboyina, B.L. Tekwani, M.A. Avery, Design, synthesis, and biological evaluation of Plasmodium falciparum lactate dehydrogenase inhibitors, J. Med. Chem. 50 (2007) 3841–3850. doi:10.1021/jm070336k.

[89] J. Zhao, X. Huang, Z. Xu, J. Dai, H. He, Y. Zhu, H. Wang, LDHA promotes tumor metastasis by facilitating epithelial-mesenchymal transition in renal cell carcinoma, Mol. Med. Rep. 16 (2017) 8335–8344. doi:10.3892/mmr.2017.7637.

[90] L. Fiume, M. Manerba, M. Vettraino, G. Di Stefano, Impairment of aerobic glycolysis by inhibitors of lactic dehydrogenase hinders the growth of human hepatocellular carcinoma cell lines, Pharmacology. 86 (2010) 157–162. doi:10.1159/000317519.

[91] Effect of Oxamate on Glycolysis and Respiration in Sarcoma 37 Ascites Cells, Cancer Res. 28 (1968) 2056–2060.

[92] J. PAPACONSTANTINOU, S.P. COLOWICK, The role of glycolysis in the growth of tumor cells. II. The effect of oxamic acid on the growth of HeLa cells in tissue culture., J. Biol. Chem. 236 (1961) 285–8.

[93] E. Goldberg, H. Nitowsky, S. Colowick, The role of glycolysis in the growth of tumor cells. The basis of glucose toxicity in oxamate-treated, cultured cells, J. Biol. Chem.

240 (1965).

[94] J.W. Jaroszewski, T. Strom-Hansen, S.H. Hansen, O. Thastrup, H. Kofod, On the botanical distribution of chiral forms of gossypol, Planta Med. 58 (1992) 454–458. doi:10.1055/s-2006-961512.

[95] K. Dodou, Investigations on gossypol: past and present developments, Expert Opin. Investig. Drugs. 14 (2005) 1419–1434. doi:10.1517/13543784.14.11.1419.

[96] M.D. Shelley, L. Hartley, R.G. Fish, P. Groundwater, J.J.G. Morgan, D. Mort, M. Mason, A. Evans, Stereo-specific cytotoxic effects of gossypol enantiomers and gossypolone in tumour cell lines, Cancer Lett. 135 (1999) 171–180. doi:10.1016/S0304-3835(98)00302-4.

[97] M. Ikeda, Inhibition kinetics of NAD-linked enzymes by gossypol acetic acid, Andrologia. 22 (2009) 409–416. doi:10.1111/j.1439-0272.1990.tb02019.x.

[98] M.S. Gomez, R.C. Piper, L.A. Hunsaker, R.E. Royer, L.M. Deck, M.T. Makler, D.L. Vander Jagt, Substrate and cofactor specificity and selective inhibition of lactate dehydrogenase from the malarial parasite P. falciparum, Mol. Biochem. Parasitol. 90 (1997) 235–246. doi:10.1016/S0166-6851(97)00140-0.

[99] D. Vander Jagt, L. Deck, R. Royer, Gossypol Prototype of Inhibitors Targeted to Dinucleotide Folds, Curr. Med. Chem. 7 (2012) 479–498. doi:10.2174/0929867003375119.

[100] S. Qian, G. Jing, X. Wu, Y. Xu, Z. Zhou, Gossypol related hypokalemia. Clinicopharmacologic studies, Chin. Med. J. (Engl). 93 (1980) 477–482.

[101] M.R. Flack, R.G. Pyle, N.M. Mullen, B. Lorenzo, Y.W. Wu, R.A. Knazek, B.C. Nisula, M.M. Reidenberg, Oral gossypol in the treatment of metastatic adrenal cancer,

J. Clin. Endocrinol. Metab. 76 (1993) 1019–1024. doi:10.1210/jcem.76.4.8473376.

[102] P. Bushunow, M.M. Reidenberg, J. Wasenko, J. Winfield, B. Lorenzo, S. Lemke, B. Himpler, R. Corona, T. Coyle, Gossypol treatment of recurrent adult malignant gliomas, J. Neurooncol. 43 (1999) 79–86. doi:10.1023/A:1006267902186.

[103] C. Van Poznak, A.D. Seidman, M.M. Reidenberg, M.M. Moasser, N. Sklarin, K. Van Zee, P. Borgen, M. Gollub, D. Bacotti, T.J. Yao, R. Bloch, M. Ligueros, M. Sonenberg, L. Norton, C. Hudis, Oral gossypol in the treatment of patients with refractory metastatic breast cancer: A phase I/II clinical trial, Breast Cancer Res. Treat. 66 (2001) 239–248. doi:10.1023/A:1010686204736.

[104] B.E. Engel, W.D. Cress, P.G. Santiago-Cardona, The retinoblastoma protein: A master tumor suppressor acts as a link between cell cycle and cell adhesion, Cell Health Cytoskelet. 7 (2014) 1–10. doi:10.2147/CHC.S28079.

[105] Y. Yu, J.A. Deck, L.A. Hunsaker, L.M. Deck, R.E. Royer, E. Goldberg, D.L. Vander Jagt, Selective active site inhibitors of human lactate dehydrogenases A4, B4, and C4, Biochem. Pharmacol. 62 (2001) 81–89. doi:10.1016/S0006-2952(01)00636-0.

[106] N. V. Rajeshkumar, P. Dutta, S. Yabuuchi, R.F. De Wilde, G. V. Martinez, A. Le, J.J. Kamphorst, J.D. Rabinowitz, S.K. Jain, M. Hidalgo, C. V. Dang, R.J. Gillies, A. Maitra, Therapeutic targeting of the warburg effect in pancreatic cancer relies on an absence of p53 function, Cancer Res. 75 (2015) 3355–3364. doi:10.1158/0008-5472.CAN-15-0108.

[107] Z.Y. Xian, J.M. Liu, Q.K. Chen, H.Z. Chen, C.J. Ye, J. Xue, H.Q. Yang, J.L. Li, X.F. Liu, S.J. Kuang, Inhibition of LDHA suppresses tumor progression in prostate cancer, Tumor Biol. 36 (2015) 8093–8100. doi:10.1007/s13277-015-3540-x.

[108] J. Billiard, J.B. Dennison, J. Briand, R.S. Annan, D. Chai, M. Colón, C.S. Dodson, S.A. Gilbert, J. Greshock, J. Jing, H. Lu, J.E. McSurdy-Freed, L.A. Orband-Miller, G.B. Mills, C.J. Quinn, J.L. Schneck, G.F. Scott, A.N. Shaw, G.M. Waitt, R.F. Wooster, K.J. Duffy, Quinoline 3-sulfonamides inhibit lactate dehydrogenase A and reverse aerobic glycolysis in cancer cells, Cancer Metab. 1 (2013) 19. doi:10.1186/2049-3002-1-19.

[109] Y. Feng, Y. Xiong, T. Qiao, X. Li, L. Jia, Y. Han, Lactate dehydrogenase A: A key player in carcinogenesis and potential target in cancer therapy, Cancer Med. 7 (2018) 6124–6136. doi:10.1002/cam4.1820.

[110] F. Farabegoli, M. Vettraino, M. Manerba, L. Fiume, M. Roberti, G. Di Stefano, Galloflavin, a new lactate dehydrogenase inhibitor, induces the death of human breast cancer cells with different glycolytic attitude by affecting distinct signaling pathways, Eur. J. Pharm. Sci. 47 (2012) 729–738. doi:10.1016/j.ejps.2012.08.012.

[111] L. Fiume, M. Vettraino, D. Carnicelli, V. Arfilli, G. Di Stefano, M. Brigotti, Galloflavin prevents the binding of lactate dehydrogenase A to single stranded DNA and inhibits RNA synthesis in cultured cells, Biochem. Biophys. Res. Commun. 430 (2013) 466–469. doi:10.1016/j.bbrc.2012.12.013.

[112] R. Rani, C. Granchi, Bioactive heterocycles containing endocyclic N-hydroxy groups, Eur. J. Med. Chem. 97 (2015) 505–524. doi:10.1016/j.ejmech.2014.11.031.

[113] C. Granchi, S. Roy, C. Giacomelli, M. MacChia, T. Tuccinardi, A. Martinelli, M. Lanza, L. Betti, G. Giannaccini, A. Lucacchini, N. Funel, L.G. León, E. Giovannetti, G.J. Peters, R. Palchaudhuri, E.C. Calvaresi, P.J. Hergenrother, F. Minutolo, Discovery of N-hydroxyindole-based inhibitors of human lactate dehydrogenase isoform A (LDH-A) as starvation agents against cancer cells, J. Med. Chem. 54 (2011) 1599–1612.

doi:10.1021/jm101007q.

[114] C. Granchi, E.C. Calvaresi, T. Tuccinardi, I. Paterni, M. Macchia, A. Martinelli, P.J. Hergenrother, F. Minutolo, Assessing the differential action on cancer cells of LDH-A inhibitors based on the N-hydroxyindole-2-carboxylate (NHI) and malonic (Mal) scaffolds, Org. Biomol. Chem. 11 (2013) 6588–6596. doi:10.1039/c3ob40870a.

[115] S. Daniele, C. Giacomelli, E. Zappelli, C. Granchi, M.L. Trincavelli, F. Minutolo, C. Martini, Lactate dehydrogenase-A inhibition induces human glioblastoma multiforme stem cell differentiation and death, Sci. Rep. 5 (2015) 15556. doi:10.1038/srep15556.

[116] E.C. Calvaresi, C. Granchi, T. Tuccinardi, V. Di Bussolo, R.W. Huigens, H.Y. Lee, R. Palchaudhuri, M. Macchia, A. Martinelli, F. Minutolo, P.J. Hergenrother, Dual targeting of the warburg effect with a glucose-conjugated lactate dehydrogenase inhibitor, ChemBioChem. 14 (2013) 2263–2267. doi:10.1002/cbic.201300562.

[117] H.Y. Lee, E.I. Parkinson, C. Granchi, I. Paterni, D. Panigrahy, P. Seth, F. Minutolo, P.J. Hergenrother, Reactive Oxygen Species Synergize to Potently and Selectively Induce Cancer Cell Death, ACS Chem. Biol. 12 (2017) 1416–1424. doi:10.1021/acschembio.7b00015.

[118] J. Lu, The Warburg metabolism fuels tumor metastasis, Cancer Metastasis Rev. (2019). doi:10.1007/s10555-019-09794-5.

[119] M. Wangpaichitr, C. Wu, Y.Y. Li, D.J.M. Nguyen, H. Kandemir, S. Shah, S. Chen, L.G. Feun, J.S. Prince, M.T. Kuo, N. Savaraj, Exploiting ROS and metabolic differences to kill cisplatin resistant lung cancer, Oncotarget. 8 (2017) 49275–49292. doi:10.18632/oncotarget.17568.

[120] G. Cannino, F. Ciscato, I. Masgras, C. Sánchez-Martín, A. Rasola, Metabolic Plasticity

of Tumor Cell Mitochondria, Front. Oncol. 8 (2018) 333. doi:10.3389/fonc.2018.00333.

[121] M.C. Maiuri, G. Kroemer, Essential role for oxidative phosphorylation in cancer progression, Cell Metab. 21 (2015) 11–12. doi:10.1016/j.cmet.2014.12.013.

[122] V. Gouirand, F. Guillaumond, S. Vasseur, Influence of the Tumor Microenvironment on Cancer Cells Metabolic Reprogramming, Front. Oncol. 8 (2018) 117. doi:10.3389/fonc.2018.00117.

[123] C. Jose, N. Bellance, R. Rossignol, Choosing between glycolysis and oxidative phosphorylation: A tumor's dilemma?, Biochim. Biophys. Acta - Bioenerg. 1807 (2011) 552–561. doi:10.1016/j.bbabio.2010.10.012.

[124] C.J. Halbrook, C. Pontious, I. Kovalenko, L. Lapienyte, S. Dreyer, H.-J. Lee, G. Thurston, Y. Zhang, J. Lazarus, P. Sajjakulnukit, H.S. Hong, D.M. Kremer, B.S. Nelson, S. Kemp, L. Zhang, D. Chang, A. Biankin, J. Shi, T.L. Frankel, H.C. Crawford, J.P. Morton, M. Pasca di Magliano, C.A. Lyssiotis, Macrophage-Released Pyrimidines Inhibit Gemcitabine Therapy in Pancreatic Cancer., Cell Metab. 29 (2019) 1390-1399.e6. doi:10.1016/j.cmet.2019.02.001.

[125] B. Ngo, J.M. Van Riper, L.C. Cantley, J. Yun, Targeting cancer vulnerabilities with high-dose vitamin C, Nat. Rev. Cancer. 19 (2019) 271–282. doi:10.1038/s41568-019-0135-7.

[126] S. Park, S. Ahn, Y. Shin, Y. Yang, C.H. Yeom, Vitamin C in cancer: A metabolomics perspective, Front. Physiol. 9 (2018) 762. doi:10.3389/fphys.2018.00762.

[127] K. Polireddy, R. Dong, G. Reed, J. Yu, P. Chen, S. Williamson, P.C. Violet, Z. Pessetto, A.K. Godwin, F. Fan, M. Levine, J.A. Drisko, Q. Chen, High dose parenteral

ascorbate inhibited pancreatic cancer growth and metastasis: Mechanisms and a phase I/IIa study, Sci. Rep. 7 (2017) 17188. doi:10.1038/s41598-017-17568-8.

[128] J.D. Schoenfeld, Z.A. Sibenaller, K.A. Mapuskar, B.A. Wagner, K.L. Cramer-Morales, M. Furqan, S. Sandhu, T.L. Carlisle, M.C. Smith, T. Abu Hejleh, D.J. Berg, J. Zhang, J. Keech, K.R. Parekh, S. Bhatia, V. Monga, K.L. Bodeker, L. Ahmann, S. Vollstedt, H. Brown, E.P. Shanahan Kauffman, M.E. Schall, R.J. Hohl, G.H. Clamon, J.D. Greenlee, M.A. Howard, M.K. Shultz, B.J. Smith, D.P. Riley, F.E. Domann, J.J. Cullen, G.R. Buettner, J.M. Buatti, D.R. Spitz, B.G. Allen, O2·− and H2O2-Mediated Disruption of Fe Metabolism Causes the Differential Susceptibility of NSCLC and GBM Cancer Cells to Pharmacological Ascorbate, Cancer Cell. 31 (2017) 487-500.e8. doi:10.1016/j.ccell.2017.02.018.

[129] J. Yun, E. Mullarky, C. Lu, K.N. Bosch, A. Kavalier, K. Rivera, J. Roper, I.I.C. Chio, E.G. Giannopoulou, C. Rago, A. Muley, J.M. Asara, J. Paik, O. Elemento, Z. Chen, D.J. Pappin, L.E. Dow, N. Papadopoulos, S.S. Gross, L.C. Cantley, Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH, Science (80-.). 350 (2015) 1391–1396. doi:10.1126/science.aaa5004.

[130] N. Mikirova, J. Casciari, N. Riordan, R. Hunninghake, Clinical experience with intravenous administration of ascorbic acid: Achievable levels in blood for different states of inflammation and disease in cancer patients, J. Transl. Med. 11 (2013) 191. doi:10.1186/1479-5876-11-191.

[131] S.J. Padayatty, Brief Communication Vitamin C Pharmacokinetics : Implications for Oral and, Ann. Intern. Med. 140 (2004) 533–538. doi:10.1111/j.1445-5994.1980.tb03725.x.

[132] Q. Chen, M.G. Espey, A.Y. Sun, C. Pooput, K.L. Kirk, M.C. Krishna, D.B. Khosh, J.

Drisko, M. Levine, Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice, Proc. Natl. Acad. Sci. 105 (2008) 11105–11109. doi:10.1073/pnas.0804226105.

[133] N. Shenoy, E. Creagan, T. Witzig, M. Levine, Ascorbic Acid in Cancer Treatment: Let the Phoenix Fly, Cancer Cell. 34 (2018) 700–706. doi:10.1016/j.ccell.2018.07.014.

[134] L. Cimmino, B.G. Neel, I. Aifantis, Vitamin C in Stem Cell Reprogramming and Cancer, Trends Cell Biol. 28 (2018) 698–708. doi:10.1016/j.tcb.2018.04.001.

[135] E.A. Minor, B.L. Court, J.I. Young, G. Wang, Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine, J. Biol. Chem. 288 (2013) 13669–13674. doi:10.1074/jbc.C113.464800.

[136] J.I. Young, S. Züchner, G. Wang, Regulation of the Epigenome by Vitamin C, Annu. Rev. Nutr. 35 (2015) 545–564. doi:10.1146/annurev-nutr-071714-034228.

[137] M. Uetaki, S. Tabata, F. Nakasuka, T. Soga, M. Tomita, Metabolomic alterations in human cancer cells by Vitamin C-induced oxidative stress, Sci. Rep. 5 (2015) 13896. doi:10.1038/srep13896.

[138] S.O. Sajadian, C. Tripura, F.S. Samani, M. Ruoss, S. Dooley, H. Baharvand, A.K. Nussler, Vitamin C enhances epigenetic modifications induced by 5-azacytidine and cell cycle arrest in the hepatocellular carcinoma cell lines HLE and Huh7, Clin. Epigenetics. (2016). doi:10.1186/s13148-016-0213-6.

[139] J. Cha, M.W. Roomi, V. Ivanov, T. Kalinovsky, A. Niedzwiecki, M. Rath, Ascorbate supplementation inhibits growth and metastasisof B16FO melanoma and 4T1 breast cancer cellsin vitamin C-deficient mice, Int. J. Oncol. (2013).

doi:10.3892/ijo.2012.1712.

[140] S.B. Vuyyuri, J. Rinkinen, E. Worden, H. Shim, S. Lee, K.R. Davis, Ascorbic Acid and a Cytostatic Inhibitor of Glycolysis Synergistically Induce Apoptosis in Non-Small Cell Lung Cancer Cells, PLoS One. 8 (2013) e67081. doi:10.1371/journal.pone.0067081.

[141] J. Du, J.J. Cullen, G.R. Buettner, Ascorbic acid: Chemistry, biology and the treatment of cancer, Biochim. Biophys. Acta - Rev. Cancer. 1826 (2012) 443–457. doi:10.1016/j.bbcan.2012.06.003.

[142] J.L. Welsh, B.A. Wagner, T.J. Van'T Erve, P.S. Zehr, D.J. Berg, T.R. Halfdanarson, N.S. Yee, K.L. Bodeker, J. Du, L.J. Roberts, J. Drisko, M. Levine, G.R. Buettner, J.J. Cullen, Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): Results from a phase i clinical trial, Cancer Chemother. Pharmacol. 71 (2013) 765–775. doi:10.1007/s00280-013-2070-8.

[143] J. Du, S.M. Martin, M. Levine, B.A. Wagner, G.R. Buettner, S.H. Wang, A.F. Taghiyev, C. Du, C.M. Knudson, J.J. Cullen, Mechanisms of ascorbate-induced cytotoxicity in pancreatic cancer, Clin. Cancer Res. 16 (2010) 509–520. doi:10.1158/1078-0432.CCR-09-1713.

[144] Z. Zhao, F. Han, S. Yang, J. Wu, W. Zhan, Oxamate-mediated inhibition of lactate dehydrogenase induces protective autophagy in gastric cancer cells: Involvement of the Akt-mTOR signaling pathway, Cancer Lett. 358 (2015) 17–26. doi:10.1016/j.canlet.2014.11.046.

[145] A. Kohlmann, S.G. Zech, F. Li, T. Zhou, R.M. Squillace, L. Commodore, M.T. Greenfield, X. Lu, D.P. Miller, W.S. Huang, J. Qi, R.M. Thomas, Y. Wang, S. Zhang, R. Dodd, S. Liu, R. Xu, Y. Xu, J.J. Miret, V. Rivera, T. Clackson, W.C. Shakespeare,

X. Zhu, D.C. Dalgarno, Fragment growing and linking lead to novel nanomolar lactate dehydrogenase inhibitors, J. Med. Chem. 56 (2013) 1023–1040. doi:10.1021/jm3014844.

[146] M. Manerba, M. Vettraino, L. Fiume, G. di Stefano, A. Sartini, E. Giacomini, R. Buonfiglio, M. Roberti, M. Recanatini, Galloflavin (CAS 568-80-9): A Novel Inhibitor of Lactate Dehydrogenase, ChemMedChem. 7 (2012) 311–317. doi:10.1002/cmdc.201100471.

FIGURES

Figure 1. Targeting tumor-metabolism and metastatic progression

Oncometabolites such as glucose or lactate, play a crucial role in tumor progression and metastasis and are reported to induce cell recruitment and migration, extracellular matrix (ECM) remodeling and epithelial-to-mesenchymal transition (EMT). Small-molecule inhibitors such as STF-31, WZB117, 2-DG and Oxamate could hinder the metastatic progression by targeting glucose transporter 1 (GLUT1), hexokinase (HK) and lactate dehydrogenase (LDH-A), respectively, leading to a decreased glucose uptake, lower production of glucose-6-phosphate (G6P) and a decrease of lactate production. Another potential inhibitor of invasiveness, cell migration and metastasis is high dose vitamin C, which can be imported into the cell as its oxidized form (DHA) via the GLUT-1 transporter and its dehydrogenated form (AscH-) by the sodium-ascorbate co-transporter (SVCT). Vitamin C can prevent metastatic niche formation mostly by increasing reactive oxygen species (ROS), which disrupt the high energy demand of tumor cells by hindering ATP and NAPDH formation. Other observed effects of vitamin C on the glycolytic pathway are: an increase of the first glucose metabolites G6P, fructose-6-phosphate (F6-P) and fructose-1,6-biphosphate (F1,6BP) and a decrease of downstream intermediates, such as 3-phosphoglycerate (3PG), phosphoenolpyruvate (PEP), pyruvate and lactate, and the crucial enzyme LDH-A, probably via HIF-1 degradation. To note, vitamin C might reverse the epithelial-to-mesenchymal transition (EMT) by increasing and reducing E-cadherin/VIM and Snail expression, respectively, and also by inhibiting matrix metalloproteases (MMPs) activity.

Figure 2. Glycolytic pathway inhibitors. Chemical structures of small molecules targeting the glycolytic pathway. The key chemical moieties necessary for the inhibition of the targets are highlighted for each molecule.

Table 1. List of agents targeting Warburg effect in cancer.

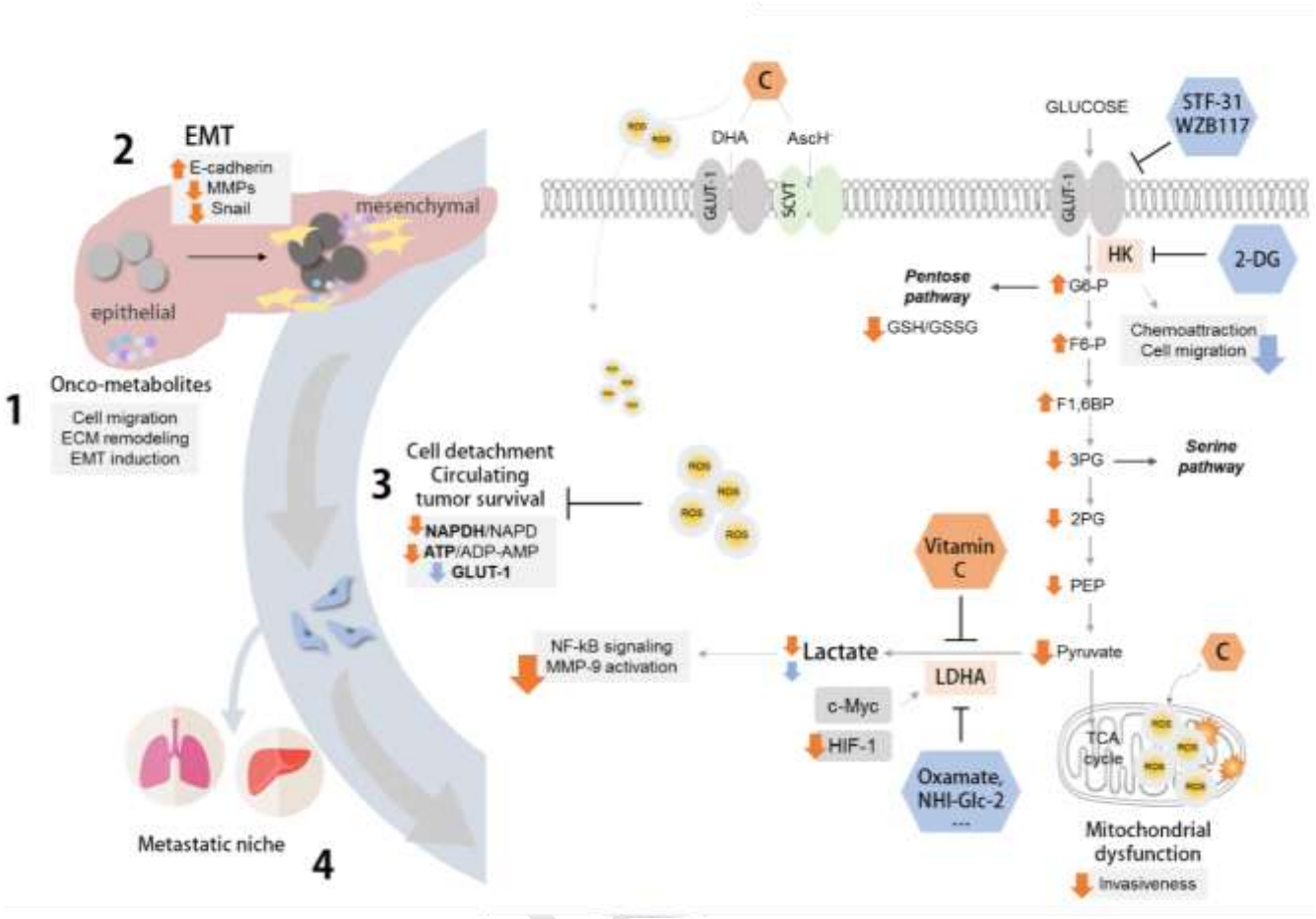

Figure 1.

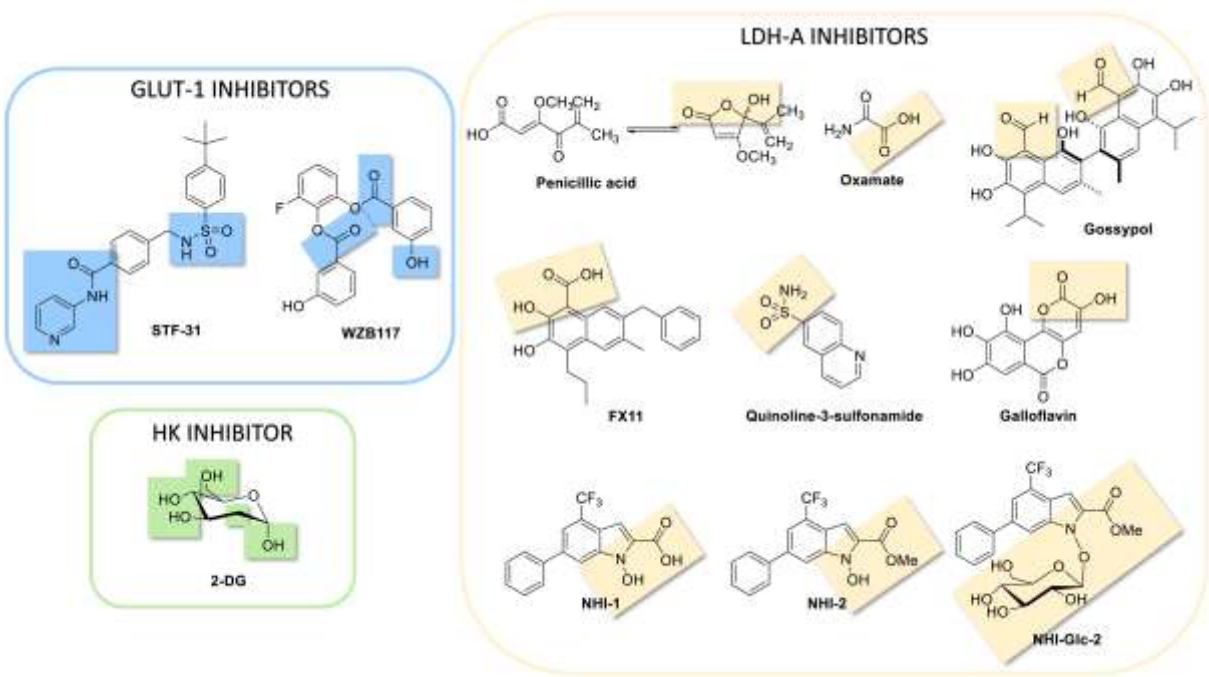

Figure 2.

Table 1.

GLUT-1 Inhibitor					
	STF-31	Renal Carcinoma	Preclinical	Narrow therapeutic range	[66]
	WZB117	Ductal breast carcinoma, non-small cell lung cancer	Preclinical	Early phase study, lack of evidence	[67]
Hexokinase Inhibitor					
	2-deoxy-d-glucose (2-DG)	Advanced Solid Tumor, Prostate Cancer, Glioma Multiforme	Phase I Clinical Trial	Low response rate, variability in recommended dose	[71–73]
Lactate Dehydrogenase Inhibitors					
Pyruvate Competitive Agent	Oxamate	Cervical Cancer, Gastric Cancer	Preclinical	Poor membrane penetration	[92,144]
NADH-competitive agent	Gossypol	Metastatic breast cancer, malignant glioma, metastatic adrenal cancer	Phase I/II clinical trial	Low response rate	[101–103]
	FX-11	Adrenal cancer	Preclinical	Reactive catechol moiety	[23,106,145]
	Quinoline 3-sulfonamide	Lymphoma, Pancreatic Cancer	Preclinical	Low clearance rate	[108]
Pyruvate and NADH-competitive agent	N-hydroxyindoles (NHI)	Hepatocellular carcinoma	Preclinical	Early phase study, lack of evidence	[9,113]
Free LDH-A Binding Agent	Galloflavin	Pancreatic, breast cancer, hepatocellular carcinoma	Preclinical	Early phase study, lack of evidence	[110,111,146]