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Mini-review

2-Deoxy-D-glucose targeting of glucose metabolism in cancer cells as a potential therapy

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ABSTRACT

Cancer cells are characterized by altered glucose metabolism known as the Warburg effect in which aerobic glycolysis is increased. Glucose is converted to lactate even under sufficient oxygen tension. Interfering with this process may be a potential effective strategy to cause cancer cell death because these cells rely heavily on glucose metabolism for survival and proliferation. 2-Deoxy-D-glucose (2DG), a glucose analog, targets glucose metabolism to deplete cancer cells of energy. In addition, 2DG increases oxidative stress, inhibits N-linked glycosylation, and induces autophagy. It can efficiently slow cell growth and potently facilitate apoptosis in specific cancer cells. Although 2DG itself has limited therapeutic effect in many types of cancers, it may be combined with other therapeutic agents or radiotherapy to exhibit a synergistic anticancer effect. In this review, we describe the Warburg effect and discuss 2DG and its underlying mechanisms and potential application for cancer treatment.

Introduction

Cancer cells differ from healthy cells because of a myriad of molecular changes, which lead to self-sufficiency in growth signals, avoidance of immuno-surveillance, insensitivity to antigrowth signals, tissue invasion and metastasis, limitless replication potential, sustained angiogenesis, and evasion of apoptosis [1]. Another hallmark of cancer is altered glucose metabolism, which was first discovered by the Nobel Prize laureate Otto Warburg in the 1920s [2]. Compared with normal cells, cancer cells ferment glucose into lactate to generate energy even in the presence of sufficient oxygen. This dependency on high levels of glycolysis is essential for energy production and the macromolecular synthesis in cancer cells. This is also correlated with decreased anticancer therapy efficiency and drug resistance [3,4]. Although the Warburg effect is not applicable to all cancers, inhibition of this sufficiently prevalent process with the glucose analog 2-Deoxy-D-glucose (2DG) is a promising treatment for many cancers [5–7]. The objective of this review is to provide a brief introduction to altered glucose metabolism in cancer cells or the "Warburg effect" and to discuss the primary mechanisms involved in the anticancer effects of 2DG.

The process of cancer glycolysis (Fig. 1)

Glucose metabolism begins with the transportation of glucose into cells primarily via glucose transporters (GLUTs). After being phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase (HK), it is either converted to fructose-6-phosphate (F-6-P) by phosphoglucose-isomerase (PGI) and further catalyzed via the remaining steps of glycolysis or metabolized by glucose-6-phosphate dehydrogenase (G-6-PD) and enters the pentose phosphate pathway (PPP). Cells lack transport systems for phosphorylated glucose; therefore, the activated anionic glucose is trapped and its accumulation inhibits HK allosterically. When HK tightly associates with the outer surface of the mitochondrial membrane via interaction with mitochondrial protein voltage-dependent anion channel (VDAC) [3,8], abundant ATP from the mitochondria can promote the acceleration of the HK-catalyzed rate-limiting glycolytic step. Phosphofructokinase (PFK) is a kinase that catalyzes the ATP-dependent phosphorylation of F-6-P. PFK-1 irreversibly converts F-6-P to fructose-1, 6-bisphosphate (F-1, 6-BP), which can be allosterically inhibited by a high level of ATP. The up-regulated PFK-2 produces F-2, 6-BP, which functions as a potent activator of PFK-1 and decreases the inhibitory effect of ATP, thus resulting in high ATP generation [2,9]. F-1, 6-BP is then converted into glyceraldehyde-3-phosphate or dihydroxyacetone phosphate, with the latter serving as a critical component for the biosynthesis of phospholipids and triacylglycerols [9]. As glycolysis proceeds, pyruvate kinase (PK) catalyzes the conversion of phosphoenol-pyruvate (PEP) to pyruvate.

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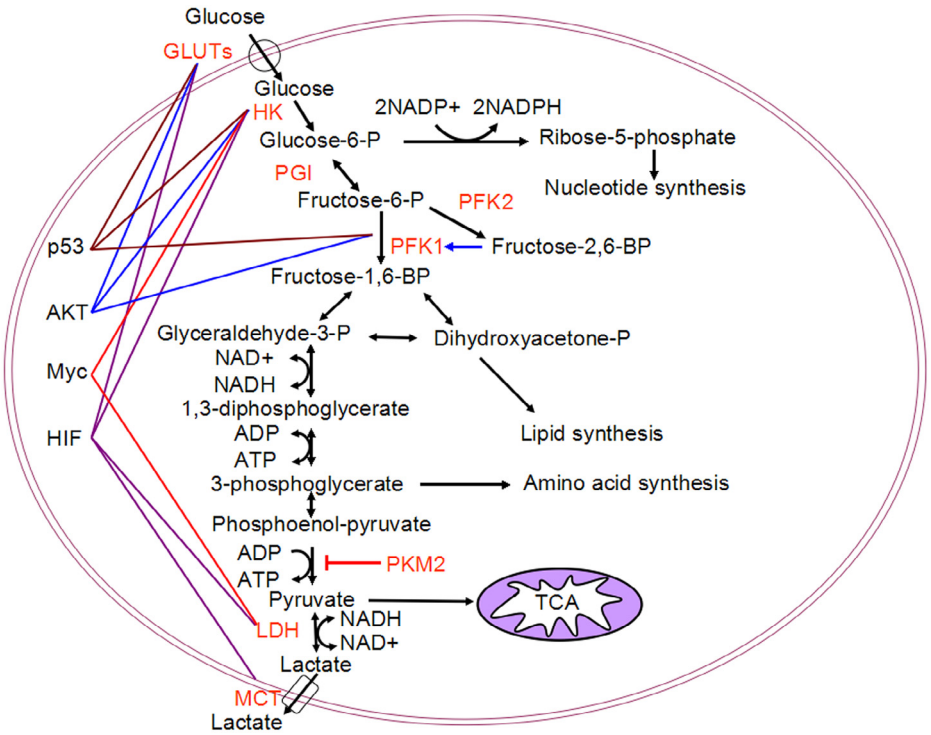

Fig. 1. Glycolysis in cancer cells. The increased glucose metabolism of cancer cells through glycolysis generates metabolic intermediates necessary for the proliferation of cancer cells. These intermediates are precursors for the synthesis of biomolecules, including lipids, amino acids and nucleotides. Highly activated PFK2 in cancer cells triggers the formation of F-2, 6-BP, which promotes the function of PFK1 and ensures a high flux of glycolysis. Because of the PKM2 bottleneck, glucose is massively shunted into the glycolytic bypasses for the generation of building blocks for cancer cells. The protooncogene Myc, the signaling pathway PI3K/Akt, the transcription factor HIF-1, and the tumor suppressor p53 are all closely related to glycolysis in cancer cells with direct/indirect effects on different targets. GLUTs, glucose transporters; HK, hexokinase; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; F-2, 6-BP, fructose-1, 6-bisphosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PK, pyruvate kinase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter.

Cancer cells express high levels of the dimeric form of the PK isoenzyme type M2 (PKM2), which has a less affinity to its substrate PEP and therefore shows less catalytic activity than PKM1 [9,10]. PKM2 leads to the accumulation and congestion of all glycolytic intermediates described above, contributing to the biosynthesis of nucleic acids, phospholipids, and amino acids. Finally, lactate dehydrogenase (LDH) generates lactate from pyruvate while oxygenizing NADH to NAD+. Over-expression of LDH maintains a high glycolytic flux by promoting NAD+ regeneration, which is necessary for the conversion of glyceraldehyde-3-phosphate to 1,3- bisphosphoglycerate [9,11].

Overall, glycolysis is a less efficient pathway for generating ATP (2 ATP per metabolized glucose) compared with mitochondrial oxidative phosphorylation (OXPHOS) (36 ATP per metabolized glucose); however, the rate of ATP production from glycolysis is more adjustable and approximately 100× faster than from OXPHOS. To meet increased bioenergetic demands for cell growth and proliferation, cancer cells consume at least 10 times more glucose than normal cells [2,12] and increase the rate of glycolysis by more than 30-fold [13].

The significance of cancer glycolysis

Cells under oxygen-depleted conditions rely on anaerobic glycolysis to generate energy, and carbon sources other than glucose are unusable. Under aerobic conditions, cells may use amino acids or fats to generate ATP. Many cancer cells grow under anaerobic or hypoxic conditions; therefore, it is reasonable to hypothesize that they use glycolysis. However, according to Warburg, cancer cells continue to rely on glycolysis even when there is sufficient oxygen.

Increased glucose metabolism promotes the production of glycolytic intermediates, which are shunted into glycolytic subsidiary pathways to supply building blocks for the biosynthesis of macromolecules [9]. NADPH generated through PPP ensures the antioxidant defense of the cell, maintains redox homeostasis and attenuates the efficiency of some chemotherapeutic agents [2]. Enhanced glycolysis also promotes acidification of the microenvironment, partly because of the extrusion of monocarboxylate acid, such as lactic acid via the monocarboxylate transporter (MCT), which favors tumor angiogenesis, promotes invasion and dissemination, induces immunosuppression against cytotoxic T cells and decreases the efficiency of some anticancer drugs, including doxorubicin and vincristine [2,14,15].

This peculiar and essential transition is advantageous to cancer cells because it allows them to survive and proliferate. However, it is also their weakness because inhibiting this process may cause death. Safe and efficient inhibition of glycolysis is a promising anticancer therapy, either by itself, or in combination with radiotherapy or chemotherapy [16].

Mechanisms involved in the Warburg effect

Although many cancer cells live under hypoxic conditions, hypoxia may not be the fundamental driving force for the glycolytic switch because this transition is observed during the early stage of carcinogenesis even before cancers experience hypoxia [17]. Warburg's initial assertion is based on the hypothesis of mitochondrial defects in cancer cells. Mitochondrial genome mutations exist in the majority of cancer cells leading to the changed structure and function of mitochondria [18]. The increased glycolytic activity is

related to but not necessarily accompanied by a reduction in oxidative phosphorylation [19,20] because serious defects in mitochondria that disrupt function are infrequent [9]. The regulating of mitochondrial function and the mechanism by which this contributes to Warburg effect remain obscure.

Molecular mechanisms, such as the activation of protooncogenes (e.g., Myc), signaling pathways (e.g., PI3K/Akt), transcription factors (e.g., HIF-1), and the inactivation of tumor suppressors (e.g., p53) are closely related to the induction of the Warburg effect [3] (Fig. 1).

Myc is a regulatory gene encoding a transcription factor, which globally activates target gene expression and plays a critical role in glucose metabolism, cell proliferation, cell cycle progression, apoptosis and cellular transformation [21]. Myc promotes energy generation by inducing genes involving in mitochondrial biogenesis and glucose metabolism, such as LDH-A [22,23].

Activation of the PI3K/Akt pathway promotes glycolysis, boosts protein synthesis and inhibits autophagy [15,24,25]. PI3K causes cancer cells to utilize glucose because it inhibits β-oxidation, a process by which fatty acids are broken down to generate acetyl-CoA and further metabolized through the citric acid cycle to generate energy [2,3]. Akt increases the surface translocation of glucose transporters, stimulates HK activation by promoting its binding to mitochondria [15,26].

Hypoxia-inducible factor (HIF) is a critical transcription factor that is activated in the majority of tumors under hypoxic conditions and even normoxic conditions (pseudo-hypoxia), and it aids in the survival of cells under hypoxic stress [27,28]. HIF-1 is heterodimeric in structure, containing HIF1α or HIF2α and HIF1β with HIF1α as the major determinant of the glycolytic phenotype. Under normoxic conditions, HIF1α undergoes rapid degradation mediated by the ubiquitin proteasome. When oxygen is sufficient, this proteasomal degradation ceases to function, resulting in the stabilization of HIF1α, which associates with HIF1β and subsequently binds to hypoxia-responsive elements (HREs) in target genes regulating glucose metabolism [13,29]. HIF1α activates GLUT1 and several key enzymes of glycolysis, such as HK, PKM2 and LDH-A and stimulates the MCT4 and pyruvate dehydrogenase kinase-1 (PDK1), thereby promoting glycolysis and enhancing lactic acid production [2,3,30].

The p53 protein, a tumor suppressor, is activated by a variety of stressors, including DNA damage, oncogene activation, telomere erosion, stromal support loss, and nutrient and oxygen deprivation [31,32]. Inactivation of p53 is suggested to directly contribute to the Warburg phenomenon [3]. Mitochondrial activity is somewhat dependent on p53 for the maintenance of mtDNA copy number and mitochondrial mass [31]. p53 promotes oxidative phosphorylation and inhibits glycolysis via down-regulation of GLUT1, GLUT3, and GLUT4 expression [33,34] and inactivation of glycolytic enzymes, such as phosphoglyerate mutase (PGM) [31,35]. The p53 transcriptionally activated TIGAR (TP53-induced glycolysis and apoptosis regulator) plays a critical part in lowering the glycolytic rate and promoting the PPP because it inhibits the function of F-2, 6-BP [3,31,36,37].

The introduction of 2DG

2DG, a synthetic glucose analog in which the C-2-hydroxyl group is replaced by hydrogen, has been extensively and thoroughly investigated in both scientific and clinical studies since the early 1950s [38]. Although 2DG is commonly thought to inhibit glycolysis, it actually has extensive metabolic effects and interferes with various biological processes, including depletion of cellular energy, intensification of oxidative stress, interference with N-linked glycosylation, and induction of autophagy [39,40], which also activates multiple signaling pathways, such as PI3K, MAPK, and AMPK [41,42]. These events are somewhat related and integrated. Toxicity following 2DG treatment is caused by more than one mechanism in many cancer cell types [40].

The mechanisms involved in the effect of 2DG on cancer cells (Fig. 2)

Inhibition of glycolysis and depletion of ATP

2DG competitively inhibits glucose uptake because they both are transferred by the GLUTs. After entering the cell, 2DG is phosphorylated by HK to form 2-Deoxy-D-glucose-6-phosphate (2DG-6-P), which cannot be further metabolized via glycolysis but accumulates and noncompetitively inhibits HK and competitively inhibits PGI [7,38,43]. Because 2DG inhibits the first critical steps at the beginning of glucose metabolism, both glycolysis and OXPHOS may be partially disrupted [44]. These events lead to decreased ATP production, blocked cell cycle, decreased and inhibited cell growth and even cell death [6,16,40].

Decreased intracellular ATP production leads to an increase of the AMP/ATP ratio, which activates and increases the AMP-activated protein kinase (AMPK), resulting in elevated catabolic metabolism via phosphorylation of downstream targets, such as the mammalian target of rapamycin (mTOR) [42]. In addition, decreased ATP sensitizes cells to extrinsic (death receptor-mediated) apoptosis [44,45] via binding of the tumor necrosis factor (TNF) ligand family members, including TNF-related apoptosis-inducing ligand (TRAIL), to their cognate transmembrane death receptors [41]. Defects of p53 are closely related to reduced ATP induced by 2DG because p53 can act as an effective energy sensor of decreased ATP and restore the ATP level by promoting oxidative phosphorylation [36]. HIF also reduces the efficiency of 2DG and induces resistance by increasing glucose transporters and several glycolytic enzymes; therefore, down-regulation of HIF sensitizes cells to the detrimental effect of 2DG [38,46].

However, energy deprivation itself is not sufficient for the antitumor effect of 2DG. First, ATP is necessary for both intrinsic and extrinsic-mediated apoptosis and apoptotic cells generally have elevated levels of ATP [38]. This suggests that 2DG may paradoxically exert a cytoprotective effect. Second, 2DG treatment, mimicking glucose deprivation, does not predispose some cells to death because they maintain OXPHOS function and utilize alternative carbon sources, such as fatty acids and amino acids to synthesize ATP under normoxic conditions [47]. In addition, treatments that rescue cells from 2DG-induced cell death, including mannose and Bcl-2 overexpression do not reverse the depletion of ATP [6].

Inhibition of antioxidation

Cellular reactive oxygen species (ROS) can cause lipid peroxidation, DNA damage and protein oxidation, thereby contributing to aberrant redox signal transmission, genomic mutation, cell immortalization, and the inability to differentiate, which are characteristics of the malignant phenotype [48,49]. Excessive ROS are detrimental; therefore, increasing ROS generation and simultaneously attenuating detoxification can kill cancer cells [50].

Increased glucose metabolism attenuates oxidative stress by detoxification of ROS and maintenance of redox homeostasis via the regeneration of NADPH from PPP and the formation of pyruvate from glycolysis [49,51]. The PPP is one of the primary pathways that create molecules with reducing power [31], and in its oxidative phase, two molecules of NADPH are generated from two molecules of NADP+, whereas the nonoxidative phase generates ribose-5-phosphate for the biosynthesis of nucleotides and nucleic acids. NADPH is a provider of reducing equivalents for all glutathione- and thioredoxin-dependent peroxidase pathways [51], both of which are major cellular thiol antioxidants responsible for the detoxification of

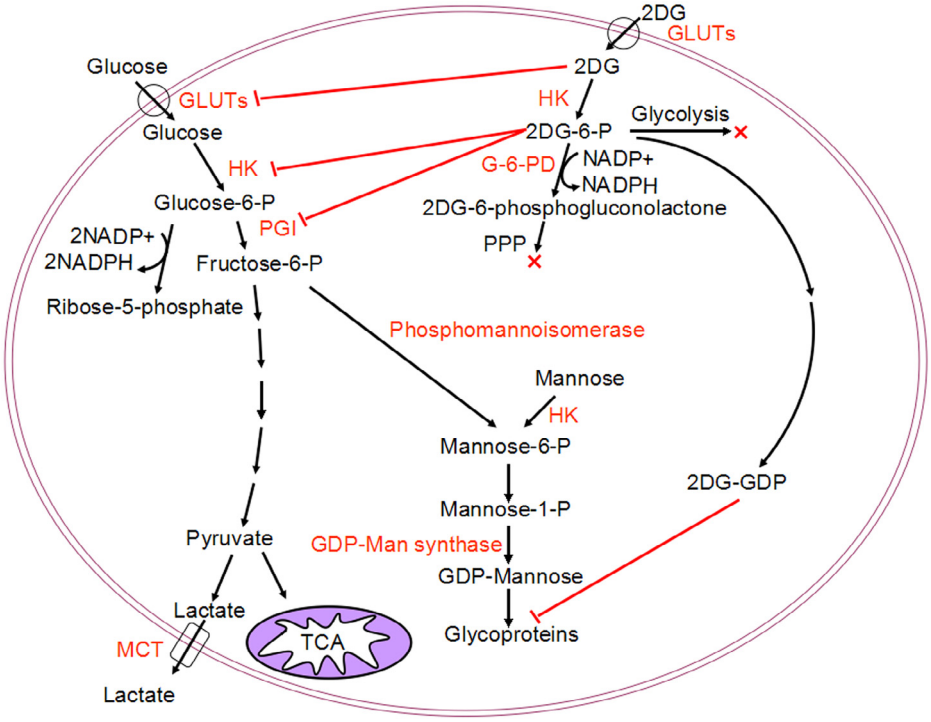

Fig. 2. The targets of 2DG. Similar to glucose, 2DG is taken up through GLUTs and then phosphorylated by HK to form 2DG-6-P, and 2DG also competitively inhibits GLUTs. 2DG-6-P cannot be further metabolized via glycolysis but accumulates in the cell and noncompetitively inhibits HK and competitively inhibits PGI. NADPH generation is inhibited. Only one molecule of NADPH can be generated from the conversion of 2DG-6-P to 2DG-6-phosphogluconolactone. 2DG structurally resembles mannose and undergoes conversion into 2DG-GDP, which interferes with the N-linked glycosylation of proteins. The inhibition of N-linked glycosylation induces the accumulation of unfold/misfolded proteins in the ER, resulting in ER stress and constant cell apoptosis. Intracellular glucose can promote glycosylation because its metabolic product F-6-P can be used in the mannose glycosylation pathway. However, the upstream glucose metabolism is inhibited by 2DG, which may not allow exogenous glucose to restore the interrupted N-linked glycosylation. GLUTs, glucose transporters; HK, hexokinase; PGI, phosphoglucose isomerase; G-6-PD, glucose-6-phosphate dehydrogenase; GDP, guanosine diphosphate; MCT, monocarboxylate transporter.

reactive oxygen and nitrogen species (ROS and RNS), maintenance cell redox potential, and the prevention and restoration of oxidative damage [31,40]. Pyruvate is crucial for maintaining the intracellular reduced glutathione level, suppressing superoxide production and direct scavenging of hydrogen peroxide [52,53].

Glucose deprivation triggers selective clonogenic cell death via increased oxidative stress from the increased production of hydrogen peroxide and superoxide [48]. Mimicking glucose deprivation, 2DG potently induces mitochondrial oxidative stress [36]. The phosphorylated product of 2DG, 2DG-6-P, can only undergo the first enzymatic reaction in the PPP by glucose-6-phosphate dehydrogenase (G-6-PD) to regenerate one molecule of NADPH from NADP+, but the product 2-Deoxy-D-glucose-6-phosphoglaconalactone cannot be further metabolized to generate the second molecule of NADPH [49,54]. In general, treatment with 2DG results in pyruvate and NADPH deficiencies, damaging the antioxidant defenses of cancer cells and may render them more vulnerable to oxidative stress induced by radiotherapy or chemotherapy. However, this effect may be impaired by p53 because p53 protects cancer cells from oxidative stress via upregulation of antioxidant enzymes, such as manganese superoxide dismutase (MnSOD) and glutathione peroxidase 1 (GPx1) [36].

Interference with N-linked glycosylation of proteins

The glycosylation of proteins is the enzymatic process that modifies proteins with a variety of structural and functional specific glycans, which promotes protein stability, proper folding and cell adhesion [55,56]. N-linked glycosylation occurs when glycans are attached to the nitrogen of asparagine or arginine side-chains of proteins, which occurs in the lumen of the endoplasmic reticulum (ER) [57,58]. During the assembly of N-linked oligosaccharides to proteins, mannose undergoes conversion to mannose-GDP by a covalent reaction with guanosine diphosphate (GDP) or dolichol phosphate (Dol-P) [58]. Serving as the precursor for the N-linked glycosylation of proteins, mannose-GDP is step-wise assembled onto N-acetyl-glucosamine residues catalyzed by GDP-mannosyltransferase and forms oligosaccharide chains. Glucose is involved in this process as it undergoes conversion to G-6-P, which can be further converted to mannose-6-phosphate by phosphomannose isomerase (PMI) and then metabolized to mannose-GDP, forming the oligosaccharide chains of proteins [38].

Because of its structural similarity to mannose, 2DG interferes with N-linked glycosylation. 2DG mimics mannose and undergoes conversion to 2DG-GDP before competing with mannose-GDP for assembly onto lipid-linked oligosaccharide chains. Furthermore, the intracellular conversion of 2DG-GDP leads to depletion of the chain-forming precursor and causes a further disrupted oligosaccharide formation. These aberrant oligosaccharides result in the disrupted synthesis of glycoproteins. Although intracellular glucose is involved in the N-linked glycosylation through its epimerization to mannose, exogenous glucose does not reverse 2DG toxicity because 2DG blocks the conversion from glucose to mannose [38].

The inhibition of N-linked glycosylation prevents the normal folding of proteins and induces the retention of unfolded/misfolded proteins in the ER, activating unfolded protein response (UPR) and leading to ER stress and subsequent demise [59–61]. The UPR acts a defensive function to relieve ER stress. On the one hand, it extensively inhibits protein translation and therefore reduces the amount of proteins entering the ER. On the other hand, it

increases the degradation of aberrant proteins [38,62]. However, with prolonged and increased ER stress, this organelle is severely impaired, and ER stress-specific apoptotic response elements, such as C/EBP homologous protein (CHOP), also known as growth arrest- and DNA damage-inducible gene 153 (GADD153), are activated [59,60,63]. Cells lacking CHOP are invulnerable to ER stress, which suggests that CHOP is essential in ER stress induced cell death and is a marker of ER stress. Other markers include glucose-regulated protein of 78 kDa, and glucose-regulated protein of 94 kDa [6,64–66]. Studies indicate that 2DG induces the up-regulation of ER stress markers, implicating glycosylation as a mechanism involved in 2DG-induced toxicity [6,51,66]. Following ER stress, the mitochondrial apoptotic pathway is triggered, involving the activation of proapoptotic Bcl-2 proteins [39]. The proapoptotic members are divided into multidomain subgroups, such as Bax and Bak, and BH3-only subgroups, such as Bim and Puma. 2DG can robustly promote Bax and Bak activation suggesting the involvement of mitochondrial apoptotic pathway in 2DG induced cell apoptosis [6,41,67]. Both Bim and Puma silencing can protect cells from ER stress caused by 2DG [6]. Many studies indicate that it is the inhibition of glycosylation rather than glycolysis that is responsible for 2DG induced cell death under aerobic conditions [5,6]. Exogenous mannose can rescue many cells from 2DG induced death but cannot reverse the significant decrease in ATP production [58,64].

Induction of autophagy

Autophagy (or "self-eating") is a ubiquitous catabolic process occurring at a basal level under normal conditions and modulates many physiological and pathological functions, such as tumorigenesis, neurodegeneration, pathogenic infection and aging [68,69]. Autophagic cell death is a type of programmed cell death distinguished from apoptosis based on the observation of autophagic structures, a lack of phagocyte recruitment, and in some cases, caspase independence [70].

Autophagy is an important cell survival mechanism for resistance to stressors, including starvation, hypoxia, hyperthermia, radiotherapy, hormones and growth factors deprivation, and cytotoxic agents. During this process, cellular organelles and bulk cytoplasm are encompassed into an isolated double membrane structure called the autophagosome, which then fuses with lysosome to form the autophagolysosome where its contents are degraded to provide an alternate energy source for use by cells [71–74]. In addition to the nutrient recycling function, autophagy mediates the degradation of defective proteins and organelles [70]. For example, autophagy rescues cells from mitochondria accumulated oxidative damage with the random and/or selective incorporation of mitochondria into autophagosome for subsequent degradation and elimination [75]. In addition, the UPR induced by misfolded, aggregated or damaged proteins activates ER stress and results in the autophagic engulfment and sequestration of the ER [76,77]. Despite the self-preservation, sustained and elevated autophagy under conditions of protracted stresses suppresses tumorigenesis [24,73,78].

A typical sign and marker of autophagy is the transformation of microtubule-associated protein 1A/1B-light chain 3 (LC3). LC3 is a soluble protein that ubiquitously exists in mammalian cells. During autophagy, the cytoplasmic form of LC3 (LC3-I) is transferred to autophagosome and concomitantly conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II), which is oriented to the autophagosomal membrane [40,79]. Significant LC3-II positivity following 2DG treatment suggests the induction of autophagy by 2DG [5,80]. Another marker, Beclin1, is also activated by 2DG because 2DG disengages Beclin 1 from Bcl-2, which acts as a key anti-apoptotic regulatory protein of the mitochondrial death pathway and also negatively regulates Beclin 1 [40,81]. 2DG increases autophagy by depleting ATP via mimicking effect of glucose deprivation [40,82]. ER stress induced by 2DG may also be an autophagic trigger because the addition of exogenous mannose, which reduces ER stress, abolishes autophagy [83,84].

The potential for 2DG use

Increased glycolysis allows cancer to acquire resistance to several drugs through acidification of the microenvironment [15]. The potent inhibition of lactate production by 2DG plays a promising role in counteracting anticancer drug resistance [20]. In addition, because ATP is decreased by 2DG treatment, ATP-dependent efflux pumps that remove cytotoxic agents cease to function, leading to intracellular drug accumulation and cell death [85]. Because cancer cells use 2DG the same way as glucose, it is possible that high glucose consuming cancer cells facilitate their own death by accumulating 2DG. Interestingly, Aft et al. found that 2DG treatment of breast cancer cells resulted in the up-regulation of GLUT-1 and the accelerated uptake of glucose and 2DG. This indiscriminate uptake is detrimental to the cells [86].

The majority of current anticancer therapies are flawed either because of high incidences of serious side effects or the gradual development of resistance through either inherent or adaptive mechanisms, which limits their clinical use. To overcome these difficulties, multiple therapies are combined to minimize side effects, maximize the therapeutic efficiency and reduce the incidence of drug resistance. Although, for the majority of cancer cells, 2DG treatment alone does not significantly induce cell death, its harmful effects on cancer cells indicate that it may cooperate with specific agents or radiation to exert a synergistic therapeutic action (Table 1). For example, simultaneous inhibition of glycolysis with 2DG and oxidative phosphorylation with either chemical (mitochondrial inhibitors), genetic, or environmental (hypoxia) methods results in the robust inhibition of cellular energy generation and synergistically induces cell death [40,85,92]. Liu et al. demonstrated that 2DG combined with mitochondrial electron transport chain blockers, such as antimycin A or rotenone effectively sensitized osteosarcoma cells to clonogenic cell killing [94]. Moreover, the combination of 2DG and metformin induced significant cell death associated with decreased cellular ATP, prolonged activation of AMPK, and sustained autophagy [20,90]. Issam et al. found that this combination was more harmful to prostate cancer cells than either agent alone, which might be because metformin inhibited 2DG induced autophagy [82].

Safety studies of 2DG

Cancer cells have a high demand for glucose, and because they uptake 2DG using the same mechanism as glucose, 2DG markedly accumulates in these cells. For the safety of 2DG, highly metabolic tissues other than cancer, such as heart and brain, may also exhibit a high uptake of 2DG; therefore, the potential toxicity of 2DG on these tissues is a practical concern and must be carefully studied. Notably, studies on rats showed that the chronic administration of 2DG at a high dose (0.2 g/kg) induced reduced food intake, cardiac toxicity and increased mortality [95,96].

In previous clinical studies, 2DG has been used to treat stress, psychiatric disorders, prostate cancer, and glioma, etc. [47,97]. Adverse events and clinical outcomes include fatigue, dizziness, restlessness, and asymptomatic QTc prolongation [98]. Clinical trials confirmed that the administration of 2DG alone or combined with other anticancer therapies, such as chemotherapy and radiotherapy was safe and well tolerated by patients (Table 2), leading to the initiation of several phase II and III clinical trials [98].

Table 1
Studies about the combination of 2DG with other therapies against cancers.

Combined therapy	Target cell lines	References
Adriamycin	Osteosarcoma and non-small cell lung cancer lines	[85]
Afatinib	Non-small cell lung cancer cell lines	[42]
Antimycin A	Colon cancer cell lines	[54]
Arctigenin	A549, HBE, Wi-38, H4, Hela and Hs578T cell lines	[87]
Ascorbate	Pancreatic cancer cell lines	[88]
Aurora kinases inhibitors: MK0457,ZM447439	Acute myeloid leukemia cell lines	[78]
Bcl-2 inhibitor: ABT263	Leukemia, cervical cancer, prostate cancer, hepatocarcinoma, breast cancer, small lung carcinomas cell lines	[41]
BH3 mimetic: ABT737	Neuroblastoma, lymphoma, leukemia, cervical cancer, prostate cancer, hepatocarcinoma, breast cancer, small lung carcinomas cell lines	[41,89]
Cisplatin	Neuroblastoma, metastatic melanoma cell lines	[89]
Efrapeptin	Breast cancer cell lines	[86]
Etoposide	Lymphoma cell lines	[50]
Ferulic acid + irradiation	Non-small cell lung cancer cell lines	[39]
Metformin	Gastric and esophageal cancer, prostate cancer cells	[80,82,90]
mTOR inhibitor: CCI779	Lung cancer cell lines	[91]
Oligomycin	Glioblastoma multiforme cell lines	[92]
Paclitaxel	Osteosarcoma and non-small cell lung cancer lines	[85]
Pyrimethamine	Metastatic melanoma cell lines	[40]
Recombinant human TRAIL	Melanoma, mantle cell lymphoma cells	[44,58]
Rotenone	Colon cancer cell lines	[54]
Staurosporine	Metastatic melanoma cell lines	[40]
Temozolomide	Metastatic melanoma cell lines	[40]
Trastuzumab	Breast cancer cell lines	[4,93]

Table 2
Several cancer clinical trials with 2DG alone or combined with other therapies.

Phases	Combined therapy	Diseases	Status	References
Phase I/II	–	Prostate cancer	Terminated (has results)	[26]
Phase I	Docetaxel	Lung cancer, breast cancer, head and neck cancers	Completed	[47]
Phase I	Radiotherapy	Glioblastoma multiforme	–	[99]
Phase I/II	Radiotherapy	Cerebral gliomas	–	[100]

Conclusion

Cancer cells have adopted a metabolic switch to aerobic glycolysis, which favors their survival and proliferation, a phenomenon known as the Warburg effect. Although the underlying mechanisms remain to be elucidated, interrupting glucose metabolism represents a novel and promising therapeutic anticancer strategy. Of the antiglycolytic agents, 2DG, a glucose analog, is a potential potent antitumor agent. It is toxic to certain cancer cells, even at a relatively low dosage. The mechanisms involved include the depletion of cellular energy, increased oxidative stress, interference with N-linked glycosylation, and induction of autophagy. Generally, 2DG only leads to growth inhibition of most cancer cells. To achieve efficacious anticancer treatment, different strategies must be combined with 2DG to overcome the defects of mono-therapy. Further investigations are underway to identify the underlying mechanisms, proper combined therapies, and clinical applications of this promising agent.

Conflicts of interest

No potential conflicts of interest were disclosed.

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