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The Warburg effect: molecular aspects and therapeutic possibilities

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Abstract It has been about nine decades since the proposal of Otto Warburg on the metabolism of cancer cells. Unlike normal cells which undergo glycolysis and oxidative phosphorylation in the presence of oxygen, proliferating and cancer cells exhibit an increased uptake of glucose and increased rate of glycolysis and predominantly undergo lactic acid fermentation. Whether this phenomenon is the consequence of genetic dysregulation in cancer or is the cause of cancer still remains unknown. However, there is certainly a strong link between the genetic factors, epigenetic modulation, cancer immunosurveillance and the Warburg effect, which will be discussed in this review. Dichloroacetate and 3-bromopyruvate are among the substances that have been studied as potential cancer therapies. With our expanding knowledge of cellular metabolism, therapies targeting the Warburg effect appear very promising. This review discusses different aspects of these emerging therapies.

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Introduction

Otto Heinrich Warburg was a renowned German scientist in physiology, biochemistry and medicine in the twentieth century [1]. In 1956, Warburg proposed a hypothesis in relation to cancer cell metabolism: unlike normal cells, cancer cells predominantly undergo aerobic glycolysis instead of oxidative phosphorylation. This particular phenomenon is referred to as the 'Warburg effect'. He suggested that this was due to a defect in mitochondria [2]. After Warburg's observation, several research groups started to work on this aspect of cancer, however, its within the past decade where our understanding of metabolism and oncogenes has increased have scientist began to investigate the Warburg effect more seriously. Since then the industry has witnessed an explosion in the development in the field of glucose metabolism in cells and cancer biology.

In normal differentiated tissues, cells undergo mitochondrial oxidative phosphorylation. One glucose molecule can generate up to 36 adenosine triphosphate (ATP). Glycolysis is an essential metabolic pathway, which takes place in the cytoplasm. Glucose uptake is facilitated by glucose transporters embedded in the cell membrane. Glucose is then converted to 2 pyruvate molecules. This process yields 2 ATP and 2 reduced nicotinamide adenine dinucleotide (NADH). In pyruvate decarboxylation, pyruvate is converted to acetyl group. Coenzyme A then comes along, forming acetyl-CoA complex. Although oxygen has no direct role in pyruvate oxidation or the Krebs cycle, its presence is required in order to complete this step. Acetyl

CoA feeds into the Krebs cycle in the matrix of mitochondria. The net result is 1 ATP, 3 NADH and 1 reduced flavin adenine dinucleotide ($FADH_2$). After the first step of the cycle, CoA is regenerated. Electrons from NADH are transferred from complex I and electrons from FADH2 are transferred from complex II to coenzyme Q, which in turn is oxidized by complex III. In complex III, two electrons are sequentially transferred to two molecular of cytochrome c, and oxidised by complex IV. Four electrons are removed from four molecules of cytochrome c, and transferred to molecular oxygen resulting in its reduction to water. The movement of electrons through complexes I–IV causes protons (H+) to be pumped out of the intermembrane space into the cell cytosol, forming the electrochemical gradient. H+ ions are subsequently returned back across the membrane through channels with ATP synthase, which converts the energy produced from this process, to form ATP. This complete process results in the yield of 36 ATP molecules.

However, in condition where oxygen is limited, glucose undergoes anaerobic metabolism, which is the partial oxidation of glucose to pyruvate, which is then reduced to lactate (in human) or alcohol (in bacteria). NADH gets oxidized and 1 NAD^+ is regenerated for glycolysis. Only two ATP are yielded in anaerobic metabolism. Warburg observed that cancer cells mostly convert glucose into lactate even in the presence of oxygen. This characteristic is shared with normal proliferating cells. The net energy yield in anaerobic glycolysis is two ATP molecules. This observation leads to the paradox: why the pathway that produces less ATP is selected in such high demanding cells?

Warburg hypothesized that there was a defect in mitochondria that directed glucose into aerobic glycolysis. However, research has shown that mitochondrial function is normal in most cancer cells and this proposal remains controversial [3]. It was then suggested that resources are scarce for tissues undergoing aerobic glycolysis, even though the evidence provided is not convincing. Proliferating tissues in mammals are continually provided with glucose and other nutrients. In addition, these cells always maintain high ratios of ATP to ADP and NADH to NAD^+ [4]. Any fluctuations in these ratios can impede proliferation and cells can either undergo apoptosis or cell cycle arrest [5].

Oxidative phosphorylation is not ideal for biosynthetic purposes since it produces a large amount of ATP while NADH and carbon productions are inefficient [3]. Proliferating cells have a great demand of nucleotides, amino acids and lipids. There need to be a compromise to balance the need of energy and substances to create biomass. Glutamine, an amino acid circulating in the blood in high concentration, is important in the provision of cellular energy, carbon and nitrogen for cell proliferation [6]. Its metabolism is also heightened in cancer along with glycolysis [7]. The conversion of glutamine to glutamate is catabolized by mitochondrial glutaminase (GLS); glutamate then enters the Krebs cycle as α-ketoglutarate. In aerobic glycolysis, most of the glucose and glutamine are processed to lactate and alanine, providing nitrogen for nonessential amino acids via transamination reactions [3]. Furthermore, even though only four ATP are generated in aerobic glycolysis, such cells are able to manage different ways to get sufficient energy for proliferation. Protein kinase B (AKT) activation provides sufficient glycolytic fluxes to sustain the level of ATP [8]. Also, as mentioned above, proliferating cells are often provided with sufficient glucose, meeting energy requirement of the tissues.

The genetic and molecular basis of cancer metabolism

The study of cancer has always put great emphasis on the genetic and molecular mechanisms involved in disease pathogenesis. For example, the key driver of tumour proliferation lies in the upregulation of oncogenes and the inactivation of tumour suppressor genes. There is growing evidence showing the importance of genes, receptors and proteins in the regulation of cell metabolism.

Glycolytic enzyme is among the most upregulated sets of genes according to microarray studies. Pyruvate kinase (PK) is a tetramer catalyzing the conversion of ADP and phosphoenolpyruvate to ATP and pyruvate. In mammals, it has four isoforms: the L and R found in liver and red blood cells, the M1 found in most tissues in adults and the M2, present in self-renewing tissues including embryonic and adult stem cells and tumour cells. When switching M2 to M1 in tumour cells, a reverse Warburg phenotype is observed, corresponding to a decrease in tumourigenesis in nude mouse xenografts. However, not all cancer cells depend on PKM2 to switch to aerobic glycolysis [4].

HIF1 upregulates glucose transporters and glycolytic enzymes. Pyruvate dehydrogenase transforms pyruvate to acetyl-CoA in the pyruvate decarboxylation. HIF1 upregulates pyruvate dehydrogenase kinases (PDKs), which phosphorylate mitochondrial pyruvate dehydrogenase complex, inhibit the activity of pyruvate dehydrogenase and prevent pyruvate from entering into the Krebs cycle [9, 10]. It was shown that PKM2 is a transcriptional cofactor of HIF1, directly interacting with HIF1α and promotes HIF1 binding. Furthermore, PKM2 is a target gene of HIF1 itself. It joins the positive feedback loop that promotes HIF1 activity and reprogramming of cancer cell metabolism, facilitating the Warburg effect [11].

One of the applications of the Warburg effect is the ^{18}F-fluorodeoxy-glucose-positron emission tomography

(^{18}FDG-PET) imaging, which allows the visualization of cancer cells that exhibit high glucose uptake phenotype. Research has shown that high ^{18}F-fluorodeoxy-glucose (^{18}FDG) uptake in human breast cancer is correlated with the overexpression of c-Myc oncogene [12]. c-Myc has an important role in cell growth, differentiation and apoptosis. In many cancers, it upregulates many glucose transporters, along with pyruvate dehydrogenase kinase, isozyme 1 (PDK1) and lactate dehydrogenase A. Lactate dehydrogenase-A converts pyruvate to lactate in normal anaerobic glycolysis and is overexpressed in tumour cells. The upregulation of LDH-A gene is necessary for cell transformation mediated by c-Myc. Glucose flux is promoted through the glycolytic pathway and pyruvate is prevented from participating the Krebs cycle [13–15].

The tumour suppressor gene p53 is well known for its ability to respond to DNA damage, initiate cell apoptosis and also regulate metabolism. The TP53-induced glycolysis and apoptosis regulator (TIGAR) decreases the levels of fructose-2,6-bisphosphate, which inhibits glycolysis and protects cells from reactive oxygen species (ROS) [16]. Moreover, p53 enhances cytochrome c oxidase-2, which is critical for the cytochrome c oxidase complex assembly of the electron transport chain. Cytochrome c oxidase is the site where most oxygen molecules are utilized. Metabolic changes observed in many tumours are correlated to the change in expression levels of subunit proteins of cytochrome c oxidase complex. Mutation in the Synthesis of Cytochrome c Oxidase 2 (SCO2) gene in wild-type p53 cells exhibits a similar glycolytic effect to the mutant p53 cells [17]. Loss of p53 function observed in many cancer patients, therefore, can increase flux through glycolysis and decrease oxidative phosphorylation [18].

Furthermore, the identification of the tumor suppressor LKB1 as a critical upstream kinase required for the activation of AMP-activated protein kinase (AMPK) by metabolic stress has been determined as critical in cancer metabolism {Woods, 2003 #1337}{Shaw, 2004 #1338}. In established tumors, the LKB1–AMPK pathway may facilitate cell survival, through its protective ability following metabolic stress. For example, re-expression of LKB1 in LKB1-deficient lung adenocarcinoma (A549) cells led to the protection of these cells against cell death induced by glucose starvation through the inhibition of fatty acid synthesis by AMPK and consequent sparing of NADPH {Jeon, 2012 #1339}.

Moreover, recently, it has found that the orphan nuclear receptor estrogen-related receptor alpha (ERRα), which facilitates mitochondrial biogenesis and oxidative phosphorylation, is importantly related to increased glucose metabolism and cell growth during the activation of effector T cell [19, 20]. Overexpression of ERRα is related to the poor outcomes of breast cancer patients. Proliferation of breast cancer cells with overly expressed ERRα is diminished by compounds antagonizing ERRα activity [21].

Phosphoinositide 3-kinase (PI3K) is associated with the growth control mechanism and glucose metabolism. The activation of PI3K via AKT induces the uptake of glucose, upregulates glucose transporters, stimulates glucose capture and enhances phosphofructokinase activity [3, 22].When PI3K signaling is interfered, there is a decline in glucose uptake, measured by ^{18}FDG-PET, and tumour regression is observed [23].

Cancer immunopathology

Despite how likely it is for a cell to get mutated and how aberrant cancers can get, the body immunity definitely plays a critical role in eliminating tumours and refining the immunogenic phenotypes of cancer that is formed in immunocompetent hosts. This is referred to as immunosurveillance [24].The idea was first proposed by Paul Erhlich. However, the concept was not supported until the presence of tumour-associated antigens (TAAs). Considering the prevalence of genetic errors, the body must evolve a way to battle these harmful mutations, which was suggested to lie in the body immune system [25].

There are numerous ways by which the immune system recognizes and responds against tumour cells. T cell with T cell receptor can interact with MHC-peptide expressed on tumour cell and deliver a lethal hit towards that cell [26]. NK cell can also interact with tumour cell via stress ligands on the surface of tumour cells [27]. Death receptors including TRAIL or Fas are used by both T cells and NK cells [28]. Another mode of recognition is the release of Danger-associated molecular patterns (DAMPs) from tumour [29]. These patterns are then seen by receptors on the surface of immune cells, like Toll-like receptor from myeloid cells. Myeloid cells can respond by performing phagocytosis or releasing superoxide, nitric oxide and cytokines (TNF-α, IFNα/β) to create an inflammatory environment to attract other cells in. Lymphocytes, on the other hand, can express pore-forming molecules and death receptors, causing death of the tumour cells. Antibodies are also able to impede cancer growth via opsonization [24].

In order to survive the body immunosurveillance, the tumour cells also evolve a way to escape the immune system by a mechanism similar to Darwinian selection via the changes to the metabolism as a response [30]. There is growing evidence showing the connection between cancer immunosurveillance and cell metabolism. Lactate, which is produced abundantly in tumours as proposed in the Warburg effect, can impact on the state of immunosurveillance towards cancer cells by intervening monocytes

differentiation to dendritic cells, inhibiting the release of cytokine from dendritic cells and cytotoxic T lymphocytes (CTLs), hindering monocyte migration and affecting on the function of cytotoxic T cells [31–33]. Also, monocarboxylate transporters that secret lactate also transport H^+ out of the cells, which decreases the extracellular pH and then reduces the function of CTL [34]. More importantly, lactate enhances the traffic of cancer cells in a concentration dependent fashion, facilitating immune escape and metastasis [30, 35].

Furthermore, when glucose supply is compromised, cells are directed to the glutaminolysis pathway which involves glutamine oxidative and requires oxidative phosphorylation for ATP production. Experiment has shown that there is a threefold increase in MHC-I expression in cells growing in glucose-free medium [36, 37]. When placing the cells back to the medium with sufficient glucose supply, MHC-I expression comes back to normal [38]. In addition, MHC-I expression is also upregulated when cells are exposed to glucose and dichloroacetate (DCA), which inhibits PDK1 and directs pyruvate into Krebs cycle [39]. More importantly, DCA shows an anti-tumoural effect. It does not only switch the metabolism from fermentation to oxidative phosphorylation but also induce cell apoptosis [40].

Epigenetics and metabolism

Epigenetic changes refer to the reversible heritable genetic modifications that do not involve DNA sequence alterations. They do not only often provide the regulation mechanism for genetic expression but also have a role in DNA replication, repair and cell proliferation. Two major components of epigenetics are the methylation of cytosine base and histone tail modification [41, 42]. The DNA methyltransferases methylate cytosine in the DNA sequence, particularly cytosine bases adjacent to guanine (CpG island). Methylated DNA then attracts methyl-binding proteins and bind to them. Histones modifying enzymes are recruited to the methylated DNA stretches and covalently modify the histones. Histone modification triggers the chromatin formation that controls the process of gene transcription. These modifications eventually affect the patterns of gene expression, thereby, have a significant role in cancer growth [43–45]. In tumour cells, hypermethylation of CpG's makes the genes transcriptionally silent, as in the repression of tumour suppressor gene p16 and 14-3-3σ [46–48]. In addition, aberrant methylation of CpG's in codon 248 of gene p53 has been suggested to form cancer mutational hot spots [49, 50]. Furthermore, the cell mechanism for DNA repair does not effectively recognize the G-T mismatch resulted from the deamination of methylated cytosine [41]. It is also reported that loss of acetylation of lysine 16 and lysine 20′s trimethylation at histone H4 are observed in many cancers [51]. Hypoacetylation of histone can inactivate tumour suppressor genes whereas histone hyperacetylation can upregulate oncogenes [52, 53].

In 1985, Peter Cerutti suggested that metabolic defects observed in tumour cells was what caused epigenetic switch in cancer [54]. Since then there has been further investigation of this epigenetic aspect. The focus of the cancer epigenetic switch is on the malfunction of several epigenetic enzymes, including DNA methytransferases (DNMTs), histone methyltransferases (HMTs), histone deacetalases (HDACs) and histone lysine demethylases (KDMs). One possible contribution to this dysregulation is the loss of the enzymes' cofactors. DNMTs and HMTs both depend on cofactor S-Adenosyl-L-methionine (SAM) for transmethylation. Regulation of SAM is associated with cell metabolism and redox state [55]. Class III HDACs utilize NAD^+ for histone deacetylation [56]. It is shown that glucose and calorie-restrictive diets affect the ratio of NAD^+/NADH and subsequently have an impact on the HDAC ability of sirtuins. Therefore, the Warburg phenotype observed in many cancer cells may decrease sirtuin activity via the alteration of NAD^+ to NADH ratio, resulting in histone hypoacetylation and aberrant gene expression eventually [55]. Additionally, α-ketoglutarate acts as an electron donor to remove methyl groups via KDMs [57]. High glutamine uptake and metabolism in cancers influence the level of α-ketoglutarate, the activity of KDMs consequently and histone methylation [55].

In addition, a high prevalence of heterozygous mutations in the isoforms of Krebs-cycle enzyme isocitrate dehydrogenase (IDH1/2) occur in glioma, malignant glioblastoma and acute myeloid leukemia {Dang, 2010 #1340}{Reitman, 2010 #1341}. The IDH mutations may inactivate IDH for conversion of isocitrate to 2-oxoglutarate {Zhao, 2009 #1343}. However most importantly, IDH mutations may also cause a gain of function, with an ability to catalyse 2-oxoglutarate to the R-enantiomer of 2-hydroxyglutarate, which may also be referred to as an 'oncometabolite' {Dang, 2009 #1344}{Gross, 2010 #1345}. Interestingly, 2-hydroxyglutarate has been shown to inhibit histone demethylation leading to the inhibition of differentiation in non-transformed cells {Lu, 2012 #1342}.

Hexokinase is catalysis of the first step of glycolysis. Elevated level of type II hexokinase (HKII) is a characteristic of hepatoma cells. There is a clear evidence for the epigenetic activation of HKII. An experiment done by Goel, Mathupala and Pederson shows in the normal hepatocytes where HKII expression is almost silent, the DNA is hypermethylated in comparison to the hepatoma cell line in which there is an elevated level of HKII. Bisulfite

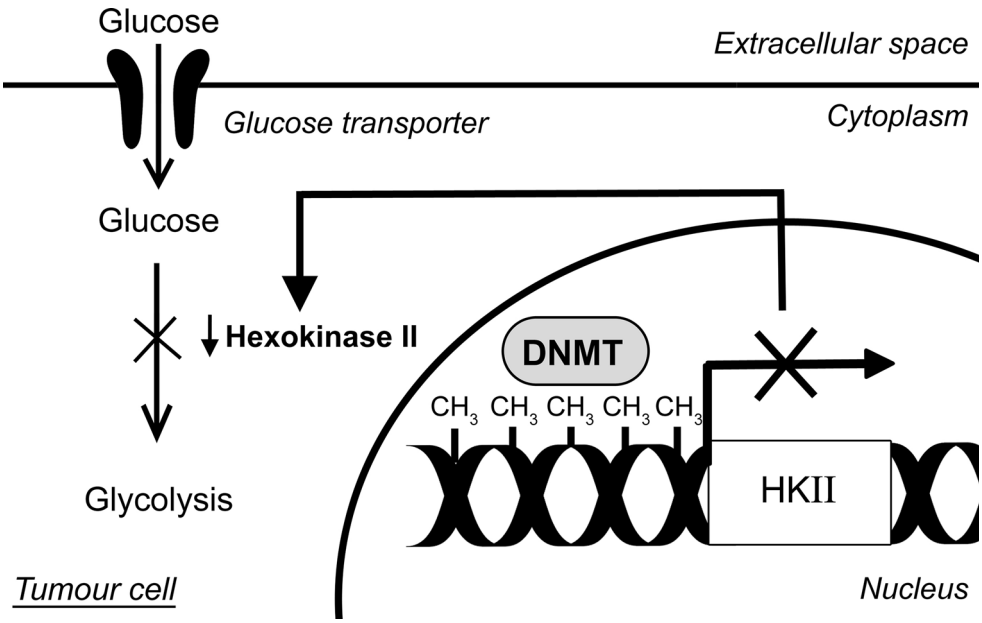

Fig. 1 Elevated levels of type II hexokinase (HKII) is a characteristic of tumour cells. DNA hypermethylation by DNA methyltransferases (DNMTs) at the CpG island of the promoter region of the HKII gene results is gene silencing and subsequent decreased protein levels of HKII enzymes resulting in the suppression of glycolysis

modification and sequence analyses of the hexokinase II CpG island detect no methylation in the hepatoma model. In addition, when treated with DNMT inhibitors 5′azaC and 5′azadC, the basal level of HKII in the hepatocytes is heightened at both mRNA and protein level. Likewise, when these cells were stably transfected with the DNA demethylase cDNA, HKII is also increased [58]. This clearly shows how DNA methylation is linked to the silencing of the HKII gene (Fig. 1).

Potential therapies targeting the Warburg effect

With the observation by Warburg, tumour cell metabolism has become one of the hallmarks of cancer. Therapies targeting this aspect of cancer are new and appear promising (Fig. 2). The very first step of glycolysis is the conversion of glucose to glucose-6-phosphate, which is catalysed by *hexokinases.* The four major types are hexokinase I, II, III and IV. The isozymes I–III exhibit a high affinity for glucose with Km value of 0.02–0.03 mM and are inhibited by the production of glucose-6-phosphate; whereas, type IV hexokinase has the Km value of 5–8 mM, much lower affinity compared to the other three and much less sensitive to glucose-6-phosphate production. When the sequence of cDNA that encodes the hexokinases was studied in various sources, it was found out that the majority of hexokinases from cancer tissues was type II. Hexokinase II (HKII) is highly expressed in tumours but present in only a small amount in normal tissues [59]. It binds to the voltage-dependent anion channel (VDAC), which is highly expressed in the mitochondrial outer membrane in eukaryotes and important in the traffic of several metabolites across membrane. There are several isoforms of VDAC and the one that mainly binds to HKII is the VDAC-I. The binding immortalizes the tumour cell by intervening in the apoptosis induction. It is hypothesized that the interaction of HKII/VDAC-I limits VDAC conductance, thereby suppresses mitochondria and activates glycolysis [60].

There have been several research studies working on therapies targeting hexokinase II. *2-deoxyglucose*, a glucose analogue, has been considered to be a metabolic block. It hinders the transfer of glucose within the cells even after insulin injection in the maximal dose [61]. 2-deoxyglucose binds and suppresses hexokinase II [62]. This suppression leads to cellular ATP depletion, impediment of the cell cycle and cell death eventually [63]. However, since 2-deoxyglucose is the analogue of glucose, its activity is affected by glucose presence, making it much less efficient in reducing the availability of glucose for glycolysis [62].

3-bromopyruvate (3-BrPA) has been shown to have the ability to dampen glycolysis by inhibiting hexokinase II [62]. An experiment on rat model with breast cancer highlighted the significant decrease of tumour FDG uptake (77 %) while that of normal cells was negligible, indicating the strong anti-glycolytic effect of 3-BrPA [64]. In another experiment on hepatocellular carcinoma rat model, it was hypothesized that due to the increased level of lactate in many cancer cells, lactate transporters are elevated and unable to discriminate the structurally similar 3-BrPA from lactate, permitting the entry of 3-BrPA into the cells and impeding the production of ATP [65]. The Bcl-2 associated death promoter protein (BAD) is a pro-apoptotic protein which regulates glycolysis and apoptosis [66]. Experiment

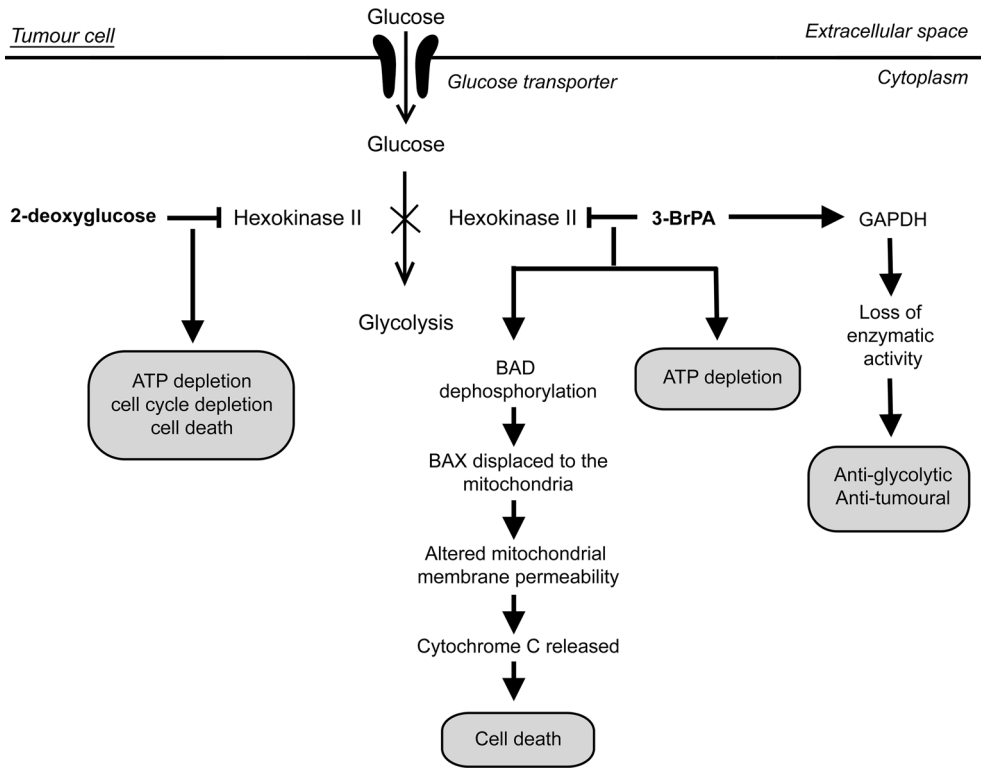

Fig. 2 Targeting hexokinase II as a potential mechanism to inhibit the Warburg effect in tumour cells. 2-deoxyglucose binds and suppresses hexokinase II leading to cellular ATP depletion, impediment of the cell cycle and cell death. 3-bromopyruvate (3-BrPA) also dampens glycolysis by inhibiting hexokinase II and impeding the production of ATP. Further, glycolysis inhibition caused by 3-BrPA leads to the dephosphorylation of Bcl-2 associated death promoter protein (BAD) at Ser^{112}. Consequently, BAX, a protein required by BAD is displaced and localised to mitochondria, changing mitochondrial membrane permeability which results in the release of Cytochrome c and subsequent cell death. In addition, a main target of 3-BrPA is the pyruvylation of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), which is associated with the loss of GAPDH enzymatic activity, resulting up the anti-glycolytic and antitumoural effects

shows glycolysis inhibition caused by 3-BrPA leads to the concentration and time-dependent BAD protein at Ser^{112} dephosphorylation. After 8 h of incubation, a complete dephosphorylation was observed. Consequently, BAX, a protein required by BAD in the formation of apoptosis preventing complexes, is displaced and localised to mitochondria, changing mitochondrial membrane permeability. Cytochrome c is released and causes cell death (67).

Further investigation shows that cancer cell death mediated by 3-BrPA also lie on the pyruvylation of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), which was identified that GAPDH was the main target of 3-BrPA. This pyruvylation is associated with the loss of GAPDH enzymatic activity, bringing up the anti-glycolytic and antitumoural effects [67]. In addition, the cytotoxicity brought up by 3-BrPA is not dependent on p53 and proved to result in massive cell death when incorporated with cisplatin and oxaliplatin [68]. More importantly, an experiment pointed out that 3-BrPA did not cross blood–brain barrier when the [^{14}C]3-BrPA uptake was measured in the brain tissue, which is crucial since the brain normally has a high glucose consumption to maintain its normal functions [69]. However, 3-BrPA is unstable and only exhibits the inhibition of glycolysis at high concentration [70].

Dichloroacetate (DCA) is a mitochondrial targeting molecule that is being proved to reverse the cancer metabolic alteration [71]. Its ability to redirect the cell metabolism from fermentation of pyruvate to mitochondrial oxidation has put it to the study of treating patients with lactic acidosis and mitochondrial diseases [72].With the recent interest in the study of cancer metabolism, research groups have started to investigate its antitumoural effects. Resistance to apoptosis in many cancer cells is contributed by high mitochondrial membrane potential ($\Delta\Psi m$) and down regulation of potassium channel Kv1.5. DCA exhibits its antitumoural effects by decreasing $\Delta\Psi m$, upregulating Kv1.5 channels via NFAT-dependent mechanism in tumour cells but not normal cells. The nuclear factor of activated T-cells (NFAT) is a Ca^{2+}-sensitive transcription factor that heightens the antiapoptotic Bcl-2 level and lowers the expression of the potassium Kv1.5. These characteristics correspond to the antiapoptotic state and also link to the suppression of mitochondrial activity.

DCA depolarizes mitochondria and returns its potential back to the level of normal cells [73]. It also inhibits pyruvate dehydrogenase kinase, contributing to the shift to mitochondrial oxidation, triggering cell death and impeding tumour growth [74]. Apoptosis induction by DCA in lung, breast cancer and glioblastoma cells was recorded in a publication by Bonnet in 2007, which was soon followed up by a similar observation in endometrial and prostate cancer [74–76]. Furthermore, DCA activates mitochondria, enhances the consumption of oxygen in cancer and increases the impact of hypoxia-specific chemotherapies in animal models [77].

In clinical testing, DCA when used with dose of 35–50 mg/kg lowers the level of lactate by 60 % and increased PDH by 3–6 folds [78, 79]. After several doses, DCA clearance declines [80]. Initially DCA half-life increases with subsequent doses, however, this rise soon becomes plateau, especially in chronic users [73, 81]. A clinical trial done in 1991 on the effects of DCA in reducing lactic acidosis stated that DCA was not significantly effective in lowering hyperlactatemia and the mortality rate was not decreased [82]. Further study of DCA has shown that DCA can cause peripheral nerve toxicity. Declined nerve conduction appears in lactic acidosis patients treated with DCA and peripheral neuropathy is a common side effect of chronic DCA users [83]. Clinical trials of DCA record muscular rigidity, tremor, lethargy and sleepiness [84].

Conclusions

As our understanding of the Warburg effect and cancer cell metabolism expands, it is becoming clearer that carcinogenesis and cancer progression involves complex interactions between different pathways, influenced by both genetic, epigenetic and metabolic factors. Therapies targeting cancer cell metabolism are emerging and promising however, they are currently limited by their broad effects on different tissues. It is anticipated that further research will be aimed at developing therapeutics that may provide improved targeting for aberrant components of cancer cell metabolism.

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