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Glucose Metabolism and Carcinogenesis: The Impact of the Tumor Suppressor p53

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Abstract

The tumor suppressor protein, p53 responds to cellular stress such as DNA damage, oncogenic activation and hypoxia by transactivating downstream genes that are responsible for apoptosis, DNA repair, senescence, cell cycle arrest and cell cycle progression. However, emerging trends show that p53 also plays multifaceted roles in regulating glucose metabolism. It promotes oxidative phosphorylation, suppresses glycolysis at multiple points as well as controlling glutamine and lipid metabolism. Current findings suggest that p53 actions have potential to influence the Warburg Effect, that is, characteristic of cancer cells. The Warburg phenomenon is characterized by their preference for glycolysis to oxidative phosphorylation for ATP generation, irrespective of adequate oxygen supply. This is often in concomitance with enhanced glucose uptake and leads to increased lactate production and anabolic processes such as lipid synthesis and *de novo* nucleic acid synthesis. The molecular underpinnings of the Warburg Effect are still poorly understood. These important differences between cancer and normal cells have induced interest in glucose metabolism as a drug target. This chapter focuses on the influence p53 exerts on glucose metabolism as well as on the implications of the Warburg phenomenon in carcinogenesis and a review of the ever-increasing number of p53 regulators.

Keywords: p53, glucose metabolism, carcinogenesis, Warburg effect, RBBP6

1. Introduction

Most normal differentiated cells rely primarily on oxidative phosphorylation and, to a lesser extent, on glycolysis for the generation of ATP. Cancer cells prefer glycolysis to oxidative phosphorylation for ATP generation from glucose even in the presence of adequate supply

of oxygen, a phenomenon that was first described by Otto Warburg in 1927 [1]. This often occurs alongside rapacious uptake of glucose and leads to increased lactate production and elevation of the pentose phosphate pathway (PPP). In addition to synthesizing nucleotides, the PPP generates large amounts of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) conferring anti-oxidant properties to cancer cells and thereby protecting them from potential damage by reactive oxygen species [2].

Previously, Hannan and Weinberg identified and reviewed six hallmarks of cancer [3]. The Warburg effect is now recognized as the seventh [4]. Given the multifaceted interventions by p53 in glucose metabolism, it would not be surprising that this potent tumor suppressor exerts substantive influence on the Warburg effect. p53 is tightly regulated in normal physiology but is activated by post-translational modifications following one or more of several cellular stresses such as DNA damage, oncogenic activation, hypoxia, ribonucleotide depletion and telomere erosion [5]. p53 activation triggers a suit of signaling cascades by either p53 transcription-dependent or -independent pathways. In normal cellular conditions, wild type p53 is kept under tight regulation mainly by the Mouse double minute (MDM2) which uses its E3 ligase activity to ubiquitinate p53 thereby tagging it for proteasomal degradation [6]. This stringent control of p53 is consistent with its well-known role in maintaining genomic and cellular integrity.

Over the years, many negative regulators of p53 have been identified further complicating the functional map of this ubiquitous tumor suppressor. Given recent findings about the importance of p53 in glucose metabolism, it is necessary to reassess the extent to which metabolic reprogramming and absence of functional p53 contributes to carcinogenesis. p53 regulates glucose metabolism at multiple points effecting outcomes such as repression of glucose transport, inhibition of glycolysis, positive influence on oxidative phosphorylation and control of glutamine and lipid metabolism **(Figure 1).**
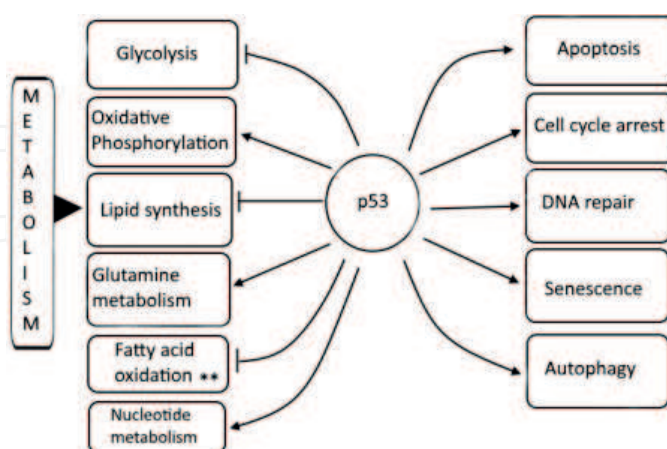

Figure 1. p53, although well-known for inducing apoptosis and cell cycle arrest for tumor suppression, it also employs several strategies via metabolic pathways for tumor suppression such as blocking glucose transport, inhibiting glycolysis, boosting oxidative phosphorylation, promoting glutamine metabolism, enhancing fatty acid oxidation in low glucose conditions and inhibiting lipid synthesis. p53 also promotes nucleotide metabolism. The asterisks indicate that the enhancement of fatty acid oxidation by p53 is only in conditions of low glucose (1 mM).

2. The impact of p53 on glucose metabolism

p53 is mutated in more that 50% of all cancers. Moreover, the development of many anti-cancer drugs often depends on the status of the *p53* gene in the targeted cancer. Recent findings show p53 intervenes in the glucose metabolic pathways at multiple points **(Figure 2).** Overall, p53 inhibits glycolysis and promotes oxidative phosphorylation **(Figure 2).**

2.1. Control of glucose transport

The first rate-limiting step for glucose metabolism is the uptake of glucose which is facilitated by glucose transporters at the cell membrane such as GLUT1-4. These receptors are often over-expressed in many cancers. Hence, carcinogenesis is associated with increased glucose uptake and glycolysis. This is mediated by glucose transporters. Consequently, glucose uptake is used for diagnosis and monitoring of cancer and metastasis in patients using positron emission tomography (PET) imaging technique that is based on consumption of the glucose analog 2-(^{18}F)-fuoro-2-deoxy-D-glucose by cells [7, 8]. Under physiological conditions, p53 monitors glucose uptake by suppressing the expression of transporters such as GLUT1 and GLUT4 by directly binding to the p53 response elements in their promoters [9]. This indicates that p53 can inhibit energy metabolism by obstructing cellular glucose uptake. p53 can also indirectly repress glucose intake via Glut3 by activating the IKK-NFκB pathway. In p53-deficient cells, there is an increase in aerobic glycolysis and upregulation of Glut3 accompanied by increased
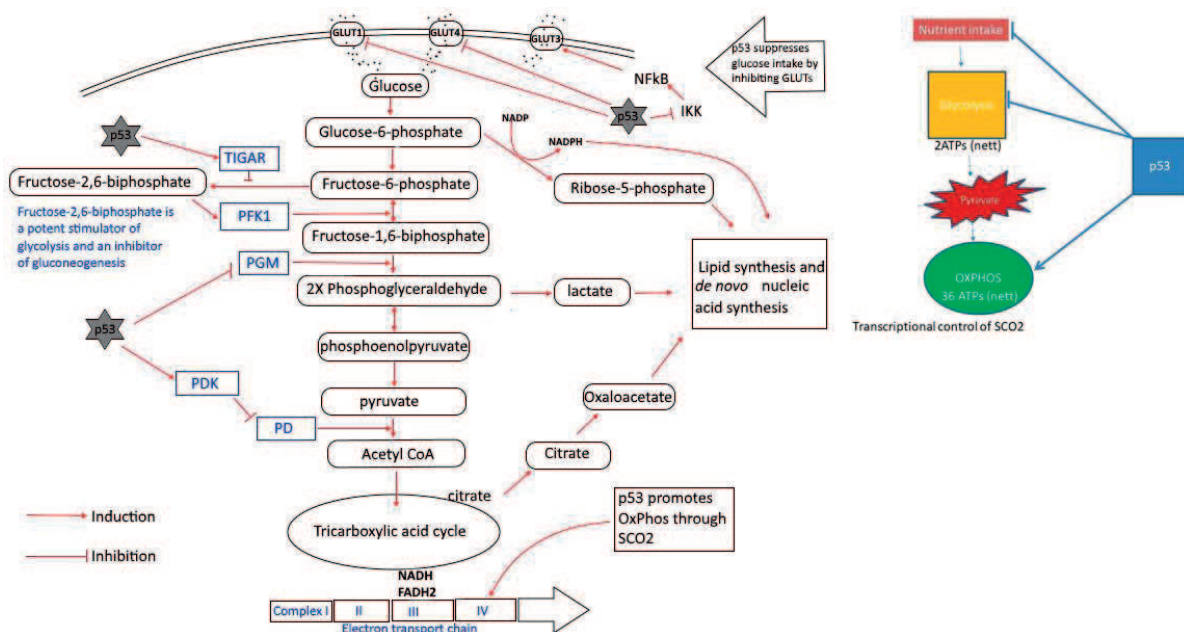

Figure 2. p53 inhibits glucose influx by directly repressing the expression of GLUT1 and GLUT4. However, p53 indirectly inhibits GLUT3 expression by repressing NFkB transcriptional activity on GLUT3 through inhibition of I kappa B kinase (IKK). p53 also represses glycolysis by inhibiting phosphoglycerate mutase expression and activating TP53-induced glycolysis and apoptosis regulator (TIGAR) which suppresses glycolysis. OXPHOS can be promoted by p53 through the activation of SCO2 at Complex IV. p53 can inhibit PDK which can phosphorylate pyruvate dehydrogenase and prevent its catalysis of pyruvate to acetyl-CoA. INSERT: p53 inhibits glycolysis and boosts OXPHOS.

activity of the IKK-NFκB axis. This accelerated glycolysis can be suppressed by elimination of the p65/NFκB complex and restored by expression of Glut3 [10]. These observations indicate that glycolysis may also influence the IKK-NFκB pathway which is activated by inflammatory molecules such as cytokines, reactive oxygen species (ROS) and by radiation.

2.2. Suppression of glycolytic pathway by p53

The p53 target gene, *TP53-induced glycolysis and apoptosis regulator* (*TIGAR*) intervenes at the third step of glycolysis by dephosphorylating fructose,2,6-phosphate, which is a potent stimulator of glycolysis and an inhibitor of gluconeogenesis, to fructose-6-phosphate **(Figure 2)**. p53 also modulates the transcription of TIGAR by binding to p53 response elements in the promoter region, thereby controlling the levels of fructose,2,6-phosphate. Hence, p53 can block glycolysis by activating TIGAR and lowering the levels of fructose-2,6-biphosphate, which is a potent activator of phosphofructokinase-1 that is responsible for converting fructose-6-phosphate to fructose1,6-bisphosphate. Thus, the activation of TIGAR inhibits glycolysis and shunts glucose breakdown through the pentose phosphate pathway [11, 12]. It is expected then that loss of p53, which occurs in most cancers, would lead to increase in fructose,2,6-phosphate and in glycolysis.

Phosphoglycerate mutase (PGM) is a glycolytic isomerase that catalyzes step 8 of glycolysis, converting 3-phosphoglycerate to 2-phosphoglycerate and is repressed by wild type p53 in mouse embryonic fibroblasts (MEFs). When expression of PGM is elevated, high glycolytic flux is observed and the MEFs are immortalized [13]. Furthermore, PGM is upregulated in p53 null cells. Evidence shows that PGM controls a critical step in glycolysis where the process is shunted to the pentose phosphate pathway (PPP) because when it is depleted by shRNA or by an small molecule inhibitor, glycolysis is reduced, PPP flux occurs and cell proliferation and tumor growth are inhibited [14, 15].

p53 also represses the transcription of pyruvate dehydrogenase kinase 2 (PDK2) thereby inhibiting the conversion of pyruvate to acetyl coA and production of lactic acid in breast carcinoma cells (MCF7). This underlines that p53 plays a pivotal role in establishing the Warburg Effect whereby pyruvate is converted to lactate even in abundant oxygen conditions [16].

Taken together, the above observations show that under normal physiological conditions, p53 inhibits glucose metabolism while boosting oxidative phosphorylation (OXPHOS). When p53 is dysfunctional, as it happens in many cancers, this scenario is reversed a situation that is consonant with the Warburg Effect.

2.3. p53 influence on lactate metabolism

Pyruvate, which is the product of glycolysis, is the key branch point at which glucose metabolism can go either to lactate production or to the tricarboxylic acid cycle (TCA). The conversion of pyruvate to acetyl-Coenzyme A, which enters the TCA, is catalyzed by pyruvate dehydrogenase complex (PDC). The enzyme pyruvate dehydrogenase kinase isoenzyme-2 (Pdk2), which inactivates acetyl-CoA production by phosphorylating the PDC, is negatively regulated by p53 [16]. Lactate is no longer regarded as merely a waste product of glycolysis, but

also as a significant regulator of carcinogenesis and metastasis, acting as fuel and as a signal molecule that promotes angiogenesis via VEGF [17, 18]. Moreover, the conversion of pyruvate to lactate is enhanced in neoplastic cells and utilizes NADH and H^+ to yield the NAD^+ that is used in the conversion of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate which refuels glycolysis. Tumor cells generally export the excessive lactate along with protons via monocarboxylate transporters (MCT) instead of utilizing it as a nutrient. This leads to an acidification of the tumor microenvironment and contributes to immune evasion. Lactate export establishes low pH and induces the production of pro-inflammatory cytokines by inflammatory cells in the tumor microenvironment. The cytokine expression is facilitated by "lactate response elements" in the promoters of the genes encoding these cytokines [19]. Wild type p53 directly interacts with the promoter of the MCT1 gene and represses it. Consequently, cancer cells that are deficient in p53 have elevated levels of MCT1 and proliferate. This phenotype also enables them to modulate import or export of lactic acid depending upon glucose availability [20]. Various isoforms of MCT including MCT1, MCT4 and chaperone CD147, are highly increased in renal cell carcinomas and also indicate poor prognosis. Furthermore, MCT has been suggested as a potential target for anti-cancer drugs, whose strategy is to reverse the Warburg Effect [21].

Lactate dehydrogenase-A (LDH-A), an important prognostic marker in several tumors, catalyzes the conversion of pyruvate to lactate in anaerobic conditions with concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH) to NAD^+. LDH-A expression is upregulated by several oncogenes, including c-Myc [22], Her2/Neu through heat shock factor 1 [23]) and hypoxia-inducible factor 1α (HIF-1α) [24] all of which are influenced by p53. p53 is also known to regulate lactate metabolism by indirectly suppressing LDH-A activity through miR-34 because p53 transactivates and upregulates them via p53 response elements on their promoters. In turn, miR-34a suppresses LDH-A activity *in vitro* and *in vivo* and has been shown to possess tumor suppressor functions [25–29]. Furthermore, miR-34a activation leads to apoptosis and to upregulation of several genes that are involved in cell cycle regulation, DNA repair and angiogenesis. These findings show that miR-34 is part of the p53 tumor suppression network.

2.4. p53 enhances oxidative phosphorylation (OXPHOS)

Oxidative phosphorylation is a metabolic process that occurs in the mitochondria to generate larger amounts of ATP from glucose as compared to glycolysis. It involves sequential transfer of electrons (electron transport chain (ETC)) facilitated by a series of enzymes located in the inner mitochondrial membrane thereby generating energy that is used to pump protons across the membrane creating an electrochemical gradient. This gradient drives the adenosine 5′-triphosphate (ATP) synthase to "almost mechanically" produce ATP by attaching a phosphate to ADP. By this method, the cell is able to produce 36 ATP molecules from a glucose molecule compared to 2 ATPs produced by glycolysis [30].

p53 displays multifaceted roles in the regulation of energy metabolism via the mitochondria. It assists in the repair or degradation of unhealthy or worn out mitochondria by inducing the expression of mitochondria-eating (mitophagic) protein (MIEAP) through binding to

the MIEAP promoter in response to mitochondrial damage, thereby facilitating good mitochondrial quality [31]. p53 is also involved in preserving mitochondrial genetic integrity by interacting with mtDNA polymerase gamma (mtDNA polymerase γ) thus improving DNA replication and enhancing the mitochondria to respond appropriately to DNA-damaging insults. Loss of p53 increases mitochondrial susceptibility to mutations, a phenotype that is rescued by wild type p53 [32]. This presents another example of p53 subcellular localization and function outside the nucleus. Mitochondrial diseases are characterized by energy depletion probably due to defects in OXPHOS since the primary function of mitochondria is to support aerobic respiration [33]. It is thus relevant to review the role of p53 in maintaining mtDNA integrity and influence in aerobic glucose metabolism.

The mitochondrion is also the key regulator of apoptosis and by extension, critical in cell cycle control and in development of tumors. p53 promotes oxidative phosphorylation by activating synthesis of cytochrome c oxidase 2 (SCO2) gene enzyme in complex IV of the electron transport chain. It transactivates SCO2 by direct DNA binding to the p53 response element at the SCO2 promoter region [34]. SCO2 then regulates cytochrome c oxidase (COX) complex or complex IV, which is the last enzyme in the ETC and regulates the major site of oxygen utilization in the cell.

Matoba et al. showed that even though the total amount of ATP produced by p53$^{-/-}$, p53$^{-/+}$ and p53$^{+/+}$ HCT116 cells was roughly the same, the relative proportion of ATP generated by glycolysis showed an inverse proportion to p53 dosage. This suggests that the Warburg Effect does not reduce the amount of ATP generated but adjusts the contributions made by glycolysis and OXPHOS. Oxidative respiration was, however, rescued in p53$^{-/-}$ cells when the SCO2 protein, which is downstream effector in OXPHOS, was expressed. This shows that the decreased OXPHOS was mediated in part by decreased levels of SCO2. Furthermore, SCO2$^{+/-}$ knockout cells showed the same metabolic profiles and proliferation rate as p53$^{-/-}$ cells [34, 35]. In contrast to glycolysis where p53 plays an inhibitory role, it has a positive effect on OXPHOS (**Figure 2** (insert)). This is consistent with p53 being a driver of the Warburg Effect because when it is mutated one expects that OXPHOS would be downregulated and glycolysis upregulated which is the glycolytic phenotype seen in cancer. However, it appears that this phenotype can occur in the background of wild type p53 suggesting that there are other factors that are crucial for the establishment of the Warburg Effect.

The Apoptosis-inducing factor (AIF) is a mitochondrial protein, encoded by nuclear DNA. It induces apoptosis when apoptotic stimuli cause it to be translocated to the nucleus where, together with cyclophilin, it executes the final events of apoptosis, nuclear chromatin condensation and large scale DNA fragmentation [36]. In the mitochondria, AIF maintains structural integrity especially of the cristae. Consequently, disruption of mitochondrial location of AIF disturbs oxidative phosphorylation [37]. AIF has an important role in the assembly and function of Complex I of the electron transport chain, as demonstrated in isolated cardiac mitochondria obtained from Harlequin and wild type mice [38]. AIF is also a direct transcriptional target of p53 and possesses p53 response elements in its promoter, although only basal levels of p53 are required with higher levels having no impact on AIF dynamics. The induction of AIF expression by p53 is therefore important in supporting cellular OXPHOS activity. AIF and

TIGAR, which are both upregulated by p53, are important examples of genes that regulate both apoptosis and metabolism; showing the diverse approaches of p53 in tumor suppression.

2.5. The role of p53 in glutamine metabolism

Glutamine is a non-essential amino acid that can be synthesized from glucose. When compared to glucose, it is a less celebrated source of energy and biosynthetic molecules. It provides nitrogen for the synthesis of nitrogenous bases and amino acids. Glutamine metabolism also generates reduced NADP which lowers anti-oxidant stress and is required for fatty acid synthesis. Glutaminases catalyze the deamination of glutamine to glutamate, which is further processed to generate α-ketoglutarate (an important metabolite in the TCA cycle) thereby boosting OXPHOS. α-Ketoglutarate can also be converted to citrate, which may be exported out of the cell for amino acid and synthesis of lipid which are important in proliferating cells. Glutamine can also be metabolized to pyruvate, which is further processed to lactate. Two types of glutaminase, encoded by two separate genes have been discovered: the kidney type glutaminase (GLS1) predominantly expressed in the kidneys and the liver type GLS2 which is highly expressed in hepatocytes. GLS1 and GLS2 show contrasting roles in carcinogenesis and seem to depend on the origin of the tumor. For example, increased glutamine metabolism, corresponding to enhanced expression of GLS1 is characteristic of several cancers including colorectal and hepatocellular carcinomas [39, 40] while downregulation in GLS2 is associated with hepatocellular cancers [41]. Moreover, GLS1 is an attractive target in glutamine-addicted tumors. A recent study showed that the small molecule glutaminase inhibitor, CB-839 exhibits anti-proliferative effects in triple negative breast cancer cells [42].

Studies by Suzuki et al. and Hu et al. showed that phosphate-activated glutaminase 2 (GLS2) is a p53 inducible gene which enhances OXPHOS and increases ATP production as well as glutathione levels [41, 43]. Two mechanisms by which GLS2 may inhibit cancer progression have been elucidated. Firstly, GLS2 represses the phosphatidylinositol 3 kinase/protein kinase B (PI3K/PkB) pathway which is upregulated in several tumors [44, 45]. It is noteworthy that the PI3K/PkB pathway, in addition to promoting cell proliferation, also boosts glycolysis [46]. Secondly, GLS2 represses the GTPase protein, Rac1, which is known to promote invasion, migration and metastasis in cancer cells through the regulation of actin dynamics [45]. Moreover, glutamine metabolism is another metabolic signature in carcinogenesis, because cancers consume large amounts of glutamine in culture and *in vivo*. In fact, the term "glutamine-addicted tumors" has been coined for such tumors and may be exploited for anti-cancer drug discovery. In addition to amino acid biosynthesis, glutamine serves other cellular functions including helping to maintain good mitochondrial membrane potential and integrity and modulating P13K-Akt and EGFR signaling which, in turn, stimulate glycolysis [47].

2.6. p53 reduces oxidative stress

p53 generally enhances OXPHOS activities which may inadvertently generate mitochondrial reactive oxygen species (ROS). ROS are key signaling molecules in cell proliferation in both cancer and in normal cells. Nevertheless, ROS can lead to damage of membranes, proteins and DNA, with DNA damage leading to genomic instability [48].

In addition to the incidental ROS production, p53 actively generates ROS by activating a network of genes that are directly involved in ROS generation. Consequently, a deficiency in p53 will results in oxidation of DNA and genomic instability which can be avoided by treatment with anti-oxidants [49]. To circumvent this problem, p53 upregulates handful of genes that protect a cell from oxidative stress. For example, GLS2-mediated glutathione synthesis as a consequence of glutaminolysis is known to protect cells from oxidative stress. A glutathione molecule gets rid of ROS by accepting an electron from it, and is oxidized to glutathione disulfide. Glutathione reductase is an enzyme that uses NADPH to catalyze the conversion of glutathione disulfide back to its reduced form, glutathione. The Tp53-induced glycolysis and apoptosis regulator (TIGAR) was shown to enhance the generation of reduced glutathione thereby protecting cells from ROS-mediated apoptosis [11]. p53 also transactivates glutathione peroxidase 1 (GPX1) [50] and aldehyde dehydrogenase 4 (ALDH4) [51], which are also important in reducing oxidative stress.

2.7. p53 influence in lipid metabolism

More than 90% of lipids required by tumors are produced by *de novo* synthesis. Precursors of these critical elements of the plasma membrane are generated by glucose metabolism. Furthermore, fatty acids play essential roles in signal transduction, phospholipid formation for the synthesis of membranes and energy storage required by rapidly proliferating cells. Metabolism of fatty acids in mitochondria through β-oxidation generates large amounts of energy. The availability of fatty acids has been shown to influence cell proliferation, and their metabolism is deregulated in several cancers. While increased fatty acid oxidation (FAO) may play a role in suppressing tumor growth, increased fatty acid synthesis which correlates with overexpression of fatty acid synthase and ATP citrate lyase connotes poor tumor prognosis [52–54]. It has been demonstrated that p53 influences cellular fatty acid metabolism. A family of transcription factors known as sterol regulatory element-binding proteins (SREBPs) transcriptionally regulates lipogenesis. p53 inhibits SREBP1c which is known to transactivate genes that promote fatty acid synthesis including FASN and ACLY [55]. However, p53 may promote FAO during glucose starvation by transactivating Lpin1a Mg^{2+}-dependent phosphatidate phosphatase enzyme that catalyzes the conversion of phosphatidate (PA) to diacylglycerol.
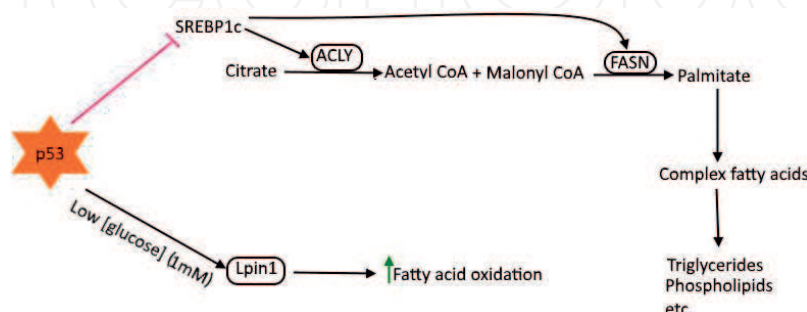

Figure 3. p53 primarily inhibits lipid synthesis by inhibiting SREBP1c which is known to transactivate ACLY and FASN which catalyze fatty acid synthesis. p53 also transactivates Lpin1 during glucose deprivation, thereby increasing fatty acid oxidation for the generation of ATP.

It is a bi-functional protein that represses and promotes FAO in mouse myoblasts at normal (25 Mm) or low (1 Mm) glucose concentrations in a ROS and ATM-dependent manner [56]. p53-mediated upregulation of Lpin1 represents a pro-survival function and confers the ability to cope with stress such as nutrient (glucose) deprivation. Inhibiting fatty acid synthesis by targeting the enzymes and transcription factors that promote lipogenesis is an attractive strategy in the development of anti-cancer drugs. For example, RNAi-mediated knockdown of FASN in prostate carcinoma resulted in decreased levels of triglycerides and phospholipids and a reduction in cell volume as well as cell-to-cell contacts [57]. Furthermore, depleting the lipogenic transcription factor SREBP1 using shRNA slowed down lipogenesis and reduced endometrial tumor growth [58] **(Figure 3).**

3. Gain-of-function mutations in p53 and carcinogenesis

Many cancers are associated with loss of p53 function often due to mutations to its DNA-binding domain. More than half of all human tumors contain mutations or deletions of p53. The remainder involves mutations in genes that regulate the p53 pathway. The hotspots, comprising about 95% of p53 mutations, are missense mutations in the DNA-binding domain. Many of these mutations have been shown to severely restrict p53 function [59]. Since p53 is a major tumor suppressor offering protection against cancers, its loss of function results in vulnerability to cancer-causing agents and aberrant growth of affected cells. Over the years, it has emerged that there is a subset of p53 mutations whereby p53 acquires new properties such as enhanced growth capacity, antiapoptotic activity, invasiveness and anti-cancer drug resistance [60]. These gain-of-function (GOF) mutants have thus become a subject of intense research.

The p53 GOF mutations were formally explored, for the first time, in cell lines whereby mutations introduced in a null p53 background were found to cause increased tumorigenicity in nude mice and enhanced growth support to the cells in soft agar [61]. Critically, these experiments showed that these mutants, in contrast to wild type p53, were able to transform p53 null cells and endow them with new properties. This property was responsible for the erroneous identification of p53 as an oncogene when it was discovered since wild type p53 does not have these characteristics [62]. Later, it was shown that one mechanism by which some of these mutations may affect carcinogenesis is by inhibiting the adenosine monophosphate (AMP)-activated protein kinase (AMPK) a key energy sensor that is activated in conditions of stress [63]. In this study, the GOF mutant p53 inhibited AMPK signaling in head and neck cancers. It was shown that it preferentially binds to the AMPKα subunit thereby increasing anabolic metabolism, causing metabolic reprogramming and inducing an invasive growth phenotype. Normally, p53 and AMPK act via a positive feedback mechanism whereby AMPK establishes a metabolic checkpoint by post-transcriptionally stabilizing p53. In turn, p53 activates AMPK transcriptionally thereby activating the gene that encodes the β subunit of AMPK [64] and sestrin [65, 66]. Among others, key pathways that are upregulated by p53 GOF mutations include the epidermal growth factor receptor (EGFR) [67], the vascular endothelial growth factor expression (VEGFR) [68] and insulin-like growth factor I receptor

(IGF-1R) [69] pathways. These are mostly mitogenic growth factor pathways that play critical roles in carcinogenesis, further underlining the clinical significance of GOF mutants. Many p53 mutations occur in the DNA-binding domain (DBD) and alter the proteins tertiary structure affecting its transcriptional activity and resulting in loss of function. Known GOF mutants include P151S, R175H, G245C and R282W.

There is also evidence showing that the GOF mutants can aggregate with wild type p53 in some cancers and that suppression of this aggregation restores the wild type p53 activity, including that of its paralogs p63 and p73. This introduces a novel mechanism of carcinogenesis involving aggregation [70]. The fact that certain cancers with p53 GOF are more aggressive than others with poor prognosis underlines their significance. Furthermore, some GOFs confer resistance to anti-cancer treatments.

4. p53 negative regulators

p53 is involved in numerous biological activities and new functions continue to be discovered. It is thus not surprising that the list of p53 regulators also continues to grow. **Table 1** shows known negative regulators of p53. It would not be surprising that the list of negative regulators will continue to grow in the near future.

The half-life of p53 is limited only to a few minutes in unstressed cells and its tight regulation in most cells is known to be controlled by Murine double minute 2 (MDM2), its prototypical negative regulator [77]. However, following DNA damage and other cellular stresses, p53 rapidly stabilizes by post-translational modifications such as phosphorylation and acetylation and the half-life extends to hours causing it to accumulate in the cell [5, 78]. The diversity in p53 negative regulators is still not fully understood but some examples show that they can be targets for anti-cancer drug discovery as they provide the mechanism for reactivating p53. This strategy, however, also comes with a critical caveat as it has been shown that in the absence of Mdm2, p53 can be spontaneously activated [79]. The main negative regulators of p53 are E3 ubiquitin ligases which tag p53 for proteasomal degradation and include MDM2, Pirh2 and COP1. These regulators generally bind to the DNA-binding domain of p53, preventing it from transactivating its target genes. Jun-N (amino)-terminal kinase (JNK) also

P53 negative regulators	Mode of action with p53 in brief	Reference
MDM2	E-3 ligase activity. Forms autoregulatory feedback loop with p53	[6]
Pihr2	E-3 ligase activity. Forms autoregulatory feedback loop with p53	[71]
COP1	E-3 ligase activity. Forms autoregulatory feedback loop with p53	[72]
Jun-N (amino)-terminal kinase (JNK) JNK	Binds to and negatively regulates p53 at G0/G1 and S/G2M cell cycle phases	[73, 74]
RBBP6	Binds p53 and E3 ligase activity. Shown to enhance MDM2-mediated ubiquitination.	[75, 76]

Table 1. p53 negative regulators.

influences p53 ubiquitination and stability probably by acting as an adaptor in the formation of the E3 ubiquitin/ligase complex [73]. There is evidence showing that p53 negative regulators can interact with each other independently of op53 and synergistically inhibit it [80].

4.1. Murine double minute 2 (MDM2)

MDM2 is an E3 ubiquitin ligase first discovered in mice. It is the prototypical negative regulator of p53 and an oncogene. It is a p53-inducible gene that binds to the 53 transactivation domain and regulates p53-mediated gene transcription. Using the E3 ligase activity, MDM2 ubiquitinates p53 and tags it for proteasomal degradation. It also transactivates p53 thereby forming an autoregulatory feedback loop [6, 81, 82]. The role of Mdm2 in glucose metabolism in not clearly understood but a Mdm2-p53-pyruvate signaling axis has been demonstrated to be activated in a diabetic situation where it links mitochondrial respiration to glucose homeostasis [83].

4.2. Pirh2

The p53-inducible gene that encodes a RING-H2 (Pirh2) protein interacts with p53 directly and catalyzes its ubiquitination through an intrinsic E3 ubiquitin ligase activity targeting it to proteasomal degradation. p53 also transactivates Pirh2 providing an autoregulatory feedback loop similar to Mdm2-p53 interaction [71]. Pirh2 has been shown to have an impact on lung tumorigenesis as increased expression corresponds to reduced levels of p53 and increased cell proliferation [84]. To our knowledge, the involvement of Pirh2 in glucose metabolism has not been reported.

4.3. Constitutively photomorphogenic 1 (COP1)

COP1 is a plant protein involved in photomorphogenesis. In Arabidopsis seedlings, for example, COP1 controls seedling development by repressing light-mediated gene expression [85]. It contains a RING-finger domain for E3 ubiquitin ligase activity. Recently, the molecular and functional importance of the human homolog of COP1 was characterized in human bone osteosarcoma (U2-OS) cells. Its molecular functions include p53-dependent activities and ubiquitination because it is encoded by p53-inducible gene and has E3 ubiquitin ligase activity. Hence, p53 transactivates COP1 which in turn targets p53 for degradation again forming an autoregulatory feedback loop that is similar to MDM2 and Pirh2. siRNA-mediated knockdown of COP1 stabilizes p53 and causes cells to accumulate in the G_1 cell cycle arrest [72].

4.4. The retinoblastoma binding protein 6 (RBBP6)

The longest RBBP6 isoform is 250 kDa protein. All RBBP6 family proteins contain the N-terminal Domain With No Name (DWNN). The shortest is isoform 3 which is essentially the DWNN. The long isoforms also possess a RING-finger with E3 ligase activity alongside an assortment of other domains and motifs. It is a member of a small class of cell cycle regulators that binds both p53 and pRb [86]. It is a negative regulator of p53 and has been shown to have properties that promote cell proliferation. It is known to act as a scaffold protein associated with nuclear matrix and telomeres of chromosomes suggesting an ability to control cellular functions to do with cell division and replication [87]. In the mouse model, it was demonstrated that RBBP6 enhances ubiquitination catalyzed by MDM2. It was, however, shown

that the phenotype exhibited by *rbbp6*^{−/−} mutants was more severe than the *mdm2*^{−/−} phenotype [75, 76]. Although the *rbbp6*^{−/−} phenotype is strikingly similar to that of *mdm2*^{−/−}, these results indicate that RBBP6 also possesses roles that are independent of Mdm2. There are many instances which show a close association of RBBP6 with carcinogenesis making it an attractive candidate for drug targeting. For example, overexpression of RBBP6 alone or in combination with mutant p53 is associated with poor prognosis in patients with colorectal cancer. Moreover, its isoforms are differentially expressed in many cancers [76, 88]. Interest in RBBP6 and glucose metabolism has been activated by an observation that, in normal cells, the various isoforms including isoform 3 which comprises the ubiquitin-like DWNN, become differentially regulated when OXPHOS is boosted in cell culture but in cancer cells only the long isoform is expressed [89]. This raises questions about its involvement in regulating mitochondrial respiration.

4.5. Jun-N (amino)-terminal kinase (JNK)

JNK is a stress activated kinase that was shown to regulate p53 levels in unstressed cells. It is a p53 inducible gene which facilitates p53 ubiquitination tagging it for proteasomal degradation p53 has a JNK binding site which when perturbed either by blocking or by mutation abrogates p53 ubiquitination. Like Mdm2-p53 complexes, JNK-p53 complexes are found at G_0/G_1 and G_2/M checkpoints [73].

5. Anti-cancer drug discovery and development targeting glucose metabolism

Preferential killing of cancer cells while protecting normal cells is the most desired outcome for anti-cancer drug discovery efforts. With this in mind, the Warburg Effect, as a distinct feature of cancer cells, offers many opportunities for drug discovery and development. Nearly a century ago, since the Warburg phenomenon was discovered in cancer cells, it is still unclear whether or not it is a cause or consequence of carcinogenesis [1, 90]. Nonetheless, this metabolic signature is shared by many cancers, making it an attractive target for preferential therapy as it is distinct from most normal cells. Using the Database for Expressed Sequence Tags (dbEST), glycolytic genes have been shown to be upregulated in 24 different types of cancer; representing more than 70% of all cases [91]. Central to the regulation of glycolysis are three transcription factors: p53, c-Myc and HIF-1α [4]. p53 functions primarily as a tumor suppressor that regulates the cell cycle and a network of genes with diverse functions related to cell proliferation and cell survival, but it also plays a pivotal role in the Warburg effect because mutant p53 (mutp35) acquires a gain-of-function phenotype in cultured cells and in knock-in mice in a GLUT1-dependent manner. Inhibition of a downstream effector in this GLUT1 pathway abolishes this GOF phenotype [92]. c-Myc (an oncogene) and HIF-1α act in complex ways to regulate glucose metabolism—sometimes cooperating but at times acting in an opposite manner [93]. A dilemma arises because targeting transcription factors is not an attractive strategy as it may result in non-specific outcomes due to the vast array of genes that they transactivate.

Extensive research efforts are underway worldwide to find strategies by which p53 is targeted for cancer therapy. In many cases, small molecules are designed to reactivate p53 [5]. The intriguing situation is that, the Warburg effect, one of the hallmarks of cancer, can occur in the presence of wild type p53. For example, non-small cell lung cancer cell lines A549 (wild type p53) and H1299 (mutant p53), both exhibit the Warburg effect [94]. Yet it has been shown that perturbation of functional p53 is sufficient to induce the Warburg effect in some cells because a mutant form of p53 (mutp53) acquired this ability [92]. Thus, drug development based on the Warburg Effect should be conducted with some consideration of the p53 status.

In the present study, we update progress made with some drugs and extend the list of drugs that are considered as anti-cancer candidates based on glucose metabolism. Studies show that cancer cells are more sensitive to inhibitors of glycolysis such as 3-bromopyruvate, oxamate and 2-deoxy-D-glucose in hypoxic tumor microenvironments [95–97]. Several inhibitors of glycolysis that target the Warburg effect as a therapeutic strategy in cancer have been reviewed elsewhere [98, 99]. Here, the drugs are divided into categories for convenience:

  i. Drugs targeting glucose transport

 ii. Drugs targeting glycolysis

iii. Drugs targeting OXPHOS

5.1. Targeting glucose transporters

Enhanced glucose intake is characteristic of several tumors, given their high glycolytic rates, and is facilitated by glucose transporters which are often overexpressed [100]. It has been shown that depletion of Glut1 transporters by shRNA knockdown and blocking the receptor with an anti-Glut1 antibody re-sensitizes cisplatin-resistant cancer cell line [101]. Similarly, a Glut1 inhibitor was used to counter 5-fluorouracil (5-Fu) resistant colon cancer [102]. Targeting the glucose transporter GLUT1 with a GLUT1-specific inhibitor, WZB117, has been shown to inhibit self-renewal and tumor initiation in ovarian, glioblastoma and pancreatic stem cells [103]. Another drug, STF1 which selectively inhibits GLUT1, preferentially killed melanoma cells and synergistically enhanced cell death induced by anti-cancer drugs melphalan, doxorubicin and bortezomib [104]. It has been demonstrated that expression of the GLUIT1 receptor is absent in sarcomas, lymphomas, melanomas and hepatoblastomas and variable in other cancers [105]. This indicates that this drug strategy would require more specific inhibitors to target the remaining glucose transporters to manage diverse cancers.

Indirectly, ritonavir, an HIV protease inhibitor, exhibits an off-target effect on GLUT4 and has been shown to inhibit GLUT4-mediated glucose transport and to induce apoptosis in multiple myeloma cells [100]. Although, we have not found drugs targeting GLUT3 receptors, it appears that inhibition of glucose transport is a feasible strategy for anti-cancer drug development. Imatinib mesylate is essentially a tyrosine kinase inhibitor targeting Bcr-Abl tyrosine kinase but also has an ability to antagonize the translocation of GLUT2 receptors to the plasma membrane thereby reducing glucose uptake leading to cancer cell death [106].

5.2. Drugs targeting glycolysis

5.2.1. Lonidamine and 3-bromopyruvate

Following glucose uptake into the cell, the next step is phosphorylation of glucose by hexokinase II. Both lonidamine and 3-bromopyruvate were identified as drugs that repress glycolysis by inhibiting hexokinase II. It was also found to selectively inhibit the electron transport chain in the mitochondria of cancer cells indicating that it might affect OXPHOS [107, 108]. Furthermore, lonidamine was shown to have an additive rather than a synergistic effect when used together with 2-deoxy-D-glucose (2-DG) [109]. Thus far, the lonidamine mechanism of action is elusive and after many studies, it is still not clear.

The primary mechanism of action for 3-bromopyruvate (3-BP) is via preferential alkylation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). This results in depletion of ATP selectively in cancer cells as they prefer glycolysis for energy production [110]. Systemic delivery of microencapsulated 3-BP to pancreatic ductal adenocarcinoma xenograft tumors showed promising results with minimal or no tumor progression observed [111]. However, safety concerns in the use of 3-BP only were observed in human trials [112].

5.2.2. Pyruvate esters

Interestingly, some pyruvate esters show anti-cancer properties, albeit via different mechanisms. Methyl pyruvate kills lung cancer cell line-A549 cells alone and in combination with irinotecan but protects the normal lung fibroblast cell line—MRC5 from irinotecan-induced apoptosis probably by inhibiting the p53/p21 axis of the apoptotic pathways during treatment and the mitochondrial pathway as well during recovery. Thus, methyl pyruvate has a potential for use as an adjunctive to chemotherapy [89]. In this case, exogenous pyruvate adds to the endogenous thereby boosting OXPHOS and reversing the Warburg Effect. It is reported that another pyruvate ester, ethyl pyruvate, preferentially killed leukemia cells by concerted mechanisms including cell death by necrosis or apoptosis/ATP depletion and by inhibition of glycolytic and para-glycolytic enzymes. Ethyl pyruvate (EP) was also shown to inhibit glyoxalase 1 (GLO1), an enzyme that detoxifies the glycolytic bi-product, methylglyoxal. It also suppresses glycolytic enzymes, LDH and pyruvate kinase [113]. In another study, ethyl pyruvate-reduced mitochondrial apoptosis and protected the murine myeloid cell line 32D c13 from radiation. It also increased survival of irradiated C57BL/6NHsd mice. In contrast, the inactive analog of EP did not enable such protection [114]. These properties of pyruvate esters are interesting as they point to a potential for this molecule as adjunctive in chemotherapy and in radiotherapy.

5.2.3.-deoxy-D-glucose (2-DG)

2-DG is a synthetic glucose analog in which a hydrogen atom replaces the C2 hydroxyl group such that it cannot be further utilized in the glycolytic pathway. 2-DG competitively inhibits glucose uptake, given that it is imported into the cell by glucose transporters (GLUTs). Following the import of 2DG into the cell, it becomes phosphorylated by hexokinase to 2-DG-6-P (2-deoxy-D-glucose-6-phosphate), rather than glucose 6-phosphate. As such, 2-DG

competitively inhibits the production of glucose-6-phosphate. 2-DG-6-P cannot be further processed, hence it accumulates in the cell and non-competitively inhibits hexokinase and competitively inhibits glucose-6-phosphate isomerase [115]. Both glycolysis and OXPHOS are partially inhibited, given that the first important steps of glycolysis are inhibited by 2-DG [116]. This leads to decreased ATP production which renders cells susceptible to death receptor-induced apoptosis. 2-DG is also known to block the cell cycle.

5.2.4. TEPP-46

Pyruvate kinase M2 (PKM2) is an alternatively spliced isoform of the key glycolytic enzyme PKMI which catalyzes the conversion of phosphoenolpyruvate (PEP) into pyruvate while concurrently producing ATP. PKM2 is expressed during embryonic development and in cancer cells where is promotes the Warburg Effect, but not in normal adult cells making it an attractive target for drug development. Negative regulation of PKM2 in cancer cells results in shunting glycolysis to the anabolic pentose phosphate pathway which benefits cancer cells. TEPP-46 reactivates pyruvate kinase M2 thereby increasing glycolytic flux and reducing lactate production in cancer cells while deleting intermediates required for entry into the pentose phosphate pathway resulting in cancer cell death [117, 118]. It has been demonstrated *in vivo* that reactivation of PKM2 activity and reduction of PPP is a plausible anti-cancer strategy. In a xenograft model for non-small lung cancer, TEPP reduced the size of the tumor [118, 119]. There is some concern about small molecule inhibitors of PKM2 as they may interfere with anabolic activity since they induce constitutive activity. It is noteworthy that PKM2 can act as a transcription factor activating transcription of genes which control other aspects of carcinogenesis such as Oct-4, hypoxia-inducible factor 1-α (HIF-1α), and β-catenin [120–122]. This indicates that small molecule inhibitors of PKM2 may also affect other pathways but this may be a positive factor since these pathways are also implicated in carcinogenesis. It also suggests that studies of these small molecule inhibitors must also include assessment of the potentially affected pathways.

5.2.5. Inhibitors of lactate dehydrogenase-A (LDH-A) and oxamate

LDH-A catalyzes the interconversion of pyruvate, the final product of glycolysis, to lactate and nicotinamide adenine dinucleotide (NAD$^+$). The NAD$^+$, thus produced, is reused to fuel further glycolysis. Depletion of endogenous LDH-A by RNAi results in death of cancer cells regardless of p53 status indicating that it supports their proliferation. However, it was shown that the p53 status is required for maintaining the NADH:NAD$^+$ ratio because acetylated p53$^{+/+}$ is important for modulating the NADH:NAD$^+$ ratio. This phenotype is dependent upon the NAD$^+$-dependent deacetylase sirtuin 1 (SIRT1). Hence, the anti-cancer activity of small molecule drug and redox-sensitive anti-cancer drug EO9 (apaziquone) was enhanced only in p53$^{+/+}$ cells. This indicates that LDH-A is a plausible therapeutic target for anti-cancer drug development and that a combinatorial approach that targets LDH-A and the redox status is even more effective [123].

EO9 is a bioreductive alkylating agent that causes DNA damage, creating single strand breaks and crosslinks [124]. Therefore, it does not target glucose metabolism but is regarded as a sensor of the NADH:NAD$^+$ redox status. These findings indicate that inhibitors of LDH-A

may have potential as anti-cancer drugs. Indeed, a small molecule inhibitor of LDH-A, FX11 (3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid) causes ATP depletion, induces oxidative stress and causes cell death which can be partially reversed by anti-oxidants such as N-acetylcysteine [125]. FX11 probably acts by upsetting the Warburg Effect because it has been demonstrated that it induces apoptosis in and reduction of patient tumor xenographs in a p53-dependnet manner whereby only mutant Tp53 tumors respond to the drug [126]. This p53-dependence seen in LDH-A-related responses is interesting as it seems to be related to the involvement of p53 in modulating the $NADH:NAD^+$ ratio. The molecular mechanism by which p53 would influence this phenotype is unclear and certainly requires more investigation. Oxamate is a related drug as it inhibits lactate dehydrogenase (LDH) and has been shown to cause death of a breast cancer cell line [127, 128].

5.2.6. Oxythiamine (OT)

The transketolase-like-1-gene (TKTL1) in urothelial and colorectal cancer is associated with poor prognosis when overexpressed and was identified as a biomarker. It encodes the enzyme transketolase that catalyzes the production of lactate from glucose via the pentose phosphate pathway in cancer cells [129]. Oxythiamine (OT) is a transketolase inhibitor that arrests growth of cancer cells. This discovery introduced the idea that in general, pentose cycle inhibitors provide yet another angle for cancer management [130].

5.3. Drugs targeting OXPHOS or mitochondrial activities

5.3.1. Dichloroacetate

Dichloroacetate (DCA) is an inhibitor of mitochondrial pyruvate dehydrogenase and its effect on metabolism is to redirect glucose metabolism from glycolysis to oxidation which effectively reverses the Warburg effect. Consequently, it inhibits proliferation and induces caspase-mediated apoptosis [131]. DCA has been taken through Phase 1 clinical trials given orally to patients with **World Health Organization (**WHO) grade III–IV gliomas or metasta-ses from a primary cancer outside the central nervous system. It was feasible and well toler-ated although genetic factors affecting tolerance were confirmed [132]. In Phase II clinical trials, DCA exhibited synergy when used with cisplatin and docetaxel leading to a sugges-tion that it might be more effective and preferable for use in combination with other drugs [133]. It is noteworthy that these studies, together with the use of methyl pyruvate mentioned earlier, support a broad principle involving the reversal of the Warburg Effect as a plausible anti-cancer strategy.

5.3.2. Rotenone

Rotenone is a natural insecticide and an inhibitor of NADH dehydrogenase complex or com-plex 1 of the mitochondrial electron transport chain (ETC). This results in the generation of reactive oxygen species and apoptosis [134, 135]. Because of its activity on ETC complex 1 which is often altered in Parkinson's disease, rotenone is sometimes used to create an animal

Drug	Mechanism of action	Status	Reference
Targeting glucose transporters			
WZB117, STF-31	Inhibits GLUT1, thereby suppressing glucose transport	Preclinical	[140, 141]
STF-31	Selectively inhibits Glut1 expression and completely induces apoptosis sin GLUT1 expressing myeloma cells.	Preclinical	[104]
ritonavir	Has an off-target inhibitory effect on GLUT4.	Approved for HIV	[100]
Imatinib	Inhibits Bcr-Abl tyrosine kinase; suppresses the activity of Hexokinase and G6PD	Approved for clinical use	[142]
Targeting glycolytic pathway			
2-DG	Inhibits HK, thereby suppressing glycolysis	Clinical trials aborted	[143, 144]
3-bromo- pyruvate	An alkylating agent that inhibits HK	Preclinical	[97, 111, 145]
Lonidamine	Suppresses glycolysis by inhibiting hexokinase II and OxPhos by dissociating HK2 from mitochondria	Clinical trials Phase II/III	[107, 108, 146]
FX11	Inhibits LDH-A	Preclinical	[125]
Oxamate	Inhibits LDH-A	Preclinical	[127, 128]
TEPP-46	Activates PKM2 and suppresses the PPP	Preclinical	[118, 147]
Oxythiamine	Inhibits transketolase thereby suppressing the PPP; inhibits pyruvate dehydrogenase	Preclinical	[130]
Targeting OXPHOS			
Dichloroacetate	Inhibits the pyruvate dehydrogenase complex	Phase II	[131–133]
Pyruvate esters	Boost OXPHOS	Experimental	[89, 113, 114]
Rotenone	Inhibits complex 1 in OXPHOS	Preclinical	[135, 148]
Targeting glutamine pathway			
L-γ-glutamyl-p-nitroanilide	Inhibits SLC1A5 (also known as (ASCT2)	Preclinical	[138]
BCH	inhibits glutamine/leucine exchange and mTOR activation	Preclinical	[139]
Benzylserine	inhibits glutamine transport by targeting both LAT1 and ASCT2	Preclinical	[139]
BPTES	inhibit glutaminase	Preclinical	[139]
CB-839	unknown	Preclinical	[139]
Compound 968	Probablt tarets by Rho GTPases	Preclinical	[139]
EGCG	inhibits GDH	Preclinical	[139]

Table 2. Potential anti-cancer drugs targeting glucose metabolism.

model of Parkinson's disease [136]. It can thus be neurotoxic. Nevertheless, rotenone, given at subtoxic amounts, was used to reverse tumor necrosis factor-related apoptosis inducing

ligand (TRAIL) resistant non-small cell lung carcinoma (NSCLC) cells in a p53-dependent manner that was also related to generation of reactive oxygen species [137]. Although, rotenone does not target glucose metabolism but rather the integral structure of the mitochondrion, it would affect glucose metabolism indirectly. It falls within a class of drugs that target the structural integrity of the mitochondrion, including resveratrol, Vitamin E analogs, arsenic trioxide, honokiol and betulinic acid [127].

5.4. Drugs targeting the glutamine pathway

Glutamine is a non-essential amino acid that can be produced from glucose. It is known that cancer cells have a propensity to consume large amounts of glutamine and undergo dramatic metabolic programming geared at production of anabolic precursors from glutamine. Glutamine, a mitochondrial substrate, enters the cell via high affinity glutamine transporters and is converted into glutamic acid and then into the tricarboxylic acid (TCA) cycle metabolite, α-ketoglutarate [47]. A window for anti-cancer drug development is therefore open at the level of glucose uptake. The Na(+)-dependent neutral amino acid transporter type 2 (ASCT2) is encoded by the *solute-linked carrier family A1 member 5 (SLC1A5)* gene and inhibited by L-γ-glutamyl-p-nitroanilide or GPNA via mTOR signaling [138]. New drugs targeting glutaminolysis have been reviewed by Jin et al. [139] and are listed in **Table 2**.

6. Conclusions

The metabolic reprogramming that occurs during carcinogenesis and also upon infections provides a window of opportunity for anti-cancer drug discovery and development. The molecular mechanisms that underlie this phenotype are being elucidated at a rapid pace further helping the development of therapeutic strategies. However, there are still key questions to be addressed with regards to the Warburg Effect. Firstly, it is still to be ascertained whether this phenomenon is a cause or a consequence of carcinogenesis. Secondly, the role of the tumor suppressor p53 which intervenes at multiple points in the glucose metabolic pathway seems essential as demonstrated by the elegant work of the Feng group at Rutgers University who showed a gain-of-function phenotype in p53 which stimulates the Warburg Effect. However, it seems that this phenomenon can occur in a wild type p53 background. Altogether, glucose metabolism offers exciting opportunities for anti-cancer drug discovery and development.

Acknowledgements
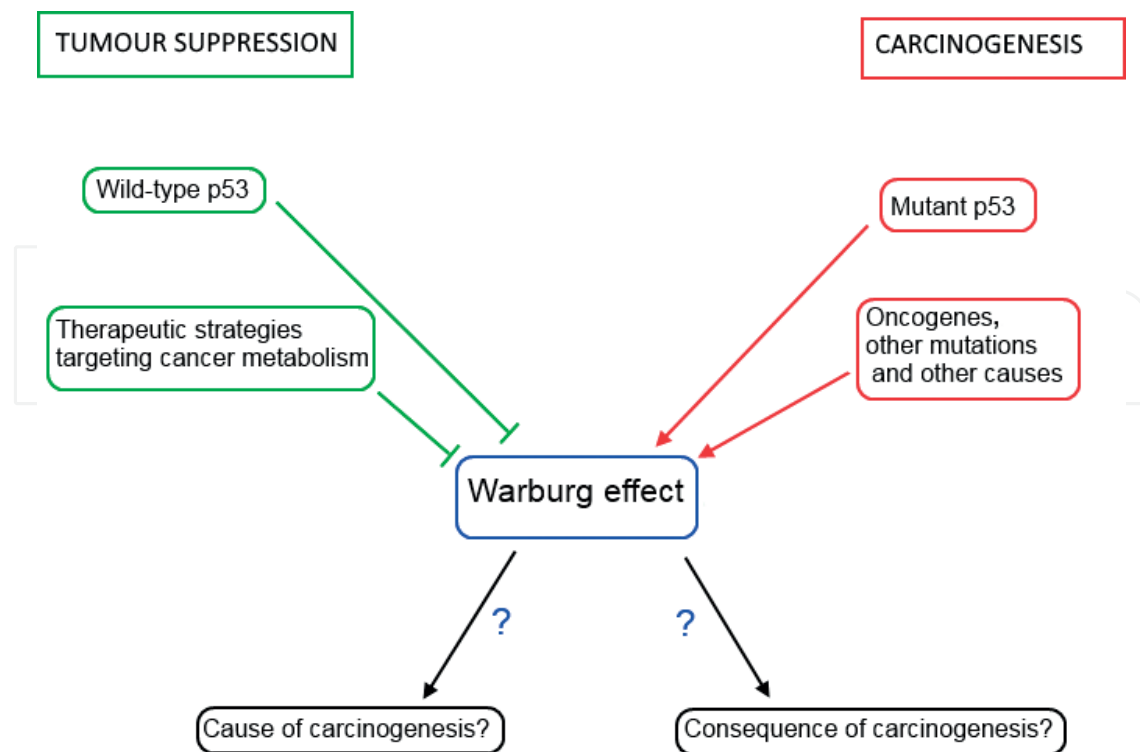
Conflict of interest

The authors declare no conflict of interest.

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References

[1] Warburg O, Wind F, Negelein E. The metabolism of tumors in the body T. The Journal of General Physiology. 1927;**8**(6):519-530

[2]   Levine AJ, Puzio-Kuter AM. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. Science. 2010;**330**(6009):1340-1344

[3]   Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**(5): 646-674

[4]   Yeung SJ, Pan J, Lee M-H. Roles of p53, Myc and HIF-1 in regulating glycolysis — The seventh hallmark of cancer. Cellular and Molecular Life Sciences. 2008;**65**(24):3981-3999

[5]   Ntwasa M. Cancer drugs targeting the p53 regulatory machinery. In: Atta-ur-Rahman F, Choudhary MI, editors. Frontiers in Anti-Cancer Drug Discovery. Bentham Publishers; 2015. pp. 199-230

[6]   Haupt Y et al. Mdm2 promotes the rapid degradation of p53. Nature. 1997;**387**(6630):296-299

[7]   Younes M et al. Overexpression of glut1 and glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer. 1997;**80**:1046-1051

[8]   Ben-Haim S, Ell P. 18F-FDG PET and PET/CT in the evaluation of cancer treatment response. Journal of Nuclear Medicine. 2009;**50**(1):88-99

[9]   Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4; gene expression. Cancer Research. 2004;**64**(7):2627

[10] Kawauchi K et al. p53 regulates glucose metabolism through an IKK-NF-[kappa]B pathway and inhibits cell transformation. Nature Cell Biology. 2008;**10**(5):611-618

[11] Bensaad K et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 2006;**126**:107-120

[12] Green DR, Chipuk JE. p53 and metabolism: Inside the TIGAR. Cell. 2006;**126**(1):30-32

[13] Kondoh H et al. Glycolytic enzymes can modulate cellular life span. Cancer Research. 2005;**65**(1):117-185

[14] Hitosugi T et al. Phosphoglycerate mutase 1 coordinates glycolysis and biosynthesis to promote tumor growth. Cancer Cell. 2012;**22**(5):585-600

[15] Tennant DA et al. Metabolic transformation in cancer. Carcinogenesis. 2009;**30**(8):1269-1280

[16] Contractor T, Harris CR. p53 negatively regulates transcription of the pyruvate dehydrogenase kinase Pdk2. Cancer Research. 2012;**72**(2):560

[17] Goodwin ML et al. Lactate and cancer: Revisiting the Warburg effect in an era of lactate shuttling. Frontiers in Nutrition. 2014;**1**:27

[18] Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. The Journal of Clinical Investigation. 2013;**123**(9):3685-3692

[19] Shime H et al. Tumor-secreted lactic acid promotes IL-23/IL-17 proinflammatory pathway. The Journal of Immunology. 2008;**180**(11):7175

[20] Boidot R et al. Regulation of monocarboxylate transporter MCT1 expression by p53 mediates inward and outward lactate fluxes in tumors. Cancer Research. 2011

[21] Kim Y et al. Expression of lactate/H+ symporters MCT1 and MCT4 and their chaperone CD147 predicts tumor progression in clear cell renal cell carcinoma: Immunohistochemical and the cancer genome atlas data analyses. Human Pathology. 2015;**46**(1):104-112

[22] Shim H et al. C-Myc transactivation of LDH-A: Implications for tumor metabolism and growth. Proceedings of the National Academy of Sciences. 1997;**94**(13):6658-6663

[23] Santagata S et al. High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer. Proceedings of the National Academy of Sciences. 2011;**108**(45):18378-18383

[24] Firth JD, Ebert BL, Ratcliffe PJ. Hypoxic regulation of lactate dehydrogenase A: Interaction between hypoxia-inducible factor1 and cAMP response elements. Journal of Biological Chemistry. 1995;**270**(36):21021-21027

[25] Kaller M et al. Genome-wide characterization of miR-34a induced changes in protein and mRNA expression by a combined pulsed SILAC and microarray analysis. Molecular & Cellular Proteomics. 2011;**10**(8)

[26] Xiao X et al. The miR-34a-LDHA axis regulates glucose metabolism and tumor growth in breast cancer. Scientific Reports. 2016;**6**:1-9

[27] Chang T-C et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Molecular Cell. 2007;**26**(5):745-752

[28] Chang T-C et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Molecular Cell. 2007;**26**(5):745-752

[29] Bommer GT et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Current Biology. 2007;**17**(15):1298-1307

[30] Saraste M. Oxidative phosphorylation at the fin de siècle. Science. 1999;**283**(5407):1488

[31] Kitamura N et al. Mieap, a p53-inducible protein, controls mitochondrial quality by repairing or eliminating unhealthy mitochondria. PLoS One. 2011;**6**(1):e16060

[32] Achanta G et al. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA pol γ. The EMBO Journal. 2005;**24**(19):3482-3492

[33] Tuppen HAL et al. Mitochondrial DNA mutations and human disease. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2010;**1797**(2):113-128

[34] Matoba S et al. p53 regulates mitochondrial respiration. Science. 2006;**312**:1650-1653

[35] Zhou S, Kachhap S, Singh KK. Mitochondrial impairment in p53-deficient human cancer cells. Mutagenesis. 2003;**18**(3):287-292

[36] Seth R et al. p53-dependent caspase-2 activation in mitochondrial release of apoptosis-inducing factor and its role in renal tubular epithelial cell injury. Journal of Biological Chemistry. 2005;**280**(35):31230-31239

[37] Cheung ECC et al. Dissociating the dual roles of apoptosis-inducing factor in maintaining mitochondrial structure and apoptosis. The EMBO Journal. 2006;**25**(17):4061

[38] Szczepanek K et al. Deficiency of apoptosis inducing factor (AIF) decreases complex I activity and increases the ROS generation in isolated cardiac mitochondria. The FASEB Journal. 2013;**27**(1 Supplement):1085.18

[39] Yu D et al. Kidney-type glutaminase (GLS1) is a biomarker for pathologic diagnosis and prognosis of hepatocellular carcinoma. Oncotarget. 2015;**6**(10):7619-7631

[40] Huang F et al. Expression of glutaminase is upregulated in colorectal cancer and of clinical significance. International Journal of Clinical and Experimental Pathology. 2014;**7**(3):1093-1100

[41] Hu W et al. Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. Proceedings of the National Academy of Sciences. 2010;**107**(16):7455-7460

[42] Gross MI et al. Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. American Association for Cancer Research. 2014;**13**(4):890-901

[43] Suzuki S et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proceedings of the National Academy of Sciences. 2010;**107**(16):7461-7466

[44] Liu J et al. Glutaminase 2 negatively regulates the PI3K/AKT signaling and shows tumor suppression activity in human hepatocellular carcinoma. Oncotarget. 2014;**5**(9):2635-2647

[45] Zhang C et al. Glutaminase 2 is a novel negative regulator of small GTPase Rac1 and mediates p53 function in suppressing metastasis. eLife. 2016;**5**:e1072

[46] Elstrom RL et al. Akt stimulates aerobic glycolysis in cancer cells. Cancer Research. 2004;**64**(11):3892

[47] Wise DR, Thompson CB. Glutamine addiction: A new therapeutic target in cancer. Trends in Biochemical Sciences. 2010;**35**(8):427-433

[48] D'Autréaux B, Toledano MB. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. Nature Reviews. Molecular Cell Biology. 2007;**8**(10):813-824

[49] Sablina AA et al. The antioxidant function of the p53 tumor suppressor. Natural Medicines. 2005;**11**(12):1306-1313

[50] Tan M et al. Transcriptional activation of the human glutathione peroxidase promoter by p53. Journal of Biological Chemistry. 1999;**274**(17):12061-12066

[51] Yoon K-A, Nakamura Y, Arakawa H. Identification of ALDH4 as a p53-inducible gene and its protective role in cellular stresses. Journal of Human Genetics. 2004;**49**(3):134-140

[52] Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nature Reviews Cancer. 2007;**7**(10):763-777

[53] Pizer ES et al. Malonyl-coenzyme-a is a potential mediator of cytotoxicity induced by fatty-acid synthase inhibition in human breast cancer cells and xenografts. Cancer Research. 2000;**60**(2):213

[54] Zaidi N, Swinnen JV, Smans K. ATP-citratelyase: A key player in cancer metabolism. Cancer Research. 2012;**72**(15):3709

[55] Yahagi N et al. p53 activation in adipocytes of obese mice. Journal of Biological Chemistry. 2003;**278**(28):25395-25400

[56] Assaily W et al. ROS-mediated p53 induction of Lpin1 regulates fatty acid oxidation in response to nutritional stress. Molecular Cell. 2011;**44**(3):491-501

[57] Schrijver ED et al. RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Research. 2003;**63**:3799-3804

[58] Li W et al. Repression of endometrial tumor growth by targeting SREBP1 and lipogenesis. Cell Cycle. 2012;**11**(12):2348-2358

[59] Hollstein M et al. p53 mutations in human cancers. Science. 1991;**253**(5015):49-53

[60] van Oijen MGCT, Slootweg PJ. Gain-of-function mutations in the tumor suppressor gene p53. Clinical Cancer Research. 2000;**6**(6):2138

[61] Dittmer D et al. Gain of function mutations in p53. Nature Genetics. 1993;**4**:42-46

[62] Oren M, Rotter V. Mutant p53 gain-of-function in cancer. Cold Spring Harbor Perspectives in Biology. 2010;**2**(2)

[63] Zhou G et al. Gain-of-function mutant p53 promotes cell growth and cancer cell metabolism via inhibition of AMPK activation. Molecular Cell. 2014;**54**(6):960-974

[64] Feng Z, Levine AJ. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. Trends in Cell Biology. 2010;**20**(7):427-434

[65] Budanov AV, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell. 2008;**134**(3):451-460

[66] Jones RG et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Molecular Cell. 2005;**18**(3):283-293

[67] Ludes-Meyers JH et al. Transcriptional activation of the human epidermal growth factor receptor promoter by human p53. Molecular and Cellular Biology. 1996;**16**(11):6009-6019

[68] Kieser A et al. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene. 1994;**9**:963-969

[69] Werner H et al. Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. Proceedings of the National Academy of Sciences. 1996;**93**(16):8318-8323

[70] Xu J et al. Gain of function of mutant p53 by coaggregation with multiple tumor suppressors. Nature Chemical Biology. 2011;**7**(5):285-295

[71] Leng RP et al. Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell. 2003;**112**(6):779-791

[72] Dornan D et al. The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature. 2004;**429**:86-92

[73] Fuchs SY et al. JNK targets p53 ubiquitination and degradation in nonstressed cells. Genes & Development. 1998;**12**:2658-2663

[74] Adler V et al. Conformation-dependent phosphorylation of p53. Proceedings of the National Academy of Sciences. 1997;**94**(5):1686-1691

[75] Li L et al. PACT is a negative regulator of p53 and essential for cell growth and embryonic development. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(19):7951-7956

[76] Ntwasa M. Retinoblastoma binding protein 6: Another p53 monitor. Trends in Cancer. 2016;**2**(11):635-637

[77] Moll UM, Petrenko O. The MDM2-p53 interaction. Molecular Cancer Research. 2003;**1**:1001-1008

[78] Fritsche M, Haessler C, Brandner G. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. Oncogene. 1993;**8**(2):307-318

[79] Ringshausen I et al. Mdm2 is critically and continuously required to suppress lethal p53 activity in vivo. Cancer Cell. 2006;**10**(6):501-514

[80] Wang L et al. Interplay between MDM2, MDMX, Pirh2 and COP1: The negative regulators of p53. Molecular Biology Reports. 2011;**38**(1):229-236

[81] Barak Y et al. mdm2 expression is induced by wild type p53 activity. The EMBO Journal. 1993;**12**(2):461-468

[82] Wu X et al. The p53-mdm-2 autoregulatory feedback loop. Genes & Development. 1993;**7**(7a):1126-1132

[83] Li X et al. The MDM2–p53–pyruvate carboxylase signalling axis couples mitochondrial metabolism to glucose-stimulated insulin secretion in pancreatic β-cells. Nature Communications. 2016;**7**:11740

[84] Duan W et al. Expression of Pirh2, a newly identified ubiquitin protein ligase, in lung cancer. Journal of the National Cancer Institute. 2004;**96**(22):1718-1721

[85] Neff MM, Fankhauser C, Chory J. Light: an indicator of time and place. Genes & Development. 2000;**14**(3):257-271

[86] Sakai Y et al. cDNA sequence and chromosomal localisation of a novel human protein, RBQ-1 (RBBP6), that binds to the retinoblastoma gene product. Genomics. 1995;**30**:98-101

[87] Gao S, White MM, Scott RE. P2P-R protein localizes to the nucleus of interphase cells and the periphery of chromosomes in mitotic cells which show maximum P2P-R immunoreactivity. Journal of Cellular Physiology. 2002;**191**:145-154

[88] Chen J et al. Overexpression of RBBP6, alone or combined with mutant TP53, is predictive of poor prognosis in colon cancer. PLoS One. 2013;**8**(6):e66524

[89] Monchusi B, Ntwasa M. Methyl pyruvate protects a normal lung fibroblast cell line from irinotecan-induced cell death: Potential use as adjunctive to chemotherapy. PLoS One. 2017;**12**(8):e0182789

[90] Warburg O. On the origin of cancer cells. Science. 1956;**123**:309-314

[91] Altenberg B, Greulich KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics. 2004;**84**(6):1014-1020

[92] Zhang C et al. Tumour-associated mutant p53 drives the Warburg effect. Nature Communications. 2013;**4**:2935

[93] Gordan JD, Thompson CB, Simon MC. HIF and c-Myc: Sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell. 2007;**12**(2):108-113

[94] Tan Allen K et al. Dichloroacetate alters Warburg metabolism, inhibits cell growth, and increases the X-ray sensitivity of human A549 and H1299 NSC lung cancer cells. Free Radical Biology and Medicine. 2015;**89**(Supplement C):263-273

[95] Liu H et al. Hypoxia increases tumor cell sensitivity to glycolytic inhibitors: A strategy for solid tumor therapy (model C). Biochemical Pharmacology. 2002;**64**(12):1745-1751

[96] Maher JC, Krishan A, Lampidis TJ. Greater cell cycle inhibition and cytotoxicity induced by 2-deoxy-d-glucose in tumor cells treated under hypoxic vs aerobic conditions. Cancer Chemotherapy and Pharmacology. 2004;**53**(2):116-122

[97] Xu R-H et al. Synergistic effect of targeting mTOR by rapamycin and depleting ATP by inhibition of glycolysis in lymphoma and leukemia cells. Leukemia. 2005;**19**(12):2153-2158

[98] Hamanaka RB, Chandel NS. Targeting glucose metabolism for cancer therapy. The Journal of Experimental Medicine. 2012;**209**(2):211

[99] Tran Q et al. Targeting cancer metabolism - revisiting the Warburg effects. Toxicological Research. 2016;**32**(3):177-193

[100] McBrayer SK et al. Multiple myeloma exhibits novel dependence on GLUT4, GLUT8, and GLUT11: Implications for glucose transporter-directed therapy. Blood. 2012; **119**(20):4686

[101] Wang Y-D, Li S-J, Liao J-X. Inhibition of glucose transporter 1 (GLUT1) chemosensitized head and neck cancer cells to cisplatin. Technology in Cancer Research & Treatment. 2013;**12**(6):525-535

[102] Liu W et al. Overcoming 5-Fu resistance of colon cells through inhibition of Glut1 by the specific inhibitor WZB117. Asian Pacific Journal of Cancer Prevention. 2014; **15**(17):7037-7041

[103] Shibuya K et al. Targeting the facilitative glucose transporter GLUT1 inhibits the self-renewal and tumor-initiating capacity of cancer stem cells. Oncotarget. 2015;**6**(2):651-661

[104] Matsumoto T et al. Inhibition of glucose transporter 1 induces apoptosis and sensitizes multiple myeloma cells to conventional chemotherapeutic agents. Leukemia Research. 2016;**41**(Supplement C):103-110

[105] Carvalho KC et al. GLUT1 expression in malignant tumors and its use as an immuno-diagnostic marker. Clinics. 2011;**66**:965-972

[106] Prenen H et al. Imatini mesylate inhibits glucose uptake in gastrointestinal stromal tumor cells by downregulation of the glusose transporters recruitment to the plasma membrane. American Journal of Biochemistry and Biotechnology. 2005;**1**(2):95-102

[107] Floridi A, Lehninger AL. Action of the antitumor and antispermatogenic agent lonidamine on electron transport in ehrlich ascites tumor mitochondria. Archives of Biochemistry and Biophysics. 1983;**226**(1):73-83

[108] Floridi A et al. Effect of lonidamine on the energy metabolism of Ehrlich ascites tumor cells. Cancer Research. 1981;**41**(11 Part 1):4661

[109] Ben-Horin H et al. Mechanism of action of the antineoplastic drug Lonidamine: ^{31}P and ^{13}C nuclear magnetic resonance studies. Cancer Research. 1995;**55**(13):2814

[110] Ko YH et al. Advanced cancers: Eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochemical and Biophysical Research Communications. 2004; **324**(1):269-275

[111] Chapiro J et al. Systemic delivery of microencapsulated 3-bromopyruvate for the therapy of pancreatic cancer. Clinical Cancer Research. 2014;**20**(24):6406

[112] El Sayed SM et al. Safety and outcome of treatment of metastatic melanoma using 3-bromopyruvate: A concise literature review and case study. Chinese Journal of Cancer. 2014;**33**(7):356-364

[113] Birkenmeier G et al. Ethyl pyruvate combats human leukemia cells but spares normal blood cells. PLoS One. 2016;**11**(8):e0161571

[114] Epperly M et al. Ethyl pyruvate, a potentially effective mitigator of damage after total-body irradiation. Radiation Research. 2007;**168**(5):552-559

[115] Wick AN et al. Localization of the primary metabolic block produced by 2-deoxyglucose. Journal of Biological Chemistry. 1957;**224**(2):963-969

[116] Robinson GL et al. Switching from aerobic glycolysis to oxidative phosphorylation modulates the sensitivity of mantle cell lymphoma cells to TRAIL. Oncogene. 2012;**31**(48):4996-5006

[117] Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature. 2012;**491**(7424):364-373

[118] Anastasiou D et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. Nature Chemical Biology. 2012;**8**(10):839-847

[119] Cheong J-H et al. Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. Molecular Cancer Therapeutics. 2011;**10**(12):2350

[120] Lee J et al. Pyruvate kinase isozyme type M2 (PKM2) interacts and cooperates with Oct-4 in regulating transcription. The International Journal of Biochemistry & Cell Biology. 2008;**40**(5):1043-1054

[121] Luo W et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell. 2011;**145**(5):732-744

[122] Yang W et al. Nuclear PKM2 regulates β-catenin transactivation upon EGFR activation. Nature. 2011;**480**:118

[123] Allison SJ et al. Identification of LDH-A as a therapeutic target for cancer cell killing via (i) p53/NAD(H)-dependent and (ii) p53-independent pathways. Oncogene. 2014;**3**(5):e102

[124] Dirix LY et al. EO9 phase II study in advanced breast, gastric, pancreatic and colorectal carcinoma by the EORTC early clinical studies group. European Journal of Cancer. 1996;**32**(11):2019-2022

[125] Le A et al. Inhibition of lactate dehydrogenase a induces oxidative stress and inhibits tumor progression. Proceedings of the National Academy of Sciences. 2010;**107**(5):2037-2042

[126] Rajeshkumar NV et al. Therapeutic targeting of the Warburg effect in pancreatic cancer relies on an absence of p53 function. Cancer Research. 2015 Aug 15;**75**(16):3355-3364. DOI: 10.1158/0008-5472.CAN-15-0108

[127] Wang F, Ogasawara MA, Huang P. Small mitochondria-targeting molecules as anticancer agents. Molecular Aspects of Medicine. 2010;**31**(1):75-92

[128] Thornburg JM et al. Targeting aspartate aminotransferase in breast cancer. Breast Cancer Research. 2008;**10**(5):R84

[129] Földi M et al. Transketolase protein TKTL1 overexpression: A potential biomarker and therapeutic target in breast cance. Oncology Reports. 2007;**17**:841-845

[130] Raïs B et al. Oxythiamine and dehydroepiandrosterone induce a G1 phase cycle arrest in Ehrlich's tumor cells through inhibition of the pentose cycle. FEBS Letters. 1999;**456**(1):113-118

[131] Kankotia S, Stacpoole PW. Dichloroacetate and cancer: New home for an orphan drug? Biochimica et Biophysica Acta (BBA) - Reviews on Cancer. 2014;**1846**(2):617-629

[132] Dunbar EM et al. Phase 1 trial of dichloroacetate (DCA) in adults with recurrent malignant brain tumors. Investigational New Drugs. 2014;**32**(3):452-464

[133] Garon EB et al. Dichloroacetate should be considered with platinum-based chemotherapy in hypoxic tumors rather than as a single agent in advanced non-small cell lung cancer. Journal of Cancer Research and Clinical Oncology. 2014;**140**(3):443-452

[134] Tada-Oikawa S et al. Mechanism for generation of hydrogen peroxide and change of mitochondrial membrane potential during rotenone-induced apoptosis. Life Sciences. 2003;**73**(25):3277-3288

[135] Li N et al. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. Journal of Biological Chemistry. 2003;**278**(10):8516-8525

[136] Kerr DS. Treatment of mitochondrial electron transport chain disorders: A review of clinical trials over the past decade. Molecular Genetics and Metabolism. 2010;**99**(3):246-255

[137] Shi YL et al. Mitochondrial inhibitor sensitizes non-small-cell lung carcinoma cells to TRAIL-induced apoptosis by reactive oxygen species and Bcl-XL/p53-mediated amplification mechanisms. Cell Death & Disease. 2014;**5**:e1579

[138] Hassanein M et al. SLC1A5 mediates glutamine transport required for lung cancer cell growth and survival. Clinical Cancer Research. 2013;**19**(3):560

[139] Jin L, Alesi GN, Kang S. Glutaminolysis as a target for cancer therapy. Oncogene. 2016;**35**(28):3619-3625

[140] Chan DA et al. Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. Science Translational Medicine. 2011;**3**(94):94ra70

[141] Liu Y et al. A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth; in vitro and in vivo. Molecular Cancer Therapeutics. 2012;**11**(8):1672

[142] Cohen MH et al. Approval summary: Imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. The Oncologist. 2009;**14**(2):174-180

[143] Raez LE et al. A phase I dose-escalation trial of 2-deoxy-d-glucose alone or combined with docetaxel in patients with advanced solid tumors. Cancer Chemotherapy and Pharmacology. 2013;**71**(2):523-530

[144] Stein M et al. Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies. The Prostate. 2010;**70**(13):1388-1394

[145] Geschwind J-FH et al. Novel therapy for liver cancer. Cancer Research. 2002;**62**(14):3909

[146] De Lena M et al. Paclitaxel, cisplatin and lonidamine in advanced ovarian cancer. A phase II study. European Journal of Cancer. 2001;**37**(3):364-368

[147] Kung C et al. Small molecule activation of PKM2 in cancer cells induces serine Auxotrophy. Chemistry & Biology. 2012;**19**(9):1187-1198

[148] Delgado T et al. Induction of the Warburg effect by Kaposi's sarcoma herpesvirus is required for the maintenance of latently infected endothelial cells. Proceedings of the National Academy of Sciences. 2010;**107**(23):10696-10701