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Review

Metabolic alterations in cancer cells and therapeutic implications

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Abstract

Cancer metabolism has emerged as an important area of research in recent years. Elucidation of the metabolic differences between cancer and normal cells and the underlying mechanisms will not only advance our understanding of fundamental cancer cell biology but also provide an important basis for the development of new therapeutic strategies and novel compounds to selectively eliminate cancer cells by targeting their unique metabolism. This article reviews several important metabolic alterations in cancer cells, with an emphasis on increased aerobic glycolysis (the Warburg effect) and glutamine addiction, and discusses the mechanisms that may contribute to such metabolic changes. In addition, metabolic alterations in cancer stem cells, mitochondrial metabolism and its influence on drug sensitivity, and potential therapeutic strategies and agents that target cancer metabolism are also discussed.

Key words Cancer metabolism, the Warburg effect, mitochondria, glycolysis, glutamine, cancer stem cells

Cancer cells have long been known to exhibit profound alterations in their metabolism, as exemplified by the Warburg effect, a phenomenon of cancer cells with elevated aerobic glycolysis [1-3]. Reprogramming of the cellular energy metabolism constitutes an emerging hallmark of cancer and may serve as a biochemical basis for new therapeutic intervention [4]. Tremendous efforts in recent years have been devoted to unveiling the underlying mechanisms for metabolic alterations in cancer, thus triggering considerable research interest in mitochondria, bioenergetics, and redox regulation in normal and malignant cells. Both glucose and glutamine are key metabolic substrates in cancer cells and are critical for cancer development, invasion, and metastases [5-9]. Glutamine is an abundant amino acid in human plasma and is present at high concentrations in the medium used for *in vitro* cell culture. Several oncogenes, including c-*Myc* and *Ras*, have been identified to promote the expression of metabolic enzymes and regulators that lead to preferential use of glycolysis over mitochondrial oxidative phosphorylation (OXPHOS). Loss of tumor suppressors, such as p53, fumarate hydratase (FH), and succinate dehydrogenase (SDH), also results in significant changes in energy metabolism and may contribute to activation of hypoxia-inducible factor (HIF)-1α-dependent pathways and adaptation to tumor hypoxia [10,11].

Mitochondria are critical cellular organelles in which many key metabolic pathways converge. Whether metabolic alterations drive tumorigenesis or are a consequence of malignant transformation is still a matter of debate. Mitochondrial dysfunction has been directly linked to alterations in gene expression profiles and significantly affects cancer development. An impaired mitochondrial respiratory chain (MRC) may significantly alter the expression of important genes such as forkhead box O family (*FOXO*) and apoptosis signal-regulating kinase 1 (*ASK-1*), leading to changes in cell cycle progression [12]. Mutations in mitochondrial DNA and nuclear DNA-encoded genes can also modulate cancer cell metabolism. Aberrant expression of specific molecules such as the serine/threonine protein kinase AKT and the mammalian target of rapamycin (mTOR) promotes increased glucose metabolism in cancer cells. In fact, AKT alone is sufficient to produce a glycolytic phenotype and glucose dependence in cancers [13]. Furthermore,

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mTOR, a downstream target of AKT, seems to function as an energy sensor that is sensitive to alterations in nutrients and amino acids[14-16] and is deregulated in many cancer types[17]. The tumor suppressor AMP-activated protein kinase (AMPK) is activated in response to stress signals, such as hypoxia and low cellular ATP levels, and regulates various critical cellular processes, including proliferation, cell cycle progression, autophagy, and cellular senescence, through its effects on key molecules such as mTORC1, p53, p27, FOXO3, and others[18,19]. In addition, metabolic reprogramming is linked to oncogenes such as *c-Myc* and *HIF-1α* and tumor suppressors such as *p53* that are important players in mediating energetic pathways[20-22]. A high dependency on glycolysis in cancers is also associated with altered glucose transporters and glycolytic enzymes such as hexokinase II (HKII) and lactate dehydrogenase (LDH)[23,24]. Therefore, many of these key molecules that are critical for maintaining cancer metabolism may be considered as potential targets for metabolic intervention in cancer treatment. The following sections provide an overview of several key metabolic alterations in cancers, their potential links to oncogenes and tumor suppressors, and the biochemical and molecular basis for targeting altered metabolism in cancers.

Metabolic Alterations in Cancers

Glycolysis and the Warburg effect

Warburg observed in early 1920s that tumor cells exhibited significant alterations in energy metabolism and mitochondrial respiration compared to normal cells[2,25]. He showed that cancer cells actively used glycolysis for ATP generation, even in the presence of an abundant supply of oxygen, a phenomenon known as the Warburg effect[1,25]. Warburg further postulated that the metabolic shift from OXPHOS to glycolysis in neoplastic cells might be due to a respiratory injury (mitochondrial dysfunction) leading to increased aerobic fermentation, a critical event that was considered as the "origin of cancer cells"[1]. Although whether metabolic alterations drive tumorigenesis or are an effect of transformation is still under debate, subsequent studies showed that increased dependence on glycolysis is observed in the majority of tumors and that glycolysis provides ATP as well as the metabolic intermediates essential for cancer cell proliferation and tumor development[8,26,27].

Aerobic conversion of glucose to lactate represents a major feature of cancer cell metabolism (Figure 1). The high flux of glycolysis results in an increased output of pyruvate, which may either be converted to lactate by LDH in the cytosol or to acetyl-CoA by pyruvate dehydrogenase (PDH) in the mitochondria. Acetyl-CoA is further metabolized through the Kreb's cycle and the MRC to generate ATP. The tumor hypoxic environment and/or oncogenes such as *Ras*, *Src*, and *HER2* stabilize *HIF-1α*, leading to up-regulation of pyruvate dehydrogenase kinase-1 (*PDK-1*), in turn inactivating *PDH* and thereby preventing the conversion of pyruvate to acetyl-CoA. Pyruvate is subsequently converted to lactate by *LDH* with a simultaneous oxidation of nicotinamide adenine dinucleotide (*NADH*) to NAD^+, which is important for the glycolytic reaction at the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). *LDH* is up-regulated not only by *HIF-1α* but also by other oncogenes such as *c-Myc*, thus ensuring sufficient NAD^+ is available for glycolysis in cancer cells.

The high rate of aerobic glycolysis confers several advantages to cancer cells. In the hypoxic microenvironment of tumor tissues, active glycolysis provides sufficient ATP for tumor cells when mitochondrial OXPHOS is limited. This seems particularly important for actively proliferating cancer cells[1,28]. Secondly, the metabolic intermediates generated during glycolysis provide important building blocks or precursors for the synthesis of DNA and fatty acids and for redox regulation[29,30]. For instance, glucose-6-phosphate, the product of the first glycolytic reaction, can be channeled to the pentose phosphate pathway (PPP) to generate ribose-5-phosphate and nicotinamide adenine dinucleotide phosphate (NADPH), which are important for nucleic acid metabolism and redox homeostasis, respectively. NADPH and 3-carbon metabolic intermediates are also important for lipid biosynthesis. Finally, the high levels of lactic acid produced during glycolysis may activate metalloproteinases and matrix remodeling enzymes, thus contributing to cancer invasion and metastasis[29,31]. It should be noted, however, although most cancer cells are highly active in glycolysis, an increase in mitochondrial OXPHOS has also been observed in some tumors.

Glutamine in cancer metabolism

Another important metabolic substrate and energy source for tumor cells is glutamine[32]. This amino acid is highly abundant in blood, and once it enters the cells, it may serve as an energy source through catabolism or as a building block via anabolism (Figure 1). In addition to its direct involvement in metabolism, glutamine seems able to affect amino acid transport and autophagy through activation of mTOR complex 1 (mTORC1)[33]. Conversion of glutamine to glutamate and its channeling into the tricarboxylic acid (TCA) cycle via α-ketoglutarate seem essential for the oncogenic *K-Ras*–induced tumor growth in colon cancer cells[5]. Studies using tumor xenograft models have shown that the expression of glutaminase, an enzyme that converts glutamine to

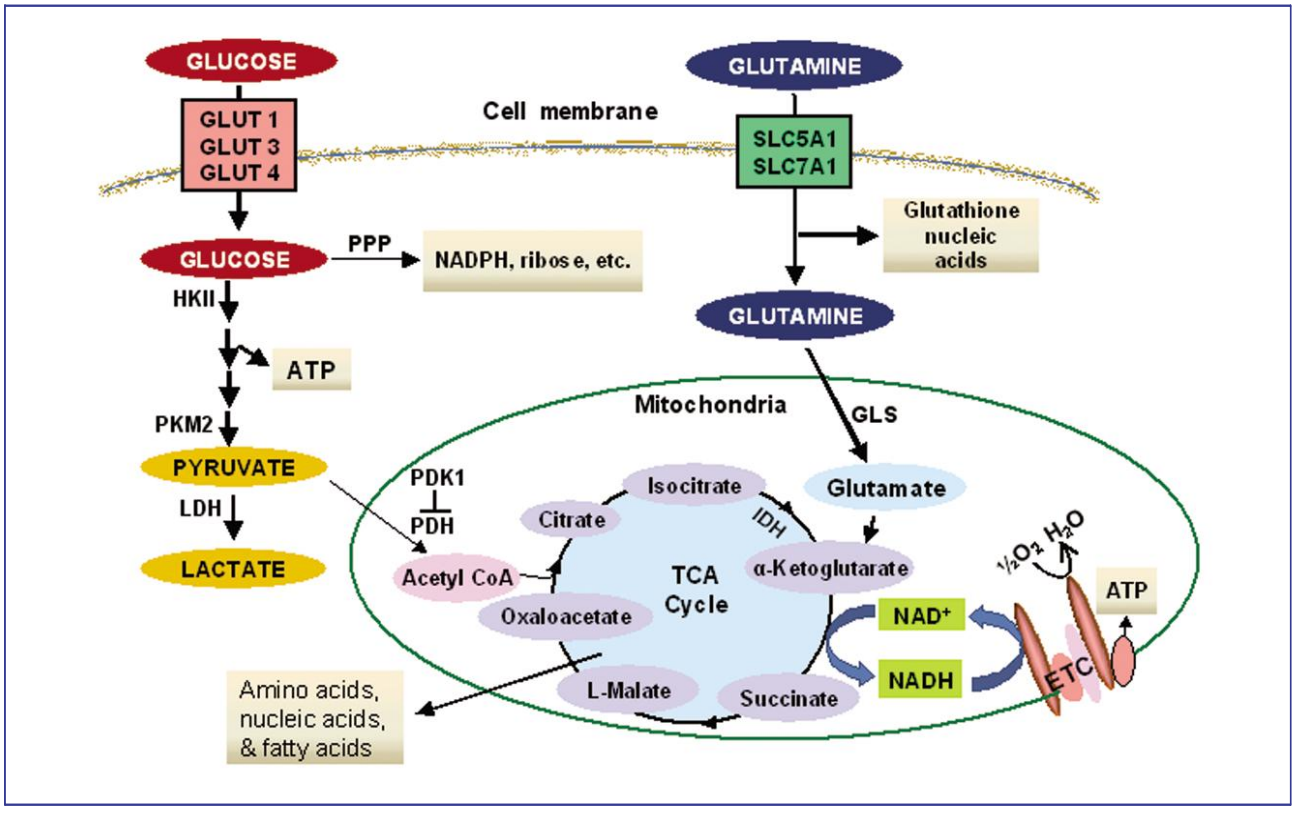

Figure 1. **Glucose and glutamine metabolism in cancer cells.** Glucose and glutamine are transported through cell membranes by their respective transporters (GLUT-1, -3 and -4 for glucose; SLC5A1 and SLC7A1 for glutamine). Glucose is metabolized in the cytosol to pyruvate, which can be either converted to lactate or transported into the mitochondria for further catabolism through the tricarboxylic acid (TCA) cycle coupled with respiration through the electron transport chain (ETC). In many cancer cells, glucose is mainly used for the glycolytic pathway, leading to a generation of lactate and important metablic intermediates such as glucose-6-phosphate for the pentose phosphate pathway (PPP) that generates NADPH and ribose for maintaining redox balance and synthesis of nucleic acids. The flow of glucose into mitochondria in the form of pyruvate is relatively low in cancer cells. Glutamine is actively metabolized in cancer cells, both in the cytosol and in the mitochondria, where it is catalyzed by glutaminase to generate glutamate, which is further converted to α-ketoglutarate for utilization through the TCA cycle. Both glycolysis and the TCA cycle provide important metabolic intermediates that serve as substrates for other pathways including the synthesis of nucleic acids, fatty acids, amino acids, and glutathione. The highly active pathways in cancer cells are indicated with bold arrows, whereas the less active metabolic flows are shown with thin arrows. Some of the important enzymes involved in cancer metabolism are indicated. HKII, hexokinase-2; PKM2, pyruvate kinase M2 isoform; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PDK-1, pyruvate dehydrogenase kinase-1; GLS, glutaminase; IDH, isocitrate dehydrogenase.

glutamate, correlates with tumor growth, and that inhibition of glutaminase in cells led to suppression of tumorigenicity and tumor growth [7,34]. A metabolic study using nuclear megnetic resonance (NMR) spectroscopic analysis in glioblastoma cells cultured with isotope-labeled glucose and glutamine demonstrated that glutamine supplied the majority of aneplerotic carbon for the TCA cycle in cancer cells[35]. The same study also showed that conversion of glutamate to α-ketoglutarate is the chief source of malate, oxaloacetate, and NADPH for fatty acid biosynthesis in tumors[35]. In fact, active glutaminolysis, the process of glutamine catabolism, is considered as a major metabolic feature of certain tumor cells[36,37]. Recent studies have shown that glutamine metabolism is regulated by the oncogene c-*Myc*, which directly stimulates the expression of the glutamine transporters *SLC5A1* and *SLC7A1* and indirectly promotes the expression of glutaminase 1 (GLS1) by repressing the expression of *miR-23A* and *miR-23B*[38,39].

Glutamine also contributes to the maintenance of the antioxidant pool in cancer cells. The glutamine-derived malate from the TCA cycle serves as a substrate for malic enzyme 1 to produce NADPH. In glioblastoma cells, the high level of NADPH has been shown to contribute to the active synthesis of glutathione (GSH), which is essential for combating reactive oxygen species (ROS)-induced stress in cancer cells and maintaining redox balance[35]. Interestingly, glutamine also seems to

play a role in cancer metastasis. Seyfried *et al*.[6] recently showed that inhibition of glutamine metabolism in a mouse breast cancer model suppressed tumor metastasis. Although suppression of the mitochondrial glutaminase activity can inhibit oncogenic transformation[40], the exact roles of glutamine in tumorigenesis remain to be elucidated. The role of glutamine metabolism in affecting tumor growth and cancer progression in patients has not also been definitely established. Further research is required to understand the mechanisms underlying the "glutamine addiction" characteristics of cancer cells.

Alterations of metabolic enzymes in cancers

The mitochondrial membrane-bound HKII has been shown to promote the high glycolytic tumor phenotype and to inhibit Bax-induced cytochrome *c*-mediated apoptosis in HeLa cells[41,42]. The pyruvate kinase isoform M2 (PKM2) seems to confer a tumorigenic advantage to cancer cells by slowing glycolysis and channeling the glycolytic intermediates to the PPP to produce NADPH and other metabolic substrates[43]. Elevated levels of PKM2 have been observed in various tumor samples[44,45], consistent with the potential oncogenic role of this enzyme. The co-factor NADPH not only fuels macromolecular biosynthesis but also functions as a major antioxidant in cells. It maintains the redox balance during cell proliferation and is a reducing agent required for the GSH and thioderoxin antioxidant systems that play a major role in quenching ROS[46].

Gain-of-function mutations in isocitrate dehydrogenase I and II (*IDH1* and *IDH2*) have been identified in several types of human cancers, especially in certain gliomas and acute myelogenous leukemia[47-49]. While the wild-type *IDH1* and *IDH2* catalyze the conversion of isocitrate to α-ketoglutarate, mutated IDH (*IDH1R132*, *IDH2R172*, *R140*) produces 2-hydroxyglutarate (2-HG), a metabolite that seems to have oncogenic properties due to its ability to affect DNA methylation[50-52]. 2-HG, which is present at low concentrations in normal cells, may reach abnormally high concentrations in glioma samples and the serum of AML patients[49,51]. It has been reported to decrease OXPHOS, thus potentiating the cellular glycolysis capacity. IDH1 overexpression has been observed in over 95% of advanced gliomas, suggesting that it might have prognostic value in gliomas[53]. Nevertheless, further study is needed to understand why IDH mutations have preferential distribution in certain cancers, as well as to specifically evaluate the mechanistic role of these mutations in cancer development and test its value as a potential therapeutic target.

Further, deactivating mutations in FH and SDH, first identified in head and neck paragangliomas, have also been reported in hereditary leiomyomatosis, renal cell carcinoma, and certain gastrointestinal tumors[54-56]. The succinate dehydrogenase subunit D (SDHD) protein is one of the four subunits composing the SDH complex (complex II). Mutations in SDHD and FH alter the function of mitochondrial complex II, which affects the electron flow involving flavine adenine dinucleotide (FADH2). These mutations seem to compromise mitochondrial respiratory function and may contribute to the Warburg effect. Loss of FH and SDH activity leads to accumulation of their substrates fumarate and succinate, possibly interfering with the function of the α-ketoglutarate-dependent dioxygenases, including prolyl hydroxylases and HIF-1α, which catalyze a host of biochemical reactions[11,29,57,58].

Metabolic alterations in cancer stem cells

The study of cancer stem cells, a rare subpopulation of malignant cells with long-term self-renewal capacity and high tumorigenic potential, has become an important and intensive research area in recent years. Owing to their special biological properties, cancer stem cells are thought to play key roles in tumor initiation, resistance to chemotherapy and radiotherapy, and disease recurrence[59]. Although extensive information on the biological properties of cancer stem cells, specifically with respect to self-renewal, tumorigenesis, maintenance of stemness, differentiation, and regulatory mechanisms, has been generated during the past decade, our understanding of the metabolic properties of cancer stem cells is rather limited. This is due in part to limited availability of proper cancer stem cell models for metabolic study. The low number of cancer stem cells that can be isolated from primary tumors also limit the scope of metabolic study in this subpopulation of cells.

Notably, certain regulatory pathways known to play important roles in governing cell metabolism and energy sensing have been shown to be involved in maintaining the self-renewal property of cancer stem cells. For instance, the tumor suppressor *PTEN* negatively regulates the AKT pathway, thus affecting cellular uptake and use of glucose. *PTEN* blockage has been shown to increase self-renewal capacity and promote clonogenicity of glioblastoma stem cells[60]. Interestingly, the tumor suppressor *p53* is also involved in metabolic regulation through its effects on mitochondrial respiratory activity[22]. Inhibition of *p53* increased the self-renewal capacity of glioma stem cells, whereas activation of *p53* promoted cell differentiation[61]. The energy censor mTOR has been shown to play an essential role in maintaining the hematopoietic stem cells at undifferentiated stage by down-regulating mitochondrial respiration[62]. The transcription factor HIFs are often up-regulated in cancer cells and have been shown to promote the maintenance

of cancer stem cells in an undifferentiated state [63,64]. Interestingly, glioma stem cells seem to be confined in the tissue niches in the brain where the oxygen concentration is restricted [65]. Such a hypoxic microenvironment would favor the stabilization of HIF-1α.

Aldehyde dehydrogenase 1 (ALDH1) is a metabolic enzyme involved in detoxification of certain toxic compounds and in the metabolism of biomolecules, such as the conversion of vitamin A (retinol) to retinoic acid. ALDH1 is often expressed in cancer stem cells and has been used as a functional marker for isolating cancer stem cells[66]. The retinoic acid signaling is believed to be important in determining the cell fate. ALDH1 has been used as a marker in detection of breast cancer stem cells [67-69] and, more recently, in lung cancer [70]. In hematopoetic stem cells, inhibition of ALDH1 activity leads to an increase in the population [71]. Interestingly, the PI3K/AKT pathway seems to play a major role in maintaining the ALDH1–positive population of breast cancer[72].

In several studies, the metabolic function in cancer stem cells has been directly assessed. Zhou *et al.* [73] reported that the stem-like glioma cells with enriched $CD133^+$ cells showed increased tumorigenic capacity, low mitochondrial respiration, and high glycolytic activity. Furthermore, these metabolic properties seemed to confer cancer stem cells the ability to survive and proliferate in a hypoxic microenvironment and render them highly sensitive to glycolytic inhibition[73]. Consistently, a recent study in human pluripotent stem cells and their differentiated counterparts showed that the stem cells expressed high levels of glycolytic enzymes and relied mostly on glycolysis to meet their energy demands[74]. In hypoxia-adapted Bcr-Abl(+) cells that exhibited stem cell-like characteristics, the expression of glyoxalase-I, an enzyme that detoxifies methylglyoxal (a toxic metabolite of glycolysis) was high, and these cells were much more sensitive to glyoxalase-I inhibitors [75]. These studies together suggest that cancer stem cells may have unique metabolic properties that can serve as potential targets for anticancer therapy. As cancer stem cells are more resistant to conventional therapeutic agents and play a major role in therapeutic failure and cancer recurrence, the development of novel agents that effectively kill cancer stem cells based on their unique metabolic properties will have major therapeutic implications.

Metabolic Alterations and Drug Resistance

Defects in the mitochondrial respiration and mutations in mitochondrial DNA (mtDNA) have been implicated to affect drug sensitivity. Several groups have used respiration-deficient ρ0 cells to investigate the role of mitochondrial respiration in drug resistance. Singh *et al.* [76] showed that the ρ0 cells were resistant to photodynamic therapy and adriamycin but remained sensitive to DNA alkylating agents and γ-radiation. Cai *et al.* [77] reported that although staurosporine treatment induced release of cytochrome *c* and activation of caspases in both ρ0 and ρ+ cells, the redox homeostasis remained unchanged in ρ0 cells upon induction of apoptosis, suggesting that apoptosis and bioenergetics are two separate events. In another study using SK-Hep1 hepatoma cells, deprivation of mtDNA resulted in ρ0 cells with elevated expression of the antioxidant enzymes manganese superoxide dismutase and glutathione peroxidase. These cells were relatively resistant to H_2O_2 and ROS-inducing agents such as doxorubicin, paraquat, and menadione, suggesting a significant role of mitochondrial respiration in drug sensitivity[78]. Furthermore, respiration-defective ρ0 prostate cancer cells were resistant to the cytotoxic effects of the synthetic retinoid 4HPR, implying that OXPHOS may be critical to mediate the effects of 4HPR in transformed human prostate cells[79]. Thus, a defect in mitochondrial respiration function may positively or negatively impact drug sensitivity, depending on the mechanisms of actions of the compounds. Ironically, the mtDNA-depleted HeLa cells (EB8 cells) lost their ability to form colonies in the anchorage-independent colony formation assay and to form tumors in nude mice, although both features were restored upon introduction of normal mtDNA into the cells[80]. It appears that a complete loss of mitochondrial respiration may compromise the cell's ability to survive and proliferate in the *in vivo* tissue environment.

Apart from mtDNA mutations and respiratory dysfunction, ROS and cellular redox homeostasis also play a critical role in determining and modulating cellular sensitivity to drugs. ROS can cause DNA mutations that may contribute to the development of a drug-resistant phenotype. However, an increase in ROS beyond a certain cellular threshold may lead to cell death through various mechanisms, such as activation of the JNK/p38-mediated signaling pathway, direct damage to the mitochondrial membrane, and release of cytochrome *c* [81-84]. Interestingly, malignant cells in advanced stage cancers may develop resistance to ROS stress by up-regulation of their antioxidant capacity. For instance, resistance to arsenic trioxide in leukemia cells has been correlated with high cellular GSH levels and elevated expression of superoxide dismutase 1 [85,86]. Similarly, resistance to paclitaxel and cisplatin has been associated with increased antioxidant levels [87-89]. Thus, abrogation of the cancer cell antioxidant defense mechanism, such as depletion of GSH using pharmacological agents, seems to be an effective strategy to overcome such drug resistance[90,91]. For drug

resistance associated with mitochondrial respiration defects and increased glycolysis, the use of glycolytic inhibitors is an effective strategy[92].

Mechanisms Underlying Metabolic Alterations

After his initial observation of increased glycolysis in cancer cells [2], Warburg later attributed this metabolic alteration to "respiratory injury" and considered this as the main cause of cancer[1]. This theory was immediately challenged by Weinhouse, who contended that mitochondrial respiration was functioning in cancer cells and that active glycolysis and OXPHOS together would result in a generation of excess ATP, thus creating a metabolic imbalance in cancer cells [93]. More recently, Moreno-Sanchez *et al.*[94] suggested that cancer cells are heterogeneous in their energy metabolic profiles, with some primarily using glycolysis whereas others use OXPHOS. Since mitochondria are the powerhouses of the cell and serve as the converging points of multiple metabolic pathways, mitochondrial dysfunction has long been suspected to play a key role in metabolic alterations in cancers. Interestingly, a recent study by Compton *et al.*[95] demonstrated that mitochondrial respiratory state can profoundly affect p53 expression and, thus, might play a role in tumorigenesis. Alterations in cancer cell metabolism have been attributed to dysfunctional mitochondria resulting in part from mtDNA mutations, and metabolic reprogramming may be linked to oncogenes and tumor suppressors that either affect mitochondrial function or regulate important molecules involved in the energetic pathways.

Mitochondria mutations in cancers

A major role of mitochondria is to generate energy in the form of ATP through OXPHOS, which is carried out through the electron transport chain (ETC) associated with the mitochondrial inner membranes [96]. The ETC consists of four respiratory complexes (I–IV), each with multiple protein components or subunits. Electrons are carried through these complexes to molecular oxygen with a simultaneous pumping of protons across the inner membranes to generate an electrochemical gradient, which is then used as the energy source to drive ATP synthesis at complex V. Each ETC complex contains subunits that are encoded by both mtDNA and nuclear DNA, with an exception of complex II, whose subunits are all encoded by nuclear DNA. Mammalian mitochondria contain a genome of 16.5 kb of double-stranded circular DNA encompassing 37 genes, of which 13 are components of respiratory chain complexes [96-98]. The lack of histone protection as well as the close physical proximity of the mitochondrial genome to the source of ROS generation makes mtDNA highly prone to mutations[99].

Accumulating evidence suggests that mtDNA mutations occur at higher frequency in cancer cells than in normal cells, perhaps due in part to the increased ROS generation in the cancer cell mitochondria [100]. Because each cell harbors numerous mitochondria with multiple copies of mtDNA, mutations at mtDNA can be heteroplasmic (variably mutated mtDNA copies co-exist in the same cells) or homoplasmic (all mtDNA copies carry the same mutations). For the heteroplasmic mutations to have a significant effect on the cells, it is estimated that such mutations should be predominant and reach a minimal threshold of 60% of the mitochondrial copies [101,102]. However, due to *in vivo* selection of mutations that confer advantage for cell survival and proliferation, the heteroplasmic mutations with functional consequences may eventually become homoplasmic or eliminated (disadvantage mutations). This may explain why the majority of mutations detected in human cancers are homoplasmic[103,104]. Figure 2 shows a summary of mtDNA mutations in the coding region of respiratory chain components detected in various types of cancer tissues. Mutations can occur in any part of mtDNA, although there may be certain "hot spots" where mutations are more concentrated. Many mutations have also been detected in the non-coding region (D-loop), where the origin of replication and the transcription promoter sequences are located. Mutations of D-loop do not directly affect the structure and function of any particular protein encoded by mtDNA but may affect mtDNA replication and transcription.

Extensive mtDNA sequencing in breast cancer conducted by Tan *et al.*[105] revealed that 74% of patient samples had mutations at 27 different sites, most of which were situated in the D-loop region. Parella *et al.*[106] detected mtDNA mutations in 61% of fine-needle aspirates of breast cancer tissues, with the majority of mutations again located in the D-loop region. Single base substitutions and deletions seem to be the most common mutations found in breast cancer mtDNA. In a colorectal cancer study, Polyak *et al.* [107] reported the occurrence of mutations in colon cancer cell lines as well as the original tumors from which the cells were derived. The mutations occurred in the coding regions including the NADH dehydrogenase 1 (ND1), ND4L, ND5, cytochrome *b*, and 12S and 16S RNA genes. Mutations in the D-loop region in colorectal cancer have also been reported[108]. Another interesting finding was the presence of mutations in the normal colonic crypt stem cells, suggesting that mutations may occur before the development of colon cancer and contribute to carcinogenesis, although this functional role has not been experimentally demonstrated[109]. In gastric cancer, the D-loop region harbored mutations in 48% of the carcinoma samples sequen ced by Wu *et al.* [110].

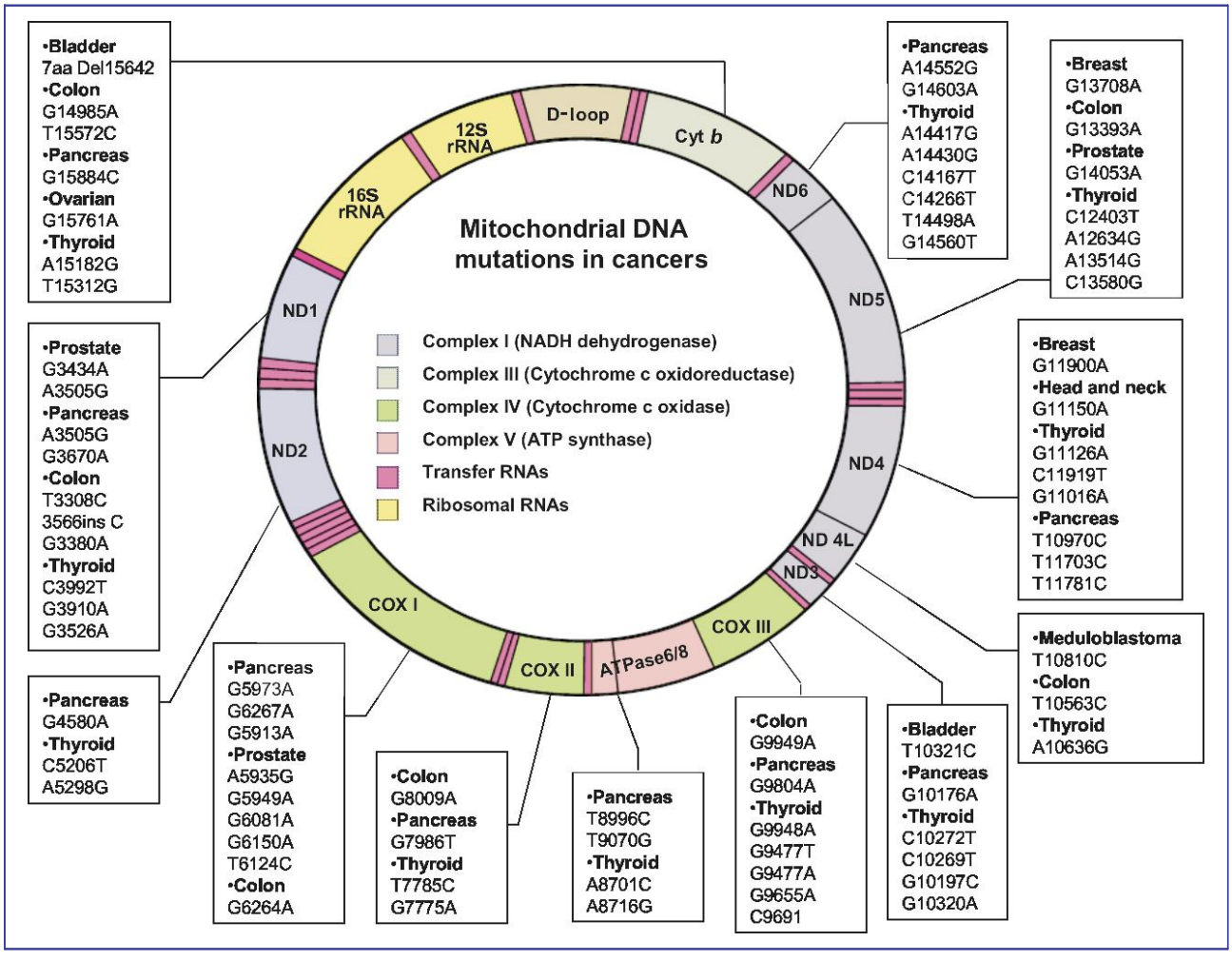

Figure 2. **Human mitochondrial DNA (mtDNA) map and mutations in cancers related to metabolism.** The human mitochondrial genome is a 16.5 kb double-stranded circular DNA, which contains 37 genes: 13 polypeptides that are components of the electron transport complexes (ETC), 22 tRNAs, and 2 ribosomal RNAs. Mutations at the 13 ETC-encoding genes may cause alterations of the respiratory chain activity and thus affect metabolism. Each box linked to a particular mitochondrial gene region contains a partial list of mutations that have been identified in that specific mtDNA in human cancers, with the specific cancer types indicated. The numbers represent the locations of the mutated bases (C, cytosine; G, guanine; T, thymine; A, Adenine). Note that the two adjacent genes for ATPase6 and ATPase8 are shown in a single box.

Additionally, mtDNA mutations have been detected in ovarian[111], hepatocellular[112,113], pancreatic[114], prostate[115], thyroid[116], brain[117], and lung cancers[118].

Role of oncogenes and tumor suppressor genes in alterations of cancer cell metabolism

Emerging evidence suggests that metabolic deregulations play a crucial role in supporting cancer cell survival, proliferation, and tumor growth and that certain oncogenes may orchestrate the activation of enzymes that are important for the glycolytic pathway and thus contribute to the Warburg effect. On the other hand, certain metabolic pathways may lead to production of metabolites that promote silencing of tumor suppressors and activation of certain oncogenes.

Akt The serine/threonine kinase AKT, a downstream effector of the insulin signaling, plays a major role in cell survival and proliferation and is an important promoter of glysolysis [119]. In the majority of tumor cells, Akt is hyperactive and seems to drive addiction to glucose metabolism for survival and proliferation [13]. The Akt glycolytic enhancer role is driven by stimulation of glucose uptake in response to insulin[13]. Akt up-regulates GLUT1 by enhancing its expression at transcription level. Moreover the AKT signaling enhances glucose metabolism within the cell by stimulating the association of HKII with the mitochondrial outer membrane, thus positioning it to phosphorylate glucose to

glucose-6-phosphate for use in glycolysis and other metabolic pathways [120]. Akt's ability to promote metabolism via glycolysis is also partly due to induced expression of glycolytic enzymes through the mTOR and HIF-1 signaling[16,121].

mTOR mTOR is an energy censor that promotes nutrient uptake and glycolysis. Deregulation of mTOR pathway is often found in cancer cells[17]. Under nutrient deprivation, mTOR forms a stable complex with raptor, leading to an inhibition of mTOR kinase activity[122]. Akt regulates mTOR through the inactivation of the protein tuberous sclerosis-2 (TSC2). TSC2 and TSC1 associate and form a complex that suppresses mTOR activity[123]. Activated AKT phosphorylates TSC2, thus affecting its interaction with the small G protein Rheb, which when loaded with GTP, activates mTORC1[122,124]. The activation of mTORC1 increases glycolysis by increasing the translation of the mRNA encoding HIF-1α, thus stimulating the expression of glycolytic enzymes [125]. Importantly, mTOR is regulated by liver kinase B1 (LKB1) and AMPK [126]. The mTOR energy sensor function is conferred in part by AMPK, a heterotrimeric serine/threonine kinase consisting of a catalytic subumit (α) and two regulatory subunits (β and γ)[127]. Nutrient depravation causes cellular stress, leading to an increase of the AMP/ATP ratio, which promotes the binding of AMP to the γ-regulatory subunit and triggers AMPK activation [128]. AMPK activation in turn induces TSC2 phosphorylation, leading to suppression of mTOR activity and inhibition of protein translation. The serine/threonine kinase LKB1 activates AMPK under low ATP conditions[129]. Therefore, the LKB1/AMPK/mTOR axis serves as an important energy sensor that links energy status and the metabolic signaling pathways[130].

HIF-1 Hypoxia inducible factor-1, a heterodimer protein complex that consists of HIF-1α and HIF-1β, has been implicated in the induction of key genes involved in cell proliferation, oxygen and nutrient delivery, and anaerobic energy metabolism [131]. HIF-1 regulation is achieved by protein degradation of the HIF-1α subunit in response to oxygen levels in the microenvironment. Oxygen promotes prolyl hydroxylation, which stimulates the association of HIF-1α with the von Hippel-Linau (VHL) tumor suppressor, thereby targeting HIF-1α for ubiquitination and proteosomal degradation. Hypoxia suppresses prolyl hydroxylation through a process involving mitochondria-generated ROS [132,133]. As such, HIF-1 is considered as the master sensor that orchestrates cellular responses to changes in oxygen homeostasis. In fact, HIF-1 has been found to be highly expressed within hypoxic tumors and at low levels within normal tissue[134].

HIF-1 up-regulates 9 of the 10 genes involved in glycolysis and, thus, plays a key role in switching glucose metabolism from OXPHOS to glycolysis when cells are in a hypoxic environment [134,135]. For instance, HIF promotes glucose uptake by up-regulating the expression of the GLUT1 and glucose metabolism by up-regulating hexokinase, the enzyme responsible for catalyzing the first glycolytic reaction. HIF-1 also promotes the expression of LDH-A at the transcription level[136,137]. Furthermore, HIF-1 enhances glycolysis by preventing the use of pyruvate by the mitochondria through the up-regulation of PDK1, which phosphorylates PDH1 and inhibits its ability to convert pyruvate to acetyl-CoA as the fuel for the TCA cycle[138].

In addition to response to environmental oxygen fluctuation, HIF-1 can also be regulated by molecules such as FH and SDH. Loss of function mutations of FH and SH lead to the accumulation of the TCA cycle intermediates furmate and succinate, resulting in inhibition of the α-ketoglutarate–dependent prolyl hydroxylase, thereby stabilizing HIF-1[139].

c-Myc *c-Myc* is an oncogene that affects many cellular functions including energy metabolism [20]. *Myc* overexpression is estimated to be associated with at least 40% of all cancers [140]. Similar to HIF-1, *c-Myc* promotes glycolysis mainly through up-regulation of glycolytic molecules including GLUT1, HKII, phosphofructokinase (PFK), enolase, and LDH [21]. It is important to note that *c-Myc* also plays a major role in promoting glutamine use in cancer cells through up-regulating the expression of glutamine transporters *SLC5A1* and *SLC7A1* and enhancing the expression of GLS1 by repressing the expression of *miR-23A* and *miR-23B*, thus releasing the suppressive effect of these miRNAs on GLS1 expression[38,39].

p53 The tumor suppressor *p53* is a stress sensor and cell cycle checkpoint regulator in mammalian cells that plays essential roles in cell cycle regulation, apoptosis, and genome stability[141]. The role of *p53* in energy metabolism was recognized by Matoba *et al.*[22], who found that loss of *p53* diminishes the synthesis of cytochrome *c* oxidase (*SCO2*), a nuclear DNA-encoding gene whose product is necessary for the assembly of mitochondrial respiratory complex IV. Thus, this study directly links *p53* to OXPHOS. The loss of *p53* shifted the cellular ATP production from mitochondrial respiration to glycolysis. Moreover, *p*53 protein represses the expression of the GLUT1 and GLUT4 transporters. Thus, the loss of *p53* enhances the expression of glucose transporters and further promotes glycolysis. Interestingly, Benssad *et al.*[21] identified a *p53* target gene known as TP53-induced glycolysis and apoptosis regulator (*TIGAR*), which is a potent glycolysis regulator. Expression of *TIGAR* decreases the intracellular level of fructose-2,6-bis-phosphate, which otherwise suppresses glycolysis by shunting glucose to the PPP[142]. In addition,

p53 also affects energy metabolism by regulating AMPK, mTOR, PTEN, and IGF-binding protein-3[143].

Ras *Ras* mutations are found in approximately 30% of all human cancers[144] and are important in promoting cancer initiation and progression[145]. *K-Ras*, the most commonly mutated oncogenic *Ras* in pancreatic cancer, has been shown to affect the shape and function of the mitochondria during fibroblast transformation[146]. Further studies showed that fibroblasts transformed by activated *K-Ras* attenuated OXPHOS by suppressing the activity of respiratory complex I, with a corresponding decrease in the expression level of complex I proteins[147]. Similarly, *H-Ras* transformed mouse fibroblasts exhibited low mitochondrial respiration, an increased dependency on glycolysis, a sensitivity to glycolytic inhibitors, and an insensitivity to OXPHOS inhibitors[148]. However, a recent study showed that *K-Ras* may affect the synthesis of the mitochondrial phospholipid cardiolipin and that the absence of *K-Ras* led to increased respiration[149], suggesting that the effect of *K-Ras* on mitochondrial respiration is likely complex and requires further study.

Therap eutic approaches targeting cancer cell metabolism

Although metabolic alterations in cancer cells are highly complex and the detailed underlying mechanisms remain to be elucidated, the unique metabolic profiles in cancer cells may serve as a biochemical basis for developing new therapeutic strategies to effectively kill the malignant cells with high selectivity. For cancer cells that are highly dependent on glycolysis, inhibiting glycolysis seems to be a logical therapeutic strategy. Similarly, interference with glutamine metabolism may significantly impact cancer cells that depend on glutamine for survival and proliferation. Due to the metabolic adaptability of cancer cells, it may be necessary to combine agents that target different metabolic pathways to achieve high therapeutic activity. The following sections describe several therapeutic strategies that target various metabolic pathways important for cancer cells. The representative compounds are listed in Table 1.

Inhibition of the glycolytic pathway

HKII has been shown to be elevated in cancers and possibly up-regulated by HIF-1[150-152]. 2-Deoxyglucose (2-DG), a non-metabolically active glucose analog that inhibits HKII and represses tumor growth, is currently in clinical trials for cancer treatment[153,154]. 2-DG preferentially kills cancer cell exhibiting mitochondrial defects or under hypoxia[155-157]. Combination of 2-DG with cisplatin has

Table 1. Therapeutic strategies to target cancer metabolism and relevant agents

Therapeutic approach	Metabolic target	Agent	References
Inhibition of glycolysis	HK	2-Deoxyglucose, 3-bromopyruvate Lonidamine	[153–158, 217, 218]
	HK-VDAC complex	Methyl jasmonate	[120, 160–162]
	LDH & lactate transport	Oxamate, shRNAs, α-cyano-4-hydroxy cinnamic acid	[3, 171, 173, 174]
	PDK	Dichloroacetate	[170, 175, 176]
	Glucose transporter	Phloretin	[177]
	Phosphofructokinase	PFKFB3-3(3-pyridinyl)-1-(4-pyridinyl)-2-p, 3PO, Clotrimazole	[178, 179, 219]
	Pyruvate Kinase	CAP-232/TLN-232	[217]
Interfering glutamine metabolism	Glutamine (analogs)	6-Diazo-5-oxo-L-norleucine, azaserine, acivicin	[182–184, 191]
	Glutamine	L-Asparaginase, phenylbutyrate,	[181, 185, 186]
	Glutamine transport	L-γ-glutamyl-p-nitroanilide (GPNA),	[33, 192]
	Glutaminase	Compound 968, 6-diazo-5oxo-norleucine	[40, 193]
	Transaminase	Amino-oxyacetic acid	[33, 195, 196]
Targeting energy sensors & regulators	AMPK	Metformin thiazolidinediones, AICAR	[199–202, 220]
	HDAC	Romidepsin, SAHA	[205, 206]
	HIF-1	Romidepsin,	[206]
	AKT	NSC 644221,	[189]
	PI3K	RX-0047	[190]
	mTOR	Genistein, celecoxib, perifosine, GST-anti-Akt1-MTS	[207, 208]
		LY294002, wortmannin	[209]
		Rapamycin, Temsirolimus (CCI-779), Everolimus (RAD-001)	[211–213]

HK, hexokinase; VDAC, voltage-dependent anion channel; LDH, lactate dehydrogenase; PDK, pyruvate dehydrogenase kinase; AMPK, AMP-activated protein kinase; HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; HIF-1: hypoxia-inducible factor-1; mTOR: mammalian target of rapamycin.

been shown to increase cytotoxicit y through oxidative stress in human head and neck cancer cells [153]. Increased cytotoxic effects were also observed when 2-DG was combined with adriamycin and paclitaxel in mice bearing human osteosarcoma and in the MV522 non–small cell lung cancer xenograft model[158].

3-Bromopyruvate (3-BrPA), another inhibitor of HKII, has been shown to be effective in killing lymphoma and colon cancer cells as well as other cancer cells with mitochondrial defects [92]. Consistent with its ability to inhibit glycolysis, 3-BrPA is particularly effective against cancer cells under hypoxia, a condition where cells become highly dependent on glycolysis. Interestingly, Chen *et al*. [159] reported that 3-BrPA triggered leukemia cell death by disrupting the association between HKII and apoptosis-inducing factor in the mitochondria. Similarly, the plant-derived methyl jasmonate or the HKII amino terminus-derived peptide TAT-HK can also disrupt the association between hexokinase and the voltage-dependent anion channel (VDAC), a phenomenon observed more in cancer cells compared to normal counterparts [120,160,161]. In a recent study, methyl jasmonate was found to activate the PI3K/Akt pathway in sarcoma cell lines, whereas its combination with 2-deoxy-D- glucose to inhibit glycolysis resulted in a synergistic cytotoxic effect[162].

Lonidamine, a derivative of indazole-3-carboxylic acid, was shown to inhibit tumor growth through inhibition of HKII, depletion of ATP, reduction of oxygen consumption, and lactate production [163-165]. Combination of lonidamine with chemotherapeutic drugs such as doxorubicin and cisplatin have been tested in clinical trials in breast cancer, ovarian cancer, glioblastoma, and non–small cell lung cancer [166-169]. In addition to its applications in cancer treatment, lonidamine was developed by Threshold Pharmaceuticals as TH-070 to treat benign prostatic hyperplasia (BPH). Unfortunately, clinical trials of TH-070 in patients with BPH did not show a significant therapeutic effect, and the development of TH-070 was discontinued in 2006. It should be noted, however, that the ability of lonidamine to inhibit glycolysis and its potent effect against cancer cells suggest that this compound may still have potential value in cancer treatment, especially for the highly glycolytic cancer types.

A logical approach to target glycolysis is to inhibit LDH, which is an NADH-dependent enzyme that converts pyruvate to lactate and has been shown to be elevated in certain cancer cells[3,170]. Inhibition of LDH has been demonstrated by use of oxamate, resulting in reduced glycolytic rate, decreased glucose uptake, and suppressed growth of HeLa cells[171]. Combination of LDH inhibition with doxorubicin resulted in enhanced ATP depletion and overall cyto toxicity [172]. Additionally, α-cyano-4-hyroxycinnamic acid is an agent that inhibits lactate transport and, thus, can affect lactate metabolism and potentially interfere with glycolysis in cancer cells [173,174].

Another strategy for inhibiting the glycolytic process is targeting PDK. PDK phosphorylates PDH, resulting in inhibition of PDH activity[170]. Dichloroacetate inhibits PDK and indirectly increases production of acetyl-CoA, which can then enter the TCA cycle, thereby switching cellular energy metabolism from glycolysis to mitochondrial OXPHOS. This compound has been previously used in treating lactic acidosis and seems to be well tolerated in young children [175]. Bonnet *et al*. [176] showed that dichloroacetate reduced tumor cell proliferation and increased apoptosis by activating the expression of the K^+ (Kv1.5) channel through nuclear factor of activated T cells and by reducing the mitochondrial membrane potential in tumor cells . The therapeutic activity of dichloroacetate for cancer remains to be evaluated in clinical trials.

Additionally, as glycolysis is dependent on glucose uptake, inhibiting glucose transporters is an alternate method to repress active glycolysis often observed in cancer cells. Phloretin is a glucose transporter inhibitor reported to induce apoptosis and overcome drug resistance in hypoxic conditions[177]. Moreover, since PFK is an important regulatory enzyme in glycolysis, it is considered a high-impact target for antitumor drugs [178]. PFKFB3-3(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one(3PO), a small-molecule inhibitor, was shown to inhibit PFK activity, reduce glucose uptake, and suppress tumor growth in several human malignant hematopoietic and adenocarcinoma cell lines[179].

Although many cancer cells are highly dependent on the glycolytic pathway, certain tumor cells, including those of the lung, mammary glands, skin, and cervix, are active in OXPHOS [94]. Therefore, it must be noted that the metabolic profiles of tumor cells are dependent on the cancer type, microenvironment, differentiation, and cancer stage. Further, inhibiting glycolysis may not be a universal strategy to target cancer metabolism.

Inhibition of glutamine metabolism

The dependency of certain cancer cells on exogenous glutamine for survival has been referred to as "glutamine addiction", which renders cancer cells highly sensitive to glutamine starvation[180,181]. Early studies have shown that glutamine analogues, such as 6-diazo-5-oxo-L-norleucine (L-DON), azaserine, and acivicin, exhibited potential anticancer activity, but the efforts to develop these compounds as therapeutic drugs were hampered due in part to potential neurotoxicity, gastrointestinal

toxicity, and myelosuppression [182-184]. Interestingly, a recent study showed that inhibition of glutamine metabol ism using L-DON suppressed cancer metastasis in a mouse model of breast cancer[6].

Another approach to inhibit glutamine metabolism is to lower the glutamine levels in blood using agents such as L-asparaginase and phenylbutyrate. L-asparaginase, an enzyme that hydrolyzes asparagine to aspartic acid, has been used successfully in treating pediatric acute lymphoblastic leukemia[185], as these particular leukemia cells are unable to synthesize asparagines. L-asparaginase can also hydrolyze glutamine to glutamic acid and ammonia, thereby depleting glutamine in the blood [181,186]. Phenylbutyrate, also known as Buphenyl (Ucyclyd Pharma) or Ammonaps (Swedish Ophan International), is an FDA-approved agent for the treatment of hyperammonemia. This compound is metabolized in the human body to phenylacetate, which is then conjugated with glutamine to form phenylacetylglutamine for excretion by the kidneys. As such, phenylbutyrate is able to deplete plasma glutamine levels. Phenylacetate has also been used in patients with various cancers and other medical conditions[187-189].

Glutamine transporters such as SLC1A5 (ASCT2) and SLC1A7 are up-regulated in cancers, making them attractive targets for the suppression of glutamine uptake[38,39,190]. Recent studies also suggest that glutamine levels affect activation of mTORC [191]. IL-γ-glutamyl-p-nitroanilide (GPNA) is a SLC1A5 inhibitor shown to inhibit glutamine uptake and attenuate glutamine-dependant mTOR activation, leading to induction of autophagy in cancer cells[33,192].

Cancer cells display increased activity in glutaminase, an enzyme responsible for hydrolyzing glutamine to glutamate and ammonia. This enzyme seems to play an important role in energy metabolism and survival of certain cancer cells, thus providing a therapeutic window through its inhibition[193]. Human B-cell lymphoma and prostate cancer cells show increased glutaminase expression, and the oncogene *c-Myc* plays a major role in promoting glutaminase expression through down-regulation of *miR-23* [39]. Similarly, breast cancer cell line MDA-MB231 showed a higher expression of glutaminase when compared to normal mammary epithelial cells[40]. A small molecule known as compound 968 has been shown to inhibit glutaminase and suppress oncogenic transformation [40]. Conversion of glutamine to α-ketoglutarate as a metabolic intermediate for the TCA cycle was demonstrated as essential for activating various pathways involving *K-Ras* that promote tumorgenesis. As the main route through which glutamine enters the TCA cycle is transamination, the transaminase inhibitor amino-oxyacetic acid (AOA) has been suggested as potential therapeutic agent for cancer [194]. AOA exerts a cytotoxic effect on glutamine-dependent Myc-amplified glioblastoma cells and inhibits breast cancer cell growth in a mouse xenograft model [33,195]. This compound has also been shown to sensitize melanoma cells to TRAIL-mediated killing by interfering with glutamine metabolism[196].

Targeting the energy sensors and regulators

AMPK activators & HDAC inhibitors AMPK, an evolutionarily conserved enzyme that plays a pivotal role in maintaining cellular energy homeostasis[197], is activated by an elevated ratio of AMP/ATP under various stress conditions, such as hypoxia, glucose deprivation, and oxidative stress. As described earlier, activated AMPK has an effect on various molecules that are involved in cancer-related cellular processes. A recent study examined the role of AMPK in breast cancer progression and showed that reduced expression of AMPK in breast cancer specimens was inversely correlated with axillary node metastasis and histological grade, suggesting an important role of AMPK signaling in cancer progression and metastasis [198]. These observations also imply that activation of AMPK might have therapeutic potential in cancer treatment. Biguanides and thiazolidinediones, two classes of compounds used in treating diabetes, can activate AMPK. A clinical study that involved treating diabetic breast cancer patients with or without the AMPK activator metformin (biguadine) showed that patients in the metformin treatment arm exhibited complete pathologic responses [199]. The antitumor effect of troglitazone (thiazolidinedione) has been demonstrated in various cancer cells involving activation of PPAR-γ[200,201]. Similarly, 5-aminoimidazole-4-carboxamide ribonucleoside reversed the sensitivity of Akt-expressing glioblastoma cells to glucose deprivation by activation of AMPK [202]. Another metabolic therapeutic approach involves the use of histone deacetylase (HDAC) inhibitors. AMPK activation promotes the phosphorylation of mitochondrial acetyl-CoA carboxylase, leading to fatty acid and cholesterol synthesis, while acetyl-CoA is necessary for histone acetylation [203]. Therefore, epigenetic regulation can be modulated by metabolic factors with therapeutic potential in cancer[204]. The HDAC inhibitor suberoylanilide hydroxamic acid was found to induce oxidative stress, autophagy, and apoptosis in imatinib-resistant chronic myelogenous leukemia [205]. Romidepsin, another HDAC inhibitor, is in clinical phase II trials for the treatment of refractory myeloma and has been shown to inhibit the HIF-1 pathway[206].

Inhibition of PI3K/AKT/mTOR axis The PI3K/AKT/mTOR signaling pathway plays a key role in energy metabolism and regulates cell growth and proliferation in a variety of tumor cells, thereby serving as a critical target for cancer therapy. Genistein, celecoxib, and

perifosine are pharmacologic agents that target *Akt* and have been tested for treating prostate cancer[207]. A cell permeable *Akt* antibody that blocks the catalytic site of AKT is effective in triggering cell death in various cancer cell lines[208]. The PI3K inhibitors LY294002 and wortmannin and other derivatives have been investigated in various types of cancer [209]. The mTOR pathway can activate the transcription factor HIF-1 and enhance angiogenesis and glycolysis while down-regulating OXPHOS, rendering tumor cells more glycolytic and aggressive[210]. Rapamycin has been shown to have an anticancer effect, and other mTOR inhibitors such as temsirolimus (CCI-779) and everolimus (RAD-001) are currently in clinical trials for cancer treatment [211-213]. Inhibition of mTOR leads to profound change in cellular metabolism and attenuation of protein synthesis.

Targeting other regulators of cancer metabolism

The *Myc* oncogene contributes to cancer development by encoding a transcription factor c-Myc, which plays a key role in cellular metabolism to carcinogenesis[20]. *Myc* affects metabolism through its ability to regulate various genes involved in glucose and glutamine metabolism, including GLUT1, HKII, PFKM, enolase, LDH-A, and glutaminase. Similarly, HIF-1 also plays a major role in sensing changes in microenvironment and promotes glycolysis through regulation of glycolytic enzymes. Various strategies are currently being explored to target these important regulatory molecules and their downstream effectors for potential use in cancer treatment[214-220].

Concluding Remarks

An increase in aerobic glycolysis, first reported by Otto Warburg more than 80 years ago, represents the most prominent metabolic alteration observed in cancer cells. Other important metabolic changes, such as glutamine addiction and active OXPHOS in certain tumor cells, were subsequently discovered. The differences between cancer cells and normal cells in their energy metabolism provide a biochemical basis for developing new therapeutic strategies to preferentially kill cancer cells with selectivity using metabolism-targeted compounds. Inhibiting glycolysis and interfering glutamine metabolism are two possible metabolic intervention approaches. Additionally, emerging metabolic profiling of cancer stem cells will also provide new therapeutic opportunities. Mitochondrial dysfunctions, activation of oncogenes, loss of tumor suppressors, and tumor tissue microenvironment conditions are known to profoundly affect the metabolism of cancer cells, rendering heterogeneous metabolic profiles among different cancer types. As such, it is important to determine the specific metabolic alterations in each particular cancer type so that effective cancer type-specific metabolic intervention strategies can be developed. Furthermore, a combination of conventional chemotherapeutic agents and metabolic modulators may enhance therapeutic activity and should be further evaluated. It should be cautioned, however, that as energy metabolism and its regulatory machinery are evolutionarily conserved and shared by various normal cells, metabolic inhibitors are likely to affect normal cells to various degrees. Despite a number of pharmacologic agents that show promising results in preclinical studies, whether the metabolic alterations in cancer cells can be targeted efficiently and safely in the clinic has yet to be determined. Thus, it is vitally important to identify the metabolic steps that are altered and critical in cancer cells but dispensable in normal cells as the valid therapeutic targets, which will enable the development of effective agents to improve cancer treatment outcomes. A comprehensive understanding of the metabolic differences between cancer and normal cells through detail investigation will pave the way to achieve this goal.

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