Review Article

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Cancer Metabolism and Its Therapeutic Implications

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Abstract

The goal of cancer therapy is to selectively kill cancer cells without significant adverse toxicity to normal cells. Tremendous progress has been made to understand the molecular mechanisms of cancer and we have realized that cancer cells have remarkable heterogeneity and adaptability, making them difficult to be targeted therapeutically. With the advanced developments in molecular biology and high throughput 'omics' technologies, metabolic alteration is revealed as the common feature of human cancer. The activation of oncogenes and/or inactivation of tumor suppressors are found to be important metabolic switch regulators. Some metabolic enzymes in cancer have gained non-enzymatic functions to facilitate tumor growth and adaptation. Metabolic reprogramming of cancer cells not only promotes survival and proliferation but also tolerates stress and induces drug resistance. Therefore, cancer metabolism might be a novel target for cancer therapy. This article reviews current understanding of cancer metabolism with major focus on glucose and glutamine metabolisms and their potential therapeutic implications.

Keywords: Pentose phosphate pathway; Hexokinase II; Reactive oxygen species

Introduction

Unlike normal cells which mainly metabolize glucose by using mitochondrial oxidative phosphorylation to generate ATP, cancer cells exhibit profound metabolic alterations by choosing aerobic glycolysis to convert glucose to lactate regardless of the presence of adequate oxygen [1]. Further studies have identified that cancer cells also have increased glutamine metabolism [2-4]. Such widespread metabolic alterations allow cancer cells to survive in adverse tumor microenvironment and under oxidative stress, to sustain higher proliferation rate through anabolic processes, to gain invasive and metastatic ability, and to resist drug treatment [5,6]. Oncogene activation and tumor suppressor inactivation are hallmarks in human cancer. Recent studies provide a plethora of evidence supporting the mechanistic link between oncogene activation, loss of tumor suppressor function, and the metabolic reprogramming of glucose and glutamine utilization. Metabolic enzymes, by expressing specific isoforms or mutations, regulate genetic and epigenetic events to favor cancer development and inhibit apoptosis. Since cancer cells have these metabolic dependencies that distinguish them from their normal counterparts, targeted inhibition of metabolic dependencies is considered a promising cancer therapeutic strategy.

Targeting Glucose Metabolism in Cancer

As a major fuel, glucose uptake in cancer cells is highly increased. This is due to increased expression of Na+ dependent Glucose Specific Transporters (GLUTs) and various glycolytic enzymes, including isoform specific Hexokinase II (HKII) and Pyruvate Kinase Muscle Isozyme 2 (PKM2). As illustrated in (Figure 1), glucose not only can be metabolized to pyruvate through the canonical glycolytic pathway, it also can branch out, generating ribose 5-phosphate for the synthesis of nucleotides and the reducing equivalent NADPH to combat Reactive Oxygen Species (ROS) induced stress and for the reductive biosynthesis of lipids and fats through the Pentose Phosphate Pathway (PPP) [7,8]. Glucose can also be metabolized to triose sugars, which are then converted to serine or glycerol for the synthesis of amino acids or lipids.

To accommodate rapid growth, cancer cells have diverse requirements. They need to replicate their genome, synthesize amino acids, fatty acids, membrane lipids, and package them into daughter cells. In addition, they need to deal with ROS stress and adverse microenvironments, such as hypoxia and acidic conditions generated by exported lactate from tumor cells. Glucose, as a key nutritional component, can fulfill these diverse cancer cell requirements through rearranging the established glycolytic pathways. There are many review articles published recently describing the pathway in great detail [6,9-12]. Here we highlight several key glycolytic enzymes and their potential roles in cancer therapy.
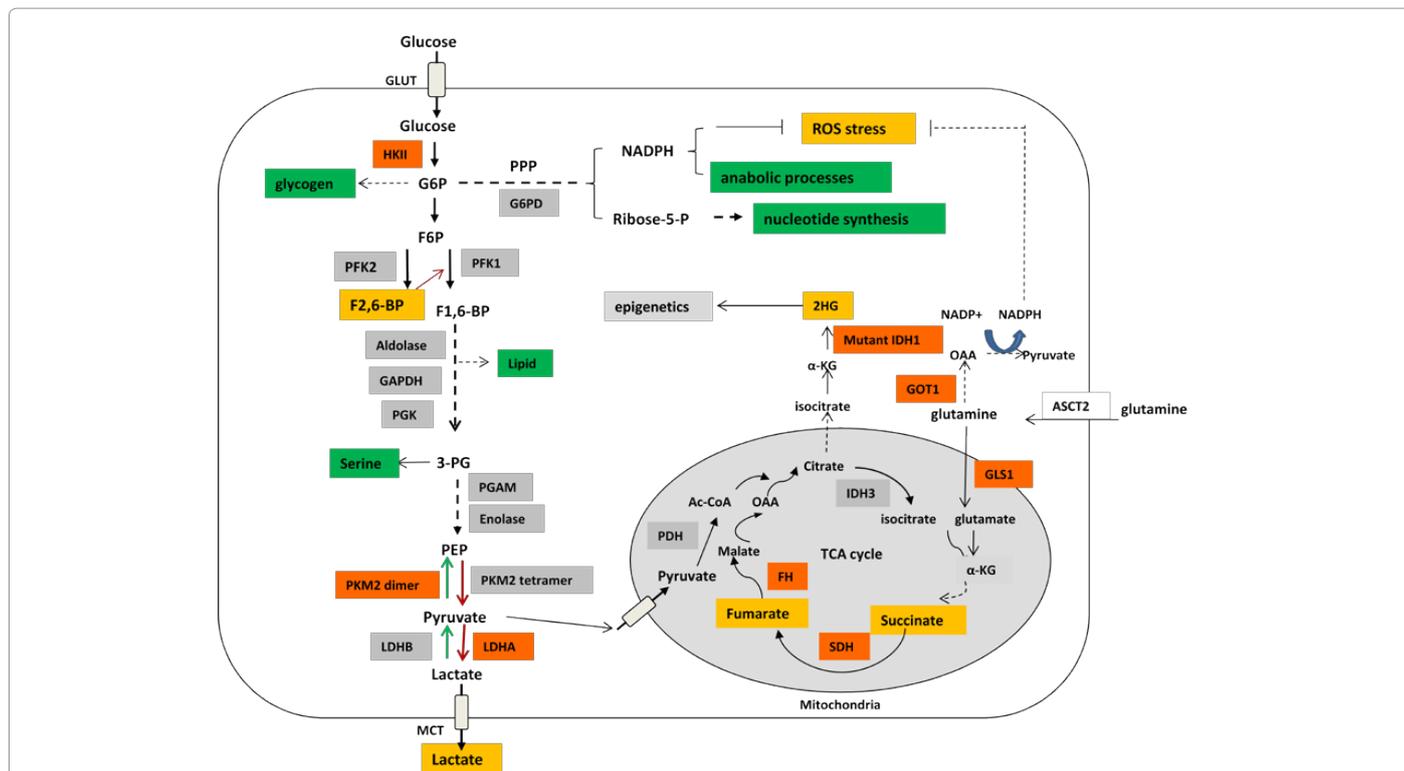
Hexokinase II (KII)

HKII is a key glycolytic enzyme that catalyzes the conversion of glucose to Glucose-6-Phosphate (G6P) and is transcriptionally enhanced in many cancers [13]. G6P generated from this reaction lies at the start point of glycolytic and the PPP pathways, thus having many possible fates. G6P may also be converted to glycogen for storage. Besides catalyzing glucose breakdown, HKII can also bind to mitochondria and interact with the mitochondrial permeability transition pore Voltage-Dependent Anion Channel (VDAC) to serve as a cell survival molecule by preventing BAX/BAK oligomerization on the mitochondrial membrane and thus suppressing apoptosis and preserve mitochondrial integrity [14,15]. HKII is also subject to epigenetic regulation. Adult normal brain with low HKII expression has methylation of CpG islands within intron 1 of HKII. In contrast, developing fetal brain and glioblastoma tissue expressing HKII have significantly lower level of methylation of CpG island [16]. By using conditional knockout of *HKII* in oncogenic Kras-driven lung cancer

Orange: key enzymes important to cancer metabolic reprogramming; yellow: key metabolites that contribute to cancer phenotype; green: anabolic products that provide building blocks in cancer.

Abbreviations: GLUT: Glucose Transporter; HKII: Hexokinase II; PFK1: Phosphofructokinase 1; PFK2: Phosphofructokinase 2; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; PGK: phosphoglycerate Kinase; PGAM: Phosphoglycerate Mutase; PKM2: Pyruvate Kinase Muscle Isozyme 2; LDHA: Lactate Dehydrogenase A; MCT: Monocarboxylate Transporter; G6PD: Glucose-6-Phosphate Dehydrogenase; SDH: Succinate Dehydrogenase; FH: Fumerase; G6P: Glucose-6-Phoaphate; F6P: Fructose-6-Phosphate; Fructose-F1:6-BP: Fructose-1:6-Bisphosphate; Ribose-5-P: Ribose-5-Phosphate; Ac-CoA: Acetyl-CoA; α-KG: α-ketoglutarate; 2HG: 2-Hydroxyglutarate; IDH: Isocitrate Dehydrogenase; ASCT2: Solute Carrier Family 1 (Neutral Amino Acid Transporter): Member 5 (also called SLC1A5); OAA: Oxaloacetate; PDH: Pyruvate Dehydrogenase; GOT1: Aspartate Transaminase 1; ROS: Reactive Oxygen Species; GSH: Glutathione

Figure 1: Overview of glycolysis: the citric acid cycle: and glutamine metabolism in cancer

Cancer cells have profound metabolic alterations in glucose metabolism: the citric acid cycle: and glutamine metabolism. By over expressing glycolytic enzymes: such as isoform specific glycolytic enzymes GLUT1: HKII: PKM2: and LDHA: cancer cells have gained increased glucose uptake: increased lactate generation: increased anabolic processes: and increased ability to combat ROS stress. By expressing mutant citric acid cycle enzymes: such as mutant SDH: FH: and IDH1/2: cancer cells have gained the ability to reprogram metabolism through genetic and epigenetic alterations. By increasing canonical and non-canonical glutamine pathways: cancer cells have increased the utilization of glutamine for the anabolic processes and to adapt to ROS stress.

and ErbB2-driven breast cancer mouse models, HKII is found to be required for tumor initiation and maintenance despite continued HK1 expression. *HKII* deletion suppresses glucose-derived ribonucleotides and impaires glutamine-derived carbon utilization in lung cancer cells [17]. Systemic *HKII* deletion is therapeutic in mice bearing lung tumors without adverse physiological consequences, providing a rationale for the development of small-molecule inhibitors of HKII for cancer therapy. 2-deoxyglucose, the pyruvate analog 3-bromopyruvate, and lonidamine are all HK inhibitors, and have been explored for cancer therapy, either used as monotherapy or combined with other anti-cancer agents [18-21]. Metformin is recently reported to inhibit HK II through an allosteric modification of its molecular structure and blocking the synthesis of G6P in a human non-small cell lung cancer cell line. Metformin treatment causes release of HKII from the outer membrane of mitochondria, thus leading to the activation of apoptotic signals [22]. A study has shown that cerulenin, a fungal metabolite, disrupts the interaction between AIF and HKII, leading to the release of AIF from the mitochondria and eventual cell death [23].

Pyruvate Kinase Muscle Isozyme 2 (PKM2)

Pyruvate kinase, a key glycolytic rate-limiting enzyme, catalyzes the conversion of Phosphoenolpyruvate (PEP) to pyruvate. It has four isozymes (type L, R, M1, and M2). PKM1 and PKM2, resulting from mutually exclusive alternative splicing of the PKM pre-mRNA, have distinct functions in glucose metabolism [24]. By re-expressing the embryonic PKM2, proliferating cancer cells acquire the ability to use glucose for anabolic processes and this metabolic phenotype provides a selective growth advantage for tumor cells, whereas the adult isoform PKM1 promotes oxidative phosphorylation [24-26]. Replacing PKM2 with PKM1 leads to reduced lactate production, increased oxygen consumption, and reduced tumor formation in nude mouse xenografts [24]. Selective expression of PKM2 over PKM1 is due to oncogenic transcription factor c-Myc mediated transcriptional upregulation of splicing factors, such as a polypyrimidine tract binding protein (PTB, known as hnRNPI), hnRNPA1, and hnRNPA2 [27]. PKM2 may exist in a tetrameric or dimeric form. The tetrameric form of PKM2 has a high affinity to its substrate PEP and favors the formation of pyruvate and ATP. The dimeric form of PKM2 has less activity and drives glucose through the route of aerobic glycolysis and facilitates anabolic processes [28]. The dynamic conversion between the tetrameric form and dimeric form of PKM2 may provide an important layer of metabolic regulation and may be exploited for cancer therapy. PKM2, but not

PKM1, can selectively bind to tyrosine-phosphorylated peptides and results in release of the allosteric activator fructose-1, 6-bisphosphate. This induces increased glycolysis and diverts glucose metabolites from energy production through the non-oxidative PPP pathway to anabolic processes [29]. TLN-232 (or CAP-232), an inhibitor of the glycolytic enzyme PKM2, is currently being explored in Phase II clinical trials for metastatic melanoma and renal cell carcinoma [30].

The PKM2 isoform is selected through alternative splicing over PKM1 by cancer cells not only to facilitate shunting of glucose to the anabolic processes as mentioned above, but also to cause chromatin remodeling through its translocation to the nucleus where it serves as a protein kinase. EGFR activation in glioblastomas results in PKM2 phosphorylation and translocation to nucleus, where it phosphorylates Histone 3 at Threonine 11 (H3-T11) [31]. This histone modification results in the dissociation of Histone Deacetylase 3 (HDAC3) from the promoters of cyclin D1 and c-Myc. Nuclear PKM2 then forms a complex with c-Src phosphorylated β-catenin at the promoter regions of cyclin D1 and c-Myc, thereby leading to acetylation of histone H3 at lysine 9 and promoting the expression of CCND1 (gene encoding cyclin D1) and Myc [31,32]. This is an example of how a cancer specific metabolic enzyme serves to regulate oncogene expression and support tumorigenesis through its non-enzymatic function. Nuclear PKM2 is also reported to phosphorylate STAT3, which then activates MEK5 transcription [28].

Lactate dehydrogenase A (LDHA)

LDHA, catalyzing the conversion of pyruvate and NADH to lactate and electron acceptor NAD+, is important to maintain continued glycolysis. Cancer cells produce more lactate and have higher LDHA activity than normal cells [33]. Lactate generated from LDHA can exit cancer cells via the Monocarboxylate Transporters (MCTs) and cause acidification of the tumor microenvironment, thus facilitating cancer cell invasion [34]. Lactate is reported to enhance HIF1α accumulation regardless of hypoxia and lactate induced acidosis often precedes angiogenesis [35]. LDHA knockdown in tumor cells stimulates mitochondrial oxidative phosphorylation, decreases cell proliferation under hypoxia, and suppresses tumorigenicity [36]. LDHA is reported to be acetylated at lysine-5 and this modified LDHA can be recognized by the HSC70 chaperone, leading to the lysosome mediated LDHA degradation [37]. Importantly, lysine-5 acetylation of LDHA is reduced in human pancreatic cancer, which provides a mechanism of LDHA upregulation and a therapeutic window for pancreatic cancer [38]. The LDHA inhibitor FX11 is found to reduce ATP levels, induce ROS mediated cell death, and inhibit lymphoma and pancreatic tumor progression [39]. By using Magnetic Resonance Spectroscopy (MRS) to real-time monitor metabolic fluxes in murine xenografts of human lymphoma, FX11 inhibition of LDHA suppresses the conversion of hyperpolarized ^{13}C-pyruvate to lactate, providing a promising technique for clinical development of imaging biomarkers to monitor metabolic pharmacodynamics [40].

Pentose phosphate pathway (PPP)

The PPP, one of the major branches of the glycolytic pathway, is essential for cancer cell survival because this pathway can generate pentose-5-phosphate for the synthesis of nucleotides and the reducing equivalent NADPH needed for cell proliferation and scavenging of ROS. Both the oxidative branch, including G6PD and the 6-phosphogluconate dehydrogenase enzymes, and the non-oxidative branch, including transketolase-like-1 and transaldolases, of the PPP have been reported to be activated during carcinogenesis [41-43]. TAp73 is a structural homologue of tumor suppressor p53, but is frequently overexpressed in human tumors. By stimulating the expression of G6PD, TAp73 increases PPP flux and directs glucose to the production of ribose and NADPH, for the synthesis of macromolecules and detoxification of ROS. The growth defect of TAp73-deficient cells can be rescued by either G6PD over-expression or the presence of nucleosides plus an ROS scavenger, suggesting the critical role of TAp73 in regulating the PPP metabolism to support oncogenic cell growth [44]. Targeted inhibition of both branches of the PPP is effective in preventing tumor metastasis [45]. Oxythiamine is a thiamine antagonist and therefore inhibits transketolase and pyruvate dehydrogenase that use Thiamine Pyrophosphate (TPP) as cofactor for their enzymatic activities. Oxythiamine has demonstrated anticancer activity both *in vitro* and *in vivo* [46,47]. 6-Aminonicotinamide (6-AN), another PPP inhibitor targeting G6PD, exhibits anticancer activity, causes oxidative stress, and sensitizes cells to anticancer agents [48,49].

Targeted metabolism	Metabolic targets	Agents	References
Glycolysis	GLUT1 HKII PGAM PKM2 LDHA Transketolase G6PD MCT1	Fasentin: WZB117: Diclofenac 2-deoxyglucose: 3-bromopyruvate: lonidamine: metformin: cerulenin MJE3 TLN-232 (or CAP-232) FX11: Diclofenac Oxythiamine 6-Aminonicotinamide (6-AN) Diclofenac	[52-54] [18-23] [50] [30] [39-40: 58-59] [46-47] [48-49] [58-59]
Glutamine	Glutaminase Glutamine ASCT2 (SLC1A5) SLC7A5/SLC3A2	Compand 968 Phenylacetate L-γ-glutamyl-p-nitroanilide (GPNA) 2-aminobicyclo-(2:2:1) heptanecarboxylic acid (BCH)	[3] [65-66] [82] [83]
Citric acid Master regulators	PDK Mutant IDH HIF1α AKT	Dichloroacetate Antibody MsMab-1: AGI-5198: KC7F2: RX-0047 MK-2206	[55-57] [145-146] [76-77] [86]

GLUT1: Glucose Transporter 1; HKII: Hexokinase II; PGAM1: Phosphoglycerate Mutase 1; PKM2: Pyruvate Kinase Muscle Isozyme 2; LDHA: Lactate Dehydrogenase A; G6PD: Glucose-6-Phosphate Dehydrogenase; MCT1: Monocarboxylate Transporter 1; ASCT2: Solute Carrier Family 1 (Neutral Amino Acid Transporter): Member 5 (also called SLC1A5); SLC7A5: Solute Carrier Family 7 (Amino Acid Transporter Light Chain: I system): Member 5; SLC3A2: Solute Carrier Family 3 (Amino Acid Transporter Heavy Chain) member 2; PDK: Pyruvate Dehydrogenase Kinase; IDH: Isocitrate Dehydrogenase; HIF1: Hypoxia Inducible Factor 1; Alpha Subunit (basic helix-loop-helix transcription factor); AKT: v-akt Murine Thymoma Viral Oncogene Homolog

Table 1: Targeting glucose/glutamine metabolism for cancer therapy.

Other strategies

A large body of evidence suggests that tumor cells have enhanced transcription of genes encoding glycolytic enzymes when compared with non-tumorigenic cells. Compounds have been developed that can directly inhibit almost every enzyme of the glycolytic pathway (Table 1). In addition to the enzymes and their inhibitors discussed above, an inhibitor of Phosphoglycerate Mutase 1 (PGAM1), called MJE3, is identified as the most potent anti-breast cancer drug from chemical screening results [50]. The Monocarboxylate Transporters (MCTs) function to export lactate out of the cells. These transporters are upregulated in various human tumor cells and can be exploited for targeted inhibition as well [51]. Disruption of glucose transporters could be another strategy to inhibit glucose metabolism. Small metabolic inhibitors such as Fasentin, Phloretin, and WZB117 have been developed to inhibit GLUT1 [52-54]. Dichloroacetate (DCA), a small-molecule inhibitor of mitochondrial pyruvate dehydrogenase kinase involved in diverting pyruvate to lactate production, is being tested in patients with metastatic solid tumors and glioma and is shown to decrease lactate levels, activate pyruvate dehydrogenase activity, and induce apoptosis in a variety of human cancer cells [55,56]. DCA also enhances the effectiveness of hypoxia-specific cytotoxic chemotherapy in solid tumors [57]. Diclofenac, a nonsteroidal anti-inflammatory drug, significantly decreases GLUT1, LDHA, and MCT1 gene expression resulting in a decrease in glucose uptake and lactate secretion, holding potential as a clinically applicable glycolysis inhibitor for cancer therapies [58,59].

Targeting Glutamine Metabolism in Cancer

To meet the high proliferation rates, cancer cells require large quantities of nucleotides, proteins, and lipids. However, for the synthesis of key cellular elements, glucose can only provide a carbon source. Since it carries two nitrogens, glutamine is an effective source of nitrogen for the biosynthesis of nucleic acids and some amino acids, such as proline, arginine and asparagine. Glutamine is the most abundant amino acid in the serum [60,61]. Glutamine is also a good source of carbon as it can be deaminated to glutamate through a phosphate-dependent glutaminase in the mitochondrion and further transaminated to α-ketoglutarate that enters the citric acid cycle. In addition, glutamate generated from deamination of glutamine is important for glutathione synthesis allowing cancer cells to survive ROS stress. High level of Glutaminase 1 (GLS1) is a common feature of cancer and rapidly dividing cells. Studies find that deficiency in glutamine but not glucose induces Myc-dependent apoptosis in human cells [62]. Further study identifies that c-Myc enhances mitochondrial GLS1 expression and glutamine metabolism through suppression of miR-23a/b [2]. These studies provide a molecular link between oncogene activation and glutamine metabolism. Another oncogene, Rho GTPase, is identified to promote glutamine metabolism through NF-κB mediated upregulation of GLS1 activity, contributing to malignant transformation [3]. Interestingly, the tumor suppressor p53 is found to regulate glutamine metabolism by transcriptionally activating liver-type mitochondrial phosphate-activated Glutaminase 2 (GLS2) to combat oxidative stress and suppress tumor growth [63].

Targeted inhibition of GLS1 by a tetrahydrobenzo derivative (small compound 968) decreases malignant transformation and tumor cell growth [3]. Inhibition of glutaminase expression by antisense mRNA decreases growth and tumorigenicity of tumor cells [64]. Phenylacetate, a drug that conjugates with glutamine to form renally excretable phenylacetylglutamine to reduce plasma glutamine levels, was in Phase II clinical testing in patient with glioma, and medulloblastoma [65,66]. Due to its relatively nontoxic features, phenylacetate is clinically appealing and early results have shown that phenylacetate can suppress tumor growth and induce differentiation of some solid tumors [65,66].

Oncogenes, Tumor Suppressors, and Cancer Metabolism

We now know that oncogene activation and loss of tumor suppressor functions have mechanistic links to cancer metabolism. One mechanism linking genetic alterations with cancer cell metabolism is through altered expression in nuclear transcription factors. For example, HIF1 transcriptionally upregulates the expression of glycolytic related genes, such as GLUT1, HKII, Phosphofructokinase (PFK), aldolase A, Phosphoglycerate Kinase (PGK), enolase 1, PKM2, LDHA, and MCT4 [67,68]. The increased glycolytic gene expression due to HIF1α stabilization leads to enhanced glycolysis [69]. HIF1α transcriptionally upregulates Pyruvate Dehydrogenase Kinase (PDK) to inhibit pyruvate entry into the citric acid cycle through phosphorylation and inactivation of pyruvate dehydrogenase [70,71]. In addition, metabolic enzyme mutations can also lead to HIF1α stabilization. Patients with inherited mutations in Succinate Dehydrogenase (SDH) and Fumarate Hydratase (FH) develop hereditary paraganglioma or skin leiomyomata and papillary renal cell cancer, respectively [72,73]. This is due to the accumulation of succinate or fumarate, resulting in the inhibition of HIF1α prolyl hydroxylases in the cytosol, leading to stabilization and activation of HIF1α [74,75]. HIF1α inhibitors are being developed for therapeutic potential. KC7F2, a lead compound with a central structure of cystamine, has been identified as a novel HIF1 inhibitor. It markedly inhibits HIF-mediated transcription in cancer cells, and exhibits enhanced cytotoxicity under hypoxia [76]. Another small molecule inhibitor, called RX-0047, inhibits the formation of human lung metastasis in xenograft mouse models and reduces tumor size in flank models [77].

Deregulated expression of the c-Myc gene is a common feature of human malignancies [78]. c-Myc expression enhances aerobic glycolysis by directly upregulating the expression of glycolytic genes such as HK II, PFK, GAPDH, enolase A, LDHA, and Glucose Transporter 1 (GLUT1) independent of hypoxia [79]. Substantial experimental data support that the pivotal role of c-Myc in glutamine metabolism. c-Myc upregulates glutamine metabolism through repression expression of miR-23a/b, which both target the 3'-UTR sequence to down-regulate glutaminase translation [2,80]. ASCT2 (also called SLC1A5) and SLC7A1, both involved in glutamine transport, are direct c-Myc target genes [80,81]. L-γ-glutamyl-p-nitroanilide (GPNA), an ASCT2 inhibitor, can inhibit glutamine uptake and inhibit glutamine-dependent mTOR activation in vitro [82]. 2-aminobicyclo-(2,2,1) heptanecarboxylic acid (BCH), an inhibitor of SLC7A5/SLC3A2, blocks the glutamine-dependent activation of mTORC1 and induces autophagy [83].

AKT activation can increase GLUT localization to the cell membrane to facilitate glucose uptake. It can also phosphorylate and activate PFK2 to generate fructose-2,6-bisphosphate for the activation of PFK1, and increase HKII association to the mitochondrial membrane promoting glycolysis [84]. A recent study has further confirmed that Akt phosphorylates HKII at Thr-473 and increases HKII association to mitochondria [85]. MK-2206, a novel allosteric Akt inhibitor, is reported to synergistically inhibit cancer cell proliferation and tumor growth *in vitro* and *in vivo* in combination with several anticancer agents [86].

Mutations of K-Ras are found in over 90% of pancreatic ductal

adenocarcinomas and are critical in pancreatic cancer initiation. Oncogenic transformation induced by K-Ras attenuates mitochondrial function, increases NADPH oxidase enzyme activity to support aerobic glycolysis, and increases ROS stress [87,88]. Studies exploring inducible expression of oncogenic K-Ras also indicate that oncogenic K-Ras serves a vital role in controlling tumor metabolism through stimulation of glucose uptake and channeling of glucose intermediates into the hexosamine biosynthesis and PPP pathways [89].

Through the transcriptional regulation of GLUT1, HKII, Phosphoglycerate Mutase (PGAM), TP53-induced glycolysis and apoptosis regulator (TIGAR), and synthesis of cytochrome c oxidase (SCO2), tumor suppressor p53 prevents a metabolic switch from aerobic respiration to glycolysis [90-94]. Mutations in p53 cause decreased mitochondrial respiration and increased glycolysis, which provide a molecular link between genetic alterations and the Warburg effect.

ROS and Cancer Metabolism

The major by-products of cellular metabolism are collectively known as ROS. Chemically, these species mainly include superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-) [95]. ROS are mainly produced from the mitochondrial electron transport chain or enzymes such as NADPH oxidases [87,96]. With unpaired electrons, ROS are highly reactive, producing chemical modifications that damage proteins, lipids, nucleotides, and carbohydrates. Moderate elevation of ROS regulates cell signaling and promotes cell proliferation [97], while highly increased level of ROS can lead to cell death [98]. ROS stress is a common feature of human cancer. In order to survive oxidative stress, cancer cells need to rearrange their metabolic flow to promote the synthesis of the major reducing equivalent NADPH and glutathione to maintain ROS at a moderate level. This promotes cancer cell proliferation and continued genomic instability.

The discovery of how ROS modifies the key glycolytic enzyme PKM2 illustrates how ROS regulates cell metabolism. This study demonstrates that nuclear translocation of PKM2 can be triggered by ROS [99]. An acute increase in intracellular ROS level is reported to decrease PKM2 activity through oxidation of PKM2 at cysteine 358, resulting in shunting glucose to the PPP, generating the reducing equivalent NADPH for the detoxification of ROS [100].

HIF1α and c-Myc are two important metabolic regulators as discussed previously. Both are subjected to ROS regulation. By oxidizing the Prolyl Hydroxylase (PHD) cofactors ferrous iron and ascorbate, ROS inhibits PHD activity under normoxic condition and stabilizes HIF1α and therefore alters metabolism into aerobic glycolysis which favors NADPH production through the PPP and allows cancer cells to adapt to ROS stress [101]. H_2O_2 is reported to induce c-Myc gene expression through ROS-mediated enhancement of IκB degradation, thus allowing NF-κB to translocate into the nucleus, where NF-κB serves as a transcription factor directing c-Myc gene expression [102].

Research conducted recently suggests that metabolic reprogramming might also help cancer cells adapt to ROS stress. 6-phosphofructo-2-kinase/fructose-2,6-biphosphases 4 (PFKBF4) is a bifunctional enzyme responsible for maintaining the cellular level of fructose-2,6-bisphosphate, which is important for the dynamic regulation of glycolytic flux by allosterically activating PFK1. By screening 222 different enzymes and other proteins involved in regulating energy production, PFKBF4 was found to be essential for balancing glycolytic activity and antioxidant production in order to maintain cellular redox balance – making sure that the cell's energy needs are met without allowing ROS to accumulate and trigger cell death [103]. Blocking PFKBF4 stalls cancer cell growth and triggers a catastrophic build-up of ROS, suggesting that it could be a suitable drug target [103,104].

Oncogenic K-Ras, the signature genetic alteration in PDAC, is known to regulate a non-canonical pathway of glutamine metabolism to favor the production of NADPH and to maintain the cellular redox status [8]. Through upregulation of aspartate transaminase (GOT1), oncogenic Kras promotes the conversion of glutamine-derived aspartate into oxaloacetate, which is converted into malate and then into pyruvate, increasing the NADPH to NADP+ ratio [8]. Targeted inhibition of the enzymes in the pathway leads to an increase in ROS and a profound suppression of PDAC growth [8]. The metabolic vulnerability of this pathway in PDAC may provide novel therapeutic strategies to treat this lethal cancer.

Mitochondria and Cancer Metabolism

Mitochondria play central roles in energy metabolism, maintenance of ion homeostasis, redox balance, and regulation of apoptosis. As the power house of the cell, mitochondrion is an important metabolic organelle intimately linked to glucose, glutamine, amino acids, and lipid metabolisms. Mitochondrial dysfunction, either due to mitochondrial DNA mutation induced defective mitochondrial electron transport chain, excessive HKII association to the mitochondrial membrane, or citric acid cycle enzyme such as FH, SDH, or Isocitrate Dehydrogenase 2 (IDH2) gene mutations, has been observed to alter cell metabolism in a wide spectrum of human cancers. A recent study has discovered that tumors harboring mutations in FH or Electron Transport Chain (ETC) with disabled oxidative mitochondrial function use glutamine-dependent reductive carboxylation rather than oxidative metabolism as the major pathway of citrate formation. This pathway uses mitochondrial and cytosolic isoforms of NADP+/NADPH-dependent isocitrate dehydrogenase, IDH1 or IDH2, to metabolize glutamine-derived α-ketoglutarate to citrate to provide the acetyl-coenzyme A for lipid synthesis [105]. Moreover, human tumors endure profound hypoxia and the stabilized HIF1α due to hypoxia inhibits the entry of pyruvate into the citric acid cycle and prevents the synthesis of citrate for the production of cytoplasmic acetyl-coenzyme A, a major source of acetyl groups for protein acetylation and lipid synthesis. Under this mitochondrial inhibition condition, glutamine-dependent reductive carboxylation by IDH1 and IDH2 is also found as the major pathway to generate citrate for lipid synthesis to support cell growth and viability [106,107].

The normal mitochondrial oxygen consumption by respiration represents a fundamental important function of mitochondria, which is to maintain redox homeostasis. Mitochondrial dysfunction causes electron leakage, which can be captured by molecular oxygen to generate superoxide, mainly by complex I and III. Superoxide can be converted to other forms of free radicals and released into the intermembrane space for the generation of cytoplasmic ROS. As mentioned previous, ROS levels can directly affect the metabolic activity of cancer cells. A cell model employing a tetracycline controlled expression of a domain-negative form of DNA polymerase gamma has been established to allow real-time monitoring of the metabolic switch from mitochondrial oxidative phosphorylation to aerobic glycolysis [87]. Increases in NADPH oxidase activity were observed to support the metabolic switch with moderate ROS generation. Further study has identified that in order to survive the mitochondrial dysfunction,

transcription factor ZNF143 was activated and mediated upregulation of the major antioxidant enzyme Glutathione Peroxidase 1 (GPX1) to keep ROS in the moderate range [108]. Compelling evidence suggests that mitochondrial dysfunction, either due to mitochondrial genetic alterations, disrupted redox balancing, specific metabolic enzyme mutations, or a hypoxic tumor environment alters mitochondrial metabolic flow by providing precursors to build macromolecules and combat ROS stress so as to promote cancer cell survival and drug resistance.

The Interplay of microRNA, Oncogenes/Tumor Suppressors, and Metabolic Enzymes

MicroRNAs (miRNAs) are small, noncoding RNAs with about 22 nucleotides in length. They regulate gene expression by interacting with the 3'-untranslated region (3'-UTR) of their target mRNAs to inhibit translation and increase mRNA degradation. More than 900 miRNAs have been annotated in the human genome and about 30% of human genes are regulated by miRNAs. miRNAs directly or indirectly regulate several key metabolic enzymes and master regulators that cause alterations in metabolic pathways, resulting in tumor progression and metastasis [109,110]. Evidence also indicates that metabolic alterations, such as ROS tress, hypoxia, glucose content, critically orchestrate miRNA activity in various pathologies [111,112].

miRNAs Directly Regulate Metabolic Enzymes

Several key glycolytic enzymes are demonstrated to be regulated by miRNAs. GLUT1 is a key glucose transporter upregulated in various human cancers. miR-130b, miR-301a, and miR-19a/b are reported to increase GLUT1 expression in renal cell carcinoma [113]. Consistent with this discovery, miR-130b and miR-301a are highly expressed in pancreatic cancer, suggesting the role of these miRNAs in regulating glucose metabolism through GLUT1 [114]. In contrast, miR-138, miR-150, miR-199a, and miR532-5p down-regulate GLUT1 expression [113]. miR-143, downregulated by Mammalian Target of Rapamycin (mTOR) activation, reduces glucose metabolism and inhibits cancer cell proliferation and tumor formation through targeting HKII [115]. miR-122 and the miR-15a/16-1 cluster target AldoA [116,117]. In glioma cells, PKM2 is found to be a target of the tumor suppressive miR-326 [118]. miR-133a and miR-133b target PKM2 in squamous cell carcinoma of tongue [119]. LDHA, the key enzyme responsible for lactate generation, is targeted by miR-34a [120]. Researchers have also demonstrated that miRNAs, such as miR-29a, miR-29b, and miR-124, modulate lactate flow of the cancer cells by regulating MCTs [121,122].

miRNAs Interact With Metabolic Master Regulators

HIF and c-Myc are master regulators of cancer metabolism. Recently, miRNAs have been identified that interact with HIF and c-Myc in tumors. miR-199a targets HIF1α in a feedback manner through Twist-1 [123]. Interestingly, HIF1 is reported as a direct target of the miR-17-92 cluster, which is under the control of c-Myc [124,125]. c-Myc is aberrantly expressed and contributes to the genesis of many human cancers [126]. Myc can not only activate miRNAs, such as miR-17-92, it also represses miRNAs, including miR-let-7, miR-23, and miR-34a [126]. Studied have also demonstrated that miRNAs can regulate c-Myc. miR-let-7a is reported to regulate c-Myc in human colon cancer [127]. miR-let-7a stably transfected cells reduces c-Myc levels and inhibits tumor growth in xenograft models, confirming the tumor suppressor function of miR-let-7a [128]. In addition, tumor suppressor miR-34c regulates c-Myc levels by directly binding the 3'-UTR region of c-Myc mRNA [129]. Halting Myc on the path to cancer

by targeting Myc-induced miRNA expression may provide novel opportunities for cancer therapies.

RAS mutations are a common feature for tumorigenesis in cancers with multiple origins. Kras is a direct target of let-7 miRNA in lung cancer [130]. In addition, both miR-96 and miR-217 target K-ras to inhibit tumor growth in pancreatic adenocarcinoma [131,132].

P53, an important metabolic regulator, regulates miRNAs and is also regulated by miRNAs. P53-inducible miR-34a is found to regulate several glycolytic enzymes, such as HKI, HKII, and LDHA to inhibit glycolysis [133]. miR-34a also directly targets PDK1 to regulate mitochondrial oxidative phosphorylation. Both miR-125a and miR-125b are reported to target p53 and they are highly expressed in pancreatic cancer for tumorigenesis [134].

miRNAs have emerged as important regulators of cancer metabolism. The potential of miRNA mediated therapy might be a novel direction for cancer treatment. Besides miRNAs, long noncoding RNAs (lncRNAs) are reported to interact with oncogenes and tumor suppressors, such as c-Myc and p53 [135]. The role of lncRNAs in cancer metabolism awaits investigation.

Metabolic Enzymes and Chromatin Remodeling in Cancer

Unique functional alterations of some metabolic enzymes are recently reported to play an important role in the reprogramming of cancer cell metabolism. PKM2 nuclear localization and IDH1/2 mutation are reported to have functions related to chromatin remodeling. PKM2 and chromatin remodeling in cancer has been discussed previously. Here, we only discuss IDH1/2 in chromatin remodeling.

Isocitrate Dehydrogenase Mutation and Gain of Function in Cancer

IDHs are a family of enzymes with three isoforms that catalyzes oxidative decarboxylation of isocitrate to α-KG. IHD1 and IHD2 are NADP+ dependent homodimers, mainly participating in reductive glutamine metabolism in the cytosol/peroxisomes and the mitochondrial compartment, respectively. IDH3 is a NAD+ dependent heterotetramer and is the main functional form of IDH in the citric acid cycle under physiological conditions. Genome-wide mutation analyses and high-throughput deep sequencing have identified that 70% of grade II-III gliomas and secondary glioblastomas as well as 10% of acute myeloid leukemia have mutations in IDH1 or IDH2 [136-138]. The mutations in IDH1 and IDH2 confer a gain of function alteration to the enzymes resulting in the production of oncometabolite 2-Hydroxyglutarate (2HG) from α-KG [139]. As α-KG competitor, elevated 2HG effectively inhibits dioxygenase enzymes, such as the 5'-methylcytosine hydroxylase TET2, resulting in increased histone and DNA methylation [140-143]. This area of study demonstrates that the aberrant metabolite 2HG that results from IDH1/2 mutations can promote cancer by changing the epigenetic landscape of the cells to alter gene expression [144]. 2HG is currently being assessed as a biomarker for cancer detection and a small molecular inhibitor called AGI-5198 and a monoclonal antibody called MsMab-1 specific to mutant IDH1/2 are developed for their therapeutic potentials [145-147]. Using MRS, 2-HG can be detected with high sensitivity and specificity in gliomas harboring IDH mutations *ex vivo* and *in vivo*, suggesting that 2-HG can be a clinical valuable biomarker [148].

Chromatin remodeling by alterations of metabolic enzymes in

cancer is a novel area of research. Continued study of the dynamic interplay between metabolism and epigenetics in cancer should lead to improved understanding of disease development and may provide novel targets for cancer therapy.

Conclusions

Metabolic reprogramming is a hallmark of cancer. Through oncogene activation, loss of tumor suppressor functions, and epigenetic alterations, cancer cells alter the metabolism of glucose and glutamine for the synthesis of nucleotides, lipids, fatty acids, and proteins to meet the proliferative needs of cancer cells and to maintain a survival advantage in the adverse tumor microenvironment. Beyond catalyzing glucose and glutamine breakdown and providing substrates for the anabolic reactions, cancer metabolic enzymes, such as HKII, PKM2, and mutant IDH, also contribute to tumor adaptation through alternative non-enzymatic functions of these enzymes. Sustained aerobic glycolysis and glutamine metabolism may also protect cancer cells from ROS induced cell death [100]. As a result of the altered metabolism of glucose, the citric acid cycle, and glutamine, targeted inhibition of these metabolic differences in cancer versus normal cells may have beneficial clinical effects. However, the full magnitude of cancer cell alterations in glycolysis, the citric acid cycle, mitochondrial respiration, glutamine utilization, ROS stress, and their interconnection with genetic and epigenetic alterations in cancer remains to be fully elucidated. In addition, the magnitude of metabolic heterogeneity occurring in cancers of different cellular origins as well as within an individual's cancer is unknown. It is hoped that by targeting altered metabolism of cancer cells, novel therapeutic strategies with high selectivity can be developed.

Acknowledgements

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