Cancer metabolism: New insights into classic characteristics

Yasumasa Kato\textsuperscript{a,\textasteriskcentered}, Toyonobu Maeda\textsuperscript{a}, Atsuko Suzuki\textsuperscript{a}, Yuh Baba\textsuperscript{b}

\textsuperscript{a} Department of Oral Function and Molecular Biology, Ohu University School of Dentistry, 31-1 Misumido, Tomita-machi, Koriyama 963-8611, Japan
\textsuperscript{b} Department of General Clinical Medicine, Ohu University School of Dentistry, 31-1 Misumido, Tomita-machi, Koriyama 963-8611, Japan

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Summary Initial studies of cancer metabolism in the early 1920s found that cancer cells were phenotypically characterized by aerobic glycolysis, in that these cells favor glucose uptake and lactate production, even in the presence of oxygen. This property, called the Warburg effect, is considered a hallmark of cancer. The mechanism by which these cells acquire aerobic glycolysis has been uncovered. Acidic extracellular fluid, secreted by cancer cells, induces a malignant phenotype, including invasion and metastasis. Cancer cells survival depends on a critical balance of redox status, which is regulated by amino acid metabolism. Glutamine is extremely important for oxidative phosphorylation and redox regulation. Cells highly dependent on glutamine and that cannot survive with glutamine are called glutamine-addicted cells. Metabolic reprogramming has been observed in cancer stem cells, which have the property of self-renewal and are resistant to chemotherapy and radiotherapy. These findings suggest that studies of cancer metabolism can reveal methods of preventing cancer recurrence and metastasis.

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1. Introduction

Initial studies of cancer metabolism in the early 1920s showed that the cancer phenotype for glucose metabolism is unique, with increased abilities to take up glucose and produce lactate, even under aerobic conditions [1]. This pathway, called aerobic glycolysis or the Warburg effect, results in extracellular fluid around tumor tissue having acidic pH [1,2]. Indeed, the extracellular pH (pH_e) of most tumor tissues is around 6.5–6.9, and may be even lower (e.g., 5.7) in some cases [3–5]. However, despite lactate production by tumor tissue, blood lactate level is often unaffected [6], suggesting that acidity is limited locally to the microenvironment around tumor tissue.

Accumulated evidence about cancer phenotypes has indicated that all cancers have in common six biological capabilities acquired during multistep development: sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis, and acquisition of invasion and metastasis [7]. Later research has revealed two additional hallmarks of cancer: reprogrammed energy metabolism and evasion of immune-mediated destruction [8]. Recent studies have shown that metabolic reprogramming regulates cancer stemness [9]. Thus, "cancer metabolism" has again become an important research topic. Here, we focus on glucose and glutamine metabolism.

2. Glucose metabolism and its regulation

2.1. Hypoxia

Tumor cells utilize glycolysis to supply energy, even under aerobic conditions, resulting in the conversion of pyruvate to lactate in the extracellular space. Hypoxia stimulates lactate production in tumors by activating hypoxia-inducible transcription factor 1α (HIF1α)-dependent expression of genes such as glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase (PK) M2, pyruvate dehydrogenase kinase 1 (PDH1), enolase 1 (ENO1), and lactate dehydrogenase A (LDHA) [10–15] (Fig. 1). LDHA converts pyruvate to lactate and PDH1 inhibits pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA to produce ATP by mitochondrial oxidative phosphorylation (OXPHOS) [11,16–18]. This pathway facilitates lactate production rather than OXPHOS. Hypoxia also induces the expression of monocarboxylate transporter 4 (MCT4), which functions as a proton-coupled transporter of lactate across cell membranes [19,20]. Thus, hypoxia enhances the Warburg effect, which is responsible for high lactate secretion by tumor cells.

2.2. Histone deacetylases (HDACs)

Sirtuins, which are mammalian homologs of the yeast histone deacetylase Sir2, are NAD⁺-dependent HDACs and consist of seven isoforms (SIRT1–7). These enzymes are involved
in resistance to cellular stress, genomic stability, energy metabolism, aging and tumorigenesis. SIRT6, which deacetylates histone H3K9, is significantly associated with glucose metabolism, elevating glucose up-take through induction of expression of GLUT1, 6-phosphofructo 1-kinase/fructose 1,6-biphosphatase (PFK1/FBPase1), aldolase c (ALDOC), PDK1 and LDHA, whose expression can also be up-regulated by HIF1 as described above [21,22] (Fig. 1).

Figure 1 Oncogene and tumor suppressor gene products regulate glucose and glutamine metabolism in cancer. Glycolysis is the main source of ATP production rather than oxidative phosphorylation (OXPHOS) in tumor cells. Glucose transporters and glycolysis metabolic enzymes are up-regulated by oncogenic product c-Myc. It was believed that mutation of p53 causes loss of function. More recently, p53’s mutation-based “gain of function” has been accepted: e.g., IκB kinase (IKK) is inhibited by wild type p53 (wtP53) but activated by mutant p53 (mutP53). Glucose transporter 4 (GLUT4) and phosphoglycerate mutase (PGM1) activities are also regulated by p53 in the same way. This means reprogramming of which metabolic pathway is directed to lactate when cellular transformation occurs. This is a significant reprogramming of metabolic pathways during carcinogenesis. Hypoxia accelerates glycolysis dependency for energy production through activation of hypoxia-inducible transcription factor 1 (HIF1). Malate and oxaloacetate (OAA) in the TCA cycle can be metabolized to pyruvate in cytosol. Especially, this pathway is important for metabolism of glutamine, rather than glucose, through α-ketoglutarate (α-KG) (see Fig. 6). Two isozymes of glutamine-OAA transaminase (GOT) are closely associated in this pathway. ASCT2, neutral amino acid transporter; SIRT6, distant mammalian Sir2 homolog (sirtuin 6); NEK2, never in mitosis gene A-related kinase 2; NF-κB, nuclear factor-κB; HK2, hexokinase 2, TIGAR, TP-53-induced glycolysis and apoptosis regulator; PFK1/2, 6-phosphofructo 1-kinase 1/2; AMPK, AMP-activated protein kinase; ALDA/C, aldolase A/C; TPI, triosephosphate isomerase; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; PGK1, phosphoglycerate kinase 1; PGM1, phosphoglycerate mutase 1; ENO1, enolase 1; PGM1/M2, pyruvate kinase M1/M2; LDHA, lactate dehydrogenase A; Nrf2, NF-E2-related factor 2; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PTEN, tensin homolog on chromosome ten; PINK1, PTEN-induced putative kinase 1; SCO2, cytochrome c oxidase assembly factor 2; GLS1/2, glutaminase 1/2; GLUD1, glutamate dehydrogenase 1; TCA cycle, tricarboxylic acid cycle.
SIRT2 directly binds β-catenin in response to oxidative stress, inhibiting the expression of Wnt target genes such as survivin, cyclin D1, and c-Myc [23]. Therefore, SIRT2 may contribute to glycolysis through c-Myc.

HDAC inhibitors promote histone acetylation and stimulation of gene expression in tumor cells; for example, they increase the expression of p21^{WAF1} and insulin-like growth factor-1 receptor, and reduce the expression of cyclin D1, AKT, and the tensin homolog on chromosome ten (PTEN) [24–26]. PTEN is a phosphatase that acts on phosphatidylinositol 3,4,5-triphosphate and antagonizes phosphatidylinositol 3-kinase (PI3K) function, thereby inhibiting signaling by PI3K/AKT/the mechanistic target of rapamycin (mTOR, formerly known as the mammalian target of rapamycin). Importantly, the complex with PTEN with PTENa, an N-terminal extended isoform of PTEN, is involved in electron transfer reactions in the respiratory-chain, producing ATP by inducing the expression of PTEN-induced putative kinase 1 (PINK1) followed by activation of the cytochrome c oxidase complex [27]. The anti-tumor agents vorinostat and romidopsin, which inhibit HDAC [28–30], have been approved worldwide for the treatment of patients with cutaneous T-cell lymphoma and also head and neck carcinoma [28,31–33].

2.3. Tyrosine and serine/threonine kinases

PKM1 and PKM2 are enzymes that convert phosphoenolpyruvate to pyruvate. PKM1 is constitutively active, whereas PKM2 can be regulated by phosphorylation. Interestingly, phosphorylation at tyrosine or serine residues has been found to differentially regulate PKM2 activity (Fig. 2). For example, fibroblast growth factor receptor 1 (FGFR1) directly phosphorylates tyrosine residues of PKM2, inhibiting the formation of active, tetrameric PKM2 by disrupting the binding of PKM2 cofactor fructose 1,6-biophosphate [34]. In contrast, the pp60^FGR kinase, which increases tyrosine phosphorylation of PKM2, inactivates the latter [35,36]. Thus, tyrosine kinase phosphorylation by growth factor signaling inhibits PKM2, resulting in the progression of anabolic metabolism in proliferating cells [34,37]. Tyrosine phosphorylation-mediated inhibition of PKM2 has been reported to result in the accumulation of 3-phosphoglycerate, resulting in the accumulation of serine followed by glycine. Glycine, along with cysteine and glutamate, are used to produce glutathione, which neutralizes the effects of reactive oxygen species (ROS), as described below.

In contrast to tyrosine phosphorylation, phosphorylation of serine residues on PKM2 by serine/threonine kinases such as A-Raf and protein kinase δ (PKCδ) induces the formation and stabilization of the tetrameric active form of PKM2 [38,39]. Pim is a serine/threonine kinase that consists of three isoforms (Pim-1, Pim-2 and Pim-3). Pim-2 directly phosphorylates PKM2, which stimulates glycolysis and reduces mitochondrial respiration [40]. In addition, Pim-2 induces the expression of genes targeted by HIF1 through the activation of mTOR complex 1 (mTORC1) as described below [41].

Figure 2 Increase in pyruvate kinase M2 (PKM2)/PKM1 ratio by phosphorylation of tyrosine residue directs to glycine production. (A) PKM2 activity is regulated by phosphorylation in contrast to constitutively active PKM1. Phosphorylation of tyrosine (Tyr) residue activates it whereas that of serine (Ser) residue inhibits it. (B) When PKM2/PKM1 ratio increases, the metabolic pathway directs to pyruvate (continues glycolysis). When the ratio decreases, glycolysis is prevented and metabolic direction changes to serine followed by glycine. Glycine condensates with γ-glutamylcysteine for glutathione synthesis (see Fig. 6).

2.4. Oncogenes and tumor-suppressor genes

2.4.1. Ras

Ras is a small G-protein that transmits signals of growth factors, such as epidermal growth factor (EGF) and hepatocyte growth factor (HGF), and enhances glycolysis through the induction of HIF1α expression. K-Ras/B-raf signal increases the transcription of Nrf2, which up-regulates the PKM2/PKM1 ratio and glycolytic enzymes [42] (Figs. 1 and 2). Nrf2 inhibits lipogenesis but increases NADPH regeneration and purine biosynthesis [43].

2.4.2. c-Myc

c-Myc is a transcription factor that up-regulates the expression of nucleotide metabolic enzymes [44] and cell cycle regulator proteins such as E2Fs and cyclins [45], and down-regulates cyclin-dependent kinase inhibitors such as p15, p21, and p27 [45]. c-Myc is one of the “Yamanaka factors” in the original protocol for production of induced pluripotent
stem cells (iPS cells); this protocol has since been modified, with c-Myc replaced by non-transforming L-Myc to prevent the risk of tumor formation by iPS-derived tissue [46,47]. c-Myc directly induces the expression of genes encoding glycolysis-related metabolic enzymes and transporters, including GLUT1, PKF2/FBPase2, PKM2, PDK1, ENO1, and LDHA; and, together with HIF1, stimulates the expression of HK2 [10,11,13–15,48] (Fig. 1). Although c-Myc can synergistically stimulate HIF1-induced HK2 expression, c-Myc alone has little effect on the induction of HK2 [10,12,48].

2.4.3. The never in mitosis gene A-related kinase 2 (NEK2)
NEK2 is a transcription factor that promotes aerobic glycolysis by increasing the PKM2/PKM1 ratio and by enhancing the expression of GLUT4, HK2, ENO1, and LDHA [49] (Figs. 1 and 2). All of these genes are also targeted by c-Myc and HIF1.

2.4.4. p53
The transcription factor p53 is a major product of the TP53 tumor suppressor gene. Although wild type p53 (wtp53) suppresses the expression of GLUT1 and GLUT4, mutant p53 (mutp53) enhances their expression which is known as the gain of function [50] (Fig. 1). Similarly, mutp53 upregulates phosphoglycerate mutase 1 (PGM1) whereas wtp53 inhibits it [51]. HK2 induction has only been seen for mutp53 [52]. On the other hand, wtp53 upregulates the expression of the TP-53-induced glycolysis and apoptosis regulator (TIGAR), which functions as PKF2 [53]. TIGAR, in turn, inhibits the production of fructose 2,6-bisphosphate, an activator of PKF1 [54], thereby inhibiting glycolysis and directing the metabolism of glucose to the pentose phosphate pathway. This results in the production of NADPH, which protects cells against ROS-associated apoptosis [53]. TIGAR knockdown has been shown to radiosensitize glioma cells by inhibiting the nuclear translocation of thioredoxin-1, a redox-sensitive oxidoreductase [55]. Nucleoredoxin, a thioredoxin-related oxidoreductase, has been reported to inhibit PKF1 activity, suggesting that nucleoredoxin is a regulator of the balance between glycolysis and the pentose phosphate pathway [56]. In mitochondria, wtp53/mutp53 induces expression of cytochrome c oxidase assembly factor 2 (SCO2), which regulates the cytochrome c oxidase complex associated with oxidative phosphorylation [57,58]. Regulation of redox state by wtp53/mutp53 has also been found to induce expression of glutaminase 2 (GLS2), which contributes to glutathione production [59]. Loss of wtp53 activates nuclear factor κB (NF-κB), thereby increasing GLUT3 expression and enhancing glycolysis [60]. Interestingly, insulin-dependent GLUT4 expression has been observed in gastric [61] and lung [62] cancers. GLUT4 expression can be increased by loss of wtp53 function [50]. Because expression of insulin receptor is higher in cancer cells than in normal cells [63,64], GLUT4 is thought to be associated with tumor development and progression.

2.4.5. c-Met and ErbB2
The Met and ERBB2, which are proto-oncogene, encode receptor tyrosine kinases known as HGF receptor (c-Met) and EGF receptor (ErbB2), respectively. As mentioned above, signaling pathway of c-Met is shared with that of erbB2 (EGF receptor): e.g., Ras/Raf signaling modulates PKM2/PKM1 ratio (Fig. 2) and PI3K-AKT-mTOR signaling upregulates HK2 through HIF1 and c-Myc expression (Fig. 1, see also Fig. 7). c-Met expression is induced by not only HIF1 [65] but also wtp53 [66]. Interestingly, mutp53[67][68], a common mutant, remains inducible function for c-Met expression but other mutants cannot [67]. In addition, c-Met is tightly associated with TIGAR expression and NADPH production [68]. Thus, growth factor signaling such as HGF and EGF are strongly associated with glycolysis. Although anti-cancer drugs targeting those receptor tyrosine kinases have been developed and obtained clinical trials, and some of them were approved in head neck cancer (e.g., cetuximab and erlotinib for EGF receptor and crizotinib for HGF receptor) [69], clinical efficacy of those drugs seems to include the effect on glycolysis.

3. Acidic metabolites
3.1. Lactate
The distribution of lactate in frozen sections of clinically obtained tumor tissue has been successfully visualized using the induced metabolic bioluminescence imaging (imBi) technique [70,71]. These studies showed that lactate concentrations in tumor tissue vary widely, from 10–20 to over 30 μmol/g-tissue weight, corresponding approximately to 10–20 mM and >30 mM, respectively. Moreover, assessments of clinical biopsy samples of primary cervical and head and neck cancers showed that survival was significantly longer in patients with low than with high median lactate levels [72,73]. These studies also showed a positive correlation between lactate concentration and the incidence of both recurrence and metastasis, suggesting that lactate not only fuels tumor growth but survival and metastasis after uptake into the cytoplasm through MCT1/SLC16A1.
Lactate is produced not only by tumor cells but by fibroblasts in tumor tissue [74] (Fig. 3). These fibroblasts are ’educated’ by tumor cells, such that their properties differ from those of ’normal’ fibroblasts. These educated fibroblasts are also called cancer-associated fibroblasts (CAFs). Because tumor cells can take up lactate through MCT1, CAFs supply energy to tumor cells via lactate and stroma-derived lactate sustains tumor progression [75,76].
Lactate also functions as a ligand that binds to G-protein-coupled receptor 81 (GPR81/HCAR1) [77] (Fig. 3). GPR81 expression is high in several tumor types and promotes the malignant phenotype of breast cancers [78]. Silencing of GPR81 was found to inhibit tumor growth and metastasis in vivo by downregulating the expression of MCT1, a receptor essential for lactate up-take [79]. GPR81 signaling induced angiogenesis in breast cancers by activating the PI3K/AKT pathway, thereby inducing the expression of several genes, including those encoding amphiregulin, platelet-derived growth factor-BB (PDGF-BB), urokinase-type plasminogen activator (uPA) and vascular endothelial growth factor (VEGF); whereas GPR81 knockdown impaired cell proliferation and increased apoptosis [78]. Thus, lactate supports survival, growth, and metastatic behavior through GPR81 signaling.
Among the CAs, CAIX has been well studied in cancers. CAIX, a CA9 gene product, has been categorized as an α class CA and exists as a homodimer. This enzyme consists of a unique extracellular proteoglycan domain, a transmembrane domain and an intracellular catalytic domain, whereas CA II exists in cytosol [93,94]. The promoter region of CA9 contains a hypoxia-responsive element, with CA9 mRNA expression upregulated by HIF1 [95]. CAIX is highly expressed in tumors and is thought to be tightly associated with primary cancer development, progression and metastasis [96–100].

TACE/ADAM17 has been found to induce the shedding of the extracellular domain of CAIX, also called soluble CAIX [101]. This molecule has been detected in the sera of cancer patients and has been shown diagnostic and/or prognostic in several cancers, including head and neck cancer [102], breast cancer, prostate cancer [103], renal cell carcinoma [104–106], ovarian cancer [107], gastric cancer [108], rectal cancer [109], and non-small cell lung cancer [103,110].

3.3. Ketone bodies

Ketone bodies consist of acetoacetate, β-hydroxybutyrate, and acetone, although β-hydroxybutyrate is not a ketone compound. Ketone bodies are abundant in the liver and are observed during diabetic ketoacidosis in children with type 1 diabetes mellitus [111]. Although lipolysis is increased in adipocytes of tumor patients, due to the high consumption of blood glucose by tumor cells, the blood levels of ketone bodies from the liver are not obviously enhanced [6]. Ketone bodies, however, may be secreted by CAFs and utilized by tumor cells, suggesting that ketone bodies are important in the microenvironment of tumor cells [74,112,113]. Moreover, similar to lactate, ketone bodies function as ligands of GPR41/FFAR3, GPR43/FFAR2, GPR81/HCAR1, and GPR109a/HCAR2 [77,114] (Fig. 3).

Although lactate enhances the malignant behavior of tumor cells, ketone bodies have the opposite clinical effect, with a ketogenic diet prolonging the overall survival rate of patients with glioma [115–118]. Administration of a ketogenic diet has been thought to reduce the consumption of glucose, as ketone bodies supply an abundant amount of acetyl-CoA. Furthermore, β-hydroxybutyrate functions as an endogenous and specific inhibitor of HDACs when incorporated into its transporter, such as MCT1/SLC16A1 and sodium-coupled MCT1 (SMCT1/SLC5A8) [119].

A study using a mouse glioma model found that a ketogenic diet reduced the expression of the HIF-1A and CA9 genes and the activation of NF-κB, as well as suppressing angiogenesis, invasive potential and vascular permeability [120]. These findings suggested that, in contrast to lactate, ketone bodies have anti-tumor activity.

4. Acidic pH_e signaling and metastasis

Hyaluronidases and cathepsins have optimal activity at acidic pH, allowing their efficient digestion of extracellular matrices in an acidic pH_e microenvironment [121–123]. Acidic pH_e also affects cellular activity through an as yet incompletely identified intracellular signaling cascade. Acid sensing ion channel 1a (ASIC1a) is an H_e^+ gated cation chan-
Activating drugs Ca\(^{2+}\) influx to the kinases melanoma

**Figure 4** Acidic pH\(_{e}\) signaling. In acidic pH\(_{e}\) signaling, Ca\(^{2+}\) influx may be common in various tumor cells. Increase in intracellular Ca\(^{2+}\) causes activation of phospholipase D and two mitogen-activated protein kinases (MAPKs) (extracellular signal-regulated kinase (ERK) 1/2 and p38) followed by nuclear factor-κB (NF-κB) activation. NF-κB is also activated by acidic sphingomyelinase (aSMase) independent of Ca\(^{2+}\) influx.

... its activation by acidic pH\(_{e}\) results in Ca\(^{2+}\) influx, thereby activating calmodulin-dependent protein kinase II [124]. Ca\(^{2+}\) influx through ASIC1a also activates PI3K/AKT signaling, which has been associated with resistant to anticancer drugs [125]. PI3K/AKT signaling, in turn, activates mTOR, which has been associated with various diseases, including cancers [126].

We have reported that acidic pH\(_{e}\)-triggered Ca\(^{2+}\) influx activates phospholipase D (PLD); two mitogen activated kinases (MAPKs), p38 and extracellular signal-regulated kinase 1/2 (ERK1/2); and the NF-κB pathway, resulting in the induction of matrix metalloproteinase-9 (MMP-9) expression [127–129] (Fig. 4). The MMP-9 induction rate was found to correlate with cellular metastatic activity in mouse B16 melanoma cells. Acidic pH\(_{e}\)-induced activation of MAPKs (p38 and ERK1/2) and NF-κB is common in mice and humans [130,131]. Moreover, acidic pH\(_{e}\) also stimulated acidic sphingomyelinase activity, the activation of which is independent of intracellular Ca\(^{2+}\), as well as contributing to NF-κB activation [132]. Acidic pH\(_{e}\) signaling was recently shown to upregulate the expression of PLD isoform type 1 (PLD1), but not type 2 (PLD2) via activation of rhoA [133]. Phosphatidate, which is a PLD product, was reported to show survival signaling by activating mTOR and inhibiting MDM2, the ubiquitin ligase of p53 [134,135].

We found that acidic pH\(_{e}\) changes the morphology of cancer cells to fibroblastic, as shown by the induction of matrigel invasion; up-regulation of MMP-9, vimentin, MMP-3, and MMP-13 gene expression; and down-regulation of E-cadherin expression [127,135]. These findings indicated that acidic pH\(_{e}\) induces epithelial mesenchymal transition (EMT), an important event in the development of a metastatic phenotype [135]. Similar, others have also reported that acidic pH\(_{e}\) induced EMT-like changes [130,137].

Acidic pH\(_{e}\) may contribute to drug resistance through ASIC1a/Ca\(^{2+}\)/PI3K/AKT/mTOR signaling. Moreover, drugs that inhibit this signaling may have efficacy in suppressing acidic pH\(_{e}\)-mediated malignant phenotype. Antitumor drugs that inhibit the PI3K/AKT/mTOR pathway are currently being tested in clinical trials, with some, such as BEZ235, approved for treatment [182]. These drugs are expected to effectively suppress the acidic pH\(_{e}\)-associated malignant phenotype of human cancer cells.

**Figure 5** Cell cycle dependent glutamine metabolism. Glutamine to α-ketoglutarate is metabolized by different enzymes depending on cell cycle status. (A) Oncogenic molecules such as c-Myc and K-ras activate glutaminase 1 (GLS1) and glutamine-oxaloacetate transaminase 2 (GOT2) in proliferating cells. K-ras inhibits glutamate dehydrogenase 1 (GLUD1). Thus, GLS1 and GOT2 are major metabolic enzymes in proliferating cells. (B) Wild type p53 (wt p53) not only increases in the cyclin-dependent kinase inhibitor p21 but also glutaminase 2 (GLS2). GLUD1 is not inhibited by K-ras in quiescent cells, thereby metabolizing by GLS2 and GLUD1.

5. Amino acid usage in cancer

5.1. Glutamine

Glutamine is most abundant amino acid in the blood, with a concentration of about 0.57 mM [139]. Following its uptake by cells, glutamine is metabolized to the non-essential amino acid glutamate by the cytoplasmic enzyme glutaminase [140]. There are two isoforms of glutaminase, namely kidney type (mitochondrial enzyme) encoded by GLS1, and liver type (cytoplasmic enzyme) encoded by GLS2 [141]. Glutamine metabolism is regulated by oncogene and tumor suppressor gene products dependent on cell cycle status
Cancer metabolism

![Figure 6](image)

**Figure 6** Glucose and glutamine metabolism in redox prevention. (The pentose phosphate pathway) and glutamine metabolism (pathway from malate to pyruvate) through part of the TCA cycle. Glutathione is a tripeptide comprising glutamate, cysteine, and glycine. OAA, oxaloacetate; Asp, aspartate; α-KG, α-ketoglutarate.

(Fig. 5). Glutamate is subsequently metabolized by glutamate dehydrogenases (GDHs) to α-ketoglutarate, which enters the tricarboxylic acid (TCA) cycle and can be metabolized to aspartate and malate (Fig. 6). Glutamine can also be used as an energy source by OXPHOS through NADH and FADH₂. Glutamine and aspartate are nitrogen donors in the synthesis of purine and pyrimidine bases and aspartate also provides the carbon skeleton for pyrimidine bases [142]. The survival of some types of cancer cells depends on glutamine, a phenomenon known as glutamine addiction that is driven by redox balance [143]. c-Myc activation induces the expression of the glutamine transporter ASCT2, glutaminase, and several glycolytic enzymes, as described above, thereby promoting glutaminolysis and triggering cellular addiction to glutamine as a bioenergetic substrate [144] (see Fig. 1).

5.2. Redox regulation

Glutathione is a tripeptide consisting of cysteine, glutamate, and glycine. Glutathione S-transferase contributes to drug resistance [145]. Glutathione peroxidase oxidizes glutathione in the presence of NADPH, with the resulting oxidized glutathione being a substrate of the enzyme glutathione reductase to neutralize H₂O₂. Thus, glutathione plays a major role in scavenging ROS [146–150]. Thioredoxin reductase is another NADPH dependent enzyme that neutralizes free radicals [151]. NADPH can be supplied by the pentose phosphate pathway and by the metabolic pathway synthesizing pyruvate from malate (Fig. 6). Glutamine can be metabolized to malate through α-ketoglutarate and aspartate [140].

Increased glutathione concentrations contribute to the absorption of free radicals and are associated with tumorogenesis, angiogenesis, and drug resistance [146–148,150,152]. Acidic pH enhances the formation of ROS by a pathway independent of MAPKs (p38 and ERK1/2) and Src family kinases, thereby increasing metastatic activity [131,153,154]. Although glutathione-mediated antioxidant-targeting therapy was expected to be useful in treating cancer patients, recent studies showed that antioxidants accelerate tumor malignant phenotypes, including those associated with metastasis [155–158]. These findings suggest that intracellular redox status is critical for tumor survival and malignant phenotype [159].

5.3. Activation of mTORC1

PI3K/AKT signaling activates mTOR, resulting in cell survival and growth [160]. mTORC1 comprises five molecules; mTOR; the regulatory associated protein of mTOR (RPTOR); the DEP domain containing mTOR interacting protein (DEPTOR); the proline-rich Akt substrate of 40-kDa (PRAS40); and the mammalian lethal with SEC13 protein 8 (mLST8). Glutamine activates mTORC1 through ADP-ribosylation factor 1 (ARF1) but leucine and arginine does through Rag small G proteins. mTORC1 promotes mRNA translation and protein synthesis through inhibition of the eukaryotic translation initiation factor eIF5B and the sterol regulatory element-binding protein 1 (SREBP1), respectively.

Glutamine, leucine, and arginine are the most potent stimuli of mTORC1 activation, resulting in autophagy [168]. Glutamine up-regulates the small G-protein ADP-ribosylation factor 1 (ARF1), thereby activating mTOR (Fig. 7). Unlike glutamine, leucine and arginine stimulate the recruitment of mTORC1 to the surface of lysosomes, with the small G-proteins RagA/B affecting kinase activation. The GTP-binding protein RheB increases mTOR kinase activity [162,169]. In contrast, wtp53 activates AMP-activated
protein kinase (AMPK)/TSC2 signaling by inducing the expression of sestrin1/2, resulting in the inactivation of the RheB/mTORC1 pathway [170]. In contrast, mutp53 inhibits AMPK, thereby activating mTORC1 [171]. Nutrient starvation-induced autophagy involves mTOR-ULK1 signaling [172,173].

6. Perspective

High glucose consumption is a common feature of several types of tumor cells. Therefore, so far, in vivo positron emission tomography (PET) imaging with the glucose analog \(^{18}\)F-fluorodeoxyglucose \((^{18}\)F-FDG\) has been used to detect various tumors. However, it has not been used for brain tumors because of high background. As discussed above, glutamine dependency is also a common feature of tumors but not for normal cells. Venneti et al. [174], successfully detected gliomas by in vivo PET imaging using the glutamine analog \(^{18}\)F-(2S,4R)-fluoroglutamine with low background. Small numbers of biologically different tumor cells with stem-like properties, such as self-renewal and tumor-initiating ability, have been detected in various tumors including head and neck cancers [175–180]. These cells, called cancer stem cells (CSCs), are less proliferative than other cancer cells and are insensitive to chemotherapy and radiotherapy. CSCs play important roles in both local recurrence and distant metastasis. CD44 variant 9 (CD44v) is a nearly universal CSC marker in various tumor types [181]. CD44v stabilizes the cysteine transporter xCT (see Fig. 6), whereas sulfasalazine inhibits xCT activity, resulting in tumor suppression by decreasing glutathione formation and increasing oxidative stress [182–184]. The anticancer activity of sulfasalazine is likely due to its targeting of CSCs, especially preventing local recurrence and metastasis. Clinical trials of sulfasalazine have yielded successful results in patients with gastric cancer, ulcerative colitis-related cancers and urogenital cancer [182,183,185]. Despite difficulties in developing a new drug targeting CSCs, the combination of sulfasalazine with conventional chemotherapy agents has been reported to selectively inhibit CSCs [186]. Although targeting CSCs may prevent local recurrence and distant metastasis, non-CSCs may acquire the properties of CSCs epigenetically, thereby complicating treatment [186]. Controlling the mechanisms underlying reprogramming should be determined to prevent non-CSCs from the de novo acquisition of CSC properties, with efforts concentrated on epigenetic regulatory networks for the development and/or stabilization of cancer stemness. Further studies are expected to develop new and selective CSC-targeting agents with high efficacy.

Conflict of interest

The authors declare no conflict of interest.

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