Japanese Dental Science Review (2018) 54, 8—21

Contents lists available at ScienceDirect

Japanese Dental Science Review

journal homepage: www.elsevier.com/locate/jdsr

Review Article

Cancer metabolism: New insights into classic characteristics

Yasumasa Kato[a,*], Toyonobu Maeda[a], Atsuko Suzuki[a], Yuh Baba[b]

[a] *Department of Oral Function and Molecular Biology, Ohu University School of Dentistry, 31-1 Misumido, Tomita-machi, Koriyama 963-8611, Japan*
[b] *Department of General Clinical Medicine, Ohu University School of Dentistry, 31-1 Misumido, Tomita-machi, Koriyama 963-8611, Japan*

Received 27 February 2017; accepted 1 August 2017

KEYWORDS
Glycolysis;
Warburg effect;
Glutamine metabolism;
Acidic extracellular pH

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.

* Corresponding author. Fax: +81 249328978.
E-mail address: yasumasa-kato@umin.ac.jp (Y. Kato).

https://doi.org/10.1016/j.jdsr.2017.08.003

Contents

1. Introduction 9
2. Glucose metabolism and its regulation 9
2.1. Hypoxia 9
2.2. Histone deacetylases (HDACs) 9
2.3. Tyrosine and serine/threonine kinases 11
2.4. Oncogenes and tumor-suppressor genes 11
2.4.1. Ras 11
2.4.2. c-Myc 11
2.4.3. The never in mitosis gene A-related kinase 2 (NEK2) 12
2.4.4. p53 12
2.4.5. c-Met and ErbB2 12
3. Acidic metabolites 12
3.1. Lactate 12
3.2. Carbon dioxide and carbonic anhydrases (CAs) 13
3.3. Ketone bodies 13
4. Acidic pH_e signaling and metastasis 13
5. Amino acid usage in cancer 14
5.1. Glutamine 14
5.2. Redox regulation 15
5.3. Activation of mTORC1 15
6. Perspective 16
Conflict of interest 16
Acknowledgments 16
References 16

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 activation 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 (PDK1), enolase 1 (ENO1), and lactate dehydrogenase A (LDHA) [10—15] (Fig. 1). LDHA converts pyruvate to lactate and PDK1 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

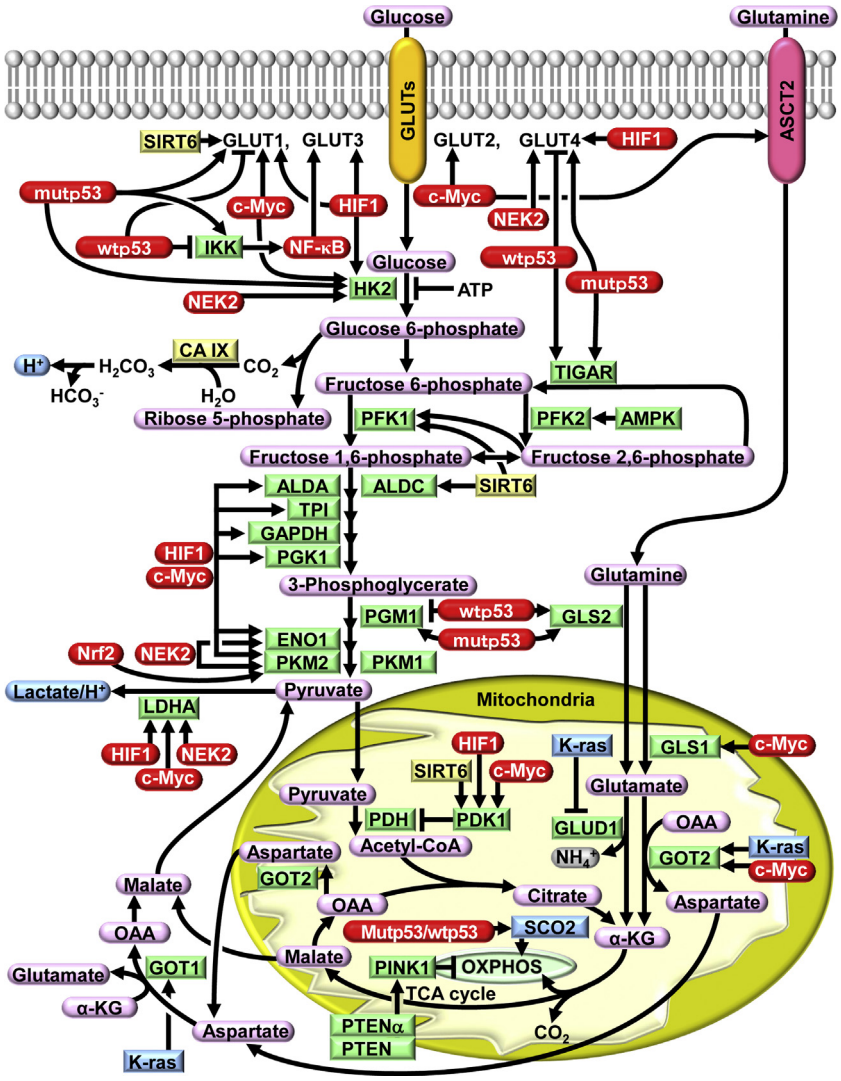

Figure 1 Oncogene and tumor suppressor gene products regulate glucose and glutamine metabolism in cancer. Glycolysis is the main source of ATP production rather than oxidative phosphorylation (OXPHOS) in tumor cells. Glucose transporters and glycolysis metabolic enzymes are up-regulated by oncogene product 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; PKM1/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.

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).

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-I 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 of PTEN with PTENα, 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 $pp60^{src}$ 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].

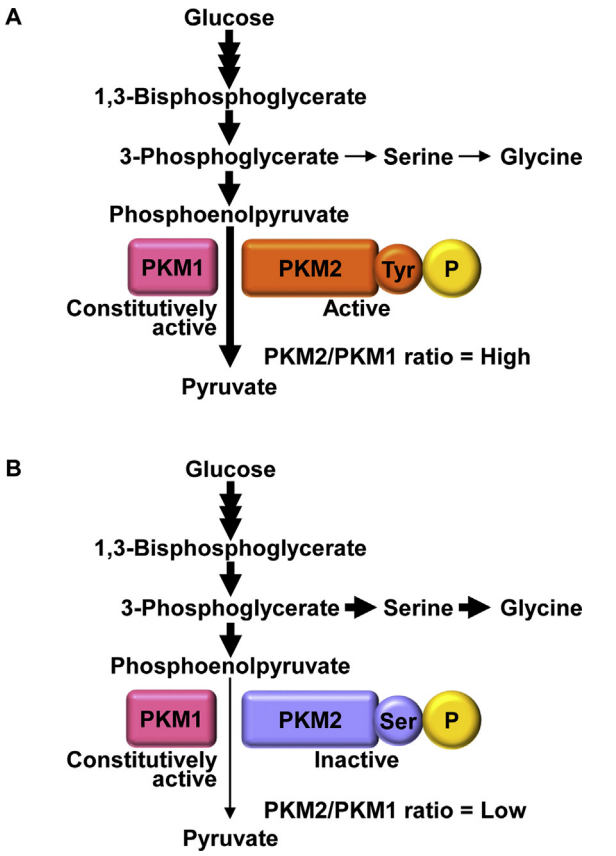

Figure 2 Increase in pyruvate kinase M2 (PKM2)/PKM1 ratio by phosphorylation of tyrosine residue directs to glycine production. (A) PKM2 activity is regulated by phosphorylation in contrast to constitutively active PKM1. Phosphorylation of tyrosine (Tyr) residue activates it whereas that of serine (Ser) 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, PFK2/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 PFK2 [53]. TIGAR, in turn, inhibits the production of fructose 2,6-bisphosphate, an activator of PFK1 [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 PFK1 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 knowing 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^{R175H}, 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.

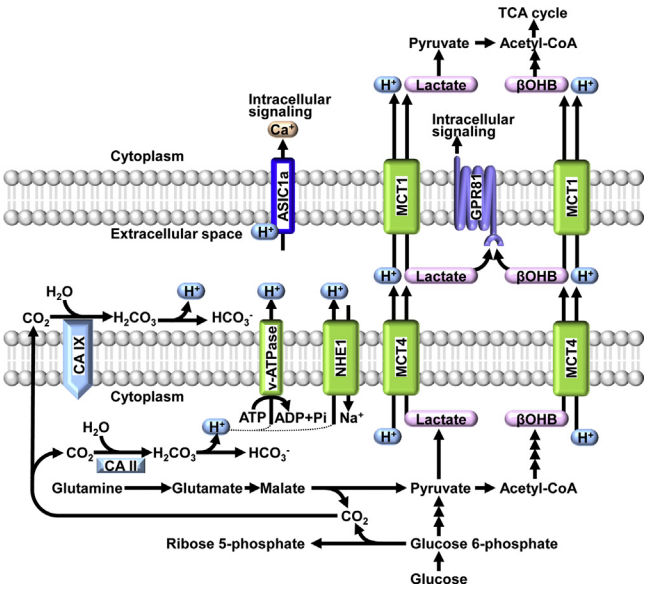

Figure 3 Cell to cell communication by proton and acidic metabolites (lactate and β-hydroxybutyrate). Carbonic anhydrase (CA) catalyzes H_2O and CO_2 yielding H_2CO_3 followed by H^+ and HCO_3^-. CA II and CA IX are located on the cytosol and plasma membrane, respectively. Intracellular H^+ is secreted by vacuolar type-ATPase (v-ATPase), Na^+/H^+ exchanger 1 (NHE1). Monocarboxylate transporter (MCT) functions as the lactate/H^+ or β-hydroxybutyrate (βOHB)/H^+ co-transporter. MCT1 and MCT4 are associated with their up-take and secretion, respectively. Intracellular HCO_3^- can be secreted by Cl^-/HCO_3^- exchanger, which is not shown in this figure. Upper cell: tumor cells in normoxia and sufficient nutrition due to proximity to blood vessels. Lower cell: cancer-associated fibroblasts (CAFs) or tumor cells in hypoxic and inadequate nutrition due to distance from blood vessels.

3.2. Carbon dioxide and carbonic anhydrases (CAs)

Once incorporated into cells, glucose is converted to glucose 6-phosphate, which is metabolized by the glycolytic and pentose phosphate pathways; the latter, called the secondary pathway of glycolysis, results in the production of ribose 5-phosphate and NADPH [80]. This pathway results in the production of one molecule of CO_2 from one molecule of glucose 6-phosphate, whereas glycolysis of glucose 6-phosphate does not produce CO_2. Tumors express high amounts of CAs, which catalyze the reaction of CO_2 with H_2O to produce H_2CO_3, which dissociates to H^+ and HCO_3^-. Experiments in glycolysis-impaired mice showed that CO_2 derived from the pentose phosphate pathway was a main cause of extracellular acidity in tumors [81]. The intracellularly yielded H^+ from the dissociation of H_2CO_3 secretes into extracellular space through a proton pump/vacuolar-type ATPase (v-ATPase) [82—84] or an Na^+/H^+ exchanger [85,86], whereas the HCO_3^{2-} is secreted through a chloride exchanger coupled with an Na^+/H^+ exchanger [87,88] (Fig. 3). Interestingly, glucose stimulates the assembly of the V0 and V1 domains of v-ATPase through PI3K, resulting in its activation [89]. Na^+/H^+ exchangers localize to the invadopodia (invasion front) [90], resulting in the front cell surface being more acidic than the rear [91,92].

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^+ gated cation chan-

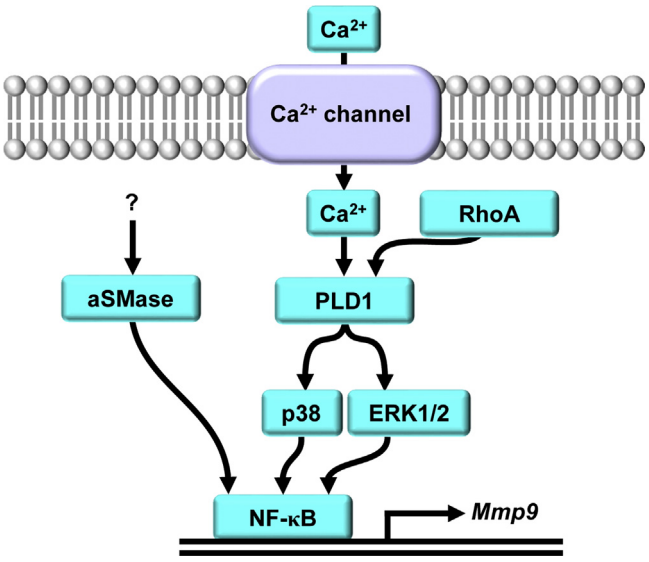

Figure 4 Acidic pH_e signaling. In acidic pH_e signaling, Ca^{2+} influx may be common in various tumor cells. Increase in intracellular Ca^{2+} causes activation of phospholipase D and two mitogen-activated protein kinases (MAPKs) (extracellular signal-regulated kinase (ERK) 1/2 and p38) followed by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

nel. 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 isozyme 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 [127,136]. These findings indicated that acidic pH_e induces epithelial mesenchymal transition (EMT), an important event in the development of a metastatic phenotype [136]. 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 [138]. These drugs are expected to effectively suppress the acidic pH_e-associated malignant phenotype of human cancer cells.

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 isozymes 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

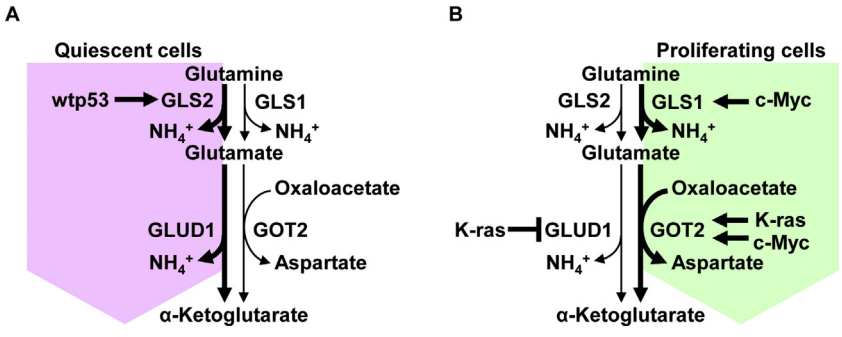

Figure 5 Cell cycle dependent glutamine metabolism. Glutamine to α-ketoglutarate is metabolized by different enzymes depending on cell cycle status. (A) Oncogenic molecules such as c-Myc and K-ras activate glutaminase 1 (GLS1) and glutamine-oxaloacetate transaminase 2 (GOT2) in proliferating cells. K-ras inhibits glutamate dehydrogenase 1 (GLUD1). Thus, GLS1 and GOT2 are major metabolic enzymes in proliferating cells. (B) Wild type p53 (wtp53) not only increase 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.

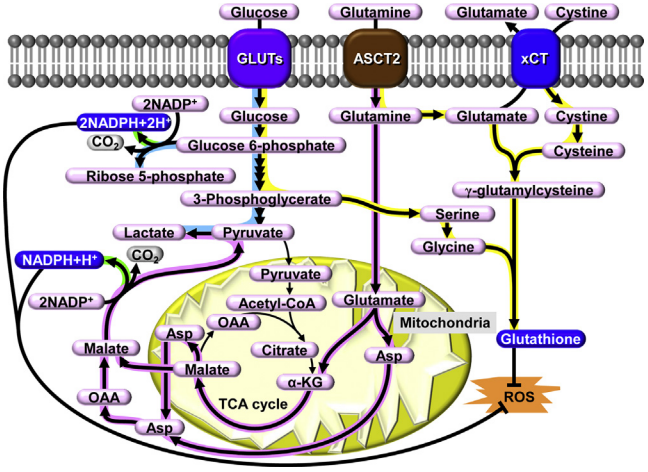

Figure 6 Glucose and glutamine metabolism in redox prevention. Production of NADPH is mainly obtained from glucose metabolism (the pentose phosphate pathway) and glutamine metabolism (pathway from malate to pyruvate) through part of the TCA cycle. Glutathione is a tripeptide comprising glutamate, cysteine,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

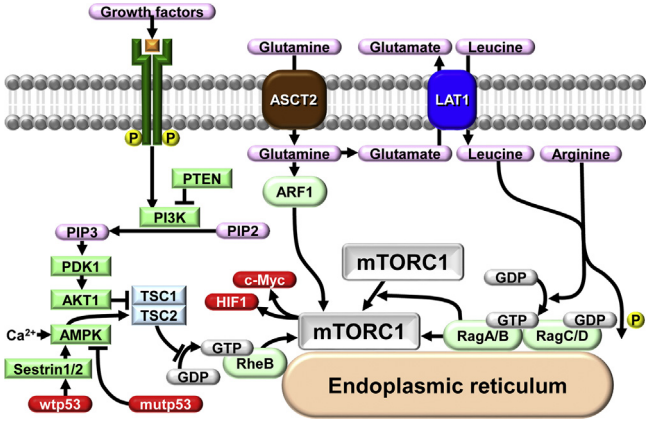

Figure 7 Regulation of mTORC1. mTORC1 comprises five molecules: mTOR; the regulatory associated protein of mTOR (RAPTOR); the DEP domain containing mTOR interacting protein (DEPTOR); the proline-rich Akt substrate of 40-kDa (PRAS40); and the mammalian lethal with SEC13 protein 8 (mLST8). Glutamine activates 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 4E-binding protein (4E-BP1) and activation of the ribosomal protein S6 kinase (S6 K). It also inhibits ULK1 and HIF1α which induce autophagy and glycolysis, respectively. mTORC1 induces inflammation and lipid synthesis through NF-κB and the sterol regulatory element binding protein 1 (SREBP1), respectively.

(Fig. 5). Glutamate is subsequently metabolized by glutamate dehydrogenases (GLUDs) 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 $FADH_2$. 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 H_2O_2. Thus, glutathione plays 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 tumorigenesis, angiogenesis, and drug resistance [146—148,150,152]. Acidic pH_e 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 (RAPTOR); 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) [161,162]. PI3K/AKT/mTOR signaling also induces expression of HIF1α and c-Myc [163—166], the activities of which are associated with glycolysis, as described above. Aberrant amino acid signaling promotes growth and metastasis through Rab1A-dependent activation of mTORC1 [167].

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 4-^{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.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number JP16K11517.

References

[1] Warburg O, Posener K, Negelein E. Über den Stoffwechsel der Tumoren (On metabolism of tumors). Biochem Z 1924;152:319—44.
[2] Warburg O. On the origin of cancer cells. Science 1956;123(3191):309—14.
[3] Volk T, Jähde E, Fortmeyer HP, Glüsenkamp KH, Rajewsky MF. pH in human tumour xenografts: effect of intravenous administration of glucose. Br J Cancer 1993;68(3):492—500.
[4] Engin K, Leeper DB, Cater JR, Thistlethwaite AJ, Tupchong L, McFarlane JD. Extracellular pH distribution in human tumours. Int J Hyperthermia 1995;11(2):211—6.
[5] Delli Castelli D, Ferrauto G, Cutrin JC, Terreno E, Aime S. In vivo maps of extracellular pH in murine melanoma by CEST-MRI. Magn Reson Med 2014;71(1):326—32.
[6] Rich AJ, Wright PD. Ketosis and nitrogen excretion in undernourished surgical patients. JPEN J Parenter Enteral Nutr 1979;3(5):350—4.
[7] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100(1):57—70.
[8] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646—74.
[9] Menendez JA, Alarcón T. Metabostemness: a new cancer hallmark. Front Oncol 2014;4:262.
[10] Osthus RC, Shim H, Kim S, Li Q, Reddy R, Mukherjee M, et al. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. J Biol Chem 2000;275(29):21797—2800.
[11] Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006;3(3):177—85.
[12] Kim JW, Gao P, Liu YC, Semenza GL, Dang CV. Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. Mol Cell Biol 2007;27(21):7381—93.
[13] Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res 2009;15(21):6479—83.
[14] Sedoris KC, Thomas SD, Miller DM. Hypoxia induces differential translation of enolase/MBP-1. BMC Cancer 2010;10:157.
[15] Teicher BA, Linehan WM, Helman LJ. Targeting cancer metabolism. Clin Cancer Res 2012;18(20):5537—45.
[16] Zimmer AD, Walbrecq G, Kozar I, Behrmann I, Haan C. Phosphorylation of the pyruvate dehydrogenase complex precedes HIF-1-mediated effects and pyruvate dehydrogenase kinase 1 upregulation during the first hours of hypoxic treatment in hepatocellular carcinoma cells. Hypoxia (Auckl) 2016;4:135—45.
[17] Huang D, Li T, Li X, Zhang L, Sun L, He X, et al. HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. Cell Rep 2014;8(6):1930—42.
[18] Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 2006;3(3):187—97.
[19] Dimmer KS, Friedrich B, Lang F, Deitmer JW, Broer S. The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochem J 2000;350(Pt. 1):219—27.
[20] Le Floch R, Chiche J, Marchiq I, Naiken T, Ilc K, Murray CM, et al. CD147 subunit of lactate/H$^+$ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. Proc Natl Acad Sci U S A 2011;108(40):16663—8.

[21] Zhong L, Mostoslavsky R. SIRT6: a master epigenetic gatekeeper of glucose metabolism. Transcription 2010;1(1):17—21.
[22] Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1α. Cell 2010;140(2):280—93.
[23] Nguyen P, Lee S, Lorang-Leins D, Trepel J, Smart DK. SIRT2 interacts with β-catenin to inhibit Wnt signaling output in response to radiation-induced stress. Mol Cancer Res 2014;12(9):1244—53.
[24] Takai N, Desmond JC, Kumagai T, Gui D, Said JW, Whittaker S, et al. Histone deacetylase inhibitors have a profound antigrowth activity in endometrial cancer cells. Clin Cancer Res 2004;10(3):1141—9.
[25] Gui CY, Ngo L, Xu WS, Richon VM, Marks PA. Histone deacetylase (HDAC) inhibitor activation of $p21^{WAF1}$ involves changes in promoter-associated proteins, including HDAC1. Proc Natl Acad Sci U S A 2004;101(5):1241—6.
[26] Sarfstein R, Bruchim I, Fishman A, Werner H. The mechanism of action of the histone deacetylase inhibitor vorinostat involves interaction with the insulin-like growth factor signaling pathway. PLoS One 2011;6(9):e24468.
[27] Liang H, He S, Yang J, Jia X, Wang P, Chen X, et al. PTENα, a PTEN isoform translated through alternative initiation, regulates mitochondrial function and energy metabolism. Cell Metab 2014;19(5):836—48.
[28] Witt O, Deubzer HE, Milde T, Oehme I. HDAC family: what are the cancer relevant targets? Cancer Lett 2009;277(1):8—21.
[29] Wang J, Kim TH, Ahn MY, Lee J, Jung JH, Choi WS, et al. Sirtinol, a class III HDAC inhibitor, induces apoptotic and autophagic cell death in MCF-7 human breast cancer cells. Int J Oncol 2012;41(3):1101—9.
[30] West AC, Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. J Clin Invest 2014;124(1):30—9.
[31] Marks PA, Breslow R. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol 2007;25(1):84—90.
[32] Zang Y, Kirk CJ, Johnson DE. Carfilzomib and oprozomib synergize with histone deacetylase inhibitors in head and neck squamous cell carcinoma models of acquired resistance to proteasome inhibitors. Cancer Biol Ther 2014;15(9):1142—52.
[33] Duvic M, Dimopoulos M. The safety profile of vorinostat (suberoylanilide hydroxamic acid) in hematologic malignancies: a review of clinical studies. Cancer Treat Rev 2016;43:58—66.
[34] Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci Signal 2009;2(97):ra73.
[35] Presek P, Reinacher M, Eigenbrodt E. Pyruvate kinase type M_2 is phosphorylated at tyrosine residues in cells transformed by Rous sarcoma virus. FEBS Lett 1988;242(1):194—8.
[36] Presek P, Glossmann H, Eigenbrodt E, Schoner W, Rubsamen H, Friis RR, et al. Similarities between a phosphoprotein ($pp60^{src}$)-associated protein kinase of Rous sarcoma virus and a cyclic adenosine 3′:5′-monophosphate-independent protein kinase that phosphorylates pyruvate kinase type M_2. Cancer Res 1980;40(5):1733—41.
[37] Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature 2008;452(7184):181—6.
[38] Le Mellay V, Houben R, Troppmair J, Hagemann C, Mazurek S, Frey U, et al. Regulation of glycolysis by Raf protein serine/threonine kinases. Adv Enzyme Regul 2002;42:317—32.
[39] Mazurek S, Grimm H, Boschek CB, Vaupel P, Eigenbrodt E. Pyruvate kinase type M2: a crossroad in the tumor metabolome. Br J Nutr 2002;87(Suppl. 1):S23—9.
[40] Yu Z, Zhao X, Huang L, Zhang T, Yang F, Xie L, et al. Proviral insertion in murine lymphomas 2 (PIM2) oncogene phosphorylates pyruvate kinase M2 (PKM2) and promotes glycolysis in cancer cells. J Biol Chem 2013;288(49):35406—16.
[41] Zhang XH, Yu HL, Wang FJ, Han YL, Yang WL. Pim-2 modulates aerobic glycolysis and energy production during the development of colorectal tumors. Int J Med Sci 2015;12(6):487—93.
[42] DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 2011;475(7354):106—9.
[43] Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci 2014;39(4):199—218.
[44] Liu YC, Li F, Handler J, Huang CR, Xiang Y, Neretti N, et al. Global regulation of nucleotide biosynthetic genes by c-Myc. PLoS One 2008;3(7):e2722.
[45] Bretones G, Delgado MD, León J. Myc and cell cycle control. Biochim Biophys Acta 2015;1849(5):506—16.
[46] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663—76.
[47] Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, et al. A more efficient method to generate integration-free human iPS cells. Nat Methods 2011;8(5):409—12.
[48] Yeung SJ, Pan J, Lee MH. Roles of p53, MYC and HIF-1 in regulating glycolysis — the seventh hallmark of cancer. Cell Mol Life Sci 2008;65(24):3981—99.
[49] Gu Z, Xia J, Xu H, Frech I, Tricot G, Zhan F. NEK2 promotes aerobic glycolysis in multiple myeloma through regulating splicing of pyruvate kinase. J Hematol Oncol 2017;10(1):17.
[50] Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters *GLUT1* and *GLUT4* gene expression. Cancer Res 2004;64(7):2627—33.
[51] Kondoh H, Lleonart ME, Gil J, Wang J, Degan P, Peters G, et al. Glycolytic enzymes can modulate cellular life span. Cancer Res 2005;65(1):177—85.
[52] Mathupala SP, Heese C, Pedersen PL. Glucose catabolism in cancer cells. The type II hexokinase promoter contains functionally active response elements for the tumor suppressor p53. J Biol Chem 1997;272(36):22776—80.
[53] Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 2006;126(1):107—20.
[54] Rider MH, Kuntz DA. Hue L Fructose 2,6-bisphosphate and its phosphorothioate analogue. Comparison of their hydrolysis and action on glycolytic and gluconeogenic enzymes. Biochem J 1988;253(2):597—601.
[55] Zhang H, Gu C, Yu J, Wang Z, Yuan X, Yang L, et al. Radiosensitization of glioma cells by TP53-induced glycolysis and apoptosis regulator knockdown is dependent on thioredoxin-1 nuclear translocation. Free Radic Biol Med 2014;69:239—48.
[56] Funato Y, Hayashi T, Irino Y, Takenawa T, Miki H. Nucleoredoxin regulates glucose metabolism via phosphofructokinase 1. Biochem Biophys Res Commun 2013;440(4):737—42.
[57] Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, et al. p53 regulates mitochondrial respiration. Science 2006;312(5780):1650—3.
[58] Won KY, Lim SJ, Kim GY, Kim YW, Han SA, Song JY, et al. Regulatory role of p53 in cancer metabolism via SCO2 and TIGAR in human breast cancer. Hum Pathol 2012;43(2):221—8.
[59] Suzuki S, Tanaka T, Poyurovsky MV, Nagano H, Mayama T, Ohkubo S, et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proc Natl Acad Sci U S A 2010;107(16):7461—6.

[60] Kawauchi K, Araki K, Tobiume K, Tanaka N. p53 regulates glucose metabolism through an IKK-NF-κB pathway and inhibits cell transformation. Nat Cell Biol 2008;10(5):611—8.
[61] Noguchi Y, Marat D, Saito A, Yoshikawa T, Doi C, Fukuzawa K, et al. Expression of facilitative glucose transporters in gastric tumors. Hepatogastroenterology 1999;46(28):2683—9.
[62] Ito T, Noguchi Y, Udaka N, Kitamura H, Satoh S. Glucose transporter expression in developing fetal lungs and lung neoplasms. Histol Histopathol 1999;14(3):895—904.
[63] Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, et al. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 1999;19(5):3278—88.
[64] Vella V, Pandini G, Sciacca L, Mineo R, Vigneri R, Pezzino V, et al. A novel autocrine loop involving IGF-II and the insulin receptor isoform-A stimulates growth of thyroid cancer. J Clin Endocrinol Metab 2002;87(1):245—54.
[65] Eckerich C, Zapf S, Fillbrandt R, Loges S, Westphal M, Lamszus K. Hypoxia can induce c-Met expression in glioma cells and enhance SF/HGF-induced cell migration. Int J Cancer 2007;121(2):276—83.
[66] Seol DW, Chen Q, Smith ML, Zarnegar R. Regulation of the *c-met* proto-oncogene promoter by p53. J Biol Chem 1999;274(6):3565—72.
[67] Grugan KD, Vega ME, Wong GS, Diehl JA, Bass AJ, Wong KK, et al. A common p53 mutation (R175H) activates c-Met receptor tyrosine kinase to enhance tumor cell invasion. Cancer Biol Ther 2013;14(9):853—9.
[68] Lui VW, Wong EY, Ho K, Ng PK, Lau CP, Tsui SK, et al. Inhibition of c-Met downregulates TIGAR expression and reduces NADPH production leading to cell death. Oncogene 2011;30(9):1127—34.
[69] Elicin O, Ozsahin M. The latest prospects of investigational drugs for head and neck cancer. Expert Opin Investig Drugs 2017;26(3):265—8.
[70] Walenta S, Mueller-Klieser WF. Lactate: mirror and motor of tumor malignancy. Semin Radiat Oncol 2004;14(3):267—74.
[71] Walenta S, Voelxen NF, Mueller-Klieser W. Lactate—an integrative mirror of cancer metabolism. Recent Results Cancer Res 2016;207:23—37.
[72] Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, et al. Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. Int J Radiat Oncol Biol Phys 2001;51(2):349—53.
[73] Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfør K, Rofstad EK, et al. High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Cancer Res 2000;60(4):916—21.
[74] Martinez-Outschoorn UE, Lisanti MP, Sotgia F. Catabolic cancer-associated fibroblasts transfer energy and biomass to anabolic cancer cells, fueling tumor growth. Semin Cancer Biol 2014;25:47—60.
[75] Whitaker-Menezes D, Martinez-Outschoorn UE, Lin Z, Ertel A, Flomenberg N, Witkiewicz AK, et al. Evidence for a stroma-epithelial lactate shuttle in human tumors: MCT4 is a marker of oxidative stress in cancer-associated fibroblasts. Cell Cycle 2011;10(11):1772—83.
[76] Sanità P, Capulli M, Teti A, Galatioto GP, Vicentini C, Chiarugi P, et al. Tumor-stroma metabolic relationship based on lactate shuttle can sustain prostate cancer progression. BMC Cancer 2014;14:154.
[77] Offermanns S. Free fatty acid (FFA) and hydroxy carboxylic acid (HCA) receptors. Annu Rev Pharmacol Toxicol 2014;54:407—34.
[78] Lee YJ, Shin KJ, Park SA, Park KS, Park S, Heo K, et al. G-protein-coupled receptor 81 promotes a malignant phenotype in breast cancer through angiogenic factor secretion. Oncotarget 2016;7(43):70898—911.
[79] Roland CL, Arumugam T, Deng D, Liu SH, Philip B, Gomez S, et al. Cell surface lactate receptor GPR81 is crucial for cancer cell survival. Cancer Res 2014;74(18):5301—10.
[80] Wamelink MM, Struys EA, Jakobs C. The biochemistry, metabolism and inherited defects of the pentose phosphate pathway: a review. J Inherit Metab Dis 2008;31(6):703—17.
[81] Helmlinger G, Sckell A, Dellian M, Forbes NS, Jain RK. Acid production in glycolysis-impaired tumors provides new insights into tumor metabolism. Clin Cancer Res 2002;8(4):1284—91.
[82] Sennoune SR, Bakunts K, Martínez GM, Chua-Tuan JL, Kebir Y, Attaya MN, et al. Vacuolar H^+-ATPase in human breast cancer cells with distinct metastatic potential: distribution and functional activity. Am J Physiol Cell Physiol 2004;286(6):C1443—52.
[83] Nishisho T, Hata K, Nakanishi M, Morita Y, Sun-Wada GH, Wada Y, et al. The *a*3 isoform vacuolar type H^+-ATPase promotes distant metastasis in the mouse B16 melanoma cells. Mol Cancer Res 2011;9(7):845—55.
[84] Pérez-Sayáns M, Reboiras-López MD, Somoza-Martín JM, Barros-Angueira F, Diz PG, Rey JM, et al. Measurement of ATP6V1C1 expression in brush cytology samples as a diagnostic and prognostic marker in oral squamous cell carcinoma. Cancer Biol Ther 2010;9(12):1057—64.
[85] Cardone RA, Casavola V, Reshkin SJ. The role of disturbed pH dynamics and the Na^+/H^+ exchanger in metastasis. Nat Rev Cancer 2005;5(10):786—95.
[86] Amith SR, Wilkinson JM, Fliegel L. Na^+/H^+ exchanger NHE1 regulation modulates metastatic potential and epithelial-mesenchymal transition of triple-negative breast cancer cells. Oncotarget 2016;7(16):21091—2113.
[87] Karumanchi SA, Jiang L, Knebelmann B, Stuart-Tilley AK, Alper SL, Sukhatme VP. VHL tumor suppressor regulates Cl^-/HCO_3^- exchange and Na^+/H^+ exchange activities in renal carcinoma cells. Physiol Genomics 2001;5(3):119—28.
[88] Walker NM, Simpson JE, Yen PF, Gill RK, Rigsby EV, Brazill JM, et al. Down-regulated in adenoma Cl/HCO_3 exchanger couples with Na/H exchanger 3 for NaCl absorption in murine small intestine. Gastroenterology 2008;135(5):1645—53, e3.
[89] Sautin YY, Lu M, Gaugler A, Zhang L, Gluck SL. Phosphatidylinositol 3-kinase-mediated effects of glucose on vacuolar H^+-ATPase assembly, translocation, and acidification of intracellular compartments in renal epithelial cells. Mol Cell Biol 2005;25(2):575—89.
[90] Reshkin SJ, Cardone RA, Harguindey S. Na^+-H^+ exchanger, pH regulation and cancer. Recent Patents Anticancer Drug Discov 2013;8(1):85—99.
[91] Stock C, Mueller M, Kraehling H, Mally S, Noël J, Eder C, et al. pH nanoenvironment at the surface of single melanoma cells. Cell Physiol Biochem 2007;20(5):679—86.
[92] Krähling H, Mally S, Eble JA, Noël J, Schwab A, Stock C. The glycocalyx maintains a cell surface pH nanoenvironment crucial for integrin-mediated migration of human melanoma cells. Pflugers Arch Eur J Physiol 2009;458(6):1069—83.
[93] Opavský R, Pastoreková S, Zelnik V, Gibadulinova A, Stanbridge EJ, Závada J, et al. Human *MN/CA9* gene, a novel member of the carbonic anhydrase family: structure and exon to protein domain relationships. Genomics 1996;33(3):480—7.
[94] Mahon BP, Pinard MA, McKenna R. Targeting carbonic anhydrase IX activity and expression. Molecules 2015;20(2):2323—48.
[95] Kaluz S, Kaluzová M, Liao SY, Lerman M, Stanbridge EJ. Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: A one transcription factor (HIF-1) show? Biochim Biophys Acta 2009;1795(2):162—72.

[96] Barathova M, Takacova M, Holotnakova T, Gibadulinova A, Ohradanova A, Zatovicova M, et al. Alternative splicing variant of the hypoxia marker carbonic anhydrase IX expressed independently of hypoxia and tumour phenotype. Br J Cancer 2008;98(1):129—36.
[97] Chiche J, Ilc K, Laferrière J, Trottier E, Dayan F, Mazure NM, et al. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. Cancer Res 2009;69(1):358—68.
[98] Choschzick M, Woelber L, Hess S, zu Eulenburg C, Schwarz J, Simon R, et al. Overexpression of carbonic anhydrase IX (CAIX) in vulvar cancer is associated with tumor progression and development of locoregional lymph node metastases. Virchows Arch 2010;456(5):483—90.
[99] Ilie M, Mazure NM, Hofman V, Ammadi RE, Ortholan C, Bonnetaud C, et al. High levels of carbonic anhydrase IX in tumour tissue and plasma are biomarkers of poor prognostic in patients with non-small cell lung cancer. Br J Cancer 2010;102(11):1627—35.
[100] Chen Y, Li X, Wu S, Xu G, Zhou Y, Gong L, et al. Expression of HIF-1α and CAIX in nasopharyngeal carcinoma and their correlation with patients' prognosis. Med Oncol 2014;31(12):304.
[101] Zatovicova M, Sedlakova O, Svastova E, Ohradanova A, Ciampor F, Arribas J, et al. Ectodomain shedding of the hypoxia-induced carbonic anhydrase IX is a metalloprotease-dependent process regulated by TACE/ADAM17. Br J Cancer 2005;93(11):1267—76.
[102] Rosenberg V, Pastorekova S, Zatovicova M, Vidlickova I, Jelenska L, Slezak P. High serum carbonic anhydrase IX predicts shorter survival in head and neck cancer. Bratisl Lek Listy 2016;117(4):201—4.
[103] Smith AD, Truong M, Bristow R, Yip P, Milosevic MF, Joshua AM. The utility of serum CA9 for prognostication in prostate cancer. Anticancer Res 2016;36(9):4489—92.
[104] Carney WP. Circulating oncoproteins HER2/neu, EGFR and CAIX (MN) as novel cancer biomarkers. Expert Rev Mol Diagn 2007;7(3):309—19.
[105] Papworth K, Sandlund J, Grankvist K, Ljungberg B, Rasmuson T. Soluble carbonic anhydrase IX is not an independent prognostic factor in human renal cell carcinoma. Anticancer Res 2010;30(7):2953—7.
[106] Gigante M, Li G, Ferlay C, Perol D, Blanc E, Paul S, et al. Prognostic value of serum CA9 in patients with metastatic clear cell renal cell carcinoma under targeted therapy. Anticancer Res 2012;32(12):5447—51.
[107] Woelber L, Mueller V, Eulenburg C, Schwarz J, Carney W, Jaenicke F, et al. Serum carbonic anhydrase IX during first-line therapy of ovarian cancer. Gynecol Oncol 2010;117(2):183—8.
[108] Fidan E, Mentese A, Ozdemir F, Deger O, Kavgaci H, Caner Karahan S, et al. Diagnostic and prognostic significance of CA IX and suPAR in gastric cancer. Med Oncol 2013;30(2):540.
[109] Hektoen HH, Flatmark K, Andersson Y, Dueland S, Redalen KR, Ree AH. Early increase in circulating carbonic anhydrase IX during neoadjuvant treatment predicts favourable outcome in locally advanced rectal cancer. BMC Cancer 2015;15:543.
[110] Carvalho S, Troost EG, Bons J, Menheere P, Lambin P, Oberije C. Prognostic value of blood-biomarkers related to hypoxia, inflammation, immune response and tumour load in non-small cell lung cancer—a survival model with external validation. Radiother Oncol 2016;119(3):487—94.
[111] Dunger DB, Sperling MA, Acerini CL, Bohn DJ, Daneman D, Danne TP, et al. ESPE/LWPES consensus statement on diabetic ketoacidosis in children and adolescents. Arch Dis Child 2004;89(2):188—94.
[112] Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, et al. Ketones and lactate fuel tumor growth and metastasis: evidence that epithelial cancer cells use oxidative mitochondrial metabolism. Cell Cycle 2010;9(17):3506—14.
[113] Salem AF, Howell A, Sartini M, Sotgia F, Lisanti MP. Downregulation of stromal BRCA1 drives breast cancer tumor growth via upregulation of HIF-1α, autophagy and ketone body production. Cell Cycle 2012;11(22):4167—73.
[114] Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: the bread and butter of the microbiota-gut-brain axis. Neurochem Int 2016;99:110—32.
[115] Scheck AC, Abdelwahab MG, Fenton KE, Stafford P. The ketogenic diet for the treatment of glioma: insights from genetic profiling. Epilepsy Res 2012;100(3):327—37.
[116] Schwartz K, Chang HT, Nikolai M, Pernicone J, Rhee S, Olson K, et al. Treatment of glioma patients with ketogenic diets: report of two cases treated with an IRB-approved energy-restricted ketogenic diet protocol and review of the literature. Cancer Metab 2015;3(3).
[117] Woolf EC, Scheck AC. The ketogenic diet for the treatment of malignant glioma. J Lipid Res 2015;56(1):5—10.
[118] Abdelwahab MG, Fenton KE, Preul MC, Rho JM, Lynch A, Stafford P, et al. The ketogenic diet is an effective adjuvant to radiation therapy for the treatment of malignant glioma. PLoS One 2012;7(5):e36197.
[119] Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science 2013;339(6116):211—4.
[120] Woolf EC, Curley KL, Liu Q, Turner GH, Charlton JA, Preul MC, et al. The ketogenic diet alters the hypoxic response and affects expression of proteins associated with angiogenesis, invasive potential and vascular permeability in a mouse glioma model. PLoS One 2015;10(6):e0130357.
[121] Bourguignon LY, Singleton PA, Diedrich F, Stern R, Gilad E. CD44 interaction with Na^+-H^+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. J Biol Chem 2004;279(26):26991—7007.
[122] Poola I, Abraham J, Marshalleck JJ, Yue Q, Lokeshwar VB, Bonney G, et al. Molecular risk assessment for breast cancer development in patients with ductal hyperplasias. Clin Cancer Res 2008;14(4):1274—80.
[123] Madan AK, Yu K, Dhurandhar N, Cullinane C, Pang Y, Beech DJ. Association of hyaluronidase and breast adenocarcinoma invasiveness. Oncol Rep 1999;6(3):607—9.
[124] Sun X, Zhao D, Li YL, Sun Y, Lei XH, Zhang JN, et al. Regulation of ASIC1 by Ca^{2+}/calmodulin-dependent protein kinase II in human glioblastoma multiforme. Oncol Rep 2013;30(6):2852—8.
[125] Zhang Y, Zhang T, Wu C, Xia Q, Xu D. ASIC1a mediates the drug resistance of human hepatocellular carcinoma via the Ca^{2+}/PI3-kinase/AKT signaling pathway. Lab Invest 2017;97(1):53—69.
[126] Laplante M. Sabatini DM: mTOR signaling in growth control and disease. Cell 2012;149(2):274—93.
[127] Kato Y, Nakayama Y, Umeda M, Miyazaki K. Induction of 103-kDa gelatinase/type IV collagenase by acidic culture conditions in mouse metastatic melanoma cell lines. J Biol Chem 1992;267(16):11424—30.
[128] Kato Y, Ozono S, Shuin T, Miyazaki K. Slow induction of gelatinase B mRNA by acidic culture conditions in mouse metastatic melanoma cells. Cell Biol Int 1996;20(5):375—7.
[129] Kato Y, Lambert CA, Colige AC, Mineur P, Noël A, Frankenne F, et al. Acidic extracellular pH induces matrix metalloproteinase-9 expression in mouse metastatic melanoma cells through the phospholipase

D-mitogen-activated protein kinase signaling. J Biol Chem 2005;280(12):10938—44.
[130] Peppicelli S, Bianchini F, Torre E, Calorini L. Contribution of acidic melanoma cells undergoing epithelial-to-mesenchymal transition to aggressiveness of non-acidic melanoma cells. Clin Exp Metastasis 2014;31(4):423—33.
[131] Riemann A, Schneider B, Ihling A, Nowak M, Sauvant C, Thews O, et al. Acidic environment leads to ROS-induced MAPK signaling in cancer cells. PLoS One 2011;6(7):e22445.
[132] Kato Y, Ozawa S, Tsukuda M, Kubota E, Miyazaki K, St-Pierre Y, et al. Acidic extracellular pH increases calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. FEBS J 2007;274(12):3171—83.
[133] Maeda T, Yuzawa S, Suzuki A, Baba Y, Nishimura Y, Kato Y. RhoA mediates the expression of acidic extracellular pH-induced matrix metalloproteinase-9 mRNA through phospholipase D1 in mouse metastatic B16-BL6 melanoma cells. Int J Oncol 2016;48(3):1251—7.
[134] Fang Y, Vilella-Bach M, Bachmann R, Flanigan A, Chen J. Phosphatidic acid-mediated mitogenic activation of mTOR signaling. Science 2001;294(5548):1942—5.
[135] Hui L, Abbas T, Pielak RM, Joseph T, Bargonetti J, Foster DA. Phospholipase D elevates the level of MDM2 and suppresses DNA damage-induced increases in p53. Mol Cell Biol 2004;24(13):5677—86.
[136] Suzuki A, Maeda T, Baba Y, Shimamura K, Kato Y. Acidic extracellular pH promotes epithelial mesenchymal transition in Lewis lung carcinoma model. Cancer Cell Int 2014;14(1):129.
[137] Peppicelli S, Bianchini F, Toti A, Laurenzana A, Fibbi G, Calorini L. Extracellular acidity strengthens mesenchymal stem cells to promote melanoma progression. Cell Cycle 2015;14(19):3088—100.
[138] Dienstmann R, Rodon J, Serra V, Tabernero J. Picking the point of inhibition: a comparative review of PI3K/AKT/mTOR pathway inhibitors. Mol Cancer Ther 2014;13(5):1021—31.
[139] Vinnars E, Bergström J, Fürst P. Influence of the postoperative state on the intracellular free amino acids in human muscle tissue. Ann Surg 1975;182(6):665—71.
[140] Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer 2016;16(10):619—34.
[141] Matés JM, Segura JA, Martín-Rufián M, Campos-Sandoval JA, Alonso FJ, Márquez J. Glutaminase isoenzymes as key regulators in metabolic and oxidative stress against cancer. Curr Mol Med 2013;13(4):514—34.
[142] Hartman SC. Purines and pyrimidines. In: Greenberg DM, editor. Metaboric pathways. New York and London: Academic Press; 1970. p. 1—68.
[143] Cetinbas NM, Sudderth J, Harris RC, Cebeci A, Negri GL, Yilmaz OH, et al. Glucose-dependent anaplerosis in cancer cells is required for cellular redox balance in the absence of glutamine. Sci Rep 2016;6:32606.
[144] Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 2008;105(48):18782—7.
[145] Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 2003;22(47):7369—75.
[146] Neumann C, Boubakari Grünert R, Bednarski PJ. Nicotinamide adenine dinucleotide phosphate-regenerating system coupled to a glutathione-reductase microtiter method for determination of total glutathione concentrations in adherent growing cancer cell lines. Anal Biochem 2003;320(2):170—8.
[147] Ganea E, Harding JJ. Glutathione-related enzymes and the eye. Curr Eye Res 2006;31(1):1—11.
[148] Ying W. NAD^+/NADH and $NADP^+$/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid Redox Signal 2008;10(2):179—206.
[149] Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature 2013;496(7443):101—5.
[150] Alfarouk KO. Tumor metabolism, cancer cell transporters, and microenvironmental resistance. J Enzyme Inhib Med Chem 2016;31(6):859—66.
[151] Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. Cancer Lett 2008;266(1):37—52.
[152] Tew KD. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 1994;54(16):4313—20.
[153] Riemann A, Schneider B, Gündel D, Stock C, Gekle M, Thews O. Acidosis Promotes Metastasis Formation by Enhancing Tumor Cell Motility. Adv Exp Med Biol 2016;876:215—20.
[154] Riemann A, Ihling A, Schneider B, Gekle M, Thews O. Impact of extracellular acidosis on intracellular pH control and cell signaling in tumor cells. Adv Exp Med Biol 2013;789:221—8.
[155] Hiramoto K, Satoh H, Suzuki T, Moriguchi T, Pi J, Shimosegawa T, et al. Myeloid lineage-specific deletion of antioxidant system enhances tumor metastasis. Cancer Prev Res (Phila) 2014;7(8):835—44.
[156] Le Gal K, Ibrahim MX, Wiel C, Sayin VI, Akula MK, Karlsson C, et al. Antioxidants can increase melanoma metastasis in mice. Sci Transl Med 2015;7(308):308re8.
[157] Piskounova E, Agathocleous M, Murphy MM, Hu Z, Huddlestun SE, Zhao Z, et al. Oxidative stress inhibits distant metastasis by human melanoma cells. Nature 2015;527(7577):186—91.
[158] Wang H, Liu X, Long M, Huang Y, Zhang L, Zhang R, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. Sci Transl Med 2016;8(334):334ra51.
[159] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011;11(2):85—95.
[160] Jewell JL, Russell RC, Guan KL. Amino acid signalling upstream of mTOR. Nat Rev Mol Cell Biol 2013;14(3):133—9.
[161] Chong ZZ, Maiese K. Mammalian target of rapamycin signaling in diabetic cardiovascular disease. Cardiovasc Diabetol 2012;11:45.
[162] Shimobayashi M, Hall MN. Multiple amino acid sensing inputs to mTORC1. Cell Res 2016;26(1):7—20.
[163] Dekanty A, Lavista-Llanos S, Irisarri M, Oldham S, Wappner P. The insulin-PI3K/TOR pathway induces a HIF-dependent transcriptional response in *Drosophila* by promoting nuclear localization of HIF-α/Sima. J Cell Sci 2005;118(Pt. 23):5431—41.
[164] Nemazanyy I, Espeillac C, Pende M, Panasyuk G. Role of PI3K, mTOR and Akt2 signalling in hepatic tumorigenesis via the control of PKM2 expression. Biochem Soc Trans 2013;41(4):917—22.
[165] Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, et al. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. Science 2014;345(6204):1250684.
[166] Miyazaki M, Miyazaki K, Chen S, Chandra V, Wagatsuma K, Agata Y, et al. The E-Id protein axis modulates the activities of the PI3K-AKT-mTORC1-Hif1a and c-myc/p19Arf pathways to suppress innate variant T_{FH} cell development, thymocyte expansion, and lymphomagenesis. Genes Dev 2015;29(4):409—25.
[167] Xu BH, Li XX, Yang Y, Zhang MY, Rao HL, Wang HY, et al. Aberrant amino acid signaling promotes growth and metastasis of hepatocellular carcinomas through Rab1A-dependent activation of mTORC1 by Rab1A. Oncotarget 2015;6(25):20813—28.

[168] Jung CH, Ro SH, Cao J, Otto NM. Kim DH mTOR regulation of autophagy. FEBS Lett 2010;584(7):1287—95.
[169] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008;320(5882):1496—501.
[170] Budanov AV. Karin M: p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell 2008;134(3):451—60.
[171] Zhou G, Wang J, Zhao M, Xie TX, Tanaka N, Sano D, et al. Gain-of-function mutant p53 promotes cell growth and cancer cell metabolism via inhibition of AMPK activation. Mol Cell 2014;54(6):960—74.
[172] Ganley IG, Lam du H, Wang J, Ding X, Chen S, Jiang X. ULK1. ATG13. FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 2009;284(18):12297—305.
[173] Egan D, Kim J, Shaw RJ, Guan KL. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. Autophagy 2011;7(6):643—4.
[174] Venneti S, Dunphy MP, Zhang H, Pitter KL, Zanzonico P, Campos C, et al. Glutamine-based PET imaging facilitates enhanced metabolic evaluation of gliomas *in vivo*. Sci Transl Med 2015;7(274):274ra17.
[175] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367(6464):645—8.
[176] Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3(7):730—7.
[177] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100(7):3983—8.
[178] Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med 2004;351(7):657—67.
[179] Kurth I, Hein L, Mäbert K, Peitzsch C, Koi L, Cojoc M, et al. Cancer stem cell related markers of radioresistance in head and neck squamous cell carcinoma. Oncotarget 2015;6(33):34494—509.
[180] Habu N, Imanishi Y, Kameyama K, Shimoda M, Tokumaru Y, Sakamoto K, et al. Expression of Oct3/4 and Nanog in the head and neck squamous carcinoma cells and its clinical implications for delayed neck metastasis in stage I/II oral tongue squamous cell carcinoma. BMC Cancer 2015;15:730.
[181] Prochazka L, Tesarik R, Turanek J. Regulation of alternative splicing of CD44 in cancer. Cell Signal 2014;26(10):2234—9.
[182] Takayama T, Kubo T, Morikawa A, Morita T, Nagano O, Saya H. Potential of sulfasalazine as a therapeutic sensitizer for CD44 splice variant 9-positive urogenital cancer. Med Oncol 2016;33(5):45.
[183] Seishima R, Okabayashi K, Nagano O, Hasegawa H, Tsuruta M, Shimoda M, et al. Sulfasalazine, a therapeutic agent for ulcerative colitis, inhibits the growth of CD44v9$^+$ cancer stem cells in ulcerative colitis-related cancer. Clin Res Hepatol Gastroenterol 2016;40(4):487—93.
[184] Wada T, Ishimoto T, Seishima R, Tsuchihashi K, Yoshikawa M, Oshima H, et al. Functional role of CD44v-xCT system in the development of spasmolytic polypeptide-expressing metaplasia. Cancer Sci 2013;104(10):1323—9.
[185] Shitara K, Doi T, Nagano O, Imamura CK, Ozeki T, Ishii Y, et al. Dose-escalation study for the targeting of CD44v$^+$ cancer stem cells by sulfasalazine in patients with advanced gastric cancer (EPOC 1205). Gastric Cancer 2017;20(2):341—9.
[186] Iliopoulos D, Hirsch HA, Wang G, Struhl K. Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. Proc Natl Acad Sci U S A 2011;108(4):1397—402.