REVIEW ARTICLE

The fuel and engine: The roles of reprogrammed metabolism in metastasis of primary liver cancer

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Received 22 October 2019; received in revised form 30 December 2019; accepted 28 January 2020
Available online 7 February 2020

Abstract

Metastasis and metabolism reprogramming are two major hallmarks of cancer. In the initiation and progression of cancer, tumor cells are known to undergo fundamental metabolic changes to sustain their development and progression. In recent years, much more attention has been drawn to their important roles in facilitating cancer metastasis through regulating the biological properties. In this review, we summarized the recent progresses in the studies of metabolism reprogramming of cancer metastasis, particularly of primary liver cancer, and highlight their potential applications.

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Introduction

Primary liver cancer (mainly hepatocellular carcinoma, HCC) is a global health problem that leading to over 780,000 deaths annually, which is the fourth common cause of cancer-related death in the world.1,2 Although many progresses in HCC diagnosis and treatment have been made during the past decades, its prognosis is still very dismal because of the high probability of metastasis, even after curative treatments.1,2 Therefore, understanding the mechanisms of HCC metastasis is of great importance in developing effective approaches to further improve HCC prognosis. In addition of metastasis, metabolic reprogramming is another hallmark of cancer,3 which is necessary for cancer cells to fulfill the bioenergetic and biosynthetic needs to sustain their faster proliferation and adapt to tumor microenvironment. Recently, accumulating studies have revealed that the plasticity in tumor metabolic programs, including glucose, amino acids as well as lipid...
metabolism, plays important roles in each step of meta-
static cascade of cancer including HCC.

**Glycometabolism reprogramming**

During the development and progression of malignancies, cancer cells are known to undergo complicated metabolic disorders, known as metabolic reprogramming. Reprogrammed glucose metabolism is the earliest and most extensively studied cancer-specific metabolic disorder, which is regarded as the highlight of this reprogramming process in cancer. In normal cells, glucose is metabolized to generate energy by two main ways: glycolysis in the cyto-
plasm and oxidative phosphorylation (OXPHOS) in mito-
chondria. Cells use both pathways but rely overwhelm-
ingly on OXPHOS. In 1920s, German biochemist Otto Warburg firstly revealed that tumor cells relied on glycolysis even in the presence of oxygen—the "Warburg effect". In this process, tumor cells take up more glucose for glycolysis and produced more lactate. Nowadays, accumulating evidences suggested that the shift in energy production from OXPHOS to glycolysis is a fundamental hallmark, not an epiphe-
nonomenon of cell transformation. The roles and related mechanisms of reprogrammed glucose metabolism in metastasis will be summarized.

**Glucose uptake and glycolysis**

Glucose uptake is the first committed step in glucose metabolism and increased glucose uptake is among the most remarkable hallmarks of cancer metabolism. Although the glycolytic rate can be constrained at many steps in the glycolytic pathway, most studies attributed the vigorous glycolytic flux in highly aggressive tumors to increased glucose uptake. The glucose transport by glucose transporters (GLUTs) and the phosphorylation of glucose to glucose-6-phosphate (G6P) by hexokinases (HKs) are the two major steps responsible for glucose uptake by trapping glucose inside cells. Clinically, the positron emission to-
mography (PET) has long been used as a cogent method for tumor diagnosis and treatment-response monitoring.

The increased glucose uptake capability in aggressive tumors is largely attributed to the up-regulation of glucose transporters (the family of glucose transporters (GLUTs) notably GLUT1, which play an important role in glucose transportation from the plasma membrane to cytoplasm and glycolysis) and type 2 hexokinase (HK2). Consistently, high expression of GLUT1 or HK2 was found to significantly correlate with invasiveness and metastasis of several can-
cers including HCC. Silencing of GLUT1 or HK2 expres-
sion or inhibition of their activity, could significantly decrease glucose uptake and glycolytic rate of cancer cells, and further inhibit cancer cell proliferation and invasion.

Mechanistically, the ectopic expression of GLUT1 or HK2 in tumors is under the regulation of a complicated genetic network, such as ras activation or TP53 inactivation. In addition, the hypoxic microenvironment of invasive tu-
mors is another critical modulator of increased glucose uptake. Under hypoxia, the stabilization of HIF1 directly target GLUT and HK genes and up-regulate their expressions, thus promoted the glucose uptake to meet the needs of increased glycolysis. Consistently, the hypoxia increases the invasive and metastatic potentials of cancer cells in both clinical and experimental observations. All these evidences establish a fundamental link between glucose uptake and cancer metastatic potential, which holds great therapeutic promises.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is commonly used as a reporter gene to estimate the amount of RNA present in Northern analyses. However, collecting reports have revealed the aberrant expression of GAPDH in malignancies, which suggest that it may play a role in tumor glycolysis. Gong et al found that GAPDH mRNA levels in HCC were significantly higher (14–16 folds) than those in adjacent non-HCC and normal liver tissues. GAPDH mRNA gene expression might serve as a diagnostic indicator for human HCC.

**Pyruvate metabolism**

In Warburg’s theory, the aerobic glycolysis of tumor cells was attributed to mitochondria dysfunction, which was frustrated by later observations that the respiration func-
tion of most tumor cells was intact. For a long time, the reason why tumor cells adopt a relatively primitive and inefficient glucose metabolism pattern remained elusive. In recent years, the pyruvate metabolism, a crucial interme-
diate process in glycolytic pathway, is found to play a pivotal role in glycolytic flux regulation of cancer cells.

The glycolytic intermediate phosphoenolpyruvate (PEP) is catalyzed to pyruvate by pyruvate kinases (PKs), which include 4 isozymes: PKLR gene encoded L (PKL) and R (PKR) isoforms, and PKM2 gene generated M1 (PKM1) and M2 (PKM2) isoforms. PKM2 is the predominant PK in embryos, which is gradually replaced by other PKs in a tissue-specific manner in adults. Ectopic expression of PKM2 has long been observed in nearly all cancers, and was associated with poor prognosis and aggressive phenotypes.

Furthermore, the non-metabolic role of PKM2 in tumor progression has also emerged. PKM2 isoform-specific dele-
tion revealed that the inactive state of PKM2 is associated with the proliferating cell population within tumors, whereas non-proliferating tumor cells require active pyru-
vate kinase. Then PKM2 was identified as a phosphoty-
orosine binding protein. Upon specific metabolites activation or some posttranslational modifications, such as phosphorylation, acetylation or oxidation, the dimeric PKM2 can translocate to the nucleus, where it can interact with β-catenin, HIF-1α or STAT-3 and thus modulate transcriptional regulation. All these metabolic and non-metabolic effects of PKM2 reveal its versatile role in tumor progression.

The regulation of pyruvate fate in mitochondria is also an important determinant of anabolic versus catabolic metab-
olism. In normal cells, pyruvate is mainly catalyzed to acetyl CoA by pyruvate dehydrogenase (PDH), thus linking glycolysis to the TCA cycle in mitochondria, or fermented to lactate in cytoplasm under hypoxia. The oncogenic activa-
tion of PDK1 is regulated by a complicated network including both intrinsic alterations and extrinsic environ-
ment. This PDK1-dependent metabolic reprogramming also
involved in cancer metastasis. The liver metastases also rely on the HIF-1α/PDK1-dependent axis for their intrinsic metabolic reprogramming. PDK1 sets in motion a glycolytic shift by inactivating PDH, which blocks the conversion of pyruvate to acetyl-CoA and suppresses the TCA cycle, which enables their efficient colonization and growth in the liver.37

The mitochondrial pyruvate carrier (MPC), which transports pyruvate from the cytosol into the mitochondrial matrix, was another critical regulator of pyruvate fate.38 MPC activity is encoded by two genes MPC1 and MPC2. Both proteins are required for activity because loss of one leads to destabilization and loss of the other and thus loss of the MPC complex.39,40 Decreased MPC activity could drive tumorigenic glucose utilization by preventing mitochondrial pyruvate uptake and oxidation, and promote cancer proliferation, which has been proved in various cancer types.41 A recent study found that the wild-type p53 promotes liver cancer metabolic switch by disrupting MPC function, resulting in decreased mitochondrial pyruvate uptake and increased glycolysis in HCCs and poor prognosis of HCC patients.42 However, another study indicated that MPC ablation markedly impairs HCC tumorigenesis by inducing metabolic competition for glutamine and limiting glutathione synthesis.43 The paradoxical findings of existing studies indicated that the association between MPC and liver cancer metastasis still needs further investigations.

In recent studies, tumors harboring mutations in the TCA cycle enzymes fumarate hydratase (FH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH) promote liver cancer epithelial-to-mesenchymal transition which is an initial stage of metastasis.44 It has also been demonstrated that mitochondrial oxidative phosphorylation is related to liver cancer metastasis.45

Lactate accumulation and reutilization

Activation of the glycolysis pathway in cancer cells not only provides sufficient ATP for tumor progression but also produces acid by-products such as lactate. Accumulation of acids in cells results in apoptosis. To avoid this, monocarboxylate transporters (MCTs) are up-regulated in cancer cells to speed up the export of lactate into the extracellular milieu. MCT4 was reported to be associated with a poor prognosis as well as cell proliferation and migration of HCC.48 Upregulation of MCT1 expression could induce autophagy and Wnt/β-catenin signaling pathway activation to promote metastasis and glycolysis in HCC cells.49

Lactate dehydrogenase (LDH) is needed for the conversion of pyruvate to lactate. LDHA plays a critical role in promoting glycolysis and reducing the oxygen dependency of cancer cells.50,51 LDHA is demonstrated to be up-regulated in HCC and promote tumor growth and metastasis.52 Several studies have indicated that LDH could serve as a biomarker for prognosis prediction and treatment selection in HCC patients.53,54

As the homozygous tetramer of LDH-A, LDH5 is mainly responsible for lactate production and elevated expression of LDH5 in tumor tissue is found to be closely related to invasive phenotypes and metastasis of multiple cancer types.55 The aberrant activation of oncogenes (c-Myc, HIF-1α, ErbB2, KRAS) can activate LDH-A transcription,56 while the K5 acetylation of LDH-A, which is decreased in pancreatic cancers, is found to facilitate LDH-A degradation.57 LDH-A plays an important role for tumor growth and invasion under hypoxia,51,58 and knockdown of LDH-A or inhibition of its activity can induce reactive oxygen species (ROS)-mediated apoptosis, and significantly attenuate the invasion and metastasis of cancer cells.50,52 which is not surprising since LDH-A knockdown can rectify the pyruvate metabolism towards TCA cycle and OXPHOS in the mitochondria. It has been revealed that mitochondrial biogenesis and OXPHOS are also essential for functional motility of cancer cells and metastasis,59 indicating that glycolysis and OXPHOS may converge to promote malignant metastasis.

Reprogrammed glutaminolysis

HCC metastasis is usually accompanied by other metabolic alterations, particularly glutaminolysis.6 This process is accomplished through the conversion of glutamine to α-ketoglutarate (α-KG) via a two-step deamination reaction catalyzed by glutaminases and glutamate dehydrogenase 1 (GLUD1).60 Glutaminase 1 (GLS1) is one key enzyme in glutaminolysis.61 Targeting GLS1 could inhibit in vivo metastasis through suppressing SNAI1.62 However, the expression level of GLS2, the mitochondrial isoform of glutaminase, was inversely correlated to tumor stage and prognosis of HCC.63 Interestingly, MYC could induce the switch from GLS2 to GLS1 to reprogram the glutamine metabolism in mouse liver tumors.64 Alanine, serine, cysteine-preferring transporter 2 (ASCT2) is a major glutamine transporter. ASCT2 is overexpressed and correlated with improved glutamine utilization in cancer.65

Lipid metabolism reprogramming

Lipids are a class of water-insoluble molecules, including triglycerides, phospholipids, sphingolipids, and sterols. The key metabolite of lipid metabolism, fatty acid, is a kind of molecule composed of a terminal carboxyl group and a hydrocarbon chain. It is an important component of lipid molecules including triglycerides, phospholipids and cholesterol esters. Lipids are important structural components of cell membrane, and they also play important roles in signal transduction and hormone synthesis.66

Fatty acid metabolism

In addition to the Warburg effect, tumor cells can also use other substrates to fuel mitochondrial metabolism and support growth and metastasis.67 Among the mitochondrial fuels, fatty acids appear as major contributors to the pool of acetyl-CoA to feed the TCA cycle and to support oxidative phosphorylation.68 As many other cancers, HCC cells show a high rate of de novo lipid synthesis.69 Sterol regulatory element-binding protein 1 (SREBP-1) is a transcriptional factor that controls the expression of various lipogenic enzymes. Large-scale gene expression profiling revealed that SREBP-1 activated lipogenic pathway, and was associated with a poor prognosis of HCC.70
Furthermore, the expression and activity of many key enzymes involved in de novo fatty acid synthesis, such as acetyl-CoA carboxylase (ACC), Stearoyl-CoA desaturase (SCD) and fatty acid synthase (FASN), are upregulated and associated with poor clinical outcomes in many types of cancers including HCC.83,69,71–73

In addition to fatty acid biosynthesis, reprogramming of fatty acid oxidation (FAO) is also associated with liver cancer metastasis. Knockout of peroxisomal acylcoenzyme a oxidase 1 (ACOX1), one key enzyme of the FAO pathway, led to HCC development in mice.74 Carnitine palmitoyl transferase 1 (CPT1), another key regulatory enzyme in FAO, was shown to regulate apoptosis and cancer development.75 It has been demonstrated that CD147 downregulated CPT1 and ACOX1 to promote HCC metastasis.76

Cholesterol metabolism

Cholesterol is an essential component of mammalian cell membrane, as well as the precursor of bile acids and steroid hormones.77,78 Many clinical and experimental evidences indicate that cholesterol plays an important role in physiological hyperproliferative conditions and in carcinogenesis.79,80 The dysregulation of key genes involved in cholesterol metabolism has been shown to be associated with HCC progression. Liver X receptor (LXR) is also an important ligand-dependent transcription factor of cholesterol metabolism, the activation of LXR is reversely associated with cellular cholesterol levels.81 Low LXR expression status in tumor samples is associated with advanced tumor stage and metastasis of HCC.82 A recent study has reported that inhibiting SREBP-2, a transcription factor that regulates expression of key genes involved in cholesterol biosynthesis, could suppress HCC progression.83 Furthermore, Acyl-coenzyme A: cholesterol acyltransferase 2 (ACAT2) was up-regulated in a subset of HCC, and functioned to eliminate excess oxysterols for tumor development.84

Cholesterol forms lipid rafts in specific regions with sphingolipids in cell membrane. Lipid rafts regulates signaling molecule assembly, membrane fluidity, protein trafficking in cell transformation and cancer progression.85–87 A recent study showed that cholesterol inhibits HCC invasion and metastasis by promoting CD44 localization in lipid rafts.88

Acetyl-CoA metabolism

Acetyl-CoA is the precursor of lipid biosynthesis to sustain the rapid proliferation of tumor cells.89 More importantly, as a substrate for protein acetylation, the level of intracellular acetyl-CoA is closely associated with the histone acetylation level, and plays an important role in epigenetic regulation.89 Acetyl-CoA metabolism is therefore essential for the regulation of various cellular processes including cell growth, cell apoptosis, cell migration and autophagy. Glycolysis, β-oxidation of fatty acids, and catabolism of glutamine are the main sources of acetyl-CoA production in mammalian cells.89,90 And acetyl-CoA can also be hydrolyzed by Acyl-CoA thioesterase 12 (ACOT12).91 While in cancer cells, acetate can also be utilized to generate acetyl-CoA.92 In our recent study, we found that the downregulation of ACOT12 was correlated with HCC metastasis and poor survival of HCC patients, and that down-regulation of ACOT12 increased acetyl-CoA levels and promoted HCC metastasis by the epigenetic induction of epithelial–mesenchymal transition (EMT).93 We also observed an elevated Acetyl-coA levels in metastatic HCC tissues in this study.93 Acetyl-CoA metabolic alterations have also been shown to be associated with cancer metastatic phenotype in several other cancers including breast cancer, glioblastoma and prostate cancer.94–96 These studies suggest that acetyl-coA may be a pro-metastasis metabolite.

Microenvironmental metabolic reprogramming and liver cancer metastasis

Tumor microenvironment (TME) is the "soil" that support tumor cell growth and metastasis. It not only acts as a scaffold, but also secretes a large number of growth factors and cytokines. It consists of stromal cells, inflammatory immune cells, vasculature, and extracellular matrix (ECM).97,98 There are a variety of stromal cells and inflammatory immune cells in TME, including cancer-associated fibroblasts (CAFs), macrophage, myeloid-derived suppressor cells (MDSC), dendritic cells, and regulatory T cells (Treg), etc. In comparison with the corresponding immune cells in peripheral lymphoid organs, these tumor-associated immune cells exhibit different activation states and functions. They secrete a variety of cytokines, act on tumor cells, activate their key signaling pathways, promote tumor cell proliferation, invasion and metastasis, and angiogenesis.99,100 The immunosuppressive state has been observed in many solid tumors including HCC.101–103

Recent studies have shown that metabolic changes of either cancer cells or TME cells play a crucial role in the activation and function of immune cells. As the major component of the tumor stroma, CAFs play critical roles in tumor initiation and metastasis by producing ECM-degrading enzymes and secreting growth factors and cytokines.104,105 Enhanced aerobic glycolysis has been demonstrated in CAFs.106,107 These CAFs support tumor cell survival and favor cancer metastasis by providing their glycolytic end-product lactate to neighboring cancer cells as fuel.108 The real mechanism of the metabolic reprogramming in CAFs is not clear yet although the PKM2 driven glycolysis and other possible signal pathways have reported.109–112

Tumor associated macrophage (TAM), which undergo abnormal polarization (M1 to M2 switch) in response to tumor microenvironment, is a key promoter of cancer metastasis by acquiring angiogenic and immunosuppressive properties.103,113 The metabolic profile of M1-like TAMs is characterized by an enhanced glycolysis, PPP and FA synthesis, and a truncated TCA cycle leading to an accumulation of succinate and citrate; M2-like TAMs exhibit a decreased glycolysis and PPP, and FA oxidation (FAO), and promotes HCC progression.114,115

Metabolic alteration is defined as a key regulatory way of T cell function. Deficiency in the glucose transporter Glut1 leads to an inability of T cells to acquire sufficient
Conversely, regulatory T cells (Tregs) are less dependent on glucose and more reliant on mitochondrial oxidative metabolism of lipids. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation.

In addition to the metabolic reprogramming of stromal cells or immune cells, the formidable glucose uptake capability of cancer cells also exacerbate the low-glucose condition in TME, which in turn, affects many stromal cells in this milieu. To perform proper effector function in different environments, activated T cells also engage aerobic glycolysis for the need of rapid proliferation by modulating mitochondrial dynamics. Many studies have showed that within the tumor niche, tumor cells and anti-tumor T cells competed for the limited glucose, and tumor cells won this game by virtue of elevated glucose uptake. Eventually, this glucose consumption by tumors metabolically restricts T cells, leading to their dampened mTOR activity, glycolytic capacity, and IFN-γ production, thereby allowing tumor progression.

Metabolite reprogramming profile and liver cancer metastasis based on metabolomics

Given that the metabolic reprogramming in primary liver cancer and its metastatic disease is a highly complicated process that requires the coordination of diverse intertwined metabolic pathways, a large-scale and comprehensive analysis of cancer cell metabolism is required to understand the mechanisms and functional consequences of metabolic alterations associated with metastasis. Metabolomics thus serves as a powerful tool combining high-throughput methodologies based on mass spectrometry or nuclear magnetic resonance spectroscopy (NMR), with multivariate data analysis, thus permitting comparison of "global" metabolite profiles between different groups of samples. Several metabolic targets have been identified by metabolomics approaches, and they could be used as biomarkers in liver cancer. Besides, metabolomics can be integrated with other omics to provide a more accurate profile of HCC. For instance, integration of metabolite and gene expression profiles identified a lipogenic network which involves stearoyl-CoA-desaturase (SCD) and palmitate signaling and was associated with HCC progression and patient outcomes. Another tissue metabolomics study yielded precise biochemical information regarding HCC tumor metabolic rewiring from mitochondrial oxidation to aerobic glycolysis by gas chromatography-mass spectrometry (GCMS)-based metabolomics. All these studies have deepened our understanding on the mechanism of metabolic reprogramming in liver cancer metastasis.

Conclusions

In conclusion, metabolic reprogramming plays critical roles in liver cancer progression, which is reflected by the fundamental changes in various metabolic genes and metabolites. Moreover, metabolic reprogramming occurs not only in tumor cells, but also in the stromal cells and immune cells in microenvironment. Based on these metabolic reprogramming, many therapies targeting specific metabolic pathway have also been explored. As liver is the most important organ for lipid metabolism, the reprogrammed lipid metabolism may be unique during primary liver cancer metastasis, compared to other reprogrammed pathways such as glucose metabolism. In addition to the reprogrammed metabolism of glucose and lipids we summarized above, recent studies found that the reprogrammed anaerobic pathways of protein and nucleic acids, as well as reprogrammed redox homeostasis also play critical roles in primary liver cancer progression. As these sub-fields are not the focus of the current review, further investigations and reviews concerning these topics is needed.

Future perspective

Metastasis is a multi-step process from primary organs to metastasizing organs. Most studies focused on metabolic reprogramming of primary tumors, few on metastatic tumors. In the future, we should pay more attention on the following two respects: First, metastatic tumors are dormant when they reach to target organs. How dormant seeds change their metabolic pattern to adapt proliferation needs in distant organ? Second, are there any metabolic changes in the microenvironment of distant organs cells to help the growth of metastatic tumors? Answers to these two questions are helpful to discover novel therapeutic targets for combating HCC metastasis.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by National Major Science and Technology Projects of China (No.2017ZX10203207) and National Natural Science Foundation of China (No. 81472677).

References

1. Villanueva A. Hepatocellular carcinoma. N Engl J Med. 2019; 380:1450-1462.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-674.
4. Lehuede C, Dupuy F, Rabinovitch R, et al. Metabolic plasticity as a determinant of tumor growth and metastasis. Canc Res. 2016;76:5201-5208.
5. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8:519-530.
6. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metabol. 2016;23:27-47.
7. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Canc*. 2004;4:891–899.

8. Wu N, Zheng B, Shaywitz A, et al. AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. *Mol Cell*. 2013;49:1167–1175.

9. Younes M, Lechago LV, Somoano JR, et al. Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers. *Canc Res*. 1996;56:1164–1167.

10. Airley R, Loncaster J, Davidson S, et al. Glucose transporter glut-1 expression correlates with tumor hypoxia and predicts metastasis-free survival in advanced carcinoma of the cervix. *Clin Canc Res*. 2001;7:928–934.

11. Botzer LE, Maman S, Sagii-Assif O, et al. Hexokinase 2 is a determinant of neuroblastoma metastasis. *Br J Canc*. 2016;114:759–766.

12. Wolf A, Agnihotri S, Micallef J, et al. Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. *J Exp Med*. 2011;208:313–326.

13. Macheda ML, Rogers S, Best JD. Molecular and cellular regulation of the human erythrocyte glucose transporter Glut-1. *Cell Physiol*. 2005;202:654–662.

14. Amann T, Haegedefrau U, Hartmann A, et al. GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *Am J Pathol*. 2009;174:1544–1552.

15. Gong L, Cui Z, Chen P, et al. Reduced survival of patients with hepatocellular carcinoma expressing hexokinase II. *Med Oncol*. 2012;29:909–914.

16. Chan DA, Sutphin PD, Nguyen P, et al. Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. *Sci Transl Med*. 2011;3:94ra70.

17. Patra KC, Wang Q, Bhaskar PT, et al. Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Canc Cell*. 2013;24:213–228.

18. Wang L, Xiong H, Wu F, et al. Hexokinase 2-mediated Warburg effect is required for PTEN- and p53-deficiency-driven prostate cancer growth. *Cell Rep*. 2014;8:1461–1474.

19. Viticchie G, Agostini M, Lena AM, et al. p63 supports aerobic respiration through hexokinase II. *Proc Natl Acad Sci U S A*. 2015;112:11577–11582.

20. Guo W, Qiu Z, Wang Z, et al. MiR-199a-5p is negatively associated with malignancies and regulates glycolysis and lactate production by targeting hexokinase 2 in liver cancer. *Hepatology*. 2015;62:1132–1144.

21. Harris AL. Hypoxia: a key regulatory factor in tumour growth. *Nat Rev Canc*. 2002;2:38–47.

22. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat Rev Canc*. 2014;14:430–439.

23. Lu X, Kang Y. Hypoxia and hypoxia-inducible factors: master regulators of metastasis. *Clin Canc Res*. 2010;16:5928–5935.

24. Guo C, Liu S, Sun MZ. Novel insight into the role of GAPDH playing in tumor. *Clin Transl Oncol*. 2013;15:167–172.

25. Gong Y, Cui L, Minuk GY. Comparison of glyceraldehyde-3-phosphate dehydrogenase and 28s-ribosomal RNA gene expression in human hepatocellular carcinoma. *Hepatology*. 1996;23:734–737.

26. Christofk HR, Vander HM, Harris MH, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*. 2008;452:230–233.

27. Yu G, Wu Y, Jin G, et al. PKM2 regulates neural invasion of and predicts poor prognosis for human hilar cholangiocarcinoma. *Mol Canc*. 2015;14:193.

28. Israelsen WJ, Dayton TL, Davidson SM, et al. PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. *Cell*. 2013;155:397–409.

29. Christofk HR, Vander HM, Wu N, et al. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature*. 2008;452:181–186.

30. Anastasiou D, Yu Y, Israelsen WJ, et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. *Nat Chem Biol*. 2012;8:839–847.

31. Yang W, Xia Y, Ji H, et al. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature*. 2011;480:118–122.

32. Iansante V, Choy PM, Fung SW, et al. PARP14 promotes the Warburg effect in hepatocellular carcinoma by inhibiting JNK1-dependent PKM2 phosphorylation and activation. *Nat Commun*. 2015;6:7882.

33. Lv L, Li D, Zhao D, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. *Mol Cell*. 2011;42:719–730.

34. Anastasiou D, Poulogiannis G, Asara JM, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science*. 2011;334:1278–1283.

35. Luo W, Hu H, Chang R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell*. 2011;145:732–744.

36. Gao X, Wang H, Yang JJ, et al. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol Cell*. 2012;45:598–609.

37. Dupuy F, Tabaries S, Andrzejewski S, et al. PDK1-Dependent metabolic reprogramming dictates metastatic potential in breast cancer. *Cell Metabol*. 2015;22:577–589.

38. Papa S, Francavilla A, Paradies G, et al. The transport of pyruvate in rat liver mitochondria. *FEBS Lett*. 1971;12:285–288.

39. Bricker DK, Taylor EB, Schell JC, et al. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. *Science*. 2012;337:96–100.

40. Herzig S, Raemy E, Montessuit S, et al. Identification and functional expression of the mitochondrial pyruvate carrier. *Science*. 2012;337:93–96.

41. Schell JC, Olson KA, Jiang L, et al. A role for the mitochondrial pyruvate carrier as a repressor of the Warburg effect and colon cancer cell growth. *Mol Cell*. 2014;56:400–413.

42. Kim J, Yu L, Chen W, et al. Wild-type p53 promotes cancer metabolic switch by inducing PUMA-dependent suppression of oxidative phosphorylation. *Canc Cell*. 2019;35(2):191–203.

43. Tompkins SC, Sheldon RD, Rauchhorst AJ, et al. Disrupting mitochondrial pyruvate uptake directs glutamine into the TCA cycle away from glutathione synthesis and impairs hepatocellular tumorigenesis. *Cell Rep*. 2019;28(10):2608–2619.

44. Grassian AR, Lin F, Barrett R, et al. Isocitrate dehydrogenase (IDH) mutations promote a reversible ZEB1/microRNA miR192-200-dependent epithelial-mesenchymal transition (EMT). *J Biol Chem*. 2012;287:42180–42194.

45. Loriot C, Burnichon N, Gadessaud N, et al. Epithelial to mesenchymal transition is activated in metastatic pheochromocytomas and paragangliomas caused by SDHB gene mutations. *J Clin Endocrinol Metab*. 2012;97:E954–E962.

46. Sciacovelli M, Goncalves E, Johnson TI, et al. Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. *Nature*; 2016;537:544–547.

47. Wu W, Zheng X, Wang J, et al. O-GlcNAcylation on Rab3A attenuates its effects on mitochondrial oxidative
phosphorylation and metastasis in hepatocellular carcinoma. Cell Death Dis. 2018;9:970.

48. Gao HJ, Zhao MC, Zhang YJ, et al. Monocarboxylate transporter 4 predicts poor prognosis in hepatocellular carcinoma and is associated with cell proliferation and migration. J Canc Res Clin Oncol. 2015;141:1151–1162.

49. Fan Q, Yang L, Zhang X, et al. Autophagy promotes metastasis and glycolysis by upregulating MCT1 expression and Wnt/β-catenin signaling pathway activation in hepatocellular carcinoma cells. J Exp Clin Canc Res. 2018;37:9.

50. Le A, Cooper CR, Gouw AM, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci U S A. 2010;107:2037–2042.

51. Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A defines metabolic reprogramming and therapeutic susceptibility. Cell Metabol. 2008;7:11

52. Shi L, Tu BP. Acetyl-CoA carboxylase-a as a novel target for cancer therapy. Front Biosci. 2010;2:515–526.

53. Yang Z, Qin W, Chen Y, et al. Cholesterol inhibits hepatocellular carcinoma invasion and metastasis by promoting CD44 localization in lipid rafts. Canc Lett. 2014;344.

54. Le A, Cooper CR, Gouw AM, et al. Inhibition of lactate dehydrogenase A suppresses tumor growth and metastasis of human hepatocellular carcinoma. FEBS J. 2012;279:3898–3910.

55. Huang J, Viswakarma N, Yu S, et al. Progressive endoplasmic reticulum stress contributes to hepatocarcinogenesis in fatty acyl-CoA oxidase 1-deficient mice. Am J Pathol. 2011;179:703–713.

56. Huang J, Viswakarma N, Yu S, et al. Progressive endoplasmic reticulum stress contributes to hepatocarcinogenesis in fatty acyl-CoA oxidase 1-deficient mice. Am J Pathol. 2011;179:703–713.

57. Le A, Cooper CR, Gouw AM, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci U S A. 2010;107:2037–2042.

58. Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A defines metabolic reprogramming and therapeutic susceptibility. Cell Metabol. 2008;7:11

59. Le A, Cooper CR, Gouw AM, et al. Inhibition of lactate dehydrogenase A suppresses tumor growth and metastasis of human hepatocellular carcinoma. FEBS J. 2012;279:3898–3910.
132. Budhu A, Roessler S, Zhao X, et al. Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. *Gastroenterology*. 2013;144(5):1066–1075.

133. Beyoğlu D, Imbeaud S, Maurhofer O, et al. Tissue metabolomics of hepatocellular carcinoma: tumor energy metabolism and the role of transcriptomic classification. *Hepatology*. 2013;58(1):229–238.

134. Ahluwalia GS, Grem JL, Hao Z, et al. Metabolism and action of amino acid analog anti-cancer agents. *Pharmacol Ther*. 1990;46:243–271.

135. Butler EB, Zhao Y, Muñoz-Pinedo C, et al. Stalling the engine of resistance: targeting cancer metabolism to overcome therapeutic resistance. *Canc Res*. 2013;73:2709–2717.

136. Tennant DA, Durán RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. *Nat Rev Canc*. 2010;10:267–277.