REVIEW ARTICLE

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

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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 attentions have 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 metastatic cascade of cancer including HCC[4]

Glycometabolism reprograming

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 cytoplasm and oxidative phosphorylation (OXPHOS) in mitochondria. Cells use both pathways but rely overwhelmingly 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".[5] 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 epiphenomenon 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.[6] 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.[7] Clinically, the positron emission tomography (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 cancers including HCC.[8–15] Silencing of GLUT1 or HK2 expression or inhibition of their activity, could significantly decrease glucose uptake and glycolytic rate of cancer cells, and further inhibit cancer cell proliferation and invasion.[16,17]

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.[9,18–20] In addition, the hypoxic microenvironment of invasive tumors 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,[21] 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.[22,23] 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.[24] 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.[25]

Pyruvate metabolism

In Warburg's theory, the aerobic glycolysis of tumor cells was attributed to mitochondria dysfunction,[7] which was frustrated by later observations that the respiration function 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 intermediate 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,[26] and was associated with poor prognosis and aggressive phenotypes.[27]

Furthermore, the non-metabolic role of PKM2 in tumor progression has also emerged. PKM2 isoform-specific deletion revealed that the inactive state of PKM2 is associated with the proliferating cell population within tumors, whereas non-proliferating tumor cells require active pyruvate kinase.[28] Then PKM2 was identified as a phosphotyrosine binding protein.[29] Upon specific metabolites activation[30] or some posttranslational modifications, such as phosphorylation,[31,32] acetylation[33] or oxidation,[34] the dimeric PKM2 can translocate to the nucleus, where it can interact with β-catenin,[31] HIF-1α[35] or STAT-3[36] 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 metabolism. 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 activation of PDK1 is regulated by a complicated network including both intrinsic alterations and extrinsic environment. 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 a initial stage of metastasis.[44−46] It has also been demonstrated that mitochondrial oxidative phosphorylation is related to liver cancer metastasis.[47]

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 reprograming

Lipids is 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.[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 aoxidase 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 reprograming 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

glucose *in vivo* which prevents inflammatory responses.[116] Conversely, regulatory T cells (Tregs) are less dependent on glucose and more reliant on mitochondrial oxidative metabolism of lipids.[117–119] PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation.[120]

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.[118–127] 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.[128,129]

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.[130] Several metabolic targets have been identified by metabolomics approaches, and they could be used as biomarkers in liver cancer.[131] 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.[132] 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.[133] 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.[124] Based on these metabolic reprograming, many therapies targeting specific metabolic pathway have also been explored.[134–136] 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 anabolic 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

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