Advances in Experimental Medicine and Biology 1136

Daniele M. Gilkes *Editor*

Hypoxia and Cancer Metastasis

Springer

Advances in Experimental Medicine and Biology

Volume 1136

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-12733-6 ISBN 978-3-030-12734-3 (eBook)
https://doi.org/10.1007/978-3-030-12734-3

Preface

I am pleased to introduce the book entitled *Hypoxia and Cancer Metastasis* in the Advances in Experimental Medicine and Biology series. The latest books on hypoxia and cancer, edited by Celeste Simon in 2010 and Giovanni Melillo in 2014, offered an excellent overview for scientists interested in the role of hypoxia in cancer biology. The current book aims to provide an update with a specific focus on hypoxia and metastasis. I am thankful that each contributor wholeheartedly welcomed the invitation to contribute chapters to provide a comprehensive (to-date) understanding of the role of hypoxia in metastasis. Each of the contributors has spent their career focused on understanding the role of hypoxia in human disease, in particular, cancer.

Metastasis is the leading cause of cancer death. Of all the processes involved in tumorigenesis, local invasion and the formation of metastases are the most clinically relevant because they result in deaths attributed to cancer. Intratumoral hypoxia is found in a majority of solid tumors and is associated with an increased risk of metastasis and cancer treatment failure. This has prompted the need for intensive investigation by cancer biologists, clinicians, and scientists, alike. Therefore, the purpose of this book is to provide a review of clinical and preclinical studies that explore the influence of hypoxia in the metastatic process. The first chapter provides an introduction and lays the groundwork for the book. Chapter 2 provides an up-to-date review on the clinical and preclinical methods used to quantify hypoxia in tumors. The chapters that follow examine the role of hypoxia in integral steps that are required for metastatic disease including the role of hypoxia in angiogenesis and lymphangiogenesis, secreted factors that influence homing and migration, and metabolic programming to enhance cell fitness and promote tumor heterogeneity. The closing chapters discuss the role of hypoxia in immune suppression and resistance to therapy. The final chapter discusses current clinical trials and emerging therapies aimed at targeting hypoxic cells.

The book is designed for researchers both new and heavily engaged in hypoxia research. The overall goal is to encourage the development of matched biomarkers and therapeutics for cancers in which hypoxia plays a detrimental role.

Baltimore, MD, USA — Daniele M. Gilkes

Contents

1 Hypoxia Mediates Tumor Malignancy and Therapy Resistance

Weibo Luo and Yingfei Wang

Abstract

Hypoxia is a hallmark of the tumor microenvironment and contributes to tumor malignant phenotypes. Hypoxia-inducible factor (HIF) is a master regulator of intratumoral hypoxia and controls hypoxia-mediated pathological processes in tumors, including angiogenesis, metabolic reprogramming, epigenetic reprogramming, immune evasion, pH homeostasis, cell migration/invasion, stem cell pluripotency, and therapy resistance. In this book chapter, we reviewed the causes and types of intratumoral hypoxia, hypoxia detection methods, and the oncogenic role of HIF in tumorigenesis and chemo- and radio-therapy resistance.

Keywords

Angiogenesis · Cell motility · Epigenetics · HIF · Hypoxia · Hypoxia imaging · Metabolism · Stem cell · Therapy resistance · Tumorigenesis

Reduced O_2 availability, known as hypoxia, is a hallmark of the tumor microenvironment. This biological phenomenon was initially observed in lung carcinoma by Thomlinson and Gray in 1950s [146]. Accumulating studies have demonstrated that hypoxia exists in all solid tumors including breast, pancreas, brain, liver, lung, stomach, cervix, ovary, head-and-neck, prostate, bladder, kidney, skin, and colon tumors [6, 7, 14, 77, 118, 146, 151, 166], and that up to 50–60% of tumor regions exhibit much lower O_2 levels than their tissues of origin [152]. For example, the median partial pressure of O_2 (pO_2) is about 10 mmHg in breast tumors, which is significantly less than 65 mmHg in normal breast tissues [152]. While the presence of intratumoral hypoxia is independent of tumor size, grade, stage, or histology [106], hypoxia regulates many pathological processes to promote tumor malignancy and is significantly correlated with poor clinical outcome in cancer patients.

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D. M. Gilkes (ed.), *Hypoxia and Cancer Metastasis*, Advances in Experimental Medicine and Biology 1136,
https://doi.org/10.1007/978-3-030-12734-3_1

1.1 Definition of Intratumoral Hypoxia

Intratumoral hypoxia is the result of an imbalance of reduced O_2 supply by abnormal tumor vessels and increased O_2 consumption by highly proliferative cancer cells. The tumor blood vessels are often occluded and leaky [19], resulting in transient perfusion-limited hypoxia within a tumor, also known as acute hypoxia. The time frame of acute hypoxia is variable in tumors, and it can last for several minutes to days [13]. Hypoxia also causes the significant morphological changes of endothelial cells in the tumor vessels, which functionally reduces O_2 diffusion to the surrounding tumor tissues [44]. As a result, the area within 70–100 μm away from the blood vessel is relatively oxygenated but oxygen diffusion is dramatically impaired beyond 100–150 μm distance to the blood vessel, leading to hypoxia or even anoxia in the necrotic regions. This type of intratumoral hypoxia has been termed 'diffusion-limited' hypoxia or chronic hypoxia. Chronic hypoxia exists from several hours to weeks in tumors [13]. Emerging studies showed that the pO_2 levels may fluctuate by cyclic hypoxia and reoxygenation within a tumor, which is defined as intermittent hypoxia [103]. Several studies have reported fluctuations of blood flow in human and murine tumors using laser Doppler and O_2 microelectrode approaches [61, 74, 115]. Intermittent hypoxia may robustly increase oxidative stress in tumors [94]. Martinive P et al. showed that intermittent hypoxia makes tumor cells and endothelial cells more resistant to apoptosis and radiotherapy [97]. Cancer cells exposed to intermittent hypoxia have been shown to be more aggressive and increase ability in tumor initiation and metastasis in xenograft mice [28, 123]. Overall, solid tumors suffer the temporal and spatial changes of pO_2, which lead to heterogeneous and gradient O_2 distribution (Fig. 1.1). Three types of hypoxia trigger the distinct intracellular signaling pathways in tumor cells by altering O_2 concentration and flux time duration and have a significant pathological role in tumorigenesis.

1.2 Detection of Intratumoral Hypoxia

As hypoxia drives tumorigenesis and also mediates resistance to chemo- and radio-therapy, the measurement of intratumoral hypoxia has great value for the basic cancer research and clinical prognosis/diagnosis in cancer patients. Many invasive and non-invasive approaches have been developed to detect and quantify the pO_2 levels within a tumor in experimental conditions as well

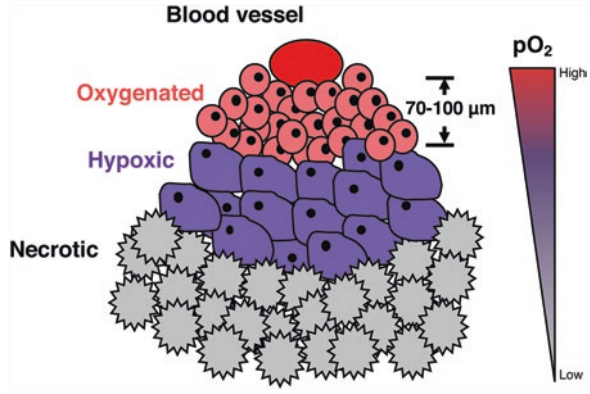

Fig. 1.1 Heterogeneity of intratumoral hypoxia Solid tumors display t he gradient pO_2 levels. The tissues surroundi n g the blood vessel are relatively oxygenated, but O_2 diffusion is significantly impaired beyond 100 μm distance to the blood vessel, which leads to hypoxia or even anoxia in the necrotic regions

Table 1.1 Methods in detection of intratumoral hypoxia

	Application	References
Invasive methods		
Polarographic oxygen electrode	Direct method	[36]
Luminescence-based optical sensor	Direct method	[33]
Pimonidazole	Immunohistochemistry	[57]
HIF-1α	Immunohistochemistry	[125]
Carbonic anhydrase IX	Immunohistochemistry	[125]
18F-2-nitroimidazolpentafluoropropylacetamiede (EF5)	Immunohistochemistry	[57]
Non-invasive methods		
18F-fluoromisonidazole (18F-FMISO)	PET	[76]
2-deoxy-2-(18F)fluoro-D-glucose (18F-FDG)	PET	[138]
18F-fluoro-erythronitroimidazole (18F-FETNIM)	PET	[34]
18F-fluoro-azomycin-arabinoside (18F-FAZA)	PET	[114]
18F-2-nitroimidazolpentafluoropropylacetamiede (EF5)	PET	[168]
18F-EF3	PET	[92]
18F-FRP170	PET	[70]
18F-flortanidazole (HX4)	PET	[150]
[(68) Ga]-HP-DO3A-nitroimidazole	PET	[159]
Cu-ATSM	PET	[64]
^{131}I-IAZGP	PET	[120]
^{89}Zr-labeled cG250-F(ab')$_2$	PET	[63]
^{123}I-Iodoazomycin arabinoside	SPECT	[113]
^{99m}Tc-cyclam-2-nitroimidazole	Planar scintigraphy	[4]
^{68}Ga-metronidazole	MRI	[101]
Oxygen-enhanced MRI	MRI	[110]
Dynamic contrast-enhanced MRI	MRI	[43]
Blood oxygen level-dependent MRI	MRI	[110]
Tissue oxygen level dependent MRI	MRI	[8]

as the clinical settings (Table 1.1). The eppendorf polarographic needle electrode is the most direct method that utilizes the electrochemical reduction of O_2 at the cathode to measure pO_2 [36]. The electrode is sensitive and can rapidly detect pO_2 changes in tumors. However, this invasive approach measures local pO_2 levels surrounding the electrode, which cannot represent the entire tumor due to the heterogeneity of intratumoral hypoxia. The electrode itself consumes O_2 during measurement, leading to the overestimation of tumor hypoxia. Therefore, this method has limitations for clinical application.

Immunohistochemical methods have been also developed to detect intratumoral hypoxia in preclinical cancer models and biopsies from cancer patients (Table 1.1). Pimonidazole, which was originally developed as a hypoxic cell radiosensitizer for clinical use [108], binds to thiol groups from proteins, peptides, and amino acids specifically in viable hypoxic cancer cells. Pimonidazole-positive cells can be visualized by an antibody-based immunohistochemical assay under microscopy [57]. Likewise, hypoxia-inducible factor (HIF)-1α, a subunit of the hypoxia regulator HIF, and its target gene carbonic anhydrase IX are also frequently used as hypoxia markers to detect hypoxic regions in tumors by the immunohistochemical approach [125]. Therefore, immunohistochemical staining generates the high-resolution hypoxic regions at a single cell level within a tumor. Although immunohistochemical methods have been widely employed to quantify hypoxic regions in solid tumors, the disadvantage of these methods is that they display a static but not dynamic hypoxia status in tumors.

The non-invasive imaging methods including positron emission tomography (PET), single photon emission computed tomography

(SPECT), and magnetic resonance imaging (MRI) have been widely employed in clinical diagnosis. These approaches are considered as the practical, sensitive, and reproducible hypoxia imaging methods. A number of hypoxia PET radiotracers have been developed for the repetitive measurement of intratumoral hypoxia in the animal models and cancer patients (Table 1.1). The current hypoxia PET radiotracers are classified into two major families, 18F-labelled nitromidazole analogs and Copper(II)-labelled diacetyl-bis(N^4-methythiosemicarbzaone) (Cu-ATSM) analogs. 18F-fluoromisonidazole (18F-FMISO) is the first PET tracer for hypoxia detection in the clinical studies [76]. Like other 2-nitroimidazole analogs, 18F-FMISO is metabolized into reactive intermediates, and these intermediates are further reduced to the nitro-radical anion that can bind to intracellular macromolecules in hypoxic cells. In contrast, 18F-FMISO intermediates are re-oxidized into the original compound under normoxia. As such, 18F-FMISO is irreversibly trapped and accumulated in viable hypoxic cells below 10 mmHg, which can be detected by a PET scanner for quantification of spatial and temporal pO_2 levels in many human cancers, including breast, brain, lung, head-and-neck, prostate, and kidney cancers [33, 65, 76, 119, 149]. However, 18F-FMISO has little clinical value in rectal and pancreatic cancers because of their non-specific accumulation in normoxic colon tissues or no accumulation in pancreatic tumors [122, 128].

Cu-ATSM is another class of hypoxia PET radiotracer that is used for clinical prognosis in cancer patients. Cu offers a series of radioisotopes (60Cu, 61Cu, 62Cu, 64Cu, and 67Cu) and 64Cu is often used for PET imaging due to its appropriate half-life (12.7 hr) and good imaging resolution [64]. 64Cu-ATSM is highly membrane permeable and requires less time to enter the cell, compared to 2-nitroimidazole analogs. After uptaken to the cell, Cu(II)-ATSM is reduced to Cu(I)-$ATSM^-$ by intracellular thiols. Reduced Cu(I)-$ATSM^-$ can be re-oxidized back to Cu(II)-ATSM by O_2 and then exits from the cell under normoxia. However, Cu(I)-$ATSM^-$ is dissociated under hypoxia and free Cu(I) is trapped within hypoxic cells, where the acidic environment facilitates destabilization of Cu(II)-ATSM and cellular Cu(I) trapping [40]. The application of the Cu-ATSM approach for prognostic evaluation has been studied in patients with head-and-neck cancer, cervical cancer and lung cancer [41, 51, 144].

18F-fluorodeoxyglucose (18F-FDG) is a D-glucose analog and has been extensively used as a hypoxia marker in preclinical and clinical cancer studies. The rationale of this radiotracer is that hypoxia increases the expression of the glucose transporters and glycolytic enzymes, and shifts glucose oxidation towards glycolysis in cancer cells [129]. Therefore, cancer cells rely on increased glucose uptake for their growth under hypoxia. 18F-FDG is accumulated in the cytosol after entering the cell through the glucose transporters because of lack of a 2-hydroxyl group and its phosphorylation by hexokinases, which blocks its degradation via the glycolytic pathway and prevents leave from cancer cells, respectively. 18F-FDG has a short half-life (110 min) and has been used for diagnosis in patients with colorectal cancer, breast cancer, head-and-neck cancer, kidney cancer, or lung cancer [52, 59, 60, 68, 107]. However, the cellular concentration of 18F-FDG is also very high in normal cells with high glucose consumption, such as normal cerebral cortex and basal ganglia in the brain, which generates a rather high background in the PET image [11].

MRI is another non-invasive imaging method that is increasingly being used to measure the temporal and spatial pO_2 levels in tumors because of its high spatial resolution and relatively low cost. It utilizes a large external magnetic field to image nuclei of atoms (such as ^1H, ^{19}F, or ^{13}C) in the body and pO_2 can be quantified in the presence of the contrast agent. Several functional MRI techniques, including blood oxygen level dependent (BOLD) MRI, tissue oxygen level dependent (TOLD) MRI, dynamic contrast enhanced (DCE) MRI, and oxygen enhanced (OE) MRI, have been developed to quantify pO_2 changes in tumors [8, 43, 110]. MRI has been applied to assess tumor hypoxia for prediction of outcome in the pre-clinical and clinical radiation

studies [35]. Please see Chap. 2 by Dewhirst and colleagues for a review of the clinical and preclinical methods for quantifying the extent of hypoxia in human tumors.

1.3 HIF Is a Master Regulator in Response to Intratumoral Hypoxia

Numerous studies have demonstrated that intratumoral hypoxia promotes angiogenesis, glycolysis, cell invasiveness, cell survival, and immune evasion, leading to cancer progression and metastasis [131]. These cellular adaptive responses to intratumoral hypoxia are mainly mediated by a family of HIFs [130]. HIF is a heterodimeric transcription factor, consisting of α and β subunits [154]. So far, three HIF family members (HIF-1, HIF-2, and HIF-3) have been identified in mammals [53, 135, 147]. HIF-1α is ubiquitously expressed in most cell types, whereas HIF-2α expression is limited to certain cells, such as endothelial cells [147]. While a total of 19 distinct HIF-3α transcripts have been identified in the human genome database due to alternative mRNA splicing mechanisms, only 8 variants may encode HIF-3α protein [91]. HIF-1 and HIF-2 are the most well-studied members and mediate many common and unique biological responses to intratumoral hypoxia [132]. The functions of HIF-3 are diverse as different HIF-3α isoforms appear to be a gene activator or repressor through distinct mechanisms [99, 165].

HIF-α is regulated by the O_2-dependent ubiquitin-proteasome mechanism (Fig. 1.2) [91].

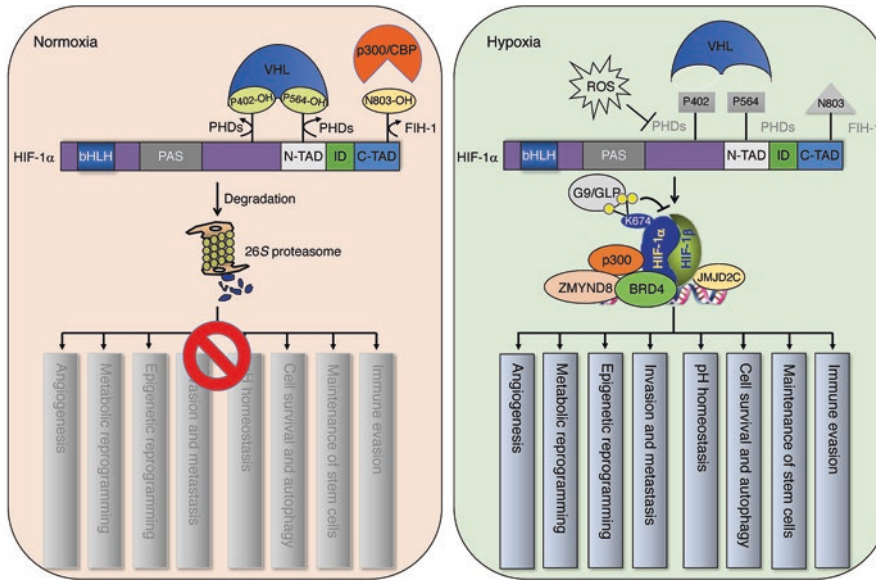

Fig. 1.2 Regulation of HIF transcriptional activity in cancer cells
HIF-α is prolyl hydroxylated by PHDs and targeted by the ubiquitin E3 ligase complex Cul2-ElonginB/C-VHL for proteasomal degradation under normoxia. HIF-α is also hydroxylated on the asparagine residue by FIH-1, which blocks the recruitment of the coactivators p300/CBP, leading to HIF inactivation. Under hypoxia, the enzymatic activity of PHDs and FIH-1 is impaired due to reduced O_2 and increased ROS. As a result, HIF-α escapes from proteasomal degradation and dimerizes with HIF-1β. The heterodimer binds to the hypoxia response element and cooperates with epigenetic regulators including p300, ZMYND8, BRD4, and JMJD2C to activate the target genes whose protein products mediate angiogenesis, metabolic reprogramming, epigenetic reprogramming, invasion/metastasis, pH homeostasis, cell survival, autophagy, maintenance of stem cells, and immune evasion. The methyltransferases G9a/GLP methylate lysine 674 on HIF-1α to inhibit HIF-1 transcriptional activity in glioblastoma cells

In well-oxygenated cells, HIF-α is hydroxylated on the proline (Pro) residues (e.g., Pro 402 and 564 on human HIF-1α) by a family of prolyl hydroxylases (PHDs) with O_2 as the substrate [66]. PHD2 is mainly responsible for prolyl hydroxylation of HIF-1α [16]. The Von Hippel-Lindau (VHL) protein recruits prolyl hydroxylated HIF-α to the ubiquitin E3 ligase complex Cullin2/Elongin-B/C, leading to HIF-α polyubiquitination and subsequent protein degradation in the 26*S* proteasome [98]. Under hypoxia, PHDs' enzymatic activity is robustly reduced and HIF-α fails to be prolyl hydroxylated, so that HIF-α protein escapes from proteasomal degradation and dimerizes with HIF-1β to enhance the transcription of hundreds of genes, whose protein products regulate angiogenesis, metabolism, epigenetics, pH homeostasis, stem cell pluripotency, cell survival, cell migration/invasion, immune evasion, and tumor growth and metastasis (Fig. 1.2). The enzymatic activity of PHDs is also inhibited by reactive oxygen species (ROS), whose production is significantly increased under hypoxia (Fig. 1.2) [23]. Therefore, multiple layers of molecular regulation have been shown to control HIF transcriptional activity in cancer cells.

1.4 HIF and Tumor Angiogenesis

Previous studies demonstrated that HIF-1α, HIF-2α, and HIF-1β knockout mice all are embryonic lethal due to defective vascularization, indicating an essential role of HIF-1 and HIF-2 in vascular remodeling [67, 78, 95]. This scenario has been also documented in tumors. HIF-1 and HIF-2 are the key regulators of hypoxia-induced angiogenesis in tumors [80]. It has been well-known that HIF-1 and HIF-2 directly induce the expression of many genes involved in multiple steps of angiogenesis, including *VEGFA*, *VEGFR2*, *PDGFB*, *bFGF, ANGPT1, ANGPT2, ANGPT3, ANGPT4, TEK, MMP-1, SDF-1,* and *CXCR4* [1, 2, 20, 47, 62, 71, 96, 109, 127, 137, 161]. Multiple cell types including cancer cells, endothelial cells, and regulatory T cells are involved in the hypoxia-induced release of angiogenic factors in tumors [47, 50, 96]. Thus, HIF-1 and HIF-2 actively participate in vascular permeability, endothelial cell proliferation, basement membrane degradation, sprouting, cell migration, and tube formation, leading to new blood vessel formation in tumors. New blood vessel formation is required to support rapidly proliferating cancer cells with O_2 and nutrients, thereby promoting tumor formation, development, and metastasis. Inhibition of angiogenesis by VEGF or VEGF receptor inhibitors impairs tumor growth in the experimental mouse models of human cancers as well as in cancer patients [124]. Please see Chap. 5 for an extensive review of hypoxic signalling in angiogenesis and lymphangiogenesis.

1.5 HIF and Metabolic Reprogramming in Tumors

Otto Warburg observed the high rate of glycolysis in tumor tissues even in the presence of oxygen, a phenomenon known as the Warburg effect [155]. HIF-1 represents one of the molecular mechanisms of the Warburg effect in renal cancer [129]. HIF-1 induces the expression of glucose transporters and glycolytic enzymes to enhance glucose uptake and oxidation (Fig. 1.3) [133]. Many glycolytic enzymes including hexokinase 2 and phosphofructokinase 1 appear to be oncogenes that mediate tumor initiation and growth [157, 163]. Pyruvate dehydrogenase kinase (PDK) 1 and PDK3 are also the direct HIF-1 target genes and induced by hypoxia to phosphorylate and inactivate pyruvate dehydrogenase complex, thereby shifting pyruvate metabolism towards lactate in cancer cells [72, 87]. Induction of lactate dehydrogenase A (LDHA) by HIF-1 further promotes glycolytic flux into lactate in cancer cells [133]. Lactate is secreted into the extracellular space by the monocarboxylate transporter (MCT) 4 [42]. High lactate levels in tumors are positively correlated with increased metastasis in head-and-neck cancer patients [18]. Interestingly, it was shown that extracellular lactate can be transported into oxygenated tumor cells by MCT1 and, as a fuel, supports the tricar-

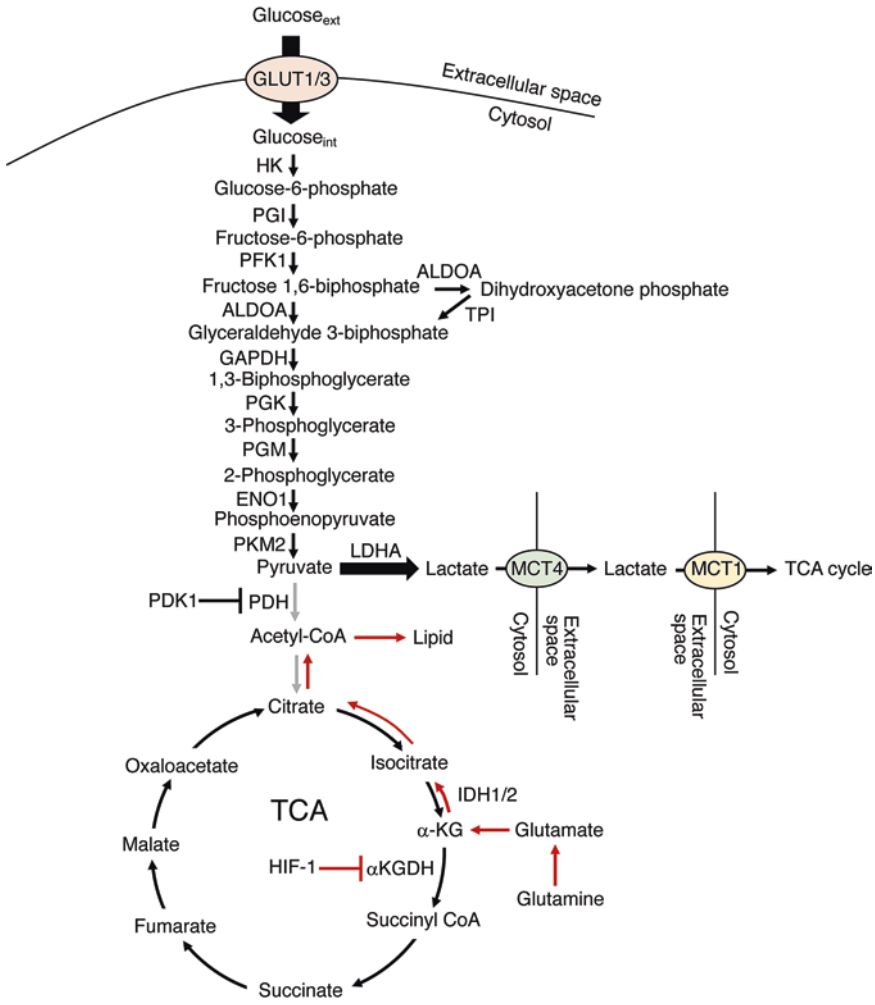

Fig. 1.3 HIF-dependent metabolic reprogramming in cancer cells
HIF directly induces the expression of the glucose transporters and glycolytic enzymes to upregulate glucose uptake and oxidation in cancer cells. PDK1 is also upregulated by HIF-1 in cancer cells and promotes the metabolic shift towards lactate production by inactivating PDH. The extracellular lactate is transported via MCT1 into the neighboring cancer cells as a fuel to support their survival. HIF also enhances reductive glutamine metabolism to sustain the TCA cycle and to increase lipogenesis in cancer cells

boxylic acid (TCA) cycle and respiration in these neighboring cancer cells [139]. These findings have been also observed in human patients with non-small-cell lung cancer [45]. Targeting MCT1 by the small molecule inhibitor blocks the growth of lung tumors in xenograft mice [116].

Recent studies uncovered the feedback role of the glycolytic enzymes in HIF-dependent glycolysis in multiple types of cancer cells. Pyruvate kinase M2 enhances the transcriptional activity of HIF-1 and HIF-2 to promote the expression of the glucose transporter GLUT1 and glycolytic enzymes LDHA and PDK1, thereby increasing glucose uptake and glycolysis in renal and cervical cancer cells [90]. Aldolase A (ALDOA) increases HIF-1α protein stability through lactate-mediated PHD inhibition to promote HIF transcriptional activity in lung cancer cells [27]. ALDOA activates HIF to increase MMP9 expression, thereby promoting lung cancer metastasis. In contrast, fructose-1,6-bisphosphatase 1 (FBP1) inhibits HIF-1 to decrease glycolysis in

renal cancer cells [83]. The *FBP1* gene is lost in human clear cell renal cell carcinoma and loss of FBP1 promotes renal cancer progression by increasing HIF-dependent glycolysis. Therefore, the glycolytic enzymes regulate HIF to control glycolysis and tumor malignancy.

Glucose donates the carbon to generate acetyl-CoA, which enters the TCA cycle for the synthesis of ATP, intermediate metabolites, and amino acids. Activation of HIF leads to increased glycolysis but decreased glycolytic flux into the TCA cycle in cancer cells (Fig. 1.3) [72, 134]. Although glucose-derived carbon source is significantly reduced in hypoxic cells, HIF enhances glutaminolysis to sustain the TCA cycle and lipogenesis, which are necessary for cancer cell survival under hypoxia [102, 156]. Isocitrate dehydrogenase (IDH) 1/2 are responsible for the reductive carboxylation of glutamine-derived α-ketoglutarate for *de novo* lipogenesis under hypoxia, although it is unclear how hypoxia promotes the reductive activity of IDH1/2 in cancer cells. HIF-1 also increases the ubiquitin E3 ligase SIAH2-mediated ubiquitination and subsequent degradation of the E1 subunit of the α-ketoglutarate dehydrogenase complex, thereby facilitating reductive glutamine metabolism in hypoxic cells [142]. Therefore, activation of HIF reprograms the cellular metabolic pathways to supplement the intracellular metabolites that are necessary for cancer cell growth under hypoxia (Fig. 1.3). Chapter 5 contains further discussion of hypoxia and metabolic reprogramming in cancer.

1.6 HIF and Epigenetic Reprogramming in Tumors

Hypoxia mediates reprogramming of the chromatin landscape by inducing many HIF-dependent epigenetic regulators in cancer cells [91]. It has been reported that hypoxia induces the global increases in histone modifications including dimethyl lysine 4 of histone H3 (H3K4me2), trimethyl lysine 4 of histone H3 (H3K4me3), dimethyl lysine 9 of histone H3 (H3K9me2), dimethyl lysine 79 of histone H3 (H3K79me2), acetyl lysine 14 of histone H3 (H3K14ac), and dimethyl arginine 3 of histone H4 (H4R3me2) in hepatoma-derived Hepa 1-6 cells, but inhibits acetyl lysine 5 of histone H4 (H4K5ac), acetyl lysine 12 of histone H4 (H4K12ac), and acetyl lysine 5 of histone H2A (H2AK5ac) in breast cancer cells [69, 158]. H3K14ac and acetyl lysine 16 of histone H4 (H4K16ac) at the local hypoxia response element is also elevated by hypoxia in breast cancer cells [32]. Hypoxia upregulates many members of the histone demethylase Jumonji domain-containing protein (JMJD) family in cancer cells in a HIF-1- and/or HIF-2-dependent manner (Table 1.2). Although O_2 is required for the catalytic activity of the JMJD family members [136], several JMJD family members including JMJD1A, JMJD2B, and JMJD2C are reported to be active in hypoxic cells, which may be due to the compensatory mechanism by increased protein levels of these enzymes under hypoxia [49, 79, 89]. JMJD2C selectively demethylates trimethyl lysine 9 of histone H3 (H3K9me3) but not trimethyl lysine 36 of histone H3 (H3K36me3) at the hypoxia response element to increase HIF-1 binding and HIF-1 target gene expression in breast cancer cells (Fig. 1.2) [89]. JMJD2C is highly amplified in breast cancer and knockdown of JMJD2C inhibits triple-negative breast tumor growth and metastasis to lungs in mice [85, 89]. Likewise, JMJD1A (also named as KDM3A) is recruited to the promoter of the *SLC2A3* gene by HIF-1α and demethylates H3K9me2 to induce SLC2A3 expression in endothelial cells [104]. JMJD1A knockdown reduces colon cancer growth in mice [79]. It was also reported that JMJD2B demethylates H3K9me3 at the promoter of hypoxia-inducible genes *SLC2A1*, *UCA1*, *ELF3*, and *IFI6* to increase their expression in HCT116 cells [49]. In contrast, the demethylase activity of JMJD3 and JARID1A/B is impaired under hypoxia, leading to increased trimethyl K27 and trimethyl K4 of histone H3, respectively [26]. Histone deacetylase 3 is also induced by HIF-1 and decreases H3K4ac in breast cancer cells, which promotes H3K4me2 and H3K4me3 by recruiting the methyltransferase WDR5 [158]. Hypoxia induces the methyltransferase G9a at both transcriptional and post-translational levels in cancer cells, which is