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Hypoxia, Hypoxia-Inducible Gene 2 (HIG2)/HILPDA, and Intracellular Lipolysis in Cancer

Davide Povero[1,2], **Scott M. Johnson**[1,3], **Jun Liu**[1,2,#]

[1]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine & Science, Rochester, Minnesota 55905, USA;

[2]Division of Endocrinology, Mayo Clinic College of Medicine & Science, Rochester, Minnesota 55905, USA;

[3]Mayo Clinic Graduate School of Biomedical Sciences, Rochester, Minnesota 55905, USA.

Abstract

Tumor tissues are chronically exposed to hypoxia owing to aberrant vascularity. Hypoxia induces metabolic alterations in cancer, thereby promoting aggressive malignancy and metastasis. While previous efforts largely focused on adaptive responses in glucose and glutamine metabolism, recent studies have begun to yield important insight into the hypoxic regulation of lipid metabolic reprogramming in cancer. Emerging evidence points to lipid droplet (LD) accumulation as a hallmark of hypoxic cancer cells. One critical underlying mechanism involves the inhibition of adipose triglyceride lipase (ATGL)-mediated intracellular lipolysis by a small protein encoded by hypoxia-inducible gene 2 (HIG2), also known as hypoxia inducible lipid droplet associated (HILPDA). In this review we summarize and discuss recent key findings on hypoxia-dependent regulation of metabolic adaptations especially lipolysis in cancer. We also pose several questions and hypotheses pertaining to the metabolic impact of lipolytic regulation in cancer under hypoxia and during hypoxia-reoxygenation transition.

Keywords

Lipolysis; hypoxia; HIF; hypoxia-inducible factor; oxygen; fatty acid; lipid droplet; ATGL; HIG2; hypoxia inducible gene 2; HILPDA

1. Introduction

Cancer cells survive within the hostile tumor microenvironment and maintain a rapid proliferation rate by acquiring alterations of metabolic pathways for carbohydrates, lipids, proteins and nucleic acids. “Aerobic glycolysis”, the so-called “Warburg effect”, is

[#]To whom correspondence should be addressed: Jun Liu, Guggenheim Building 14-11A, 222 3rd Avenue SW, Rochester, MN 55905. Phone: 507-284-8120; liu.jun@mayo.edu.

considered as a hallmark of nearly all highly expanding solid tumors [1, 2]. By generating ATP and lactate via the glycolytic pathway even under normoxia, this metabolic adaptation was hypothesized to benefit cancer cells with mitochondrial defects. However, later studies showed that most cancer cells are not impaired in mitochondrial function. The aerobic glycolysis not only supports rapid ATP production, but also provides carbons for biomass generation to sustain cell growth and produces reducing equivalents required for preserving redox balance [3, 4].

Maintenance of oxygen homeostasis is a key feature of adapting cancer cells. Several studies have shown that hypoxia-inducible factors (HIFs) mediate multiple protective mechanisms by reducing mitochondrial oxidative capacity and oxygen consumption in hypoxic cells [5–10]. Fatty acid (FA) oxidation consumes a large amount of oxygen and in a hypoxic environment, produces reactive oxygen species (ROS) that further exacerbate oxygen insufficiency. Elevated oxidative stress in turn causes damages to membrane lipids, deactivation of proteins and enzymes, and apoptotic cell death [11–13]. In response to limited oxygen supply, cancer cells often switch off FA oxidation through downregulation of intracellular lipolysis, while increasing accumulation of triglyceride lipid droplets (TG-LDs) [14–16]. LD accumulation and lipolytic inhibition not only provide fuel and sources of building blocks for the future growth, but also protect cancer cells against oxidative stress and lipotoxicity in hypoxia. Recent studies have yielded critical insight into a novel anti-lipolytic mechanism that is key for cancer cells to survive hypoxia. This mechanism represents a departure from the canonical metabolic pathways that have been targeted therapeutically to deprive cancer cells of necessary fuel/building blocks. In this review, we outline recent discoveries on reprogramming of FA metabolism in hypoxic cancer cells as well as the roles of lipolytic inhibition and LD formation as adaptive mechanisms to hypoxia.

2. Hypoxia and HIF signaling in cancer

Since the biochemical purification of the subunits of the heterodimeric HIF-1 (HIF-1α and HIF-1β) was reported by Semenza and colleagues in 1995 [17], oxygen sensing has been recognized to influence a host of physiologic processes including cell growth, energy metabolism, redox homeostasis, organ development and angiogenesis [18]. Changes in oxygen availability and adaptation to low oxygen environment at cellular, tissue and organismal levels also influence the development of several disorders such as cardiovascular disease, pulmonary hypertension, stroke, bacterial infections, inflammation and wound healing. A large body of existing evidence indicates that due to scarce oxygen diffusion through the abnormal and inefficient tumor vascular architecture, hypoxia occurs in 90% of solid tumors and plays a critical role in shaping the fate and aggressiveness of cancer cells [19, 20]. Tumor microenvironment is subject not only to chronic hypoxia (continuous low oxygen supply for several hours), but also to cycling (intermittent) hypoxia characterized by numerous spatial and temporal fluctuations of oxygen levels, alternating cycles of severe, moderate hypoxia followed by reoxygenation episodes when blood flow is restored [21]. Tumors exposed to cycling hypoxia have been reported to exhibit greater extent of pathological angiogenesis, intratumor inflammation, metastatic activity and drug resistance compared to chronic hypoxia [22–24]. Chronic hypoxia usually is the first type of hypoxia

to occur during tumor development because of rapidly proliferating tumor cells whose distance from a functional blood vessel exceeds 100 μm [25, 26]. Under these oxygen-deprived conditions, HIFs play a crucial role in mediating cancer cell growth, migration, invasion and increased resistance to pharmacotherapies [27, 28].

Fluctuations in external oxygen gradients directly influence global nuclear gene expression programs in cancer cells, where the HIF-prolyl hydroxylase domain enzyme (PHD)-von Hippel-Lindau protein (pVHL) axis plays a crucial role as oxygen sensor at the molecular level [29–31]. In normoxia, the HIF-α subunits (HIF-1α; HIF-2α and HIF-3α) are hydroxylated on proline residues by PHD, permitting their ubiquitination by pVHL and consequent proteasomal degradation. Under hypoxic conditions where PHD hydroxylation is diminished, the stabilized HIF-α proteins translocate to the cell nucleus and dimerize with constitutively present HIF-1β. The HIF-α/β heterodimers bind specific DNA hypoxia response elements (HRE), activating the transcription of over 1,000 different genes and mediating a diverse array of signaling pathways in multiple disease processes [32]. The three HIF-α isoforms are encoded by different gene loci and differ in promoter usage and splicing patterns. Despite similar domain architecture and proteolytic regulation, HIF-α proteins exhibit different tissue distribution patterns. While HIF-1α is ubiquitously present in a variety of tissues, HIF-2α is selectively expressed in liver, duodenum, and to a lesser extent in kidney, heart and brain [33, 34]. HIF-3α, with a poorly characterized function, is expressed mainly in pulmonary alveolar epithelial cells [35]. In cancer cells, activities of HIFs are not only regulated by tumor oxygen levels, but also by mutations in oncogenes and tumor suppressor proteins. A notable example of HIF activation independently of tumor oxygen levels is loss-of-function mutations of VHL tumor suppressor gene associated with clear cell renal cell carcinoma (ccRCC), which accounts for 70–80% of all renal malignancies. A defining hallmark of ccRCC cells is the “clear” phenotype caused by the extensive cytoplasmic accumulation of lipids and glycogen [36].

3. Hypoxia and remodeling of glucose and glutamine metabolism in cancer

Metabolic adaptation to the hypoxic microenvironment has become a hallmark of cancer. In response to scarcity of oxygen and nutrients, cancer cells often fine-tune their metabolism to secure the amounts of energy and building blocks required for sustaining their growth and survival. The first and best characterized metabolic adaptation in cancer cells is the Warburg effect, which consists of switching ATP generation from oxidative phosphorylation to glycolysis even in the presence of sufficient oxygen supply [2]. In cancer cells with intact mitochondrial function, aerobic glycolysis is often favored over oxidative metabolism to gain three key metabolic benefits: rapid energy production, provision of metabolic intermediates and precursors for the biosynthesis of macromolecules, and generation of NADPH to help maintain the redox status [3, 4, 37]. These characteristic glycolytic phenotypes are largely controlled by HIF-1 [8], which activates expression of not only genes responsible for glucose uptake and glycolysis, but also enzymes involved in the inhibition of glucose oxidation such as pyruvate dehydrogenase kinase 1 (PDK1). PDK1 phosphorylates and inhibits pyruvate dehydrogenase (PDH) [38], thereby promoting lactate

production through limiting the entry of glycolysis-derived pyruvate into the oxidative TCA cycle. In response to hypoxia, HIF-1 activation also acts to reduce oxygen consumption through downregulating expression of genes critical for mitochondrial oxidative metabolism [5, 6].

Cancer cells require large amounts of lipids for the synthesis of cellular membranes to sustain high proliferation rates. Over the last decade an increasingly number of reports have described how alterations in lipid metabolism contribute to tumor growth, survival, local invasion and metastatic development [39, 40]. FAs, either of exogenous origin or generated de novo, are paramount for anabolic and catabolic metabolism of cancer cells as either building blocks for more complex lipids or substrates for energy production [41]. For example, FAs were identified as a key regulator of pancreatic cancer cell proliferation in vitro, and n-6 polyunsaturated FAs were found to induce liver metastasis in a xenograft model [42]. A study conducted shortly after by Okumura et al. further showed that FAs derived from lipolysis in the peripancreatic adipose tissue promotes the invasiveness and chemoresistance of pancreatic ductal adenocarcinoma (PDAC) cells [43]. A more recent report by Iwamoto et al. demonstrated that hepatocellular carcinoma (HCC) cells implanted in fatty livers of mice fed a high fat diet accumulated LDs, and switched their metabolism from glycolysis to oxidative FA metabolism when treated with antiangiogenic drugs and under intermittent hypoxia. These orthotopic tumors were able to grow even in the presence of minimal number of microvessels [44].

While normal cells preferentially use exogenous FAs for synthesis of structural lipids and as energy substrates, cancer cells upregulate the expression of fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), thereby producing most of cellular FAs through de novo synthesis to support membrane phospholipid biosynthesis [45]. ACC catalyzes the carboxylation of acetyl-CoA - converted from glycolytic end-product pyruvate – to form malonyl-CoA.

Malonyl-CoA is further converted by FASN to long-chain FAs. Under normoxic conditions, citrate and acetyl-CoA used for *de novo* lipogenesis are primarily generated from glucose-derived pyruvate through the citrate shuttle and ATP citrate lyase in the cytosol. Hypoxic cancer cells, however, are able to increase de novo lipid synthesis using citrate produced from reductive carboxylation of glutamine. In particular, it was discovered that this reductive metabolism of glutamine is dependent on isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) [46, 47], which catalyzes the key step of isocitrate decarboxylation into α-ketoglutarate (α-KG). RCC cells with VHL deficiency and resultant pseudohypoxic response preferentially utilize reductive glutamine metabolism for lipid biosynthesis even in normoxic conditions [46, 48]. Furthermore, in a report by Bjornson et al., a global gene profiling of 361 hepatocellular carcinoma (HCC) tissues identified the mitochondrial acetyl-CoA synthetase 1 (ACSS1) as the top expressed gene in tumors exposed to hypoxia. ACSS1 is a key enzyme that converts acetate into acetyl-CoA. This finding suggests that in hypoxic conditions, cancer cells utilize mitochondrial acetate as carbon source to generate FAs and structural lipids required for membrane biosynthesis [49].

4. Hypoxia and lipid droplet accumulation in cancer

In most cell types, FAs can be oxidized to produce energy, esterified into neutral and phospholipids, or converted to various signaling molecules. LDs are a key organelle responsible for storing cellular surplus of FAs in esterified neural forms such as TGs and sterol esters. Studying the LD biology has revealed fundamental functions of LDs and has led to identification of numerous LD-associated structural proteins, enzymes and proteins regulating membrane trafficking [50–52]. Since the first reports describing LDs in mammary carcinoma and malignant lymphomas published in 1960s [53, 54], major breakthrough in cancer LD research has been achieved in providing evidence to demonstrate that LDs not only serve a neutral lipid storage depot, but also are implicated in all major steps of cancer development [15, 55]. The effects of LDs include advancement of cancer cell growth and proliferation, suppression of cancer cell death, evasion of tumor growth suppressing mechanisms, as well as promotion of angiogenesis, genome instability, inflammation, metabolic deregulation and evasion of host cancer immune suppression [56].

LD formation and TG synthesis are two tightly coupled processes under energy-rich conditions. The existing evidence is in support of a model that TG-LDs forms through *de novo* synthesis of TGs as a lens within the ER bilayer [57]. The initial step of TG synthesis is the conversion of exogenous or de novo synthetized FAs to fatty acyl-CoA by acyl-CoA synthetase (ACS). Next, fatty acyl-CoA and glycerol 3-phosphate are combined into TG in the ER via the classic glycerol phosphate pathway that consists of a series of key enzymes including glycerol-3-phosphate acyltransferase (GPAT), acylglycerolphosphate acyltransferase (AGPAT), phosphatidic acid phosphohydrolase (PAP or lipin), and diacylglycerol acyltransferase (DGAT). Subsequently newly formed LDs bud from the ER bilayer towards the cytosol. During nutrient shortage, LD-stored TGs can be hydrolyzed by LD-associated lipases to release FAs for energy production via mitochondrial β-oxidation and other metabolic pathways [58]. Regulation of intracellular lipolysis has been recently reported in various cancers and will be further discussed in this review. An alternative mechanism of LD breakdown is called lipophagy, and it consists of engulfment of portions of the LD by lipoautophagosomes and transport to the lysosomes for further degradation by lysosomal acid lipases (LAL) [59–61].

In 1977, Bush and colleagues first reported that cultured mouse fibroblasts accumulate TG-LDs in response to severe oxygen restriction [62]. Fast forward to now, increased accumulation of LDs is being recognized as a hallmark of hypoxic cancer cells of various origins [55]. A direct link between hypoxia and LDs has been extensively reported as HIFs regulate the transcription of various genes involved in FA metabolism [63]. Other studies found that in cancer cells, hypoxia increases FA uptake via upregulating FA binding proteins FABP3 and FABP7 [15], and to promote scavenging of FAs from serum lysophospholipids [64]. FABPs are a large family of cytoplasmic proteins essential for intracellular transport of FAs and other lipophilic molecules such as eicosanoids and retinoids. In addition to down-modulation of overall mitochondrial oxidative capacity, HIF-1 activation has been implicated in suppressing FA oxidation by decreasing the expression of carnitine palmitoyltransferase 1A (CPT1A) [65] as well as increasing TG synthesis by inducing Lipin

1 [66]. These events, which in combination lead to increased accumulation of TG-LDs, are strongly associated with greater tumor malignancy [67].

5. Regulation of cancer lipolysis by hypoxia

During instances of heightened metabolic demand, TA stores in LDs undergo a hydrolytic process termed lipolysis to release free FAs. In adipocytes, complete lipolysis is delicately regulated and is catalyzed sequentially by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL) [58, 68–70]. A majority of the free FAs and glycerol derived from white adipocyte lipolysis are delivered to other peripheral tissues for energy production. Compared to the white adipocytes that store a single large TG-LD, cells from other tissues types (e.g. liver, skeletal muscle) and cancer only possess a limited capacity for TG synthesis and only contain many smaller LDs. In these nonadipocyte cells, FAs derived from intracellular lipolysis usually are oxidized internally to meet the local energy demand [58, 71].

5.1 ATGL-mediated lipolysis and its protein regulators

ATGL is the rate-limiting intracellular TG hydrolase that catalyzes the first step of lipolysis, converting TG to diacylglycerol (DG) and one free FA [58]. ATGL exhibits low or no lipase activity for other substrates, such as DG, MG or cholesteryl esters [68]. The human and mouse genes for ATGL/ Patatin Like Phospholipase Domain Containing 2 (PNPLA2) encode proteins that share 84% of amino acids. ATGL is expressed broadly in all tissues with the highest levels in white and brown adipose tissue. Studies using knockout mouse models have demonstrated the essential function of ATGL in adipose lipolysis as well as in the whole body lipid and energy metabolism [72–74]. In brown adipose tissue, liver and striated muscles, ATGL selectively channels hydrolyzed FAs to β-oxidation in mitochondria and for synthesis of lipid ligands for PPARα and PPARδ/β [75–77]. Consistently, ATGL deficiency causes accumulation of TG-LDs and impairs mitochondrial FA oxidation in these oxidative tissues [73, 77, 78].

Aside from the LD localization, the enzyme action of ATGL is decided by the presence of its coactivator CGI-58 (comparative gene identification-58; also known as ABHD5 (α/β hydrolase fold-containing protein 5))[79]. The patatin-like domain of ATGL interacts with CGI-58, while the C-terminal domain of this enzyme is responsible for its localization to LDs. In humans, mutations in ATGL and CGI-58 genes are causal for a group of neutral lipid storage disease characterized by excessive TG deposition in multiple nonadipose tissues [80, 81]. Earlier work by our laboratory identified a small protein encoded by G0/G1 Switch Gene 2 (G0S2) as a potent endogenous inhibitor of ATGL [82], providing another important piece to this complex lipolysis puzzle. G0S2 is a basic protein that contains two potential α helices separated by a hydrophobic domain (HD). Direct binding between the HD of G0S2 and the patatin-like catalytic domain of ATGL has been shown to convey inhibition of ATGL's TG hydrolase activity *in vitro* and ATGL-mediated lipolysis in various cell types [83, 84]. An enzymatic study by Oberer and colleagues has further shown that a peptide derived from the human G0S2 HD inhibits ATGL noncompetitively in the nanomolar range [85]. Importantly, recent animal studies have yielded compelling evidence

demonstrating the *in vivo* role of G0S2 as a major metabolic and energy regulator in adipose tissue, liver and cardiac muscle through its inhibitory action on ATGL [86–91].

5.2 Tumor suppressive role of intracellular lipolysis

A large body of evidence suggests that inhibition of intracellular lipolysis may promote cancer development. ATGL expression was found to be decreased in the tissues of human lung adenocarcinoma and lung squamous cell carcinoma compared to normal epithelium. Consistently, ablation of ATGL induces spontaneous pulmonary neoplasia in mice and a more aggressive phenotype in lung carcinoma cells [92]. Intestine-specific deletion of ATGL coactivator CGI-58 resulted in colorectal carcinogenesis [93]. Adipose-specific ATGL and HSL double-knock out induced development of liposarcoma in brown adipose tissue [94]. HSL was originally identified in white adipose tissue as a fasting-induced TG hydrolase [95]. However, later studies have shown that HSL mainly acts as a diacylglycerol lipase downstream of ATGL-mediate TG hydrolysis [69, 70]. Moreover, deficiency of HSL was recently reported to accelerate the development of pancreatic cancer in a conditional KrasG12D mouse model [96].

5.3 HIG2/HILPDA as a key regulator of ATGL-mediate lipolysis in hypoxic cancer

In the report by Bensaad et al., convincing data are provided to demonstrate that hypoxia induces HIF-1α-dependent accumulation of LDs in various tumor cell lines and that storage of neutral lipids in LDs constitutes an essential mechanism for survival during the hypoxia-reoxygenation transition [15]. This adaptive mechanism appears to require FA binding proteins FABP3/7 and LD structural protein Perilipin 2 as their singly depletion significantly decreased LD formation under hypoxia. Consequently, hypoxic cells became impaired in their ability to handle lipolytic products, leading to increased ceramide production and cell death [15]. Evidence derived from separate studies of glial and neural stem cells further implies that the capacity to accumulate LDs is positively linked to the ability of cells to survive oxidative stress in hypoxia [97, 98]. Given that FA oxidation and hypoxia both promote mitochondrial generation of reactive oxygen species (ROS) [11–13], a potential mechanism emerged in which TG-LDs accumulation in hypoxia is a result of lipolytic inhibition to limit the amount of free FAs available for oxidation. By using various ATGL CRISPR knockout CRC and RCC cell lines, we were able to obtain first piece of evidence that ATGL-mediated lipolysis is suppressed in hypoxic cancer cells [14]. A subsequent proteomic search for specific lipolytic regulators in hypoxic cancer cells led to our discovery of hypoxia-inducible gene 2 (HIG2) as a selective inhibitor of ATGL [14]. HIG2 is a 63-amino acid (~7-kDa) peptide encoded by a HIF-1 target gene named Hilpda (Hypoxia Inducible Lipid Droplet Associated).

Early reports described HIG2 as a PPARα target gene in liver and acts to promote hepatic TG deposition by reducing TG secretion [99]. In addition, HIG2-ablated hepatocytes were previously shown to exhibit increased TG turnover under normoxic conditions [100]. However, knockout mouse studies conducted later yielded results arguing against a direct involvement of HIG2 in lipolysis in the absence of oxygen shortage [101, 102]. Other studies suggested that HIG2 represent a LD associated protein upregulated during hypoxia [103]. Indeed, HIF-1α was shown to bind the promoter region of the Hilpda gene to activate

transcription of HIG2 [103]. As a result, HIG2 expression pattern is consistent with that of HIF-1α. In macrophages, HIG2 was shown to be localized to the ER-LD interface, and HIG2 deletion abolished hypoxia-induced accumulation of TGs, cholesteryl esters and oxidized LDL. Conditional HIG2 knockout mice exhibited reduced plaque areas in aorta and macrophage content when crossed onto an apoE-deficient background [104]. These results suggest that HIG2 promotes lesion formation and progression of atherosclerosis. Furthermore, a most recent study by van Dierendonck et al. showed that HIG2 is essential for LD accumulation in adipose tissue macrophages, though the lipid storage mediated by HIG2 does not seem to influence adipose tissue inflammation or obesity phenotypes in mice fed with high fat diet [105].

Early studies have revealed that HIG2 contributes to the production of intact LDs in various cancer cells, including those from colorectal cancer (CRC), ovarian cancer, RCC and HCC [100, 103, 106]. The first hint on HIG2 protein function came from the alignment that revealed a homology between HIG2 and G0S2 HD sequences. Subsequent biochemical analysis showed that like G0S2, HIG2 inhibits ATGL through direct physical association [14, 107]. This inhibition can occur under both basal as well as CGI-58-stimulated conditions. Both interaction and co-localization with ATGL at the LD surface require a LY(V/L)LG motif in the HD of HIG2 [14]. In cells of various cancer types including RCC and CRC, hypoxia promotes TG-LD accumulation through HIG2-mediated ATGL inhibition [14, 16]. Lipolytic inhibition in turns triggers downregulation of PPARα target genes for mitochondrial activity and FA β-oxidation, leading to decreased ROS generation and apoptotic cell death (Figure 1). Consistently, when exposed to oleate and hypoxia, increased FA oxidation and excessive ROS generation caused most of HIG2 knockout cells to undergo capsase-3 dependent apoptosis. HIG2 knockout also led to a profound delay in tumor growth along with increased lipid peroxidation and apoptosis in xenograft tumor tissues. Though it was shown to be a weaker ATGL inhibitor than G0S2 in in vitro enzymatic assays [107], HIG2's function appears to be ATGL-specific in vivo as co-deletion of ATGL was able to completely reverse the phenotypes caused by HIG2 deficiency [14]. In addition to hypoxia, nutrient deprivation was recently shown to upregulate HIG2 post-transcriptionally in CRC cells by a mechanism requiring autophagic flux and LD turnover [16]. This finding raises the possibility that HIG2 mediates the storage of autophagy-generated FAs as TGs in LDs. Additional insight into the function of HIG2 has been uncovered by lipidomic analysis showing that aside from promoting TG storage under hypoxia, HIG2 affects FA length, saturation and amounts of major phospholipids classes [16]. These results indicate a role of HIG2 in the regulation of fluidity, homeostasis and metabolism of membrane phospholipids.

Analysis of the Cancer Genome Atlas (TCGA) datasets showed that the expression of HIG2 mRNA is strongly associated with various solid tumors in humans including CRC and RCC [14]. HIG2 protein was found to be highly abundant in human RCC samples relative to the matched adjacent normal kidney tissues. The expression pattern of HIG2 also correlates with the tumor TG content [14]. Recently, Zou et al. reported a critical relationship between HIF2-dependent HIG2 expression and the vulnerability of clear cell RCC to ferroptosis [108]. One key piece of supporting evidence is that HIG2 expression promotes enrichment of polyunsaturated lipids, the rate-limiting substrates for lipid peroxidation. As G0S2 expression failed to elicit a similar effect, the involvement of HIG2 in ferroptosis of ccRCC

is thought to be ATGL-independent. However, the fact that enrichment of polyunsaturated FAs increased in both TGs and phospholipids seems to argue against the possibility that HIG2 inhibits a separate TG lipase other than ATGL. Interestingly, both G0S2 and HIG2 were recently shown to possess an ATGL-independent role in promoting TG synthesis. While G0S2 harbors an intrinsic lysophosphatidic acid acyltransferase (LPAAT) activity [109], HIG2 appears to be capable of stimulating the function of DGAT1 [110]. However, it remains to be determined if HIG2 plays a similar lipogenic role in the settings of clear cell carcinoma.

6. Implications of lipolytic inhibition by HIG2/HILPDA

Hypoxia and pseudohypoxia associated with VHL deficiency potently induces expression of HIF-1α during the expanding growth of solid tumors. Increased HIF-1 activity is now known to trigger three major metabolic switches that support cell growth, proliferation and survival: (1) decreased glucose oxidation and increased glycolysis, (2) increased dependence on glutamine, and (3) inhibition of intracellular lipolysis. While glycolytic intermediates are channeled into pathways for synthesis of macromolecules (such as lipids, nucleotides, and proteins), glutamine is often favorably used through reductive carboxylation for *de novo* FA synthesis to support membrane biogenesis [46–48]. Lipolytic inhibition, on the other hand, leads to decreased FA oxidation and associated oxidative stress.

6.1 Impact on glucose and glutamine metabolism

Recently, increased storage of FAs as TGs is being recognized as another prominent feature of hypoxic and pseudohypoxic cancer cells [15, 55]. We and others discovered that the HIF-HIG2 antilipolytic pathway is critical for mediating this process [14, 16]. Specifically, HIG2 was found to directly binds to and inhibits ATGL at the LD surface, thereby blocking lipolytic breakdown of TGs. Although lipolytic liberation leads to increased FA oxidation and ROS production, HIG2 deletion incurs no effect on glucose consumption and glycolytic phenotypes in hypoxic cancer cells [14]. This departs from how glucose and FA metabolism is coordinately regulated in normoxic conditions. In the classical Randle cycle, increased FA oxidation occurs alongside inhibition of glucose uptake and utilization [111]. On the other hand, these results complement the existing model illustrating that HIF-1 represses glucose flux to the TCA cycle through mediating expression of PDK1, which inhibits pyruvate oxidation through phosphorylating and inactivating PDH [38]. In cancer cells located within the hypoxic regions of solid tumors, concurrent inhibition of ATGL by HIG2 and PDH by PDK1 should provide a survival advantage as inhibition of FA and glucose oxidation together should lead to substantially reduced oxygen consumption and mitochondrial ROS generation.

Whether and how lipolytic inhibition mediated by HIG2 impacts glutamine metabolism in hypoxic cancer cells remain to be determined. To this end, an arising question concerns why cancer cells would choose to synthesize more FAs from glutamine in hypoxia during the same time when FA uptake and storage in TG-LDs is upregulated. One explanation may be that FAs derived from different sources are metabolized in a highly partitioned manner. According to previous studies of normal oxidative cell types, exogenously acquired

FAs are generally channeled through the LD-lipolysis-mitochondria route for oxidation [58]. In this context, LD storage and lipolytic action of ATGL constitute a buffering system to balance the uptake of exogenous FAs and their oxidative utilization. In proliferating cancer cells, however, membrane phospholipid (PL) biosynthesis was shown to mostly require FAs generated endogenously through *de novo* synthesis [112]. Moreover, a recent study found that lipogenesis through glutamine reduction is tightly regulated by intracellular citrate levels [48]. Based on these results, it is tempting to hypothesize that along with the prevention of ROS production to sustain cell survival, a simultaneous inhibition of FA oxidation by HIG2 and glucose/pyruvate oxidation by PDK1 leads to decreased generation of acetyl-CoA and citrate, thereby driving the reductive flux of glutamine by mass action to maintain lipogenesis and cell growth under hypoxia. Further investigation is needed to elucidate the interactions between the metabolic pathways of these three major energy substrates in hypoxic cancer cells.

6.2 Energy production upon reoxygenation following hypoxia

The abnormal vascularization greatly contributes to the fluctuation of oxygen levels inside solid tumors. The cyclic changes between hypoxia and reoxygenation due to the opening and closing of blood vessels have been associated with cancer metastasis [5, 56, 113]. While cancer cells exposed to hypoxia at the primary lesions mainly rely on glycolysis for energy production, detached cells are exposed to reoxygenation when they enter circulation and can potentially switch to reliance on mitochondrial substrate oxidation. In fact, FA oxidation was found recently to be essential to the development of anoikis resistance and metastatic phenotypes in various cancers [114–118]. Conceivably, tumor cells with a higher capacity to store TG-LDs in hypoxia and to mobilize them upon reoxygenation would be in a more advantageous position during detachment, invasion and metastasis. In accordance with this concept, the study by Bensaad et al. has shown that FAs stored in LDs during hypoxia are essential for ATP production in hypoxia-reoxygenation transition [15]. We speculate that the oxygen-sensing HIF-1-HIG2 pathway may be a crucial part of a mechanism conferring such a capacity. By inhibiting ATGL-mediated lipolysis, HIG2 acts to enhance TG stores downstream of HIF-1 activation in hypoxia. Upon reoxygenation, downregulation of the HIF-1-HIG2 pathway could lead to the liberation of ATGL activity, enabling TG hydrolysis to supply free FAs for β-oxidation in support of the energy need of invasion and metastasis. In human CRC, HIG2 expression was indeed found to be upregulated in liver and lung metastases as compared to the primary tumors [119].

6.3 Possible functional interaction between HIG2/HILPDA and G0S2

ATGL-mediated intracellular lipolysis is tightly coupled to FA oxidation, a catabolic process that depends on the supply of both FAs and oxygen. G0S2 is mainly expressed in normal metabolic tissues such as liver, adipose tissue and striated muscles [83]. In comparison, HIG2 is abundantly expressed in various solid tumors as well as macrophages [104, 105]. While G0S2 expression is controlled by PPARγ and LXRα and is responsive to increased FA levels [120, 121], endogenous level of HIG2 is low under normoxia and its expression is activated by HIF-1 in response to oxygen depletion [14, 103]. In macrophages but not cancer cells, mRNA expression of HIG2 can also be upregulated upon exposure to ectopic lipids [16, 104, 105]. Given the short half life of these small inhibitory proteins [122], the

available data are in support of a proof-of-concept that ATGL together with G0S2 and HIG2 constitute a gauge and valve system capable of reacting to the rapid changes in the ratio of intracellular free FAs to O_2. With the ability to adjust the rates of ATGL-mediated lipolysis and downstream FA oxidation, such a system renders cells capable of storing TGs during exposure to excessive amounts of FAs as well as evading oxidative stress when oxygen becomes scarce. Currently, no data is available with regard to the functional relationship between G0S2 and HIG2, or a context in which they are co-expressed and contribute to common phenotypes. Although G0S2 was originally thought to be a cell cycle regulator [123, 124], evidence is still lacking to establish its function in cancer settings. An earlier study by Kioka et al. showed that G0S2 promotes hypoxia tolerance by positively regulating oxidative phosphorylation and ATP synthesis in cardiomyocytes [125]. However, G0S2 expression does not appear to increase in hypoxic cancer cells [14]. Whether HIG2 plays a similar role in cancer also remains to be determined.

7. Conclusions

A large volume of evidence has been reported on the crucial role of metabolic reprogramming during cancer development. Cancer cells often are exposed to nutrient-deprived and poorly oxygenated tumor microenvironment. As a consequence, cancer cells rely on metabolic adaptations for maintaining energy production, acquiring building blocks to support cell growth, and synthesizing signaling molecules for various tumor-promoting activities. Rewiring of FA metabolism under hypoxia is particularly important in lipid-rich solid tumors such as RCC, CRC and fatty liver-associated HCC as FA oxidation consumes a large amount of oxygen and is a major contributor to ROS generation. The recently obtained evidence supports a novel concept that inhibition of TG breakdown or lipolysis by HIG2 is one of the crucial metabolic adaptations that hypoxic cancer cells deploy to maintain redox homeostasis through limiting FA. We propose that HIG2 is a metabolic oncogenic factor, which exerts tumor-promoting activity by inhibiting the complex ATGL/CGI-58 under hypoxia. Despite the progress, however, several major questions remain to be answered: whether and how is TG accumulation linked to glucose and glutamine metabolism in cancer cells? Why would hypoxic cancer cells need to simultaneously upregulate LD storage and *de novo* lipogenesis? Is TG-LD accumulation controlled by HIF-1-dependent mechanism relevant in tumor development *in vivo*? Do TG storage in hypoxia and hydrolysis in reoxygenation impact the energy supply required for cancer invasion and metastasis? Do TG-LDs, because of their anti-oxidant role, contribute to the resistance of cancer cells to pro-oxidant and anti-angiogenic therapeutics? Future research is needed to uncover the full spectrum of the interplay between lipolytic and antilipolitic mechanisms in specific tumor settings, and how it influences the overall metabolic behavior of cancer in hypoxia. Novel insight if produced will likely open up additional diagnostic and therapeutic avenues that improve outcomes for patients with solid tumors.

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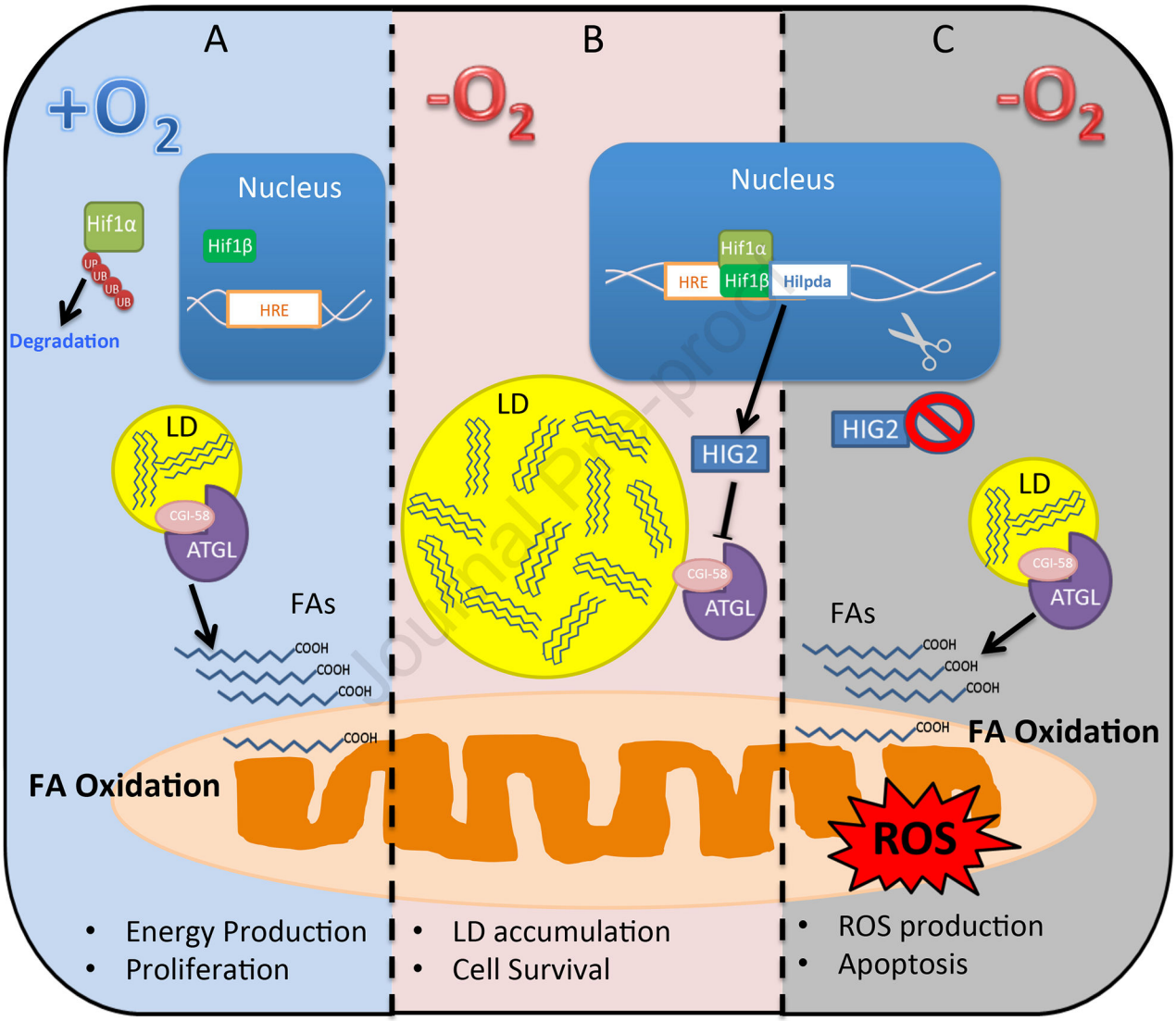

Figure 1. Lipolytic inhibition by HIG2 is central for a survival strategy employed by hypoxic cancer cells.

(A) In normoxic cells where HIF-1α protein stability is low due to increased ubiquitination and proteasomal degradation, ATGL-mediated lipolysis is active and downstream FA oxidation contributes to the overall energy production. **(B)** Hypoxic stabilization of HIF-1α protein leads to expression of the ATGL inhibitor HIG2, and subsequent lipolytic inhibition results in decreased FA oxidation along with improved oxygen homeostasis and cell viability. **(C)** HIG2 deficiency, induced either genetically or pharmacologically, forces increased lipolysis and FA oxidation that exacerbate ROS generation and apoptotic death in hypoxic cancer cells.