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Review

Regulation of lipid droplet homeostasis by hypoxia inducible lipid droplet associated HILPDA

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ABSTRACT

Nearly all cell types have the ability to store excess energy as triglycerides in specialized organelles called lipid droplets. The formation and degradation of lipid droplets is governed by a diverse set of enzymes and lipid droplet-associated proteins. One of the lipid droplet-associated proteins is Hypoxia Inducible Lipid Droplet Associated (HILPDA). HILPDA was originally discovered in a screen to identify novel hypoxia-inducible proteins. Apart from hypoxia, levels of HILPDA are induced by fatty acids and adrenergic agonists. HILPDA is a small protein of 63 amino acids in humans and 64 amino acids in mice. Inside cells, HILPDA is located in the endoplasmic reticulum and around lipid droplets. Gain- and loss-of-function experiments have demonstrated that HILPDA promotes lipid storage in hepatocytes, macrophages and cancer cells. HILPDA increases lipid droplet accumulation at least partly by inhibiting triglyceride hydrolysis via ATGL and stimulating triglyceride synthesis via DGAT1. Overall, HILPDA is a novel regulatory signal that adjusts triglyceride storage and the intracellular availability of fatty acids to the external fatty acid supply and the capacity for oxidation.

1. Introduction

Fatty acids are a major fuel for many different types of cells. Indeed, nearly all cells have the ability to oxidize fatty acids in mitochondria, leading to the production of ATP. Fatty acids enter cells via fatty acid transporters. After uptake, they are bound by fatty acid-binding proteins, which direct the fatty acids to their different metabolic fates. When uptake of fatty acids exceeds the requirements and capacity for oxidation, the excess fatty acids are esterified into triglycerides and stored in specialized organelles called lipid droplets [1]. The ability to store energy as triglycerides in lipid droplets endows cells with the ability to overcome periods of limited nutrient availability. In addition, storing fatty acids as triglycerides permits cells to cope with fluctuations in fatty acid supply and avoid lipotoxicity. Nearly all types of cells contain lipid droplets. Whereas an adipocyte contains one large lipid droplet, most cell types have multiple small lipid droplets. Under normal circumstances, these lipid droplets only take up limited space in the cell. However, in certain physiological and pathological conditions, lipid droplets may expand and occupy substantial cell volume [2]. For example, alcoholic and non-alcoholic steatohepatitis are characterized by proliferation and expansion of lipid droplets in hepatocytes [3,4]. A similar phenomenon is observed in hepatocytes during fasting.

A unique feature of lipid droplets is that they are very dynamic and can quickly expand or shrink depending on cellular demands. The synthesis of triglycerides, their storage in lipid droplets, and the subsequent breakdown of triglycerides into fatty acids is regulated by a complex set of enzymes and lipid droplet-associated proteins. These lipid droplet-associated proteins serve structural roles in lipid droplets and regulate the activity of key lipogenic and lipolytic enzymes [5]. The three enzymes responsible for intracellular lipolysis are adipose triglyceride lipase (ATGL; PNPLA2), hormone sensitive lipase (HSL; LIPE), and mono-glyceride lipase (MGLL) [6]. The activity of these lipases is carefully controlled at the transcriptional and especially post-translational level, which, besides phosphorylation, involves physical interactions with specific regulatory proteins. For example, the activity of ATGL is activated by ABHD5 (CGI-58) and inhibited by G0S2 [7]. Other important groups of lipid droplet-associated proteins are the five members of the perilipin family, PLIN1-PLIN5, and the three members of the Cell Death Inducing DFFA Like Effectors (CIDE) family. Detailed information on these groups of lipid droplet-associated proteins can be found elsewhere [8–10]. Recently, the Hypoxia Inducible Lipid Droplet Associated (HILPDA) protein emerged as a novel modulator of intracellular lipid droplet homeostasis in cancer cells, macrophages, and other cell types. This review provides a detailed overview of the role of HILPDA in lipid homeostasis in various cell types.

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MKHVLNLYLLGVVLTLLSIFVRVMESLEGLLESPSPGTSWTTRSQLANTEPTKGLPDHPSRSM   Human
MKHVLNLYLLGVVLTLLSIFVRVMESLEGLLESPSPGTSWTTRSQLANTEPTKGLPDHPSRSM   Pan Paniscus
MKHMLNLYLLGVVLTLLSIFIRVMESLEGLLENASPGTSWTTRSQLANTEPTKGLPDHPSRSM   Macaque
MKQVLQLYLLGVVLTLLSVFVRLMETLGGLLESPSPGSFWTTRGQLANTEATKSLPEHPSRGV   Sus scrofa
MKYMLNLYVLGVMLTLLSIFVRLMESLGGLLENPLPGSSWITRGHLANTQPPRGLPDHPSRGVQ  Rattus norvegicus
MKFMLNLYVLGIMLTLLSIFVRVMESLGGLLESPLPGSSWITRGQLANTQPPKGLPDHPSRGVQ  Mouse
MK  LNLY+LGV LTLLSIFVR+MESL GLLE P PG SW TR QLANT P KGLPDHPSR
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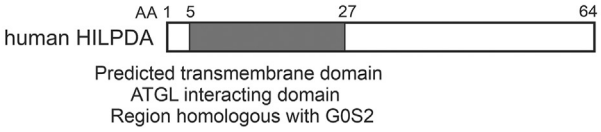
B



C

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METVQELIPLAKEMMAQKRKGKMVKLYVLGSVLALFGVVLGLMETVCSPFTAARRLRDQEAAVAELQAALERQALQKQALQEKGKQQDTVLGGRALSNRQHAS Human G0S2
                + LY+LG VL L   + + +ME++
                MKHVLNLYLLGVVLTLLSIFVRVMESLEGLLESPSPGTSWTTRSQLANTEPTKGLPDHPSRSM                                  Human HILPDA
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Fig. 1. Primary sequence of HILPDA. A) Comparative analysis of the amino acid sequence of HILPDA in different species. B) Graphical presentation of the primary sequence of HILPDA, highlighting the N-terminal domain involved in interactions with lipid droplets and ATGL. C) Comparative analysis of the amino acid sequence of human HILPDA and G0S2, showing significant homology in the N-terminal region of HILPDA.

2. Cell-specific expression and regulation of HILPDA

2.1. HILPDA gene

HILPDA was originally discovered in a subtractive hybridization screen to identify novel genes upregulated by hypoxia in cultured human cervical epithelial cells [11]. The human *HILPDA* gene is located on chromosome 7 and consists of two exons and one intron, covering a genomic region of 2.6 kb. The human cDNA is almost 1.4 kb and gives rises to a protein of 63 amino acids. The mouse HILPDA protein is slightly larger at 64 amino acids. The primary structure of HILPDA in several mammals is shown in Fig. 1A.

According to Genevestigator, *HILPDA* mRNA is expressed at high levels in numerous tissues and cell types [12]. In humans, high *HILPDA* mRNA expression is reported in peripheral blood CD8 activated T cells, adipocytes, esophagus, blood vessels, dendritic cells, and various epithelial cells, while in mice, the highest *Hilpda* mRNA expression is observed in various types of immune cells, including dendritic cells, neutrophils, eosinophils, macrophages, B cells, and T cells, as well as cultured hepatocytes. At the protein level, high HILPDA levels are found in murine white and brown adipose tissue, heart, and lung [13–15].

2.2. Regulation of HILPDA expression

The expression of HILPDA in different cell types is controlled by several stimuli. As reflected by its full name, HILPDA is induced by hypoxia. Induction of HILPDA mRNA and protein by hypoxia was first observed in human cervical epithelial cells [11], and was subsequently confirmed in numerous cancer cells [11,16–18], and macrophages [14,19]. Induction of *Hilpda* by hypoxia is mediated by HIF-1α via a number of hypoxia-response elements located 300 nucleotides upstream of the transcription start site of the *Hilpda* gene [16].

Other potent activators of *Hilpda* mRNA expression are fatty acids, which has been observed in various types of macrophages [14,19], hepatocytes [20], mouse embryonic fibroblasts [17], and in the HCT116 human colon cancer cell line [17]. The relative increase in HILPDA protein by fatty acids is more pronounced than the increase in *Hilpda* mRNA, which is consistent with the observation that fatty acids protect HILPDA from degradation [16]. Induction of *Hilpda* mRNA is likely mediated in part by the fatty acid-inducible PPAR transcription factors. In human and mouse hepatocytes, *Hilpda* mRNA is upregulated by PPARα via a conserved PPAR response element located 1200 nucleotides upstream of the transcription start site [21]. Regulation by PPARα is not responsible for the induction of hepatic *Hilpda* mRNA and protein during fasting [21,22]. In human and mouse adipocytes, *HILPDA* mRNA is upregulated by PPARγ via a prominent PPARγ superenhancer covering about 8 kb upstream of the *HILPDA* gene and containing several conserved PPARγ binding sites.

Other stimuli that increase HILPDA mRNA and protein levels are several β-adrenergic agonists and forskolin. These effects were observed in adipocytes and suggest a stimulatory effect of cAMP [13,15]. Fasting induces *Hilpda* mRNA and protein levels in mouse adipose tissue, which may be mediated by cAMP [13]. In bone marrow-derived macrophages, HILPDA protein is highly induced by lipopolysaccharide [19].

Lastly, HILPDA mRNA and protein levels were found to be upregulated in different types of tumor cells, including renal cell carcinoma, ovarian clear cell adenocarcinoma, colorectal adenoma and carcinoma, and in different solid tumors [18,23,24]. In renal cell carcinoma cell lines, *HILPDA* expression is under positive transcriptional control of the β-catenin/Wnt pathway. Induction of *HILPDA* expression by β-catenin/Wnt is mediated by a TCF/LEF binding site about 2.1 kb upstream of the transcription start site [25].

3. Intracellular localization of HILPDA

HILPDA was shown to localize to lipid droplets in several different cell types [13,16,22]. However, not all lipid droplets are surrounded by HILPDA. In contrast to PLIN2, a ubiquitously expressed and constitutive lipid droplet-associated protein, HILPDA only colocalizes with a subpopulation of the lipid droplets [16,20]. Apart from lipid droplets, HILPDA has also been shown to localize to and migrate through the endoplasmic reticulum, the site of lipid droplet synthesis, where it correlated significantly with newly synthesized triglycerides [19,20]. In addition, HILPDA preferably accumulates in lipid droplets undergoing remodelling (shrinking and expansion) [20]. Consistent with this observation, HILPDA was shown to colocalize with the lipolytic and lipogenic enzymes ATGL and DGAT1/2 respectively [18,20,26,27]. Together, these observations suggest that HILPDA is coupled to a functionally distinct subpopulation of lipid droplets that is undergoing

active remodelling, which fits with its alleged role as small molecule inhibitor and activator of lipolysis and triglyceride synthesis, respectively (see below).

4. Molecular mechanism of action of HILPDA

HILPDA is a small protein that bears no homology with any other protein, except for a conserved region that is predicted to be a transmembrane domain. This stretch of 24 amino acids, which is close to the N-terminus and targets HILPDA to lipid droplets, is highly homologous to a region in the protein G0S2 (Fig. 1B, C). G0S2 is an inhibitor of ATGL and is active in several tissues, including adipose tissue, liver, and skeletal muscle [28]. Recent research suggests that HILPDA also inhibits ATGL. In HTC116 cells, mouse embryonic fibroblasts, and bone marrow-derived macrophages, HILPDA deficiency caused a decrease in lipid droplets and triglyceride storage, which was restored by ATGL inactivation [14,17,18]. By contrast, ATGL inactivation had a minimal effect on lipid storage in wildtype cells. Moreover, the increase in lipolysis in HILPDA-deficient cells was nullified by ATGL inactivation [17,18]. These data indicate that HILPDA is an endogenous and physiological inhibitor of ATGL. Biochemical studies indicate that HILPDA directly inhibits ATGL triglyceride hydrolase activity via a direct physical interaction between the two proteins [18,26]. This interaction was also observed intracellularly in live cells using FRET-FLIM [26]. The ATGL interaction and inhibition by HILPDA are mediated by the conserved N-terminus of HILPDA and crucially depend on residues 7–11 [17,18,26]. The HILPDA interacting region in ATGL covers the N-terminal patatin domain-containing region, which also mediates triglyceride hydrolysis and the interaction with CGI-58/ABHD5, Atglistatin, acyl-CoA, and G0S2.

Interestingly, in a cell-free system, the interaction between ATGL and HILPDA was found to be substantially weaker than the interaction between ATGL and G0S2 [26], which may also explain why initial studies failed to provide support for ATGL inhibition by HILPDA [21]. Nevertheless, studies in HILPDA-deficient cells indicate that HILPDA effectively inhibits ATGL-mediated lipolysis. Additional research is needed to determine why HILPDA is less potent in cell-free systems compared to live cells, but it can be hypothesized that HILPDA requires interaction with an auxiliary factor to gain full activity. It should be noted that apart from binding and inhibiting ATGL, HILPDA also seems to reduce ATGL protein levels, possibly by destabilizing ATGL protein [13,14].

Besides inhibiting lipolysis, in a recent preprint, HILPDA was shown to promote intracellular lipid accumulation by enhancing triglyceride synthesis [20]. The final rate-limiting step in the formation of triglycerides is catalyzed by diacylglycerol acyltransferases (DGAT), consisting of two evolutionarily unrelated enzymes DGAT1 and DGAT2. It has been shown that DGAT2 preferentially catalyzes triglyceride synthesis from de novo synthesized fatty acids, whereas DGAT1 catalyzes triglyceride synthesis from exogenous fatty acids or fatty acids released by lipolysis [29]. In the preprint, HILPDA was shown to co-localize and physically interact with DGAT1 and DGAT2 in HepG2 cells and promote DGAT activity. Although HILPDA colocalized with DGAT2, HILPDA needs exogenous fatty acids to increase triglyceride accumulation, suggesting that DGAT1 likely is the preferred target of HILPDA [20]. In addition, HILPDA increased protein levels of DGAT1, possibly by serving as a protein stabilizer. Collectively, HILPDA may thus promote intracellular lipid storage by suppressing lipolysis via inhibition of ATGL and inducing triglyceride synthesis via activation of DGAT1 [20].

5. Role of HILPDA in lipid metabolism in various cells and tissues

Numerous studies have demonstrated that HILPDA promotes lipid storage in various cells and tissues. Below, an overview is given of the functional data on HILPDA in different cell types.

5.1. Hepatocytes

Overexpression of HILPDA in primary hepatocytes or hepatoma cells significantly increases intracellular lipid accumulation [20,22]. Similarly, in vivo AAV-mediated HILPDA over-expression markedly increases liver triglyceride content, which is at least in part due to a decrease in VLDL-TG secretion [21]. Conversely, hepatocyte-specific HILPDA deficiency was found to lower liver triglycerides, yet only in mice fed chow, a semi-purified low fat diet, and a choline and methionine deficient high fat diet [20,22]. The overall magnitude of the decrease in hepatic triglycerides by HILPDA deficiency is modest. No significant decrease in liver triglycerides was found in hepatocyte-specific HILPDA deficient mice fed a regular semi-purified high fat diet or mice in the fasted or refed state [20,22]. In vitro, HILPDA deficiency caused a very pronounced decrease in cellular lipid accumulation in primary hepatocytes and liver slices. The modest effect of HILPDA deficiency on triglyceride levels in mouse liver compared to primary mouse hepatocytes and mouse liver slices may be explained by the relatively low expression of *Hilpda* in mouse liver as compared to ex vivo [20].

Mechanistically, it was shown that HILPDA deficiency in hepatocytes increases triglyceride turnover and lipolysis, as well as fatty acid β-oxidation [22]. The elevated triglyceride turnover in HILPDA-deficient hepatocytes is likely mediated by an inhibitory and stimulatory effect of HILPDA on ATGL and DGAT1 activity, respectively. [20,22]

Neither hepatocyte-specific HILPDA overexpression nor deficiency has any influence on plasma cholesterol, triglyceride, ketone body, or non-esterified fatty acid (NEFA) levels [20–22]. Interestingly, hepatocyte-specific HILPDA deficiency significantly improved glucose tolerance in mice fed chow or a high fat diet, while no significant effect of HILPDA deficiency was observed on insulin tolerance [22]. Currently, the mechanism underlying the changes in glucose tolerance remains unclear.

5.2. Adipocytes

HILPDA is expressed at high levels in brown and white adipocytes and increases during mouse 3T3-L1, mouse primary brown fat, and human SGBS adipogenesis [13,15,30]. Nevertheless, HILPDA does not seem to be directly implicated in adipogenesis [13]. The effect of HILPDA deficiency in adipocytes has been studied in vivo and in vitro. In vivo, adipocyte-specific HILPDA deficiency significantly decreased weight of the epididymal fat depot in mice fed a high fat diet [30]. In contrast, no effect of HILPDA deficiency on weight of adipose tissue depots was observed under conditions of 24-h fasting, 10-day cold exposure, acute injection of a β3-adrenergic agonist, and when mice were fed a high fat diet at thermoneutrality [13,30]. In all of the above conditions, HILPDA deficiency in adipocytes did not alter plasma NEFAs and glycerol, two systemic measures of lipolysis, nor did it influence plasma triglycerides [13,30]. The effects of HILPDA deficiency on ex vivo and in vitro lipolysis are mixed. HILPDA deficiency did not influence basal or isoproterenol-induced ex vivo NEFA and glycerol release from adipose tissue explants and primary adipocytes [13,15,30]. Also, *Hilpda* silencing did not influence fatty acid and glycerol release in 3 T3-L1 adipocytes, despite lower triglyceride storage. By contrast, AV-mediated HILPDA overexpression significantly decreased fatty acid release by 3T3-L1 adipocytes, concurrent with a marked reduction in ATGL protein levels. Interestingly, adipocyte-specific HILPDA deficiency raised liver triglycerides in mice after fasting, which may reflect increased delivery of NEFAs from adipose tissue [13]. Collectively, these data lead to the tentative conclusion that HILPDA is not a major physiological regulator of adipocyte lipolysis, although additional studies are warranted.

Interestingly, in vivo adipocyte-specific HILPDA deficiency was associated with worsened glucose tolerance in mice fed a high fat diet at thermoneutrality, but improved glucose tolerance at 23 °C [30]. The

latter result appears to be due to deficiency of HILPDA in brown adipocytes. How deficiency of HILPDA in brown adipocytes may improve glucose tolerance is unknown. By contrast, whole body *Hilpda*−/− mice fed chow and kept at 22 °C exhibited no change in glucose tolerance [15].

5.3. Macrophages

The functional role of HILPDA in macrophages has been studied by crossing *Hilpda*$^{flox/flox}$ mice with mice expressing Cre under the Tie2 promoter (endothelial and hematopoietic cells) or LysM promoter (myeloid cells). These models, which both lead to a very pronounced reduction in HILPDA levels in bone marrow-derived macrophages, consistently show a marked reduction in intracellular lipid droplets and total lipid levels, which are not caused by decreased fatty acid uptake but rather by decreased triglyceride retention [14,19]. The decrease in triglyceride retention was shown to be mediated by elevated ATGL-mediated lipolysis. Enhanced lipolysis without efficient disposal of the released fatty acids may be expected to lead to lipotoxicity. Indeed, fatty acid-loaded HILPDA-deficient macrophages exhibited increased formation of reactive oxygen species, decreased cell viability, and elevated fatty acid-dependent gene regulation [14,19]. However, lipotoxicity may be limited in HILPDA-deficient macrophages by increased oxidative disposal of fatty acids [14].

Lipid-laden macrophages (also known as foam cells) are abundant in atherosclerotic plaques and are a key feature of atherosclerosis. Immunohistochemical studies located HILPDA to foam cells in human atherosclerotic plaques, suggesting a potential role for HILPDA in atherogenesis [16]. In agreement with this notion, in atherosclerosis-prone *Apoe*−/− mice, Tie2-driven HILPDA-deficiency was associated with a marked reduction in atherosclerotic lesions and plaque lipid content [19]. These data suggest that HILPDA may be a potential therapeutic target for atherosclerosis. Another tissue enriched in lipid-laden macrophages is adipose tissue, where foam cells are believed to contribute to the inflammatory phenotype during obesity. Despite reducing lipid storage in adipose tissue macrophages, LysM-driven HILPDA-deficiency did not alter the inflammatory status of adipose tissue in diet-induced obesity, nor did it influence any metabolic perturbations associated with obesity [14]. This study suggests that the contribution of foam cells in obesity-induced inflammation and metabolic dysregulation may be less important than previously envisioned.

5.4. Tumor cells

Expression of *HILPDA* is upregulated in several cancers, including renal [25], ovarian [24], uterine [31], and colon cancers [23]. The functional role of HILPDA has been studied in HTC116 cells, a human colon cancer cell line. In these cells, hypoxia increased intracellular triglycerides by inhibiting lipolysis in a HILPDA-dependent manner [17,18]. Similarly, fatty acids increased lipid droplet abundance and intracellular triglycerides in HTC116 cells via HILPDA [17]. HILPDA deficiency in hypoxic HTC116 cells led to enhanced apoptosis, elevated ROS production, and increased fatty acid oxidation, concomitant with upregulation of PPAR target genes [17,18]. Co-ablation of ATGL was able to rescue the effects of HILPDA deficiency, indicating that the pro-survival property of HILPDA against apoptosis during hypoxia is mediated by inhibition of lipolysis [18]. Interestingly, the growth of HCT116 tumors in nude mice was slower for the HILPDA deficient cells than for wildtype cells [17,18]. Consistent with HILPDA acting via ATGL, deficiency of ATGL along with HILPDA restored tumor growth and rescued the effects elicited by HILPDA deficiency alone [18]. Overall, these data suggest that HILPDA promotes cancer cell survival via inhibition of lipolysis by decreasing lipotoxicity, ROS production, and oxidative damage.

5.5. Whole body

Experiments using whole body *Hilpda*−/− mice have shown that HILPDA is not required for viability, growth and reproduction [15]. However, at 22 °C, *Hilpda*−/− mice were less active, had lower energy expenditure, and lower food intake. In addition, *Hilpda*−/− mice had lower body temperature after fasting, suggesting that HILPDA is required for maintaining body temperature during fasting. *Hilpda*−/− mice did not exhibit any significant change in plasma cholesterol, triglycerides, creatine kinase, lactate dehydrogenase, and in the triglyceride content of adipose tissue, liver and muscle [15].

6. Why are there two ATGL inhibitors: HILPDA and G0S2?

HILPDA shares a lot of functional and structural resemblance with G0S2. Similar to HILPDA, G0S2 is a relatively small protein, consisting of 103 amino acids. Compared to *G0S2,* the *HILPDA* gene is evolutionarily younger [26]. G0S2 was demonstrated by Yang et al. to be an interacting partner and selective inhibitor of ATGL [32]. G0S2 and HILPDA interact with ATGL through the conserved LY(V/L)LG motif present in their hydrophobic domain [18]. Despite the structural resemblance between HILPDA and G0S2, in a cell-free system HILPDA showed a lower inhibitory potency toward ATGL than G0S2 (IC_{50} value 2 μM vs. 22 nM for HILPDA and G0S2, respectively) [26]. *Hilpda* and *G0s2* are both induced by fatty acids, and are regulated by PPARs [21,33,34]. In addition, HILPDA and GOS2 are both highly expressed in white and brown adipose tissue, as well as in a number of immune cells [12]. However, the overall expression patterns and regulation of *Hilpda* and *G0s2* are quite disparate, suggesting that they are required under different circumstances and accordingly may not be functionally redundant. We found that when HILPDA and G0S2 are co-expressed in the same cell, they decorate different lipid droplets, suggesting they may regulate distinct subpopulations of lipid droplets (our unpublished data). How these lipid droplet subpopulations differ from each other is unclear. By harboring distinct types of lipid droplets and regulating their lipolysis via different mechanisms, cells may be able to control the availability and possibly the type of fatty acids for particular cellular functions. Further research is necessary to better define the specific functional roles of HILPDA and G0S2 in cellular lipid metabolism.

7. Concluding remarks and future perspectives

Studies over the past decade have shown that HILPDA has a profound impact on intracellular fatty acid metabolism. Cell-specific *Hilpda*-deficient mouse models suggest that the importance of HILPDA in regulating cellular triglyceride storage is cell-type specific and is likely dictated by the level of HILPDA expression, as well as by the expression of related proteins such as G0S2. Mechanistically, HILPDA promotes triglyceride storage by inhibiting ATGL-mediated lipolysis and stimulating DGAT1-mediated triglyceride synthesis (Fig. 2). It can be hypothesized that HILPDA may be part of a larger triglyceride turnover complex (“lipolysome”) that includes enzymes involved in triglyceride synthesis and triglyceride breakdown, including ATGL and DGAT1, as well as regulatory proteins such as ABHD5 and G0S2 [35–37]. Exactly how HILPDA regulates the activity of these enzymes is still unknown and should be the subject of future biochemical studies. It is conceivable that HILPDA functions as small protein binding partner of ATGL and DGAT1, causing their destabilization (ATGL) or stabilization (DGAT1).

One of the unique features of HILPDA is its marked upregulation by several external stimuli, including hypoxia, fatty acids, and β-adrenergic agonists. It thus seems that HILPDA functions as an adaptive signal that is called into action under specific circumstances. The induction by fatty acids is likely part of a feed forward mechanism to properly dispose of intracellular fatty acids by promoting their storage as triglycerides, either by activating the last step in triglyceride

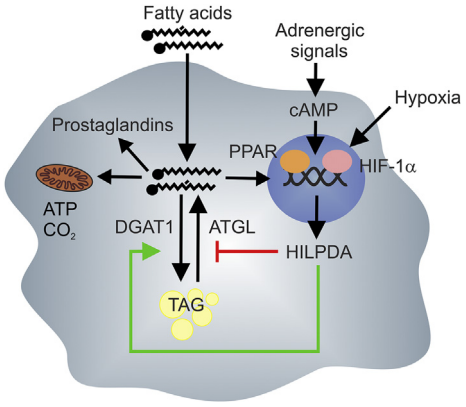

Fig. 2. Graphical overview of the role of HILPDA in cellular lipid metabolism. Fatty acids increase HILPDA levels partly via PPAR-mediated transcriptional regulation, hypoxia increases HILPDA levels via HIF-1α-mediated transcriptional regulation, and adrenergic stimulation increases HILPDA levels via cAMP. HILPDA suppresses lipolysis via inhibition of ATGL and induces triglyceride synthesis via activation of DGAT1. Induction of HILPDA by fatty acids and hypoxia can be considered an adaptive mechanism to lower the intracellular concentration of fatty acids.

synthesis and/or inhibiting the first step in triglyceride breakdown (Fig. 2). The induction of HILPDA by hypoxia likely serves to shift fuel utilization toward glucose by lowering the intracellular concentration of fatty acids, which cannot be used as fuel under low oxygen conditions.

A major effect of HILPDA deficiency on lipid metabolism has been observed in macrophages, showing markedly reduced lipid storage as a result of enhanced lipolysis. So far, the functional impact of HILPDA deficiency in macrophages has only been studied in relation to atherosclerosis and adipose tissue inflammation. In the future, it would be of interest to investigate the role of macrophage HILPDA in non-alcoholic steatohepatitis. In addition, HILPDA may be involved in other diseases characterized by foamy macrophages, including tuberculosis, multiple sclerosis, and kidney disease. Finally, it would be interesting to explore whether HILPDA plays an important role in lipid storage in microglial cells, especially since it was recently proposed that lipid accumulation in microglial cells is connected with aging in mouse and human brains [38].

As mentioned previously, HILPDA is not only highly expressed in macrophages but also in numerous other immune cells, including dendritic cells, neutrophils, eosinophils, B cells, and T cells. Currently, the role of HILPDA in these immune cells is unknown. Inactivation of HILPDA may be an effective tool to study the functional roles of lipid droplets in immune cells, which are still poorly characterized.

Overall, it can be concluded that HILPDA is a novel regulator of intracellular lipid metabolism. Specific stimuli such as hypoxia and fatty acids promote intracellular lipid storage at least partly via upregulation of HILPDA. HILPDA thus adjusts triglyceride storage and the intracellular availability of fatty acids to the external fatty acid supply and the capacity for oxidation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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