Lipid droplets, potential biomarker and metabolic target in glioblastoma

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Abstract

Lipid droplets (LDs) are subcellular organelles that store large amounts of the neutral lipids, triglycerides (TG) and/or cholesteryl esters (CE). LDs are commonly formed in adipocytes, liver cells and macrophages, and their formation has been shown to be associated with the progression of metabolic diseases, i.e., obesity, fatty liver and atherosclerosis. Interestingly, LDs are also found in some tumor tissues. We recently showed that LDs are prevalent in glioblastoma (GBM), the most deadly brain tumor, but are not detectable in low-grade gliomas and normal brain tissues, suggesting that LDs may serve as a novel diagnostic biomarker for GBM. This short review will briefly introduce LD biology, summarize recent observations about LDs in several types of cancer tissues, and discuss LD formation in GBM. Moreover, we will highlight the role of SOAT1 (sterol-O transferase 1), a key enzyme regulating CE synthesis and LD formation in GBM, in the regulation of SREBP (sterol regulatory-element binding protein) activation. The therapeutic potential of LDs and SOAT1 will be discussed.

Introduction

Lipid droplets (LDs) are subcellular organelles that are the major storage site of neutral lipids, i.e., triglycerides (TG) and cholesteryl esters (CE), which form the lipid core that is surrounded by a monolayer of phospholipids (1-3). LDs have been found in most cells, from bacteria (4, 5), yeast (6, 7), and plant (8, 9) to mammals (10-12). Many terms have been used to refer to LDs, such as lipid bodies, adiposomes, spherosomes, oil bodies and fat bodies. Nevertheless, LDs have only begun to gain significant attention in the last few decades.

Cell biology of LDs

LDs are very small organelles found in many cells. The number and size of LDs vary significantly in different cell types, with a diameter usually around 1 μm, but rarely larger than 10 μm, although it can be >50 μm in white adipocytes (11, 13). LDs are very dynamic organelles, and their number and size can change rapidly.

LDs arise from the endoplasmic reticulum (ER), where the enzymes DGAT1 and DGAT2 (diacylglyceride acyltransferase) (14, 15), and SOAT1 and SOAT2 (sterol O-acyltransferase), also named ACAT1 and ACAT2 (acyl-coenzyme A:cholesterol acyltransferase) (16-20), convert excess cellular fatty acids and cholesterol to TG and CE in the interspace between the bilayer leaflets of the ER membrane (1, 2, 21). It has been hypothesized that the cytoplasmic leaflet of the ER membrane encloses the neutral lipids and buds from the ER into the cytoplasm to form LDs (22). Interestingly, several groups have reported that LDs are also observed in the cell nucleus (23-25), suggesting that LDs may have some function in the nucleus.

Hundreds of proteins have been found in purified LD fractions, although some of them may be included as a result of contamination due to the close association of LDs with other organelles including the ER, endosomes, peroxisomes and mitochondria (26, 27). Some proteins were identified as peripherally associated proteins and shown to regulate LD size and number, including the PAT-family of lipid droplet proteins, such as perilipin1, perilipin2 (ADRP), perilipin3 (Tip47), perilipin4, CIDE (Cell Death Inducing DNA Fragmentation Factor) proteins and several lipases (28-33). Perilipin1 is expressed primarily in adipose and steroidogenic cells (34), whereas perilipin2 and perilipin3 are ubiquitously expressed and serve as the predominant LD coat proteins in other tissues (35).

LDs and cancers

Deregulation of LD metabolism has been shown to correlate with various metabolic diseases including obesity, fatty liver, and atherosclerosis (1, 36-38). Interestingly, LDs are also observed in several types of tumor tissues from cancer patients (39-42). Accioly *et al.* found that LDs are present in biopsy samples from colon cancer patients (40). They reported that LDs were associated with the generation of prostaglandin E2 in colon adenocarcinoma cell lines. Yue *et al.* observed that CE and LDs are formed in high-grade human prostate cancer tissues, but not in normal prostate or benign prostatic tumor tissues (42). Guillaumond *et al.* reported that CE are present in pancreatic ductal adenocarcinoma (PDAC) tumors (43). Moreover, LDs were also observed in clear-cell renal cell carcinoma (ccRCC) patient samples. The study showed that overexpression of perilipin2 promoted lipid storage and tumor growth in ccRCC xenografts model (41). Nevertheless, while LDs are found in many cancers, their role in tumor pathophysiology has only started to be explored.

LDs and glioblastoma

Recent progress in the understanding of cancer biology has revealed that metabolism reprogramming is a new hallmark of

malignancies (44-47). Our group was the first to report that lipid metabolism is rewired in glioblastoma (GBM) and promotes tumor growth (48-53). GBM is the most aggressive brain tumor and is also referred as grade IV astrocytoma. Despite extensive therapies, including surgical resection, radiation and chemotherapy, the median survival for GBM patients remains only 12-15 months from the initial diagnosis (54). The biggest challenge for treating GBM is the quickly developing resistance of tumor cells to therapies, leading to inevitable tumor recurrence and treatment failure (55, 56).

We recently uncovered that SREBP-1 (sterol regulatory element-binding protein-1), a master transcription factor in the regulation of lipid metabolism (57-59), is highly upregulated in GBM and promotes fatty acid synthesis (49, 51, 53, 60-62). Moreover, we found that GBM cells take up large amounts of cholesterol through LDLR (low-density lipoprotein receptor) that is also upregulated by SREBP-1 (63). It has been shown that significant increase in free fatty acids and cholesterol can cause ER stress and lipotoxicity that ultimately lead to cell death (41, 64-71). Interestingly, it is unclear how GBM cells are able to prevent the lipotoxicity potentially induced by the increased fatty acid synthesis and cholesterol uptake.

Most recently, using electron microscopy and fluorescence imaging, we observed that tumor tissues from GBM patients contain large amounts of LDs (72), suggesting that GBM cells may store excess fatty acids and cholesterol into LDs to prevent lipotoxicity and ER stress. Our data further showed that LDs are only present in GBM, and are not detectable in normal brain tissues and low-grade gliomas, demonstrating that LDs may serve as novel diagnostic biomarkers for GBM (72). Interestingly, when analyzing tumor tissues from a large cohort of GBM patients, we found that higher LD prevalence in tumor tissues was inversely correlated with overall survival (72), suggesting that LDs may play an important role in GBM growth.

SOAT1 and SREBPs

Our data showed that SOAT1 is highly expressed in GBM tumor tissues and that the levels of SOAT1 expression correlated with the prevalence of LDs (72). In contrast, SOAT2 was not detected in GBM (72), which is consistent with a previous report showing that SOAT2 is predominantly expressed in fetal liver and intestine tissues (17). Moreover, our data showed that genetic or pharmacologic inhibition of SOAT1 markedly reduced CE synthesis and LD formation in GBM cells, and suppressed GBM growth both *in vitro* and in orthotopic xenograft mouse models (72).

Interestingly, we further demonstrated that inhibition of SOAT1 significantly down-regulated SREBP activation and lipogenesis (72). SREBP activation is tightly regulated by ER cholesterol (57). Report shows that even an increase in ER cholesterol as low as 5% could significantly impede the trafficking of SREBP from the ER to the Golgi, leading to the reduction of SREBP-mediated lipogenesis (73, 74). Our data suggest that targeting SOAT1 to inhibit CE synthesis may cause the significant accumulation of cholesterol in the ER, leading to the suppression of SREBP activation (Figure. 1). It would be important to be able to measure ER cholesterol upon SOAT1 inhibition; unfortunately, we have not been successful, as we encountered difficulty to isolate pure ER fractions. Moreover, the cholesterol level in the ER is very low, corresponding to around 1-2% of total cellular cholesterol (75-77); thus, it is challenging to make accurate measurements for the changes in ER cholesterol with current fraction methodology.

The potential of SREBP being a molecular target in metabolic syndromes and cancer is supported by strong evidence (61, 78-80). Nevertheless, although SREBPs have been discovered around twenty years ago, the development of clinically effective inhibitors targeting SREBP has not been successful. Our study showing that restriction of SREBP in the ER via enhancing ER cholesterol level through suppressing SOAT1 may be a promising strategy to treat metabolic syndromes and cancer (72). In fact, it has been shown that the SOAT inhibitor, avasimibe, which has already been tested in a Phase III clinical trial in atherosclerosis patients (81), significantly suppressed growth *in vitro* and *in vivo* in GBM and prostate cancer (72, 82, 83). Therefore, developing more effective inhibitors targeting SOAT1 may bring great hope for targeting GBM and other late-stage malignancies.

Future directions

Lately, LDs have attracted significant attention from researchers in the cancer field. The list of the different cancers containing LDs is quickly growing. Current evidence shows that LDs are mainly formed in the tumor tissues from patients at an advanced disease stage (42, 72). It would be worth examining the prevalence of LDs across cancer types and stages, which could provide important information about the correlation between LD formation and cancer progression. Moreover, there are many questions regarding the role of LDs in tumor tissues: 1) what are the underlying mechanisms regulating LD formation in tumor cells? 2) what is the exact role of LDs in cancer cells? 3) are LD formation and hydrolysis correlated with nutrient levels in cancer cells? 4) are LDs playing a role in tumor resistance to therapies? Undoubtedly, addressing these important questions will provide us great insights for understanding of cancer biology and metabolism reprogramming. Furthermore, investigating LD metabolism may identify promising metabolic targets and novel therapeutic approaches for cancer treatment.

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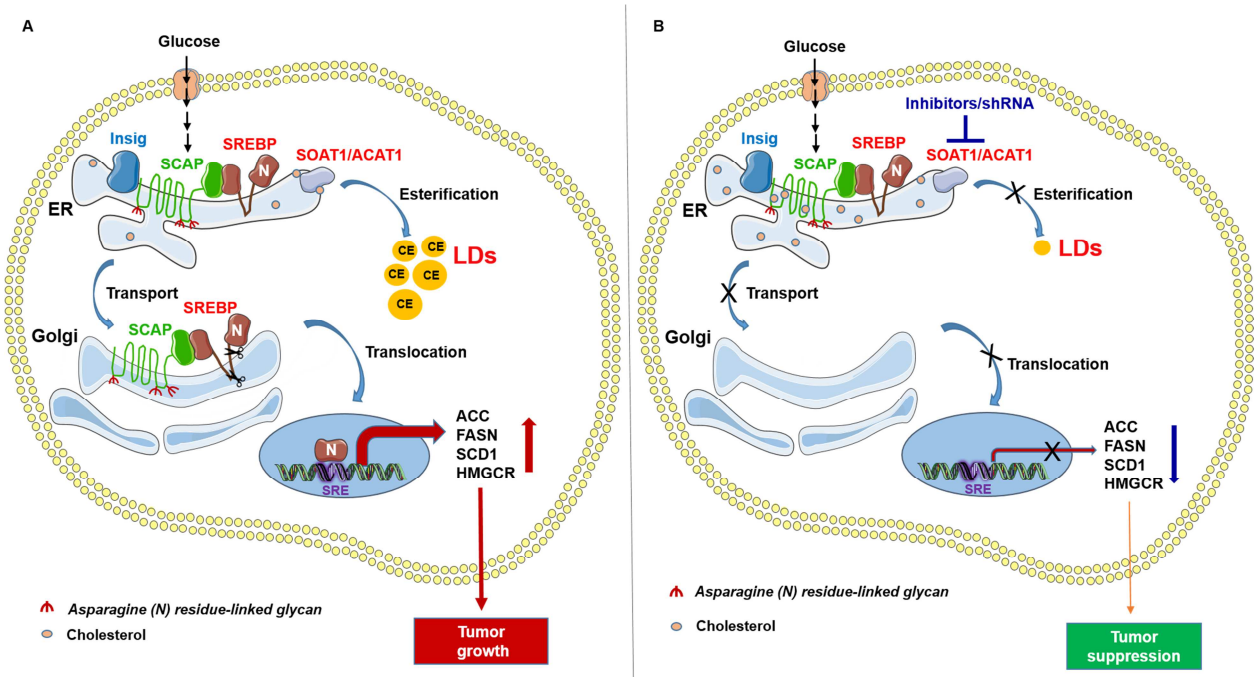

Figure 1. SOAT1/ACAT1 regulates SREBP activation and GBM growth by promoting cholesterol esterification and LD formation.

A) SOAT1/ACAT1 esterifies excess cellular cholesterol to form CE and LDs, thereby maintaining ER cholesterol homeostasis. This reduces the association of *N*-glycosylated SCAP (SREBP-cleavage activating protein) and Insig (insulin-induced gene protein), an ER-anchored protein, promoting SCAP/SREBP trafficking from the ER to the Golgi. In the Golgi, two proteases sequentially cleave SREBPs and release their N-terminal active forms, which then enter into the nucleus to activate lipogenesis gene expression for tumor growth (57, 72).

B) Inhibition of SOAT1/ACAT1 suppresses cholesterol esterification and LD formation, resulting in the accumulation of cholesterol in the ER. This enhances the binding of SCAP and Insig, thereby retaining the SCAP/SREBP complex in the ER, and leading to the reduction of lipogenesis and tumor suppression.

CE, cholesteryl esters; LDs, lipid droplets; ER, endoplasmic reticulum; SRE, sterol regulatory element; ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; HMGCR, HMG-CoA reductase.