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Review Article

Dropping in on lipid droplets: insights into cellular stress and cancer

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Lipid droplets (LD) have increasingly become a major topic of research in recent years following its establishment as a highly dynamic organelle. Contrary to the initial view of LDs being passive cytoplasmic structures for lipid storage, studies have provided support on how they act in concert with different organelles to exert functions in various cellular processes. Although lipid dysregulation resulting from aberrant LD homeostasis has been well characterised, how this translates and contributes to cancer progression is poorly understood. This review summarises the different paradigms on how LDs function in the regulation of cellular stress as a contributing factor to cancer progression. Mechanisms employed by a broad range of cancer cell types in differentially utilising LDs for tumourigenesis will also be highlighted. Finally, we discuss the potential of targeting LDs in the context of cancer therapeutics.

Introduction

Lipids constitute a class of biomolecules with species as diverse as they are functionally versatile. Not only are these molecules integral to cellular structure, but they are also vital in key biological processes such as energy production, cell signalling and gene regulation among many others [1]. However, despite their physiological importance and implication in many disease states, only in recent years has the study of lipid homeostasis and dynamics found its renaissance.

Lipid droplets (LD) are intracellular storage organelles of neutral lipids (NL). Although prominently found in adipose tissue, LDs can exist virtually in all cell types and tissues [2-4]. While these cytoplasmic structures have long been thought to be inert reservoirs for surplus lipids, it became clear more than half a century ago that LDs are dynamic and are exploited by cancer cells [5]. Moreover, only in recent years has the interest on the indispensable role of LDs in cancer progression and therapeutics emerged. Based on their inherent structural properties, cellular lipids may broadly be classified as membrane or non-membrane lipids. The former is primarily composed of amphipathic glycerophospholipids, sphingolipids and sterols, while fatty acids (FAs) and their derivatives are categorised under the latter. Maintenance of cellular lipid levels is governed by the constant flux between its various forms as mediated by several biosynthetic enzymes. However, a distinct class known as NLs forms a non-reactive pool of lipid molecules that serve both as a reserve for intermediates or repository for the surplus of lipids [1,6]. The defining characteristic of LDs as an organelle is its core of NLs. Due to their highly non-polar nature, NLs such as triacylglycerols (TG) and steryl esters (SE) are insoluble in the polar cytoplasmic environment and are not readily incorporated into amphipathic cellular membranes (Figure 1). They instead coalesce and form the core of LDs subsequently after synthesis within the double-membrane leaflet of the endoplasmic reticulum (ER) [7-10]. Thus, the structure of these dynamic cellular organelles is relatively simple, wherein the NL core is surrounded by an ER-derived phospholipid monolayer, with phosphatidylcholine as the major phospholipid component followed by phosphatidylinositol and phosphatidylethanolamine
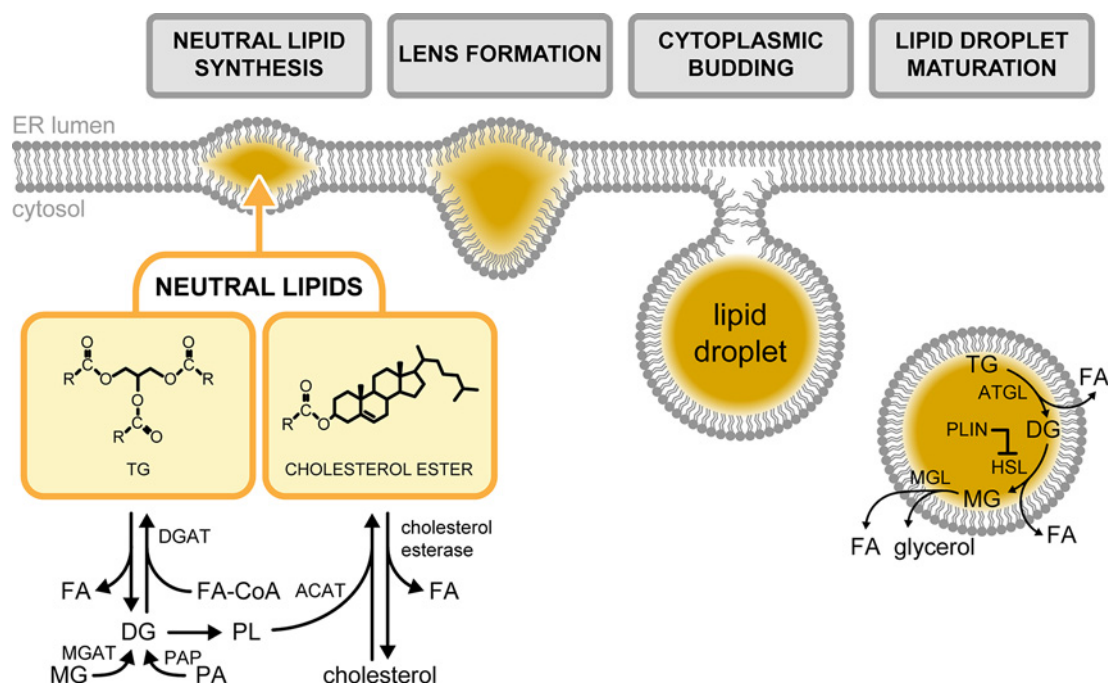
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Figure 1. Lipid droplet biogenesis

The neutral lipids, TG and cholesterol ester, are synthesised by ER membrane proteins. These lipid molecules then coalesce to form the core of a lens-shaped structure that protrudes towards the cytoplasm. The nascent LD buds off from the ER membrane to form the mature cytoplasmic organelle. Abbreviations: ATGL, adipose triglyceride lipase; DG, diacylglycerol; DGAT, diacylglycerol acyl-transferase; FA-CoA, fatty acyl-CoA; HSL, hormone-sensitive lipase; MG, monoacylglycerol; MGAT, monoacylglycerol acyl-transferase; MGL, monoacylglycerol lipase; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PL, phospholipids.

[11]. Moreover, the single phospholipid coat of LDs selectively houses a subset of proteins. Most of these are involved in lipid metabolism such as sterol biosynthetic enzymes and lipases [12-15], while other non-conventional proteins have also been identified [16].

Over the last decade, scientific advancements have conversely provided evidence on the dynamic nature of LDs. An increasing body of study has identified the close physical and functional apposition of LDs to other organelles across different model organisms, as *in vitro* and *in vivo* studies have established the connection between LDs and cellular compartments such as the mitochondria [17-19], the proteasome and the autophagic machinery [20,21]. The association between LDs and various cellular organelles lends support to the role of LDs in a broad range of cellular processes and protein quality control should LD homeostasis be dysregulated [22-25]. Although the appreciation for LDs have grown significantly, apart from studies detailing proteins that influence LD formation [7,26-28], definitive insight on the fundamental events that govern its biogenesis and functioning remains largely enigmatic to this day. Furthermore, these mechanistic studies have been conducted primarily in the unicellular model organism, *Saccharomyces cerevisiae*. Although this may be due to the robust genetic tools available for biochemical manipulation in the budding yeast, it also alludes to the increasing complexity of LD biogenesis in higher eukaryotes. Interestingly, while mutations in proteins associated with LD formation in *S. cerevisiae* yield modest phenotypes under physiological conditions, gross and more severe defects were associated in higher organisms with the corresponding genetic background. For example, deletion of seipin (*SEI1*) in yeast caused a delay in *de novo* LD formation with aberrant morphology, but otherwise yielded minimal effect on cell growth [27]. However, human seipin, also known as the Berardinelli-Seip congenital lipodystrophy 2 gene (*BSCL2*), was found to not only be critical for adipocyte differentiation and lipogenesis induction using *in vitro* cell cultures [29], but is also linked to a more severe form of congenital general lipodystrophy characterised by insulin resistance, hepatic steatosis and extreme reduction in both metabolically active and mechanical adipose tissue in patient studies [30]. Similarly, loss of the fat storage-inducing 2 (*FIT2*) yeast gene homologues impaired LD maturation without affecting general cellular fitness, but a corresponding loss of homologues resulted in early death in both the nematode *Caenorhabditis elegans* and mouse models [28,31]. All these lend support to the role of LDs in both organismal development and metabolic disease predisposition.

As mentioned earlier, LDs have been strongly implicated in cancer progression. However, the current inseparability of LD formation from the synthesis and turnover of its constituent NLs and phospholipids remains to be a caveat that needs to be addressed to ascertain the contribution of LD to tumourigenesis as a fully functional organelle. To date, most studies only focused on the partial functions of the highly dynamic and complex nature of LDs. This review presents different models on the direct and stress-regulatory roles of LDs in cancer cells based on our current understanding of LD biology.

Cellular stress en route to tumourigenesis: the LD connection

The altered metabolic activity in highly proliferative cancer cells warrants the need for understanding adaptive remodelling of key players in bioenergetics. LDs are among the most integral organelles in this process, and are increasingly identified in various cancer cell types [32]. Furthermore, cancer cells are characterised by elevated cellular stress factors and the activation of their corresponding adaptive response pathways. Concomitantly, the occurrence of LDs is increased under the same stress conditions [33-36]. This then presents the question of whether LD formation potentially aids in stress adaptive responses or contributes to consequences of disrupted cellular homeostasis. Furthermore, how LDs impact stress response regulation in cancer cells is less understood.

Unfolded protein response in cancer

The unfolded protein response (UPR) is a stress response pathway canonically activated from the accumulation of misfolded proteins within the ER lumen, but has since been shown to be similarly triggered upon exposure to exogenous free fatty acids (FFAs) and phospholipid perturbation [37-39], especially that of the ER membrane. This adaptive response pathway aims to restore ER homeostasis by modulating the expression of downstream target genes, and alternatively drives pro-apoptotic pathways should the stress condition remain unresolved. In metazoans, the UPR is mediated by signalling cascade events affected by three distinct ER transmembrane proteins: inositol-requiring enzyme 1α (Ire1α), PRKR-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6), the most evolutionarily conserved and well-studied from yeast to humans being the Ire1 axis (Figure 2). Although there are variations in the intensity of UPR activation as well as differential regulation of downstream target genes dependent on the cause of stress [40-43], both protein- and lipid-induced UPR activation similarly result in increased lipogenic markers and subsequently LD formation [33,34,44], and mutants incapable of LD formation up-regulate the UPR, thus strongly indicative of a role for LDs under the UPR programme. However, the dispensability of NL synthesis for viability under ER stress conditions [33] suggests that the constituent LD core may not be the sole contributor to the homeostatic response and that LDs have another function in protein-induced ER stress.

Consistent with this, the ablation of LD formation by genetic or pharmacological disruption of NL synthesis is dispensable for ER-associated protein degradation (ERAD) function in both yeast and mammalian cell models [45,46]. Proteins failing to fold correctly in the ER are retained and targeted towards ERAD, the terminal phase of ER quality control (Figure 2) [44]. When the pathway is saturated, it leads to the activation of the UPR from an accumulation of misfolded proteins. The ERAD-associated protein ancient ubiquitous protein 1 that mediates substrate ubiquitination has both been found to localise to the LD membrane and to regulate LD abundance [47]. A potential function of LDs in response to protein stress is by serving as temporary depot for proteins destined for degradation [48]. In line with this, HMG-CoA, the rate-limiting enzyme in cholesterol biosynthesis, is shuttled onto LDs as a part of its endogenous degradation mechanism [49]. Similarly, ubiquitinated apoB localises to LDs at sites in contact with the proteasome, and is further supported by the co-fractionation of LDs with ubiquitinated apoB and 20S proteasomal subunits [20]. From these, it may be hypothesised that apart from its NL core, the LD phospholipid membrane may serve a proteostatic function, specifically by housing client proteins that are marked for degradation.

Most recently, increased transcripts of the critical ERAD-associated ubiquitin ligase derlin 3 (DERL3) have been reported in metastatic patient-derived breast cancer samples, correlating with poor disease prognosis [50]. Inhibition of DERL3 expression substantially abrogated proliferation and invasiveness in breast cancer cell lines, while pharmacological impairment of two other steps in ERAD by the anti-cancer agents Eeyarestatin I or Bortezomib-promoted cell death in the JeKo-1 mantle cell lymphoma cell line [50,51]. From these, it could be argued that precluding the clearance of aberrantly misfolded proteins induces apoptosis by increasing the proteotoxic load beyond manageable levels. Consequently, the up-regulation of the proapoptotic factor ATF4 under the PERK-axis of the UPR, upon blocking ERAD [51], provided a close link between the UPR and cancer progression but not a definitive rationale for the increase in LDs that correlate with UPR activation.
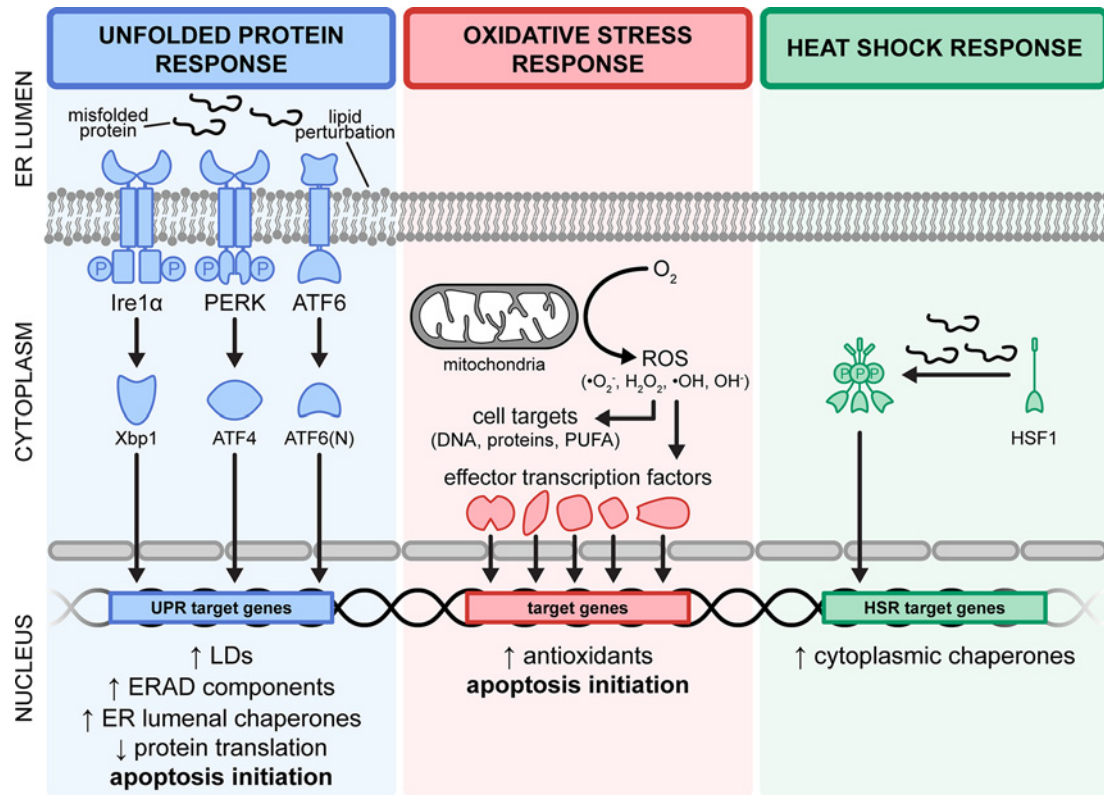
Figure 2. Fundamental activation mechanism of major cellular stress responses
(**Left panel**) The UPR is activated by ER stress conditions (i.e. ER membrane perturbation and aberrant protein folding within the ER lumen). These stressors are affected by three distinct axes, namely Ire1α, PERK and ATF6. The cognate transcription factors, Xbp1, ATF4 and ATF6(N), then translocate into the nucleus to modulate gene expression including those of ER luminal chaperones and ERAD components. UPR activation concurrently attenuates global protein translation and induces LD formation to restore homeostasis. (**Middle panel**) Oxidative stressors in the form of reactive oxygen species are generated through energy metabolism. Accumulation of ROS potentially induces a cascade of events that damages DNA, proteins and long-chain PUFAs. This cellular threat is mitigated by the up-regulation of antioxidants that neutralise ROS. The failure to restore homeostasis under conditions of ER and oxidative stress ultimately results in apoptosis. (**Right panel**) In contrast with the UPR, the presence of misfolded proteins in the cytoplasm as well as elevated temperatures activates the heat shock response (HSR). Under these conditions, the monomeric HSF1 sensor forms the homotrimeric HSF1 transcription factor that translocates into the nucleus to increase expression of cytoplasmic protein chaperones to subsequently aid in the refolding and processing of misfolded client substrates. Abbreviations: ERAD, ER-associated degradation; PUFA: polysaturated fatty acid; ROS, reactive oxygen species.

Blocking proteasomal degradation by MG132 causes ubiquitinated HMG-CoA to associate with LDs [49]. Despite the non-essentiality of LDs in ERAD, preventing LD formation by the fatty acid synthesis inhibitor triacsin C has been shown to cause increased apoptosis along with the up-regulation of the proapoptotic IRE1- and PERK-axes of the UPR [46]. Taken together, LDs may serve as a safety net for the proteotoxic stress, albeit with an unclear mechanism, should the ERAD network be limited and without which the cell is unable to cope with the proteotoxic load resulting in cell death.

Interestingly, the disruption of the circadian clock by genetic mutation of the Clock and Bmal1 transcriptional regulators have been shown to decrease lipolysis and cause the accumulation of TG in white adipocytes [52], in agreement with low mRNA levels of *Bmal1* and the related regulatory genes *Per2, Rev-erbα* and *Cry1* in obese mice models that develop severe hepatosteatosis [53]. These are strongly indicative of the role of circadian clock as a key regulator of lipid homeostasis. Moreover, the circadian clock is tightly coordinated with temporal UPR activation [54], and is therefore capable of modulating the protein quality control effectors of the UPR. In line with this, it has recently been shown that PERK-mediated UPR activation led to temporal suppression of both *Clock* and *Bmal1* and promoted cancer cell survival by attenuating protein synthesis [55], while the overexpression of *Bmal1* sensitised colorectal

cancer cells (CRCs) to chemotherapeutics [56]. Perhaps, pathways that increase LD formation additionally orchestrate a general protein homeostatic response (i.e. ERAD or inhibition of protein synthesis) to prevent proteotoxic stress in cancer cells.

Despite the number of studies that suggest of its protective effects against ER stress, the abundance of cytoplasmic LDs appears to be selectively advantageous for cancer cells and is otherwise cytotoxic in normal cells. The incretin mimetic drug exendin-4 has been shown to reduce accumulation of ER stress-induced LDs with a concomitant decrease in UPR markers in cancer and non-cancer cells [57,58]. It exhibited anti-tumour effects [59] but promoted survival of normal cells [60-62]. Therefore, LDs may not confer an all-encompassing protective effect, but instead buffer against a form of cellular aberration that is exclusively present in cancer cells. An increase in lipogenic enzyme markers has been extensively reported in a variety of cancer cells [63-65], and the resultant flux of lipids warrants a heightened capacity to store them in the form of LDs, a possible route to prevent ER stress induction [38]. However, it was recently reported that while ablation of lipogenic factors prevented cancer progression *in vitro*, it conversely increased tumourigenesis in an *in vivo* hepatocarcinoma mouse model [66]. Although this does not dismiss the plausible contribution of LDs in stress-adaptive tumourigenic processes, it suggests of alternative LD functions and similarly provides a strong impetus to investigate the role of LDs under the complex environment of *in vivo* systems.

Oxidative stress and cancer

Increased oxidative stress has been observed in several disease states [67-70], a condition characterised by elevated levels of reactive oxygen species (ROS). The generation of ROS naturally occurs through the course of cellular metabolism but could lead to detrimental effects upon accumulation beyond physiological levels. To mitigate this threat, antioxidants (e.g. superoxide dismutases; SODs) sequester and convert ROS into innocuous forms which are further neutralised by the cell [71,72]. Introduction of oxidative stressors and the reduction in antioxidant capability of cells similarly resulted in the increased formation of LDs [22,73,74], suggestive of the buffering capacity of LDs under these conditions. Conversely, knockdown of the LD-stabilising protein perilipin-5 (PLIN5) in an ischaemia-reperfusion mouse model not only resulted in decreased LD pool in myocardial tissue, but also impaired SOD levels and elevated ROS along with severely damaged mitochondria [75]. Similarly in clear-cell renal cell carcinoma (RCC), LD-associated perilipin-2 (PLIN2) expression was found to be regulated by ROS-related hypoxia-inducible factor 2α, resulting in a decrease in FA transport from LDs into the mitochondria [76].

Mitochondria are key sites of lipid metabolism, critical for both energetics and conversion of key lipid species. Being the hub for aerobic respiration, it is also a major site of ROS generation (Figure 2) [77,78]. Without the protective effect of antioxidants, this would be deleterious as lipids, especially polyunsaturated fatty acids (PUFAs), are highly susceptible to peroxidation by ROS [79], and the latter consequently generates toxic by-products that further cause cellular dysfunction. The antioxidant capacity of LDs was demonstrated in *Drosophila* glial cells by the modulation of ROS levels and prevention of lipid peroxidation [80]. Accordingly, incorporation of FFA into NL and storage into LD can be a protective mechanism against peroxidative damage.

Paradoxically, high ROS levels are one of the defining characteristics of various cancer types and is opportunistically utilised by cancerous cells to drive its proliferative and metastatic capacities [81,82]. The current model is that highly metabolic cancer cells generate increased levels of ROS in the mitochondria, as well as up-regulate antioxidant levels. Although higher than non-cancerous cells, ROS levels in cancer cells do not drive apoptosis but instead fuel cell growth and survival through various pathways [83]. This may reflect a general increase in oxidative stress tolerance inherent within cancer cells *per se*, or alternatively a specific consequence of their bolstered antioxidant capacity.

In support of the latter, a wide variety of cancer types significantly up-regulate the antioxidants Sod2, glutaredoxin and glutathione peroxidases [84]. Recent studies also suggest that this increase in oxidative stress tolerance could be extended to adjacent cells and the tumour microenvironment. Cancer cells could alter the proteome of neighbouring cells to its advantage, as evidenced by up-regulation of antioxidants among other proliferative factors in normal fibroblasts co-cultured with breast cancer cells [85]. Similarly, extracellular vesicles in the form of exosomes in conditioned media from pancreatic cancer cells have been reported to house and carry transcripts of *SOD2*, and decreased ROS production in cells treated with these exosomes [86]. Curbing this antioxidant advantage by preventing their expression conversely resulted in increased therapeutic susceptibility [87,88]. As reservoirs of cellular lipids, LDs serve not only to store but also to protect their contents from potential peroxidation, thereby providing yet another avenue for oxidative stress resistance in cancer cells. This is in line with what has been reported for breast cancer cells wherein induction of LD biogenesis conferred resistance to and reduced oxidative stress levels [89]. Mechanistically, this was proposed to be due to the protection of PUFAs from peroxidation by sequestering these toxic lipid by-products into LDs.

A strong evidence for the increased antioxidant capacity of cancer cells came with the recent report of heightened hepatocarcinoma aggressiveness coupled with increased resistance to oxidative agents *in vivo* following the ablation of FA synthesis by *ACC1/2* depletion [66]. While devoid of *de novo* FA biosynthesis, liver-specific *ACC1/2* null mice unexpectedly increased TG accumulation and heightened hepatosteatosis, which was further linked to the decrease in lipid oxidation pathways [90]. This in turn greatly emphasises that the retention of LDs is advantageous not merely for bioenergetics, but likely for antioxidant properties, as evidenced by the decreased levels of oxidant-induced lipid and DNA damage reported in the same study [66]. As information on protein effectors for LD formation as a biophysical process remains elusive, most studies that characterised the function of LDs have been done with ablation of FA synthesis that elicit broader effects on the metabolic network. Liver-specific *ACC1/2*-ablated mice exhibit a greater shift in the activity of the pentose-phosphate pathway, a bioenergetic pathway that has previously been shown to attenuate oxidative stress-induced cell death [91,92]. Therefore, the increased oxidative stress resistance conferred by *ACC1/2* deletion may not solely be dependent on the persistence of cytoplasmic LDs but could possibly be a secondary effect of metabolic remodelling.

Interestingly, ATF4-mediated UPR activation has been shown to increase protein synthesis and oxidative stress, which ultimately promoted cell death [93]. Similarly, an LD-binding thalidomide analogue exhibited cytotoxicity specifically in cancer cell lines concurrently with the induction of both oxidative and ER stress [94]. It would be important to determine if the LD localisation of this drug affects the biosynthesis or stability of LDs, as this would give invaluable information on how LDs participate in its mechanism of action and more importantly, on how LDs are relevant in the modulation of ER and oxidative stress responses in a cancer-specific manner.

Heat shock response and cancer

Perhaps less appreciated among stress response activation pathways in cancer cells is the heat shock response (HSR). Originally identified to be triggered by heat stress, the HSR is also activated under conditions of cytoplasmic-misfolded protein accumulation [95,96]. In brief, recognition of the stress stimulus activates the master transcriptional regulator of the HSR, heat shock factor 1 (HSF1), which then up-regulates the expression of proteins to promote protein folding and turnover (Figure 2) [97]. These factors include molecular chaperones and their cognate partners termed as heat shock proteins (HSP) that aid in the maturation, folding and refolding of client proteins.

These elements in the HSR signalling pathway have also been reported to have distinct contributions to cancer progression. The transcriptional regulator HSF1 has been reported to not only be critical to sustain cancer proliferation [98], but also up-regulate a subset of genes exclusively in patient-derived malignant cell specimens that are distinct from the canonical HSR targets. This signature gene expression profile has been associated with colon, lung and breast cancer patients with poor prognosis and survivability [99]. Furthermore, cancer cells can up-regulate HSF1 activity in non-cancer cells within the tumour microenvironment to promote the expression of proliferative and metastatic factors [100]. Whether the contribution of HSF1 to cancer progression is restricted to this specialised transcriptional profile remains to be fully understood, as competitive binding of a synthetic compound to known HSF1 targets was sufficient to reduce cell viability [101]. Downstream in the pathway, various forms of Hsp70 and Hsp90 are also up-regulated in hepatocellular carcinoma (HCC) [102], thought to be essential for mitigating proteotoxicity resulting from the highly aberrant genetic profile of cancer cells. To this end, several molecular inhibitors of these HSPs have been developed, which in turn have exhibited anti-cancer properties [103-105].

On a fundamental level, TG synthesis has been reported to be important in coping with mild heat stress in *Schizosaccharomyces pombe* [106], suggesting a general adaptive role for LDs in response to heat shock. Furthermore, the expression of the HSR-associated HSP70 chaperone that is integral to protein folding both under basal and heat stress conditions has been found to be lower in diseased individuals [107] and up-regulated upon exposure to heat stress in an *in vitro* adipocyte model [108]. It was then hypothesised that Hsp70 localises to the LD membrane to facilitate denatured proteins for folding, consistent with that of previous studies [48].

In cancer cells, induction of heat stress resulted in membrane remodelling involving key changes in membrane fluidity to maintain cellular viability and integrity under non-physiological temperatures [109]. Among many factors, cholesterol content is a key determinant of membrane fluidity [110-113]. With this, cholesterol esters (CE) within the core of LDs could be accessed by lipases to liberate their constituent cholesterol moiety for subsequent incorporation into membranes. More recently, the metabolic regulator peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) has been identified to serve an anti-proliferative function in prostate cancer cells [114] and repress HSF1 transcription, thereby preventing the otherwise up-regulation of HSR target genes in hepatocellular cancer cells [115]. Interestingly, PLIN5 on the surface of LDs form an active transcriptional complex with PGC-1α upon lipolytic induction [116]. It could therefore be hypothesised that PLIN5 may activate PGC-1α activity to repress HSF1 function.

However, with LD accumulation in cancer cells, PLIN5 remains sequestered on the LD membrane, thereby allowing the unrestrained HSR to exert its cancer-promoting functions.

The direct association between the HSR and cancer has long been established with early reports of increased tumour chemoresistance following prolonged or pre-administration of cells to non-lethal heat stress [117,118]. For instance, the prominent downstream Hsp70 effector of the HSR was reported to drive lipogenic pathways in HepG2 cells and increase intracellular LDs [119]. The phospholipase A2 enzyme localises to the surface of LDs from which it catalyses the release of arachidonic acid [120], a strong driver of HSR activation [121]. Arachidonic acid release in cancer cells was also observed to increase from phospholipase activity following exposure to high temperatures [122]. The induction of Hsp70 and the phospholipase-mediated release of arachidonic acid from LDs may then constitute a positive feedback mechanism to increase cancer cell survival under chemotherapeutic stress, as disruption of either phospholipase A2 or Hsp70 improved drug efficacy [123,124].

Undoubtedly, LDs not only exert various functions in cellular stress response pathways, but in turn are also intricately regulated by the activation of the latter. Adding to their complex regulation, LDs interact and communicate with other organelles involving key biological processes. Therefore, it is not surprising that LDs have emerged as critical factors in disease pathogenesis in recent years. However, one of the greatest obstacles to reach a definitive conclusion for the contribution of LDs to cellular stress adaptation is the limitation on methods to uncouple LD formation from FA synthesis. Key scientific findings such as investigating the function of LDs as an independent physical organelle and not as a consequence of lipid biosynthesis *per se*, as well as the identification of definitive regulators for its formation are necessary to further advance the field of LD biology. Nevertheless, the functional roles of LDs are becoming a significant focus of cancer research in anticipation to exploit LD dynamics to curb cancer progression.

Cancer: a LD-driven metabolic disease
Correlation of LDs and cancer aggressiveness

Tumour development can be characterised by well-defined hallmarks of cancer that include sustenance of proliferative signalling, evasion of growth suppressors, invasion and metastasis, enabling replicative immortality, angiogenesis and resisting cell death among others [125]. LDs may potentially influence these processes, thus contributing to tumour development and aggressiveness, with potential for LD accumulation as an additional hallmark of cancer. This can be illustrated by reports studying breast cancer cell lines [126,127]. Here, LD abundance was shown to correlate with degree of aggressiveness from the non-malignant MCF10A cells to the highly malignant MDA-MB-231 cells, while MCF7 cells were found to be intermediately aggressive. The higher lipid content in malignant breast cancer cells may arise from the increased rate of FA and phospholipid synthesis, which were found to be essential to drive proliferation in unfavourable harsh environments [128-130]. Furthermore, it has been shown that to prevent nutrient stress and promote proliferation, breast cancer cells import FFAs to either generate energy through β-oxidation or subsequently store them into LDs when present in excess [89]. In another report, the migratory capability of the malignant cell line MDA-MB-231 was also shown to correlate with lipid accumulation [131]. Additionally, high levels of LDs in circulating tumour cells have been associated with cell invasiveness [132]. The CE constituent of LDs was also shown to be associated with human prostate cancer invasiveness and metastasis, while inhibition of CE ester synthesis and storage reduced cancer aggressiveness [133]. Reports have shown cholesterol biogenesis to mostly occur in hepatocytes and adipose tissue [134,135]. Since regulators of cholesterol metabolism, specifically the sterol regulatory element binding proteins (SREBP-1 and SREBP-2) and Δ24-sterol reductase, are present at low levels in renal cells, cholesterol biogenesis is thought to be minimal [136]. However, SREBPs have been reported to be up-regulated in RCC. The *TRC8* gene which encodes a multimembrane-spanning ER protein with E3-ubiquitin ligase activity [137,138] is associated with hereditary RCC as well as ovarian dysgerminoma [139,140]. In RCC, TRC8 destabilises SREBPs at the ER membrane resulting in high levels of soluble SREBPs. Membrane-bound SREBPs sense cholesterol at the ER and are cleaved by sequential enzymes at the Golgi apparatus, releasing soluble SREBPs that function as a transcription factor. In addition to increasing cholesterol biogenesis through stearoyl CoA desaturase (*SCD*), SREBPs also regulate fatty acid synthase (*FASN*). Thus, TRC8 contributes to the increase of LDs in RCC and possibly to its aggressiveness as inhibiting LD synthesis supresses malignancy. Similarly, SREBP-1 has been reported to be overexpressed in prostate cancer cells, inducing an increase in LD biogenesis [141,142]. LD formation has been previously associated with cell survival and cell migration in prostate cancers [142]. Interestingly, androgen treatment increased the number of LDs in prostate cancer cells where SREBP-1 might be implicated [143]. Thus, LDs appear to play an active role in enhancing cancer aggressiveness and could be used as a potential biomarker for metastatic and high-grade cancers. Taken together, all these suggest a role for LDs as a promising target for cancer therapy.
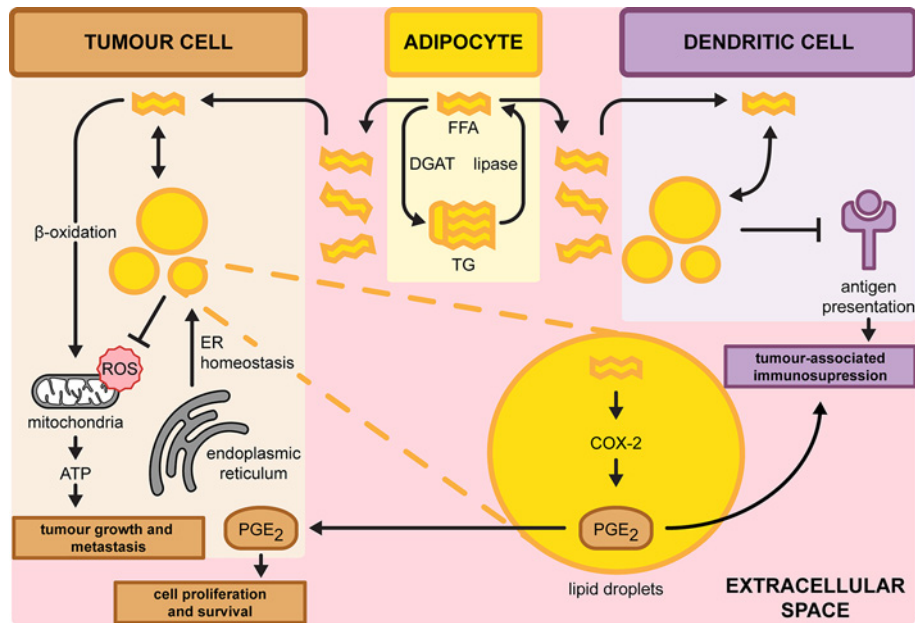
Figure 3. Overview on the role of LDs in regulating cancer progression

Tumour cells import FFAs from the extracellular space and adipocyte. Subsequently, these can be stored into LDs to prevent the accumulation of ROS or degraded through β-oxidation to promote tumour growth and metastasis. LD accumulation is also triggered from ER stress to alleviate stress. In dendritic cells (DCs), accumulation of LDs dysregulates antigen presentation of tumour-associated antigens which may inhibit immune response. In parallel, LD-localised prostaglandin E_2 (PGE_2) might be secreted causing immunosuppression. In tumour cells, PGE_2 promotes cell proliferation and cell survival. COX-2, cyclooxygenase-2.

Function of LDs: interplay between tumour microenvironment, bioenergetics and inflammation

Cancer cells often acquire characteristic changes in their metabolic status [144]. Accordingly, one such change is the enhancement of glycolysis to fuel cancer cell proliferation by supplying energy and precursors for anabolism [145,146]. Lipid metabolism is also altered in highly proliferating cancer cells [147]. The lipogenic genes acetyl-CoA carboxylase (*ACC*), ATP citrate synthase (*ACLY*) and *FASN* have been shown to be up-regulated in various tumours [148-150]. In agreement, increased number of LDs has been reported in many cancer types as excess lipids are stored in LDs to prevent lipotoxicity [5,126,151,152]. Analogous to the role of lipid synthesis in promoting cancer, the breakdown of LD-localised lipids can function as either the primary or alternative source of energy and precursors for phospholipids, the building blocks of biological membranes, in the course of cancer cell proliferation (Figure 3) [152,153]. The connection between LD accumulation and cancer bioenergetics was elegantly demonstrated both *in vitro* and *in vivo* in ovarian cancer cells adjacent to adipocytes [154]. Ovarian cancer cells induce the release of FFAs from TG in adipocytes by intercellular stimulation of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) mediated lipolysis (Figure 1). Adipocyte-derived FFAs were also shown to be transported into cancer cells by the fatty acid-binding protein 4 and routed to β-oxidation, contributing to tumour growth and metastasis [155]. Similar findings of FFA transfer from adipocytes were reported in breast and prostate cancer cells [156,157]. Thus, cancer cells opportunistically exploit the surrounding tumour microenvironment to fuel their energetic needs. Consequently, the abundance of lipids stored in adipocytes of overweight patients could be a driver of cancer progression that merits further exploration in future studies.

In addition to adipocytes, the lipid-rich tumour microenvironment could also potentiate defective anti-tumour properties in dendritic cells (DCs) (Figure 3) [158]. DCs with high LD content failed to process or present tumour-associated antigens to stimulate allogeneic T cells in tumour-bearing mice. The accumulation of LDs is a direct consequence of the increased uptake in extracellular lipids. Even in the absence of a lipid-rich microenvironment, a study found that the abnormal build-up of LDs in tumour-associated dendritic DCs could be triggered by the intrinsic ER stress-dependent XBP1 pathway [159]. In both scenarios, suppression of lipid accumulation by 5-(tetradecyloxy)-2-furoic acid (TOFA) restored the functional capacity of DCs and enhanced the activity of the

anti-tumour T-cell response [160]. Molecular mechanisms underlying the defective antigen presentation by high LD content remains to be elucidated. Excessive accumulation of LD may interfere with antigen presentation in DCs by sequestering antigenic fragments into LDs, thereby preventing its cell surface display [161]. Protein-LD co-localisation analyses in tumour-associated DCs verified this phenomenon. From these, LD accumulation in cancer cells may modulate the effectiveness of anti-tumour immune responses and therefore pose as a viable target for therapy.

Adenocarcinoma and HCC cell lines were found to contain a significant level of LD-localised cyclooxygenase-2 (COX-2). The enzyme COX-2 is the rate-limiting step in prostaglandin synthesis, which promotes tumour growth as well as coordinates crosstalk with the surrounding stromal cells [162]. LDs then serve as a distinct site for the production of prostaglandin E_2 (PGE$_2$), a potent lipid inflammatory mediator (Figure 3) [163,164]. It is noteworthy that PGE$_2$ is the most abundant prostaglandin found in several malignancies including colon, lung and breast tissues [165,166]. PGE$_2$ not only promotes tumour growth in a paracrine manner but could also perturb anti-tumour immunity by regulating the activity of the surrounding immune cells in the microenvironment [167,168]. Recent findings reveal that tumour-derived PGE$_2$ impaired the viability of natural killer cells and the subsequent recruitment of DCs, resulting in immune evasion [169]. Furthermore, PGE$_2$ was also found to suppress natural killer cell activity through the induction of myeloid-derived suppressor cells from the tumour microenvironment [170,171]. These observations suggest that LD accumulation in either tumour or immune cells promotes immunosuppression. Thus, LD could serve as an alternative inflammatory machinery apart from the ER and Golgi apparatus that houses a readily available source of lipids to synthesise vast amounts of pro-inflammatory mediators. LDs could also process lipids acquired from the extracellular environment and efficiently convert them into potent inflammatory products. A better understanding of the role of LDs in regulating the immune response against tumour cells is critical to address cancer-related immunosuppression. Identifying the components of isolated LD and its associated proteins in LD-laden cells by lipidomic and proteomic approaches will unravel integrated lipid biosynthesis pathways responsible for the production of lipid mediators [172,173]. Similar approaches could also be employed to identify other critical LD-associated factors that contribute to cancer aggressiveness in malignant breast cancer cell lines [126,127].

LD links viral infection to cancer

Hepatitis C virus (HCV) infection is one of the major drivers of HCC [174]. Chronic HCV infection increases inflammation to progressively cause liver damage by stimulating fibrosis and cirrhosis, contributing to HCC development. HCV, through its core protein, dysregulates lipid metabolism, resulting in the development of fatty liver [175]. Although it remains unclear if HCV is involved in tumour initiation or the subsequent inflammatory response, LDs have been shown to play a crucial role in the generation of HCV in infected cells [176]. The HCV core protein promotes the localisation of HCV non-structural proteins from the ER to LDs [176], suggesting that LDs promote the production of the infectious virus particle. HCV triggers the accumulation of LDs which function as a recruitment site for virus assembly, and failure to associate the essential viral proteins and RNAs with LDs impaired the production of the infectious virus [176,177]. The role of LDs in promoting HCV-induced carcinogenesis is also supported by the up-regulation of the PLIN2 biomarker that correlates with proliferation rate during early tumourigenesis [178]. Furthermore, the disruption of LD biogenesis by genetically attenuating the TG-synthesis enzyme diacylglycerol acyltransferase-1 severely reduced viral replication [179]. Collectively, these suggest that oncovirus-induced LDs are indispensable in the development of HCC.

Interaction between viruses and LDs as well as LD formation are not unique to HCV. Dengue virus, part of the *Flaviviridae* single-stranded positive-sense RNA virus family along with HCV, associate with LDs for assembly and RNA replication [180-183]. Viruses of other families can also exploit LDs for their replication. Rotavirus and orthoreovirus interact with LDs as a part of their infection strategy [184,185], while Hepatitis B virus (HBV) X protein expression has been reported to promote LD accumulation [186-188]. Interestingly, HBV was found to induce the up-regulation of transcription factor SREBPs, FASN, as well as FA storage and glucose metabolism regulator PPARγ, all of which contribute to lipid biogenesis and storage. Thus, the role of various viruses in promoting tumourigenesis through the exploitation of LDs could be further studied and exploited therapeutically, especially for chronic infections.

LDs in chemotherapy

LDs in cancer patients with cachexia

Cachexia is a complex metabolic syndrome characterised by severe body wasting from the decline in muscle mass and adipose tissue during cancer progression [189]. A hallmark of cachexia is the loss of skeletal muscle and lean tissue together with adipose tissue which consequently leads to physical weakness in patients [190]. Chemotherapy is frequently the underlying cause of cachexia in cancer patients [191]. It was recently proposed that

chemotherapy-induced cachexia is driven by oxidative stress-associated MAPK activation in mitochondria of muscle tissues, as in the case of Folfox or Folfiri drug treatments [192]. Elevated levels of plasma FFAs, TG and cholesterol correlate with muscle and adipose tissue wasting in cancer patients with cachexia [193]. Moreover, expression levels of LD-associated proteins are altered in cancer-associated cachexia resulting in changes in adipocyte morphology. This is further supported by the increase in ATGL-dependent lipolysis found in white adipose tissue (WAT) during cancer cachexia [194]. WAT characteristically stores a single oversized LD for energy storage that occupies the bulk of the cytoplasm, while brown adipose tissue (BAT) contains small LDs and an unusually high abundance of mitochondria consequently releasing heat through FA β-oxidation [195]. Increased lipolysis in WAT is also modulated by HSL [193,196]. Although lipid breakdown is the major determinant of cancer cachexia, impaired lipogenic and lipid storage pathways have been closely associated with adipose tissue atrophy [197]. Furthermore, loss of WAT can be due to its gradual conversion to BAT-like cells also known as beige adipocytes [198]. The increase in beige adipocytes may exacerbate the metabolic dysfunction by enhancing lipid mobilisation and driving high energy expenditure in cancer cachexia [189]. The dramatic loss of WAT and its enhanced lipolysis precede the onset of skeletal muscle atrophy and result in the overall depletion of LDs in adipocyte tissue [190]. In contrast, increase in the number and size of intramyocellular LDs had been observed throughout cachexia progression, specifically upon weight loss, in cancer patients [199]. Although the mechanism leading to LD accumulation in muscle cells in cachectic patients is unclear, the dysregulated storage and catabolism of lipids may be the primary underlying cause [189].

LDs contribution to cell death and therapy-induced senescence

In addition to its role in mediating cancer cell development and proliferation, accumulation of LDs has been widely reported to correlate with chemotherapeutic treatments. For example, LDs have been shown to be a consequence of metabolic changes during etoposide-induced apoptosis, where the inhibition of FA β-oxidation in defective mitochondria re-directs FFAs towards lipid storage (Figure 3) [200]. Indeed, inhibition of β-oxidation or oxidative phosphorylation is sufficient to drive LD formation in cancer cells [201]. Treatment of N-myc proto-oncogene protein-amplified neuroblastoma cells with 10058-F4, an inhibitor of c-MYC/Max protein, a member of the basic helix–loop–helix leucine zipper family of transcription factors, triggers the accumulation of intracellular LDs as an outcome of mitochondrial dysfunction. The chemotherapeutic drugs doxorubicin and 5-fluorouracil (5-FU) in human colon carcinoma cells induce the accumulation of LDs as a result of up-regulated TG biosynthesis [192,202]. Hence, this points to a potential role for LDs as part of a general stress response to different classes of chemotherapeutic treatments. Increased LD formation during chemotherapy may also be a consequence of non-apoptotic cell death (NCD) as ferroptosis-induced NCD perturbed FA metabolism [203,204]. Lipid biosynthesis stimulated the accumulation of FA and initiated NCD in fibrosarcoma HT1080 cell lines [205]. Suppression of either LD or FA accumulation by TOFA reduced lipid dysregulation-associated cell death [206]. Elucidating how LDs assist in mediating cell death, and isolating factors that directly contribute to LD accumulation could be the focus of future studies.

Conventional anticancer treatment in tumour cells may induce a stable cell cycle arrest, a cytostatic phenotype known as 'therapy-induced senescence' (TIS). Irreparable DNA damage, chemotherapeutics and ionising radiation all contribute to TIS. Thus, TIS cells have emerged as an exploitable side effect of chemotherapy to prevent cancer progression while reducing toxicity [207]. Recently, autophagy was suggested to be a mechanism contributing to anti-mitotic drug TIS [208]. Although the mechanism was not elucidated, one could envision that lipid metabolism may play a role in promoting autophagy-dependent TIS. Indeed, TIS cells were recently shown to accumulate LDs in contrast with their proliferative counterpart [209]. Here, the accumulation of LDs in TIS from enhanced lipid import was found to contribute to the onset and maintenance of senescence. Excess lipids that could not be stored in overloaded LDs may promote the generation of lipotoxic diacylglycerol and ceramides that potentially trigger stress-induced cellular senescence [210]. This is further supported by the notion that TG or ceramide alone is sufficient to induce senescence in cancer cells. Therefore, accumulation of LDs may be exploited as a means to identify TIS and potentially promote clinical remission through senescence induction.

LDs contribution to chemoresistance

In contrast with the role of LDs in contributing to cell death, increased LD formation in certain cancer cells correlate with chemotherapy resistance. Chemoresistance is a persistent problem in treating tumour invasion along with the evasion of drug-induced tumour growth inhibition. Resistance varies tremendously among different cancer types, but lipid metabolism can be a common underlying contributor. As such, in oncogene-inactivated residual breast cancer cells, there is a metabolic shift that is associated with the accumulation of LDs [211]. Oncogene ablation-resistant pancreatic cancer cells were shown to accumulate LDs to maintain energetic balance [212]. Recently, LD accumulation in

5-FU and oxaliplatin-treated CRC was also shown to drive cell death resistance by perturbing the caspase activation cascade and ER stress response [213]. Both drugs induce apoptosis by activating the caspase-12 pathway through ER stress. LD accumulation in chemotherapy-induced CRC is dependent on the expression of lysophosphatidyl-choline acyltransferase 2 (LPCAT2), an LD-localised enzyme essential for phosphatidylcholine synthesis. The high expression of LPCAT2 is sufficient to prevent the chemotherapy-induced ER stress, underlining the role of LD accumulation in reducing ER stress [214]. More remarkably, it was also reported that LD accumulation correlates with a reduction in immunogenic cell death and CD8^{+} T cell infiltration in mouse xenograft and metastatic tumours of CRC patients, further reinforcing the involvement of LDs in promoting an immunosuppressive tumour environment. The present study reaffirmed the multifaceted role of LDs in mediating chemoresistance by concurrently alleviating chemotherapeutic-induced stress and regulating the tumour microenvironment.

The contribution of LDs to chemoresistance could arise from *de novo* formation following treatment with chemotherapeutic agents or from LDs inherently present in cancer cells. An example of the latter is shown in androgen-sensitive prostate cancer cells that rely on LD degradation and lipolysis for survival during androgen deprivation therapy [215]. Enhanced lipolysis of LD may provide fatty acids for energy production through fatty acid β-oxidation or supply lipid intermediates, particularly CE to promote prostate cancer cell survival [216]. Put into perspective, LD formation is not merely a consequence of the apoptotic cell death response but a hallmark of cell resistance contributing to chemoresistance or disease relapse.

Conclusion and perspectives

Lipids are essential members of the cellular machinery to regulate proliferation, migration and survival of cancer cells. As part of the equation, LDs from cancer or neighbouring cells play a role in regulating as well as dysregulating some of these processes necessary for cancer proliferation. In cancer cells, the UPR, HSR and oxidative stress response are exploited for survival, resulting in elevated LDs. The field is still in its infancy, and it is imperative that recent promising leads be further explored. Importantly, our current knowledge of how cancer cells exploit LD for critical growth for the development of new therapeutic treatments is still limited and requires in-depth studies. Thus far, no direct inhibitors of LD biogenesis have been developed as anti-proliferative targets.

Indirectly connected to LD regulation, several lipid biogenesis inhibitors are currently under preclinical development including those that specifically target FA regulation [147,217]. Among these are inhibitors of biosynthetic enzymes for the acetyl-CoA precursor (ACLY) [218], FA synthesis (FASN) [219], FA elongation [220], FA desaturation (SCD1-5) [221] and FA β-oxidation (CPT1) [222-224], which could potentially be used to deter cancer cells that exploit LDs for growth. These inhibitors will need to attenuate tumourigenesis without dysregulating body weight. However, as mentioned earlier, no specific LD inhibitors have been developed to date and should be an avenue of exploration. Inhibition of LD formation results in toxic accumulation of FFAs promoting cell death [225,226]. To inhibit LD formation, genetic attenuation or small molecules targeting FIT1, FIT2 or PLIN should be considered. As LD biogenesis is still poorly understood, the urgent need to identify novel protein factors will be critical for the development of new inhibitors to prevent tumour growth. A combination with LD inhibitors might enhance the efficacies of existing treatments. Drugs that specifically target LD formation hold greater therapeutic potential compared with general lipid biosynthesis inhibitors as the accumulation of LDs confer survival advantages in cancer cells. Specifically reducing LD accumulation in tumour might reduce drug toxicity on non-malignant cells. In contrast, drugs targeting precursors of FAs, phospholipids, cholesterol, sphingolipids or ceramides might lead to undesirable side effects as these lipids are involved in a large variety of cellular processes. All in all, specific inhibitors against LD biogenesis or the utility of LD as biomarkers in certain cancers could open up a new class of therapeutics or prognostication in cancers.

Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

5-FU, 5-fluorouracil; ACC, acetyl-CoA carboxylase; ACLY, ATP citrate synthase; ATF6, activating transcription factor 6; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; CE, cholesterol ester; COX-2, cyclooxygenase-2; CRC, colorectal cancer; DC, dendritic cell; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; FA, fatty acid; FFA, free fatty acid; FASN, fatty acid synthase; HBV, hepatitis B virus; HCV, hepatitis C virus; HSL, hormone-sensitive lipase; HSR, heat shock response; Ire1α, inositol-requiring enzyme 1α; LD, lipid droplet; LPCAT2, lysophosphatidylcholine acyltransferase 2; MAPK, mitogen-activated protein kinase; NCD, non-apoptotic cell death; NL, neutral lipid; PERK, PRKR-like endoplasmic reticulum kinase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; PGE$_2$, prostaglandin E$_2$; PUFA, polyunsaturated fatty acid; RCC, renal cell carcinoma; ROS, reactive oxygen species; SCD, stearoyl CoA desaturase; SE, steryl ester; SREBP, sterol regulatory element biding protein; SOD, superoxide dismutase; TIS, therapy-induced senescence; TG, triacylglycerol; TOFA, 5-(tetradecyloxy)-2-furoic acid; UPR, unfolded protein response; WAT, white adipose tissue.

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