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Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment

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Abstract

Solid tumors typically develop hostile microenvironments characterized by irregular vascularization and poor oxygen (O₂) and nutrient supply. While normal cells modulate anabolic and catabolic pathways in response to changes in nutrient availability, cancer cells exhibit unregulated growth even under nutrient scarcity. Recent studies have demonstrated that constitutive activation of growth-promoting pathways results in a dependence on unsaturated fatty acids for survival under O₂ deprivation. In cancer cells, this dependence represents a critical metabolic vulnerability that could be exploited therapeutically. Here we review how this dependence on unsaturated lipids is affected by the microenvironmental conditions faced by cancer cells.

Keywords

unsaturated lipids; hypoxia; metabolism; SCD1; ER stress

Metabolic and microenvironmental challenges affecting cancer cells

Oncogenic events, such as the loss of tumor suppressors or activation of oncogenes, imbue cancer cells with a dysregulated growth rate, leading to uncontrolled proliferation. Consequently, solid tumors can quickly outgrow existing vasculature and experience decreased access to blood-borne nutrients and O₂. While the induction of angiogenesis is common during cancer progression, the resulting tumor blood vessels are often disorganized and leaky. Despite their vascularity, many solid tumors exhibit high levels of tissue hypoxia [5], which can increase cellular reactive oxygen species and cause endoplasmic reticulum (ER) stress. In addition to hypoxia, abnormal blood vessels limit the delivery of blood-borne nutrients to tumor cells [6-8]. While normal cells can adapt to these circumstances by

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adjusting their rate of proliferation, neoplastic transformation disrupts the signaling pathways that control cell division and growth. Cancer cells therefore confront the compound challenges of high growth rates and limited and unreliable supply of O₂ and nutrients. Recent studies suggest that the metabolic challenges of malignant growth lead to vulnerabilities associated with discontinuous nutrient supply. The results of these investigations could reveal new treatment strategies for targeting poorly vascularized tumor microenvironments.

Compared to non-malignant cells, cancer cells exhibit significant metabolic alterations with respect to several critical nutrients and substrates, including important changes in the metabolism of both glucose and glutamine [12]. Cancer cells also exhibit increased demand for fatty acids, which are derived endogenously from citrate or taken up from exogenous sources. The elevated rates of lipid synthesis occur through increased expression of various lipogenic enzymes. There is ample evidence that increased lipid production is critical for cancer cell survival, while expression of a central lipogenic enzyme, fatty acid synthase (FASN), is strongly correlated with cancer progression [13, 14]. Fatty acids can be incorporated into membranes as phospholipids, stored in lipid droplets and used for the production of signaling lipids. In prostate tumors, which are known to import less glucose than other tumor types [15], β -oxidation of FAs may be an important alternative energy source [16, 17].

Although the requirement for fatty acids is mostly met by synthesis from glucose-derived carbon, fatty acid uptake can also be an important source of lipids in some settings. To obtain free fatty acids from blood, triglycerides in circulating chylomicrons and very low-density lipoprotein particles are hydrolyzed to fatty acids by lipoprotein lipase (LPL) and then imported via the fatty acid channel protein CD36. Both are widely expressed in clinical breast, liposarcoma and prostate tumor samples [19], indicating that cancer cells may obtain fatty acids from circulating, diet-derived lipoprotein particles. *In vitro* studies demonstrate that cancer cells can utilize these exogenous lipids and in some cases depend on exogenous lipid for survival [13]. The critical role of lipids in cancer cell proliferation has led to a number of proposed strategies for treating cancer through inhibiting lipid availability [20].

Recent studies have identified unsaturated lipid deprivation as an important challenge for rapidly dividing cancer cells. In this review, we focus on mechanisms that may underlie this vulnerability and discuss the role of different lipid species in mediating this phenotype.

Cancer, ER stress and lipids

To satisfy the requirements of doubling biomass for rapid cell division, cancer cells increase macromolecular synthesis through oncogenic mutations in numerous signaling pathways. One pathway commonly activated in cancer is regulated by the serine/threonine kinase complex mTORC1 [21]. mTORC1 contributes to unrestrained proliferation through its effects on ribosome biogenesis, protein synthesis and lipogenesis via numerous downstream effectors [22, 23]. While mTORC1 activation stimulates growth, hyperactivation of mTORC1 can have negative effects on cell function [24]. In mouse embryonic fibroblasts (MEFs), constitutive mTORC1 activity can be modelled through depletion of the TSC

complex, which negatively regulates mTORC1. Loss of either TSC1 or TSC2 leads to an enhanced growth rate, increased protein synthesis and numerous other changes in macromolecular biogenesis. However, elevated rates of mRNA translation also increase ER unfolded protein load, which leads to ER stress and activation of the unfolded protein response (UPR; see Box 1) [24].

Whether the high growth rate of cancer cells affects their survival under complex tumor microenvironmental conditions is central to the study of cancer metabolism. Recently, highly proliferative MEFs exhibiting mTORC1 dysregulation (*Tsc2*^{-/-}, *p53*^{-/-}) and grown under tumor-like conditions of serum and O₂ deprivation were found to undergo programmed cell death due to a specific deficiency in unsaturated lipid [25]. Indeed, the requirement for unsaturated lipids applies to multiple cancer cell lines and is supported by *in vivo* data in *Tsc2*^{-/-} mouse kidney cystic adenomas [22]. This cell death occurred because the desaturation of *de novo* synthesized lipids by stearyl-coA desaturases, such as SCD1, requires O₂. O₂ deprivation inhibits this enzymatic reaction, rendering cells dependent on exogenous unsaturated lipids. Restricting the supply of exogenous lipid to hypoxic cells therefore leads to a critical unsaturated lipid deficiency and causes cell death by eliciting ER stress and activating the UPR. UPR-mediated cell death under these conditions is dependent on mTORC1 and is likely caused by an mTORC1-driven increase in protein synthesis [24, 25]. IRE1 α is required for apoptosis upon unsaturated lipid deprivation in *Tsc2*^{-/-} MEFs, indicating that decreased cell survival is a consequence of terminal UPR signaling. Taken together, these findings suggest that proliferating cells need to balance their growth rate and unsaturated lipid availability to prevent ER stress, terminal UPR activation and cell death.

SCD1-mediated lipid desaturation was also found to be a critical determinant of cancer cell survival downstream of SREBP transcription factors [26]. SREBPs have important roles in regulating lipid metabolism. When activated, they induce expression of a lipogenic program thought to play a critical role in cancer cell metabolism [22, 27]. SREBPs are regulated by mTORC1 [22, 28] and thus may be essential effectors of its role in promoting cancer cell growth. SREBP-mediated lipid synthesis is elevated in human glioblastoma multiforme (GBM), for example, and loss of SREBP and lipid synthesis blocks growth of glioblastoma cells in xenograft models [26, 27, 29]. SREBP inhibition results in decreased cellular unsaturated lipid levels and cell death when exogenous lipid supplies are limited [26]. This phenotype can be rescued by addition of unsaturated oleic acid or by re-expressing SCD1 [26, 29], indicating that the effects of SREBP loss are attributable to the regulation of SCD1 expression by SREBP. SREBP ablation is also accompanied by significant ER stress and activation of the IRE1 α and PERK branches of the UPR. Moreover, UPR induction is abrogated by the addition of exogenous unsaturated lipids. These data confirm that loss of SCD1 activity, which can occur through hypoxia or loss of *Scd1* gene expression after SREBP inhibition, can lead to cancer cell death by induction of ER stress.

If the proliferation of cancer cells occupying hypoxic tumor domains is limited by a lack of unsaturated lipid, then understanding how cancer cells overcome this limitation would likely open up new therapeutic strategies. A recently published study [30] found that hypoxic cells exhibit increased uptake of unsaturated lipid from their environment, thus bypassing the requirement for fatty acid desaturation. Cancer cell lines with activating mutations in Ras

also elevate fatty acid uptake, possibly making them more independent of their O₂ supply. Using Ras-driven human cancer cells and immortalized baby mouse kidney (iBMK) epithelial cells, it was found that oncogenic Ras renders cells less sensitive to SCD1 inhibition compared to other oncogenes, presumably through elevating lipid import. LC-MS analysis of lipid species in spent media identified lysophospholipids as the predominant lipid form taken up by these cells. Details of mechanisms for uptake of unsaturated lipid are currently unknown and are an area of active research.

These recent lines of investigation paint an interesting picture of cancer cells' metabolic dependence on their microenvironment (see **Figure 1**). A normal cell's capacity to regulate its growth rate in response to nutrient availability and retain a pool of unsaturated lipid allows it to maintain homeostasis and avoid ER stress. In contrast, a cancer cell's dysregulated growth rate requires high levels of protein synthesis, creating a metabolic requirement for unsaturated lipid, much like that of *Tsc2*^{-/-} MEFs. As a tumor grows and outstrips its O₂ supply, its source of unsaturated lipid shifts from endogenous desaturation to uptake from the microenvironment. If exogenous unsaturated lipids also become limited, cells experience ER stress and activation of the terminal UPR, ultimately causing cell death. Evidence suggests that Ras-driven cells are more able to import lipid from media than Akt-driven cells, demonstrating that different oncogenic events can alter a tumor cell's ability to survive under harsh microenvironmental conditions [30].

Effects of the ratio of saturated to unsaturated phospholipids on ER stress

Changes in cellular lipid composition, such as the accumulation of free cholesterol in the ER [31] and exogenous addition of saturated fatty acids [32, 33], have previously been shown to induce ER stress and trigger the UPR. In particular, the effect of changes in the saturated:unsaturated lipid ratio on cell function has been investigated extensively due to the role of lipotoxic saturated fatty acids in diabetes and non-alcoholic fatty liver disease [34-36].

Preventing ER stress appears to require balancing saturated and unsaturated lipid species, since the lipotoxic effects of saturated lipids can often be reversed through the addition of unsaturated lipids. Shifting the cellular lipid profile towards saturated lipids by administration of saturated lipid or loss of desaturase activity causes ER stress and cell death [37-39]. *In vivo* models of hepatic steatosis exhibit a similar relationship between saturated lipid uptake, apoptosis [40] and ER stress [41]. ER stress caused by lipid saturation can be ameliorated or rescued by addition of exogenous, unsaturated lipid or by overexpressing SCD1. ER stress following administration of saturated lipids is known to lead to insulin resistance [42] and can also be overcome by addition of unsaturated fatty acids [39, 43, 44]. Even dietary changes in levels of unsaturated lipid consumption have been shown to improve insulin sensitivity in humans [45-47]. Studies on the effects of lipid saturation on insulin resistance therefore support the view that ER homeostasis requires the correct balance of unsaturated to saturated lipids.

Although a link between the ratio of saturated to unsaturated lipid and ER stress has been shown, identifying the lipid species and cellular compartments that are critical for this effect

is more difficult. Recent work supports the theory that lipid-induced ER stress is caused by disruption of ER structure by over-incorporation of saturated phospholipids into the ER membrane [37, 39]. Administering oleic acid may reverse this process by shifting the ratio back towards unsaturated phospholipids. The composition of phospholipid in the ER membrane affects membrane fluidity and the assembly of membrane subdomains [48]. Studies on LPCAT3, an enzyme capable of altering the composition of the ER membrane, support the view that changes in phospholipid saturation can trigger ER stress. The saturation of the phospholipid pool can be influenced by several factors, such as the degree of saturation of the cellular fatty acid pool. Additionally, the Lands cycle is capable of remodeling membrane composition through fatty acyl deacylation and reacylation. LPCAT3 contributes an increased unsaturated phospholipid fraction within membranes by preferentially incorporating arachidonic and linoleic acid into phospholipids. Recent work has shown that loss of LPCAT3 leads to increased phospholipid saturation and elevated ER stress. LPCAT3 inhibition in HeLa cells sensitizes them to UPR activation and cell death under these circumstances [38]. The transcription factor LXR regulates both LPCAT3 and SCD1 and plays an important role in maintaining membrane fluidity and homeostasis through a number of downstream processes [48].

In summary, there is growing evidence that an unsaturated lipid pool is necessary for the maintenance of cell viability, especially in cells with high rates of protein synthesis, because of the essential role of these lipids in maintaining ER homeostasis by counteracting the effects of saturated lipids (see **Figure 2**). That ER stress occurs because of a shift in phospholipid saturation specifically is supported by the effects of LPCAT3 loss; however, an additional role for unsaturated fatty acids in preventing ER stress cannot be excluded.

Sensing ER membrane lipid saturation: A role for IRE1 α and PERK

ER stress can be a consequence of high levels of protein synthesis in malignant cells and also exacerbated by a hypoxic tumor microenvironment. Hypoxia has long been known to cause ER stress, possibly due to the elevated levels of reactive oxygen species in hypoxic cells and the role of O₂ as the terminal electron acceptor for disulphide bond formation [49]. During conventional ER stress, three primary UPR sensors detect the accumulation of unfolded proteins via direct binding to their ER-luminal domains or through binding of the chaperone protein Grp78.

Furthermore, changes in phospholipid composition of ER membranes have been shown to trigger the UPR. Indeed, electron microscopy of *Tsc2*^{-/-} MEFs demonstrates a physical distension of the ER during unsaturated lipid deprivation [25], which is often accompanied by engaging the IRE1 α and PERK branches of the UPR. Mechanisms of IRE1 α and PERK activation through changes in membrane lipid composition are currently being elucidated [48] and appear to be distinct from UPR activation through misfolded proteins.

Several lines of investigation indicate that lipid-induced and conventional ER stress trigger the UPR through separate mechanisms. Fluorescent labeling of IRE1 α during ER stress also reveals clear differences in IRE1 α activation. Under conventional ER stress, fluorescently labeled IRE1 α proteins aggregate in distinct foci in the ER, but these are not visible under

lipid-induced ER stress [50]. Furthermore, it was found that the luminal domains of IRE1 α and PERK, essential for the detection of misfolded proteins, are mostly dispensable for activation by saturated lipids [51, 52]. These data indicate that IRE1 α and PERK are capable of directly sensing biophysical changes in the membrane following changes in saturation. How this is achieved is unclear, although several theories have been postulated. For example, changes in membrane saturation could affect the proteins' propensity to homodimerize and autophosphorylate or change the tilting angle of transmembrane protein domains, thus leading to changes in activity. Alternatively, some of the known interacting partners of IRE1 α may play a role in sensing membrane properties [52].

Persistent ER stress triggers a "terminal" UPR that leads to cell death via several pathways. The question of how ER stress downstream of unsaturated lipid deprivation causes cell death has not been resolved and the answer is likely to be condition-dependent. For example, in non-alcoholic liver disease models, JNK was found to be essential for cell death, but CHOP is not required for saturated fatty acid-dependent apoptosis [34]. In contrast, JNK appears to be dispensable in mTORC1-driven MEFs, where apoptosis was caused by IRE1 α activation [25]. Conversely, HeLa cell death from SCD1 loss is suppressed by PERK knockdown and unaffected by IRE1 α loss [38]. Increased lipid saturation through SCD1 and SREBP inhibition has also been shown to elevate reactive oxygen species (ROS) levels [29], which can also promote cell death. The source of ROS under lipid-induced stress is unclear: unsaturated lipid deprivation may cause mitochondrial dysfunction, which can lead to ROS generation and ER stress. Alternatively ER stress can lead to oxidative damage directly through release of ROS from the oxidizing environment of the ER lumen [53]. How the mechanisms of a terminal UPR differ and how saturated fatty acid-dependent apoptosis is induced in distinct cell types during variable conditions still needs to be more thoroughly investigated. While there is reason to believe that changes in lipid saturation affect ER stress through changes in ER phospholipid saturation, the involvement of other organelles, such as mitochondria, should also be explored.

Supplying cancer cells with exogenous lipid: Microenvironmental and dietary factors

As evidence accumulates that cancer cells, especially those driven by oncogenic Ras, benefit from exogenously supplied lipids, it remains to be determined how circulating lipids affect tumor growth. Understanding the extent to which cancer cells gain access to unsaturated lipid species from their microenvironment could have therapeutic implications. Adipocytes are a major component of tumor microenvironments [54] and adipocyte-secreted factors have been shown to enhance cell motility and promote expression of genes associated with a more aggressive phenotype, although the exact nature of these secreted factors is under dispute [54, 55]. Adipocyte-derived lipids appear to impact cancer progression in ovarian cancer metastases to the omentum [56]. Metastasis to this site occurs at least partly because adipocytes secrete cytokines that attract cancer cells. Through unknown mechanisms, the malignant cells then cause adipocytes to increase lipolysis and secrete fatty acids that are taken up by the cancer cells to be used for energy production. This work showed that omental adipocytes transfer lipids to ovarian cancer cells and thus promote tumor growth.

Moreover, the fatty acid binding protein FABP4 was found to play a critical role in the transfer of lipids and in metastasis in a murine ovarian cancer model.

These data suggest that cancer cells are capable of inducing stromal cells to modify their metabolism and secrete lipid species that are subsequently imported to promote tumor growth. Such a model is reminiscent of cachexia, in which patients with advanced stages of cancer exhibit adipose tissue atrophy. Adipocytes undergo increased lipolysis in cachectic patients and release more fatty acids, which may be triggered by tumor-derived factors [57-61]. The importance of exogenous lipid availability to tumor growth suggests that broader questions of diet and obesity could influence cancer cell metabolism by altering the availability of lipid species to cancer cells. Obesity has long been known to increase the risk of some cancer types, with up to 5% of cancer incidence being attributable to being overweight [62-64]. Whether this increase in cancer risk can be partially explained through lipid availability remains to be seen, as obesity not only affects circulating lipid levels, but is also associated with hormonal changes, cytokine release and inflammation [54].

Concluding remarks

Reflecting the lipogenic nature of many cancers as well as the need for unsaturated lipid in cancer cell proliferation, SCD1 expression levels are frequently elevated in cancer [65, 66]. Several studies on the effects of SCD1 inhibition on xenograft tumor growth have found a modest growth decrease in ccRCC (clear cell renal cell carcinoma) cells [67, 68], gastric carcinoma cells [69] and colon cancer cells [70]. *In vitro*, SCD1 inhibition leads to induction of ER stress and apoptosis in multiple cell lines [67, 69], which is an expected consequence of increased lipid pool saturation. As predicted, given that unsaturated lipid deprivation has its most pronounced effect on cells that exhibit dysregulated growth [25], SCD1 inhibition has been found to affect cancer cells, but not normal cells in several cases [67, 71]. That SCD1 inhibition fails to have larger effects on tumor growth is not surprising given cancer cells' ability to take up unsaturated lipid from their microenvironment [30].

While it is established that tumor cells fulfill their ravenous demand for glucose by increasing the expression of surface glucose transporters, recent work has identified macropinocytosis as another route by which nutrient uptake is enhanced in cancer cells. During this process, engulfed extracellular material is internalized in macropinosome vesicles and degraded by the cell's lysosomal compartment. It was recently demonstrated that oncogenes such as Ras and Src increase macropinocytotic rates and that uptake of soluble extracellular protein by cancer cells fulfills a metabolic need for amino acids [72]. Inhibition of macropinocytosis reduces the growth of Ras-driven human pancreatic cancer cell xenograft tumors, demonstrating that this uptake route is an important nutrient source for some cancers [72]. Ras-driven cells have been described as possessing a "scavenging" phenotype and their increased import of lysophospholipids has been shown to render them less sensitive to SCD1 inhibition [30]. It remains to be determined whether Ras-driven cells' increased rate of macropinocytosis also explains their increased lipid uptake or whether this route is reserved for protein transport.

The studies discussed here suggest the possibility that unsaturated lipid deprivation could be used as a therapeutic intervention to target cancer cells, since dysregulated growth sensitizes cells to terminal UPR pathways. On its own, SCD1 inhibition is unlikely to be effective, as exogenous lipid uptake renders cells resistant to SCD1 inhibition. Delineating the dynamics of lipid uptake will likely open novel therapeutic options for exploiting cancer cells' dependence on exogenous unsaturated lipid. This approach could be used to target hypoxic tumor domains or used in combination with available SCD1 inhibitors to cause cell death from ER stress due to unsaturated lipid deprivation. A better understanding of the mechanisms by which ER stress and UPR activation are elicited and how they trigger cell death during unsaturated lipid deprivation could also provide important new strategies to sensitize cancer cells to this type of stress.

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Box 1: The unfolded protein response (UPR)

The unfolded protein response is a highly conserved signaling program that monitors ER stress and resolves it by decreasing mRNA translation, triggering an expansion of ER membrane, increasing protein degradation and enhancing protein folding capacity. These responses are mediated by three separate pathways and initiated by separate sensors (ATF6, PERK and IRE1 α) that reside in the ER membrane and detect accumulation of misfolded and under-glycosylated proteins. ER stress activates ATF6 by causing this transmembrane protein to be transported to the Golgi apparatus in vesicles where it is cleaved by two proteases. The remaining cytosolic fragment enters the nucleus following cleavage and alters gene expression. PERK engagement by ER stress occurs by oligomerization and autophosphorylation. Once active, it phosphorylates eIF2 α and inhibits mRNA translation, thus reducing ER protein load. PERK stimulation also leads to the preferential translation of specific proteins, including the transcription factor ATF4, a critical mediator of PERK activity. The bifunctional transmembrane protein IRE1 α acts as the third branch of the UPR. Like PERK, detection of unfolded protein leads to autophosphorylation and oligomerization. IRE1 α exerts much of its effects via RNase activity (which is capable of degrading mRNAs to reduce peptide synthesis [1]), splicing specific mRNAs [2], and degrading miRNAs [3, 4]. A key component of the IRE1 α controlled response is the transcription factor XBP1. Active IRE1 α removes an exon from Xbp1 mRNA leading to the accumulation of a more stable XBP1s protein under ER stress. XBP1s enhances the expression of proteins that help improve ER capacity [9]. IRE1 α also interacts with cytosolic proteins, such as ASK1 and TRAF2 to modulate p38MAPK and JNK activation and also impacts the ERK and NF- κ B pathways. While mild ER stress typically elicits a cytoprotective UPR response, persistent ER stress can induce the UPR to initiate programmed cell death [10]. PERK activation triggers cell death by stimulating the expression of the pro-apoptotic C/EBP-homologous protein (CHOP) [11]. IRE1 α can also trigger cell death by JNK activation via TRAF2 recruitment and ASK1 phosphorylation. Once activated, JNK induces apoptosis via phosphorylation of various Bcl-2 protein family members and modulation of their pro-apoptotic and anti-apoptotic activity in favor of cell death [18]. In addition to its kinase activity, IRE1 α nuclease activity can trigger cell death. The process of regulated IRE1-dependent decay of mRNAs (RIDD), leads to the degradation of mRNA and the reduction of ER-protein burden. RIDD initially helps resolve ER-stress, and can promote cell death under irremediable conditions. IRE1 α activity can also induce apoptosis relieving inhibition of pro-apoptotic factors by degradation of key miRNAs [3, 4]. Cleavage of miRNAs occurs at sites that are distinct from DICER sites as well as sites of IRE1 α cleavage in Xbp1 mRNA. IRE1 α thus plays an integral role in committing the cell to an apoptotic program under persistent and irremediable ER-stress.

Highlights

- Cancer cells inhabit a challenging microenvironment
- Malignant cells do not adjust their rate of proliferation in response to nutrient availability
- Rapid growth in the context of limited nutrients and oxygen results in metabolic challenges
- These challenges create metabolic vulnerabilities for cancer cells

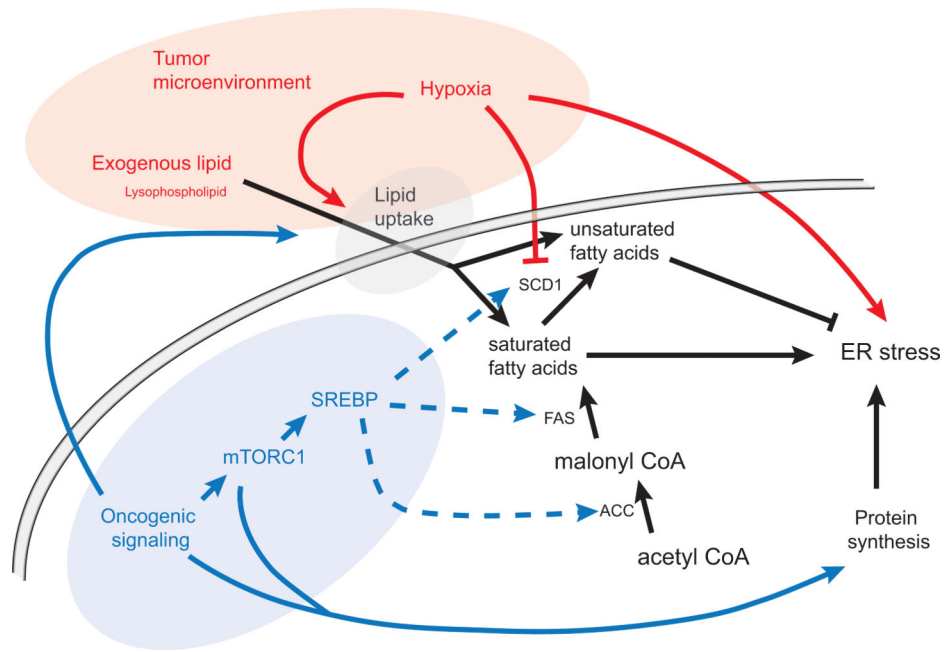


Figure 1.

Unsaturated lipids can be acquired by desaturation of *de novo* synthesized fatty acids or by uptake of exogenous lipid. Saturated fatty acids have been shown to promote ER stress while unsaturated fatty acids counteract the effect of saturated lipid. The tumor microenvironment can contribute to ER stress by inhibiting optimal protein folding and SCD1 desaturase activity. Hypoxia can also increase lipid uptake, which counteracts the effects of SCD1 inhibition. Oncogenic signaling increases fatty acid synthesis and protein synthesis. Elevated rates of protein synthesis have been shown to increase ER stress.

Oncogenic changes, such as activating Ras mutations, also promote lipid uptake.

SCD1: stearoyl-Coenzyme A desaturase 1

FAS: fatty acid synthase

ACC: acetyl-CoA carboxylase

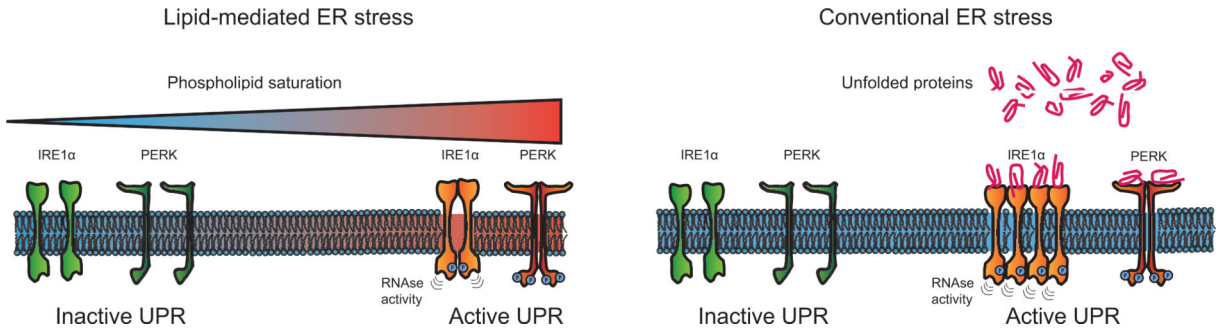


Figure 2.

Increases in phospholipid saturation have been shown to activate the IRE1 α and PERK branches of the UPR. The mechanisms of activation by lipid saturation are distinct from those triggered by unfolded protein accumulation. Conventional ER stress triggers IRE1 α and PERK through either direct binding of unfolded proteins to the luminal domains of the sensors or through their interaction with the BiP chaperone protein [9]. While UPR activation by increased unfolded protein load occurs via the luminal domains of IRE1 α and PERK, these domains are not required for activation by elevated lipid saturation. In addition, IRE1 α activation by saturated lipids does not lead to the characteristic formation of IRE1 α “foci” indicating that lipid-induced IRE1 α activation does not lead to oligomerization.