Review

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

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Solid tumors typically develop hostile microenvironments characterized by irregular vascularization and poor oxygen (O_2) and nutrient supply. Whereas 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 dependence on unsaturated fatty acids for survival under O_2 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.

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 the existing vasculature and experience decreased access to blood-borne nutrients and O_2. Although 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 [1], which can increase cellular reactive oxygen species (ROS) and cause endoplasmic reticulum (ER) stress. In addition to hypoxia, abnormal blood vessels limit the delivery of blood-borne nutrients to tumor cells [2–4]. Whereas normal cells can adapt to these circumstances by 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 O_2 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 with nonmalignant 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 [5]. 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 and expression of a central lipogenic enzyme, fatty acid synthase (FASN), is strongly correlated with cancer progression [6,7]. 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 [8], β-oxidation of fatty acids may be an important alternative energy source [9,10].

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 [11], 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 [6]. The critical role of lipids in cancer cell proliferation has led to several proposed strategies for treating cancer through inhibiting lipid availability [12].

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

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Box 1. The UPR

The UPR 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 IRE1α) 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 eIF2α 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 IRE1α acts as the third branch of the UPR. Like PERK, detection of unfolded protein leads to autophosphorylation and oligomerization. IRE1α exerts much of its effect via RNAse activity (which is capable of degrading mRNAs to reduce peptide synthesis [65]), splicing specific mRNAs [66], and degrading miRNAs [67,68]. A key component of the IRE1α-controlled response is the transcription factor XBP1. Active IRE1α removes an exon from Xbp1 mRNA leading to the accumulation of the more stable XBP1s protein under ER stress. XBP1s enhances the expression of proteins that help improve ER capacity [69]. IRE1α also interacts with cytosolic proteins such as ASK1 and TRAF2 to modulate p38 MAPK and JNK activation and also impacts the ERK and nuclear factor kappa B (NF-κB) pathways. Whereas mild ER stress typically elicits a cytoprotective UPR response, persistent ER stress can induce the UPR to initiate programmed cell death [70]. PERK activation triggers cell death by stimulating the expression of the proapoptotic CHOP [71]. IRE1α 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 proapoptotic and antiapoptotic activity in favor of cell death [72]. In addition to its kinase activity, IRE1α nuclease activity can trigger cell death. The process of regulated IRE1-dependent decay of mRNAs (RIDD) leads to the degradation of mRNA and reduction of the ER protein burden. RIDD initially helps resolve ER stress and can promote cell death under irremediable conditions. IRE1α activity can also induce apoptosis, relieving inhibition of proapoptotic factors by degradation of key miRNAs [67,68]. Cleavage of miRNAs occurs at sites that are distinct from DICER sites as well as sites of IRE1α cleavage in Xbp1 mRNA. IRE1α thus plays an integral role in committing the cell to an apoptotic program under persistent and irremediable ER stress.

through oncogenic mutations in numerous signaling pathways. One pathway commonly activated in cancer is regulated by the serine/threonine kinase complex mTORC1 [13]. mTORC1 contributes to unrestrained proliferation through its effects on ribosome biogenesis, protein synthesis, and lipogenesis via numerous downstream effectors [14,15]. Although mTORC1 activation stimulates growth, hyperactivation of mTORC1 can have negative effects on cell function [16]. In mouse embryonic fibroblasts (MEFs), constitutive mTORC1 activity can be modeled through depletion of the tuberous sclerosis complex (TSC), 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 the ER unfolded protein load, which leads to ER stress and activation of the unfolded protein response (UPR) (Box 1) [16].

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 O_2 deprivation were found to undergo programmed cell death due to a specific deficiency in unsaturated lipid. 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 [14,17]. This cell death occur because the desaturation of *de novo* synthesized lipids by stearoyl-Coenzyme A (CoA) desaturases (SCDs), such as SCD1, requires O_2. O_2 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 to be caused by an mTORC1-driven increase in protein synthesis [16,17]. IRE1α is required for apoptosis on 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 [18]. 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 [14,19]. SREBPs are regulated by mTORC1 [14,20] 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 [18,19,21]. SREBP inhibition results in decreased cellular unsaturated lipid levels and cell death when exogenous lipid supplies are limited [18]. This phenotype can be rescued by addition of unsaturated oleic acid or by re-expressing SCD1 [18,21], 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 IRE1α 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, understanding how cancer cells overcome this limitation