The Heterogeneity of Lipid Metabolism in Cancer

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Keywords
Cancer metabolism · Tumor heterogeneity · Lipid synthesis · Fatty acid oxidation · Fatty acid uptake · Metastasis · Lipidomics

Abbreviations

4-HNE	4-Hydroxy-nonenal
ACC	Acetyl-coenzyme A carboxylase
ACLY	Adenosine triphosphate citrate lyase
ACSL3	Acyl-coenzyme A synthetase long-chain family member 3
ACSS2	Acyl-coenzyme A synthetase short-chain family member 2
AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
BMI	Body mass index
BTA	Benzene-tricarboxylate
CAV1	Caveolin 1
CD36	Cluster of differentiation 36 protein
CoA	Coenzyme A
CPT1	Carnitine palmitoyltransferase 1
CTP	Citrate transporter protein
DNA	Deoxyribonucleic acid
DNLS	De novo lipid synthesis
EMT	Epithelial-mesenchymal transition
ERS	Endoplasmic reticulum stress
FADH2	Flavin adenine dinucleotide
FAO	Fatty acid oxidation
FAS	Fatty acid synthase
FATP	Fatty acid transport protein
GBM	Glioblastoma multiforme
HCC	Hepatocellular carcinoma
HFD	High-fat diet
HIF-1α	Hypoxia-inducible factor 1-alpha
HMGCR	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
IDH	Isocitrate dehydrogenase
LD	Lipid droplet
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein kinase
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1

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NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NSCLC	Non-small cell lung cancer
PDAC	Pancreatic ductal adenocarcinoma
PE	Phosphatidylethanolamine
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PIP2	Phosphatidylinositol-4,5-bisphosphate
PTEN	Phosphatase and tensin homolog
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SCD	Stearoyl-coenzyme A desaturase
TCA	Tricarboxylic acid
TG	Triglyceride
TME	Tumor microenvironment
TSC	Tuberous sclerosis protein
ω-3/6	Omega-3/6 fatty acid

Key Points

- Fatty acid synthesis is upregulated in cancer.
- The mitochondrial citrate transporter protein (CTP) protects mitochondrial function in cancer.
- ATP citrate lyase (ACLY) is upregulated in cancer.
- Inhibiting acetyl-CoA carboxylase (ACC) has multifaceted effects on cancer.
- The first fatty acid synthase (FAS) inhibitor, TVB-2640, is in clinical trials for cancer.
- Markers such as cell type, oncogene mutations, expression/activity of lipid synthesis enzymes, and metabolic profiles can be used to predict cancer cell sensitivity to lipid synthesis inhibition.
- The tumor microenvironment influences the sensitivity of cancer cells to lipid synthesis inhibitors.
- The efficacy of inhibiting cholesterol synthesis with adjuvant statins is variable.
- Fatty acid uptake is associated with metastasis.
- Targeting fatty acid oxidation (FAO) for cancer therapy may be achieved by inhibiting carnitine palmitoyltransferase 1.
- Carnitine palmitoyltransferase 1 (CPT1) inhibitors are now in clinical trials for cancer treatment.
- FAO occurs at peroxisomes, where peroxisome proliferator-activated receptors (PPARs) act as ligand-activated transcription factors.

1 Introduction

The study of cancer cell metabolism has traditionally focused on glycolysis and glutaminolysis. However, lipidomic technologies have matured considerably over the last decade and broadened our understanding of how lipid metabolism is relevant to cancer biology [1–3]. Studies now suggest that the reprogramming of cellular lipid metabolism contributes directly to malignant transformation and progression [4, 5]. For example, de novo lipid synthesis can supply proliferating tumor cells with phospholipid components that comprise the plasma and organelle membranes of new daughter cells [6, 7]. Moreover, the upregulation of mitochondrial β-oxidation can support tumor cell energetics and redox homeostasis [8], while lipid-derived messengers can regulate major signaling pathways or coordinate immunosuppressive mechanisms [9–11]. Lipid metabolism has, therefore, become implicated in a variety of oncogenic processes, including metastatic colonization, drug resistance, and cell differentiation [10, 12–16]. However, whether we can safely and effectively modulate the underlying mechanisms of lipid metabolism for cancer therapy is still an open question.

As discussed in previous chapters, inter- and intra-tumoral heterogeneities are major causes of treatment failure in clinical oncology because tumor subclones with either intrinsic or acquired resistance to therapy can be selected by Darwinian mechanisms and allowed to drive disease relapse [17–20]. An alarming number of parameters seem to be capable of inducing this diversity, including (epi)genetic lesions, microenvironmental constraints, stromal interactions, and treatment effects [21–23]. Perhaps unsurprisingly then, translational strategies targeting lipid metabolism have reported mixed or even diverging responses in

preclinical models of cancer. These results hint at differential tumor cell dependencies on lipids, but we are far from understanding the extent to which this heterogeneity arises. Moreover, how this nonuniformity of lipid metabolism undermines patient treatment is unclear. To better understand the clinical potential of this emerging discipline, we will have to address both the spatial and temporal heterogeneities of cellular lipid metabolism.

Here, we provide a brief synopsis of novel findings on the lipid metabolism of cancer cells, with an emphasis on heterogeneity across and/or within tumors. Given the rapid pace of this field, we focus on central pathways involving fatty acid synthesis, uptake, and oxidation.

2 Fatty Acid Synthesis Is Upregulated in Cancer

Endogenous fatty acid synthesis is frequently upregulated in cancer because fatty acids can serve as substrates to produce lipid signaling molecules, modify protein functions through lipidation, synthesize phospholipids for cell membranes, or store energy as triglycerides. The primary source of carbons for fatty acid synthesis in cancer cells comes from glucose. Glucose carbon is incorporated into acetyl-CoA, which then forms citrate in the mitochondria. The mitochondrial citrate transporter protein (CTP) carries citrate from the mitochondria to the cytosol. ATP citrate lyase (ACLY), a key enzyme of de novo fatty acid synthesis (DNLS), cleaves cytosolic citrate into acetyl-CoA and oxaloacetate. Cytosolic acetyl-CoA is used to form fatty acids. Hence, the localization of acetyl-CoA within a cell can determine its metabolic fate.

2.1 The Mitochondrial Citrate Transporter Protein (CTP) Protects Mitochondrial Function in Cancer

The Avantaggiati research group has extensively studied the mitochondrial citrate transporter protein (CTP) and demonstrated that CTP plays an important role in preventing mitochondrial damage and preserving its function, such as in cellular bioenergetics [24]. The inhibition of CTP resulted in anti-tumorigenesis in vivo. Although the authors observed a decrease in fatty acid synthesis from glucose due to the suppression of CTP-dependent transport of citrate by a benzene-tricarboxylate analog (BTA), they believe that this effect only played a partial role in tumor reduction because the total FA levels were not drastically affected. Moreover, CTP levels were associated with cancer aggressiveness [24].

2.2 ATP Citrate Lyase (ACLY) Is Upregulated in Cancer

ATP citrate lyase (ACLY) is found to be elevated in many types of cancers, including breast [25], lung [26], liver [27], and bladder cancers [28]. Migita et al. found that ACLY expression is significantly higher in human lung adenocarcinoma samples as compared to normal lung tissue. It also correlated with stage, differentiation grade, and a poorer prognosis. ACLY inhibition arrested lung cancer cell growth in vitro and in vivo. ACLY knockdown compromised de novo lipogenesis, but intracellular lipids were increased, suggesting alternative mechanisms of lipid accumulation [26]. A study by Schlichtholz et al. similarly demonstrated upregulations of ACLY, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and citrate synthase, which are enzymes involved in fatty acid synthesis, in bladder cancer [28].

2.3 Multifaceted Effects of Inhibiting Acetyl-CoA Carboxylase (ACC) in Cancer

After ACLY produces cytosolic acetyl-CoA, the enzyme acetyl-CoA carboxylase (ACC) irreversibly converts acetyl-CoA into malonyl-CoA. Malonyl-CoA is required for fatty acid synthesis and elongation and negatively regulates

β-oxidation of long-chain fatty acids by inhibiting the enzyme carnitine palmitoyltransferase 1 (CPT1) [29]. ACC exists as two isozymes (ACC1/2, genes *ACACA/B*) [30]. ACC1 is preferentially expressed in lipogenic cells, such as adipocytes [30]. The two ACC isozymes catalyze the same reaction, and one can compensate for the loss of function of another as malonyl-CoA levels only decrease in hepatocytes if both ACC1 and ACC2 are inhibited [31]. This demonstrates that inhibiting both ACC1 and ACC2 isozymes may be more efficacious than inhibiting either isozyme alone for the treatment of cancer. ACC is now receiving greater attention as a therapeutic target against cancer because the formation of malonyl-CoA catalyzed by ACC is the rate-limiting step of fatty acid synthesis.

The expression of ACC1 is highly enriched in breast [32], prostate [33], liver [34], and renal cancers [35]. The expression of ACC1 also increases with tumor grade in liver cancer, and its overexpression increases liver cancer cell viability while decreasing apoptosis [34, 36]. ACC1 expression is also prognostic for some cancers. High expression of ACC1 is correlated with worse survival in renal cancer [35]. Inhibition of ACC1 with siRNA reduced cell viability in breast [37] and liver cancers [36]. Furthermore, simultaneous inhibition of both ACC1 and ACC2 with a small chemical molecule or siRNA reduced tumor growth in the prostate [38], brain [39], and pancreatic cancers [40].

While ACC inhibition appears to arrest the growth of certain cancer types, it has paradoxically been shown to promote breast cancer invasion and metastasis by promoting epithelial-to-mesenchymal transition (EMT) [41]. ACC-deficient hepatocytes are also more susceptible to diethylnitrosamine-induced hepatocellular carcinoma. ACC-deficient mice exhibited a reduction in hepatic lipogenesis, a decrease in glutathione, and an increase in NADPH [34]. Collectively, these preclinical studies demonstrate the duality of ACC inhibition: it could attenuate tumor growth in some cancer types, but it could also contribute to carcinogenesis or promote metastasis in others.

Long-term regulation of ACC occurs at the level of transcription, while short-term regulation of ACC occurs through allosteric binding and reversible phosphorylation. Short-term regulation allows ACC activity to rapidly adapt to the microenvironment. For instance, AMP-activated protein kinase (AMPK) can inactivate ACC via phosphorylation (p-ACC). Metformin is a widely prescribed first-line treatment for type 2 diabetes that activates AMPK. Preclinical studies in mice have demonstrated that metformin can reduce cancer growth, in part by increasing p-ACC levels [42, 43]. There are currently hundreds of clinical trials investigating whether metformin can be repurposed to treat cancer as an adjuvant monotherapy or in combination with other drugs. However, a potential adverse effect of metformin may be an increase in the metastasis of certain cancer types, given that both metastatic breast and lung tumors have increased levels of p-ACC1 [41]. Protein phosphatase 2A (PP2A) can reactivate p-ACC by dephosphorylation. The tumor suppressor known as breast cancer susceptibility gene 1 (BRCA1), which is deactivated primarily in breast and ovarian cancers, prevents dephosphorylation of p-ACC [44]. Cancers with loss-of-function mutations in BRCA1 have increased ACC activity due to less phosphorylation of ACC and thus may be more susceptible to ACC inhibition [44, 45].

2.4 The First Fatty Acid Synthase (FAS) Inhibitor TVB-2640 Is in Clinical Trials for Cancer

A large number of studies have now documented an increase in the expressions of lipogenic enzymes across several cancers. For instance, Szutowicz et al. revealed that the activity of citrate lyase, an important enzyme in lipogenesis, is elevated in breast carcinoma and fibrocystic disease compared to healthy breast tissue [25]. As such, it is reasonable that key enzymes involved in de novo fatty acid synthesis could be potential targets for cancer therapy. One such enzyme is fatty acid synthase (FAS) encoded by the *FASN* gene [46].

FAS is a multienzyme protein complex that catalyzes the final reactions converting malonyl-CoA and acetyl-CoA into a saturated long-chain fatty acid composed of 16 carbons known as palmitic acid. Palmitic acid can be used as a precursor to produce lipid signaling molecules, modify protein functions through palmitoylation, store energy as triglycerides, or form structural lipids for cell membranes. NADPH is the reducing agent for fatty acid synthesis, and 14 molecules of NADPH are used to synthesize each molecule of palmitic acid starting with acetyl-CoA. The pentose phosphate pathway (PPP) generates NADPH through the oxidation of glucose into pentose sugars and ribulose-5-phosphate. Overexpression of *FASN* is usually accompanied by the overexpression of enzymes in the PPP to supply NADPH for fatty acid synthesis. Increased expression of FAS and PPP enzymes is associated with worse survival in renal and breast cancers [35, 47]. Increased FAS expression is also associated with tumor grade in prostate cancer [48]. Inhibition of FAS reduces cell proliferation and increases cell death in human breast [37, 43], prostate [49], and colon cancers [50]. FAS inhibitors can also be used in combination with chemotherapy taxane to improve anticancer efficacy [51]. Colorectal cancer metastasis is also mitigated by FAS inhibition in mice [50]. While inhibition of FAS reduces tumor growth and metastasis in most cancers, it has also been demonstrated to reduce survival rates in mice with lung cancer by increasing metastasis [52], demonstrating how FAS inhibition can sometimes worsen cancer outcomes as seen with ACC inhibition.

The FAS inhibitor TVB-2640 has been tested on cancer patients in clinical trials (Clinical Trial ID: NCT02223247). Inhibiting FAS did not result in severe side effects, and all of the mild side effects were reversible after discontinuation [53]. Moreover, side effects were not worsened by its combined application with the chemotherapy drug paclitaxel [53]. Monotherapy with TVB-2640 stabilized cancer progression in three out of six patients with *KRAS*-driven non-small cell lung cancer (NSCLC) [53]. There are now two clinical trials (phase II) testing the efficacy of TVB-2640 in combination with additional chemotherapy drugs for $HER2^+$ breast cancer (Clinical Trial ID: NCT03179904) and astrocytoma (Clinical Trial ID: NCT03032484). The third clinical trial is a phase I study investigating the pharmacodynamic effects of TVB-2640 in patients who require surgery for colon cancer (Clinical Trial ID: NCT02980029).

2.5 Which Markers Can Predict Cancer Cell Sensitivity to Lipid Synthesis Inhibition?

Phosphatidylinositol (3,4,5)-trisphosphate (PIP3) is produced by phosphoinositide 3-kinase (PI3K) activation, which is mutated in many types of cancers [54–56]. PI3K, which is a key regulator of phosphoinositide metabolisms, is considered a potential target in preclinical and clinical settings to suppress advanced solid tumors, including malignant glioma, NSCLC, and breast cancer [57] (NCT00485719, NCT00777699, NCT00704080, NCT00907205, NCT00600275, NCT00876109, and NCT00726583).

Two highly studied intracellular signaling pathways that oncogenes activate to drive tumorigenesis and increase expression of lipid synthesis enzymes are the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway and the RAS/mitogen-activated protein kinase (MAPK) pathway [58, 59]. Constitutive activation of the PI3K/Akt/mTOR pathway results from activation of receptor tyrosine kinases (RTKs), loss-of-function mutations in the tumor suppressors phosphatase and tensin homolog (PTEN) and tuberous sclerosis protein (TSC), or gain-of-function mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and AKT1 [60]. The RAS oncogene family including HRAS, KRAS, and NRAS can also activate the PI3K/Akt/mTOR pathway [61]. Cancer cells that are driven by the overactivation of the PI3K or RAS pathways are more susceptible to inhibitors that target lipid synthesis than cancers that are driven by pathways not associated with lipid synthesis regulation [62, 63]. Inhibitors of ACLY, ACC, and FAS have been shown to be efficacious in cancers with PI3K and RAS pathways [64–67].

Cancer cells that overactivate RTKs, such as epidermal growth factor receptor (EGFR, also known as HER) family members 1–4 and c-MET [68–70], are also sensitive to lipid synthesis inhibition because these RTKs activate PI3K and RAS pathways. ACLY and ACC inhibitions are efficacious in HER1- and HER2-driven cancers [39, 66, 71–73], and FAS inhibition is efficacious in HER1-, HER2-, and c-MET-driven cancers [68, 74, 75]. Additional oncogenic signaling pathways that may be susceptible to FAS inhibition are MYC, beta-catenin, and steroid-responsive tumors because FAS inhibition downregulates these pathways [64, 76, 77]. Cancers with a loss of function in BRCA1 and p53 are also sensitive to FAS and ACC inhibition [45, 67, 78]. Preclinical studies testing ACLY, ACC, and FAS inhibitors may reveal the oncogenes and RTKs that confer susceptibility to DNLS inhibition and guide the design of future clinical trials. To date, several mTOR/PI3K inhibitors, such as idelalisib, copanlisib, rapamycin, temsirolimus, everolimus, and ridaforolimus, have been approved by the FDA.

Nevertheless, not all cancers with oncogene-mediated overactivation of RAS and PI3K pathways appear to be susceptible to lipid synthesis inhibition. For instance, *KRAS* mutations correlated with FAS inhibition sensitivity in lung cancer cell lines but not in colon cancer cell lines [64], which demonstrates that cancer cell susceptibility to lipid synthesis inhibition is not always driven by oncogenes. In other studies, oncogenes conferred resistance to FAS inhibition. Hepatocytes that are transformed into malignant cancer cells by the overactivation of c-MET and Akt are susceptible to FAS inhibition, but hepatocytes that are transformed by the overactivation of c-MET and Wnt/beta-catenin signaling are unresponsive to FAS inhibition [63]. The c-MET/beta-catenin-driven cancer cells may be unresponsive because beta-catenin activation in hepatocytes reduces FAS expression and lipid synthesis [63, 79, 80]. Interestingly, while beta-catenin decreases lipid synthesis in hepatocytes, beta-catenin signaling can increase lipid synthesis in B-cell lymphoma. Beta-catenin-driven B-cell lymphoma is susceptible to FAS inhibition [81]. This demonstrates that cancer cell type is relevant to determining susceptibility to FAS inhibition since oncogenic signaling pathways can result in different phenotypes depending on the cell type. In order for oncogenes to be reliable markers of lipid synthesis inhibition sensitivity, it will be important to consider the cell type of the cancer being discussed.

Of note, protein expression or enzyme activity may be better predictors of susceptibility to lipid synthesis inhibitors than genetic markers. For example, mRNA expression of ACLY, ACC, and FAS may not correlate with protein expression and activity [64, 82]. Increased expression of FAS and ACC at the protein level can occur without an increase in mRNA expression by increased translation of FAS and ACC mRNA via mTOR signaling [82].

Metabolic profiling may be a valuable method for determining susceptibility to FAS inhibition. One study examined 38 pancreatic cancer cell lines and classified them as lipogenic or glycolytic depending on their metabolic profile, which was determined by the amount of lipogenic or glycolytic metabolites [83]. Glycolytic cancer cells were significantly more susceptible to glycolytic inhibitors than those that were lipogenic. However, lipogenic cancer cells were not significantly more susceptible to lipogenic inhibitors, such as FAS inhibitors, than glycolytic cancer cells [83]. Only half of the lipogenic cancer cell lines were sensitive to FAS inhibition, suggesting that broad lipogenic profiling is not an accurate predictor of susceptibility to FAS inhibition. While no single marker is able to definitively predict which cancers are susceptible to lipid synthesis inhibition, using a combination of markers, such as cell type, oncogene mutations, expression/activity of lipid synthesis enzymes, and metabolic profiles, may provide a reliable means to identify cancers that are sensitive to lipid synthesis inhibitors.

2.6 Tumor Microenvironment Influences the Sensitivity of Cancer Cells to Lipid Synthesis Inhibitors

As mentioned in the chapter "Different Tumor Microenvironments Lead to Different Metabolic Phenotypes," TCA cycle activity is reduced under hypoxic conditions, which results in reduced citrate and acetyl-CoA production. However, cancer cells manage to generate acetyl-CoA for fatty acid synthesis by different mechanisms, including reliance on glutamine for citrate synthesis and acetate for acetyl-CoA via acetyl-CoA synthetase (ACSS2). Evidence suggests that ACSS2 expression can be increased to maintain growth under microenvironmental stress, such as hypoxia [84].

Bensaad et al. showed that while DNLS is repressed in hypoxia, lipid droplet accumulation and expression of fatty acid uptake proteins, such as fatty acid-binding protein 3 and 7 (FABP3 and FABP7), are induced by hypoxia-inducible factor 1-alpha (HIF-1α). Lipid synthesis was restored in cancer cells after reoxygenation or the removal of antiangiogenic therapy [85]. Other studies have corroborated that hypoxic tumor cells may be extraordinarily dependent on fatty acid uptake compared to those in normoxia [86, 87]. However, this can be differentially driven by oncogenic mammalian target of rapamycin complex 1 (mTORC1), RAS, and/or HIF-1α signaling [85–87]. Moreover, triple-negative breast cancer is reliant on lipid droplet-derived substrates for β-oxidation and ATP generation after hypoxia-reoxygenation, whereas glioblastoma multiforme (GBM) is more dependent on glycolytic pathways [85]. This implies that FA uptake is not a universal feature of hypoxic cancer cells; therefore, inhibiting FA uptake may be a strategy for targeting tumor cells in hypoxic microenvironments for certain types of cancers but not others.

The availability of metabolic nutrients can also greatly impact the susceptibility of cancer cells to inhibition of DNLS. FAS expression was observed to be the highest at the edge of tumors, suggesting that DNLS is preferred in cancer cells that are vascularized and have access to oxygen and glucose [88]. Tumors in low-lipid environments increase de novo fatty acid synthesis and thus may demonstrate increased sensitivity to FAS inhibition. The fact that lipoprotein supplementation can override DNLS inhibition emphasizes the importance of nutrient availability and, again, the role of exogenous lipid uptake [89, 90]. The availability of glucose for glucose-dependent lipogenesis is also important for cancer cell sensitivity to ACLY inhibitors. Low-glucose environments result in cancer cells that are less susceptible to ACLY inhibition because cancer cells can use acetate instead of citrate to produce acetyl-CoA for DNLS [90, 91].

3 Targeting Fatty Acid Elongation

Once palmitic acid is produced by de novo lipid synthesis, it can be modified by having its fatty acid chain elongated. Elongation of fatty acids is important for creating lipid precursors that are involved in cellular signaling and for producing phospholipids of cell membranes. Fatty acids that consist of 16 carbons or more, such as palmitic acid, are elongated in the smooth endoplasmic reticulum, while fatty acids consisting of fewer than 16 carbons are elongated in the mitochondria. Elongation of fatty acids in the smooth endoplasmic reticulum is regulated by four enzymes. These enzymes elongate fatty acids by using malonyl-CoA. The first step is the rate-determining reaction regulated by the enzyme β-ketoacyl-CoA synthase (elongase). There are seven types of elongases in humans, known as ELOVL1–7. ELOVL7 was identified to be overexpressed in prostate cancer, and feeding mice a diet high in long- and very-long-chain fatty acids increased the growth of ELOVL7-expressing tumor cells [92]. Meanwhile, inhibiting ELOVL7 with siRNA attenuated prostate cancer growth [92]. ELOVL1 is another elongase implicated in cancer growth. ELOVL1 was observed to be overexpressed in breast cancer, and inhibition of ELOVL1 with siRNA reduced breast cancer cell viability in some cell lines [37].

While inhibiting elongases appears to be a therapeutic strategy for cancer treatment, inhibiting ACC1 as described previously may be more promising because ACC1 inhibition reduces both fatty acid synthesis and fatty acid elongation, while elongase inhibition only targets elongation. ACC1 inhibition can reduce fatty acid elongation by decreasing the availability of malonyl-CoA [93]. This is suggested by a study in which silencing of ELOVL1 with a silencing efficiency of 70–80% decreased cell viability by greater than 50% in one breast cancer cell line while silencing of ACC1 with a lower silencing efficiency of 30% decreased cell viability by greater than 50% in two breast cancer cell lines [37]. Neoadjuvant chemotherapy-resistant breast cancer has been associated with increased expression of fatty acid elongation proteins in the mitochondria [94]. Whether inhibiting mitochondrial fatty acid elongation is a potential therapeutic strategy against cancer remains to be determined.

4 The Efficacy of Inhibiting Cholesterol Synthesis with Adjuvant Statins Is Variable

Another anabolic pathway associated with lipid metabolism is the mevalonate pathway, which synthesizes cholesterol. Cholesterol is a major component of cell membranes, influencing membrane fluidity, and function. It also forms detergent-resistant microdomains called lipid rafts that coordinate the activation of signal transduction pathways. The enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) catalyzes the rate-limiting step of cholesterol synthesis. Increased expression of HMGCR and other cholesterol synthesis enzymes is associated with reduced survival rates in breast cancer [95]. HMGCR is the target of a class of cholesterol-lowering drugs called statins. Numerous epidemiological studies have demonstrated that patients who use statins have a reduced risk of cancer and cancer mortality [96–98]. This has raised the question as to whether statins can improve treatment outcomes in cancer patients. There are many clinical trials currently investigating whether statins can be prescribed to reduce the progression of cancer.

Results from preclinical studies suggest that the efficacy of statins can be predicted based on the status of gene expression, such as that of HMGCR [99]. Breast cancer cells with overactive HER2 are also sensitive to statins because of HER2 signals through the RAS pathway [100]. On the other hand, estrogen receptor-positive breast cancer cells appear to be less responsive to statins. *MYC* is another transcription factor that regulates cholesterol synthesis. Cancers with overactive *MYC* have been observed with increased expression of HMGCR and sensitivity to statins [101, 102]. Statins have also been shown to reduce metastasis in colon and ovarian cancer and selectively induce apoptosis in cancer cells [103, 104]. Along with monotherapy of statins being efficacious in preclinical studies, statins are also efficacious in combination therapy by increasing sensitivity to radiation therapy [105].

The ability of statins to bind to HMGCR greatly affects their efficacy. Genetic variations in HMGCR have been found to modify the therapeutic effect that statins have on colorectal cancer [106]. A single-nucleotide polymorphism (SNP) in the HMGCR–statin-binding domain reduced the protective association between statins and colorectal cancer. An in vitro experiment demonstrated that the SNP in the HMGCR gene reduced the ability of statins to inhibit HMGCR and cholesterol synthesis. The anticancer activity of statins is also dependent on the ability of statins to enter cancer cells. For instance, the statin pravastatin was found to inhibit tumor growth preferentially in cancers that express sodium-independent organic anion transporter protein-1B1 (OATP1B1), such as liver cancer, because this transporter is necessary for cellular uptake of pravastatin [107].

While preclinical studies have provided promising results for statins, clinical trials have not been as successful. A phase II clinical trial demonstrated that combining the statin simvastatin with the chemotherapy drug afatinib did not improve treatment efficacy compared to using afatinib in monotherapy [108]. Two additional

phase II clinical trials found that statins were unable to resensitize cancers harboring *KRAS*-activating mutations to the chemotherapy drugs cetuximab and panitumumab [109, 110].

5 Fatty Acid Uptake Is Associated with Metastasis

As discussed previously, enhanced lipogenesis is a frequent alteration of lipid metabolism in cancer cells, and therapies targeting it are promising. However, studies show that this strategy can be undermined by the supplementation of exogenous fatty acids, suggesting that extracellular lipids in the microenvironment may functionally substitute for endogenously derived FA [111]. After all, the scavenging of circulating nutrients is another hallmark of cancer cell metabolism [112]. Recently, it was reported that tumors, including those of breast cancer and liposarcoma, may rely on extracellular lipolysis in addition to lipogenesis to fuel cellular lipid requirements [113]. Lipoprotein lipase (LPL) is a rate-limiting enzyme of this mechanism, hydrolyzing circulating triglyceride-rich lipoprotein, such as very-low-density lipoproteins and chylomicrons, into free FAs and monoacylglycerol molecules. Free FAs are then imported into cells by FA transporters such as a cluster of differentiation (CD36) or those of the fatty acid-binding protein (FABP). Both LPL and CD36 expressions have been associated with aggressive cancers, including hepatocellular carcinoma (HCC) and pancreatic ductal adenocarcinoma (PDAC), and negatively correlated with patient prognosis [114–117], but how this phenomenon varies among tumors and whether it can be inhibited for therapeutic effect remain uncertain.

An estimated 90% of all cancer-related deaths are attributed to metastasis, but the detailed mechanisms of metastasis remain unclear [118]. Recently, metastasis was associated with enhanced lipid metabolism [14, 119, 120]. One study identified an altered gene signature associated with fatty acid uptake (e.g., caveolin 1 (CAV1), CD36) in metastatic tumors across cancer types [121]. Moreover, this genetic signature had a significant effect on patient survival rates, suggesting prominent roles of extracellular fatty acids, specifically on metastatic progression. Corroborating this is a recent report describing abnormally high expression of CD36 in metastasis-initiating oral cancer cells [12]. Treating orthotopic xenografts with CD36-neutralizing antibodies inhibited metastasis initiation. These studies suggest that tumor cells of high metastatic potential have an outsized need for FA uptake compared to those displaying less aggressive phenotypes. Interestingly, however, an earlier study observed up to a 100-fold *lower* expression of CD36 in breast cancer cells with high metastatic potential compared to their less aggressive counterparts [122]. This inconsistency may be due to alternative mechanisms of CD36 related to cell adhesion [123]. Nevertheless, these data support the overarching concept of asymmetrical CD36 expression and fatty acid uptake even within cancers of the same type.

6 Fatty Acid Oxidation Encompasses a Diverse Set of Molecular Mechanisms

Lipids are important for cancer proliferation not only because of their ability to provide structural support as a component of the cell membrane but also because they can be broken down to provide energy. Lipids can be catabolized after cellular uptake via the β-oxidation pathway, also known as fatty acid oxidation (FAO). FAO has not been examined as thoroughly as glycolysis or glutaminolysis, but recent advances have shed light on the role of FAO in cancer cells. Recently, lipids were also identified as a carbon source for nucleotide synthesis and histone acetylation in nonmalignant cells, and emerging evidence suggests that these mechanisms are relevant to tumor cells as well [124, 125]. The tumor microenvironment is often depleted of nutrients like glucose, so cancer cells often rely on FAO to generate ATP. Lipids are energetically dense molecules that cancer cells can exploit as an alternative source of energy. FAO yields ATP and NADPH, which support cellular energetics and redox homeostasis,

respectively. Several studies have demonstrated that certain malignancies, such as those in the prostate, breast, and lung, and B-cell lymphoma heavily depend on FAO for growth and survival [126–128]. Similarly, acetate is a 2-carbon fatty acid that is avidly oxidized in tumors, including GBM [129, 130].

6.1 Targeting FAO for Cancer Therapy May Be Achieved by Inhibiting Carnitine Palmitoyltransferase 1

The inhibition of the FAO pathway could prevent cancer progression. An example of this strategy is the inhibition of carnitine palmitoyltransferase 1 (CPT1), which is the rate-limiting enzyme of FAO. CPT1 is a membrane protein that removes an acyl group from a fatty acyl-CoA and attaches the acyl group to carnitine. This results in the formation of acylcarnitine, including palmitoylcarnitine, and thereby facilitates the shuttling of fatty acids, such as palmitate, into the mitochondrial matrix for FAO [124]. There are three subtypes of CPT1. CPT1A is expressed throughout several tissue types, but CPT1B is restricted mostly to muscle tissue. In physiological settings, all isoenzymes are inhibited by malonyl-CoA, but due to the greater binding efficiency of CPT1A to malonyl-CoA, CPT1A is found to be the isoform with the greatest capacity to perform the rate-limiting step of FAO [131]. The third and final isoform of CPT1 is CPT1C, which is normally found only in the brain [132]. However, many cancers also express CPT1C [133]. CPT1C is thought to confer resistance to oxidative stress in many tumors. CPT1C promotes resistance to rapamycin, an mTOR pathway inhibitor [133].

Physiologically, it is crucial to note that successful inhibition of CPT1 is dependent on the source and location of malonyl-CoA. Malonyl-CoA produced via acetyl-CoA carboxylase 1 (ACC1) is localized in the cytosol and thus will not inhibit CPT1. The malonyl-CoA produced via the mitochondrial ACC2 enzyme, however, is capable of this inhibitory action. Thus, the relative concentrations of acetyl-CoA to malonyl-CoA can influence whether the cell is in a state of FAS or FAO [134]. AMP-activated protein kinase (AMPK) inhibits both ACC1 and ACC2 and increases reactive oxygen species (ROS) in doing so. The increase in ROS leads to depletion of NADPH and induces oxidative stress on the cell, eventually leading to cell death [8]. This finding is in accordance with other studies that have noted the role of AMPK activation in cancer states. For example, metformin exerts anticancer effects and activates AMPK, but in tumors lacking CPT1C, the effect of metformin is less pronounced. This suggests that the action of metformin on AMPK is upstream of its effect on CPT1C [133].

The upregulation of CPT1 in several cancer types makes it a potential therapeutic target [131]. However, this upregulation does not appear to be a universal feature of all tumors, as demonstrated by a recent study showing that, in clear cell renal cell carcinoma (ccRCC), transcriptional repression of CPT1A is mediated by hypoxia-inducible factors (HIF-1α and HIF-2α) [135]. However, conflicting reports regarding the role of HIFs in FAO have also emerged, and one may speculate that this is again due to the heterogeneity of metabolism across cancer subtypes. Although HIFs are known to inhibit FAO, one study performed in liver cancer cells determined that HIF-1 also decreases ROS levels and maintains redox homeostasis, thereby promoting cell proliferation [136]. This effect is thought to be mediated by the action of HIF-1 on medium- and long-chain acyl-CoA dehydrogenases (MCAD and LCAD, respectively). This study further pointed to correlations between decreased LCAD expression and patient mortality rates [136]. Thus, we see that the precise role of HIFs varies across cancer types, and as such, therapies targeting HIF-related pathways may need to be tailored to specific cancers to maximize their impact.

6.2 CPT1 Inhibitors Are Now in Clinical Trials

As far as pharmacological interventions for FAO are concerned, some CPT1 inhibitors are being developed for other conditions such as diabetes

[137]. Therefore, the possibility of repurposing them for cancer therapy is an intriguing possibility requiring further clinical trials [137]. One CPT1 inhibitor, etomoxir, has been difficult to advance through clinical trials due to its toxicity. A clinical study examining etomoxir in healthy adults found elevated levels of transaminases of some patients, and the study had to be terminated early [138]. The issue with etomoxir arises from its inability to distinguish CPT1 across tissue types. However, it has been applied to preclinical studies of breast cancer, where an interesting degree of heterogeneity has been noted. In one study, etomoxir was compared across two triple-negative breast cancer (TNBC) lines. One line expressed high amounts of the oncogenic transcription factor *MYC*, whereas the other expressed low amounts of *MYC*. In the high-*MYC*-expressing line, the application of etomoxir decreased levels of ATP, and this effect was not observed in the low-*MYC*-expressing line [128]. Furthermore, this effect was observed in no other breast cancer subtypes besides TNBC. This provides further evidence of the ways in which cancer heterogeneity should be appreciated and exploited for the development of viable treatments.

6.3 FAO for Very-Long-Chain Fatty Acids Occurs at the Peroxisome Where Peroxisome Proliferator-Activated Receptors (PPARs) Act as Ligand-Activated Transcription Factors

FAO also occurs in peroxisomes. Oxidation at the peroxisome is restricted to very-long-chain fatty acids. The peroxisome breaks these very long chains into smaller chains, which may then be further oxidized in the mitochondria. Peroxisomes are built via peroxins, the products of the Pex genes. So far, 3 of the 30 known peroxins, Pex3, Pex16, and Pex19, have been shown to be necessary for proper peroxisome assembly [139]. One of these peroxins, Pex19, was shown in a series of experiments to be involved in the transition to malignancy in prostate cancer through monocarboxylate transporter 2 (MCT2). MCT2 is upregulated in prostate cancer and, like other MCTs, serves to facilitate the transport of lactic acid in glycolytic tumors. Immunoprecipitation experiments demonstrated that colocalization of MCT2 with peroxisomes was strongest at disease initiation and decreased as metastasis increased; furthermore, colocalization was absent in nonmalignant prostate cancer lines [140].

Other components of peroxisomes are the peroxisome proliferator-activated receptors (PPARs). Three PPARs (PPARα, PPARγ, PPARβ/δ) are known and have been described as ligand-activated transcription factors [141]. These three PPARs differ predominantly in tissue distribution, and their exact functions in cancer remain ambiguous. It has been shown that PPARs are key regulators that integrate lipid metabolism and inflammation [142]. Furthermore, the PPARs have been directly implicated in cancers as well as in cancer-related processes, including carcinogenesis and chemoresistance [143, 144].

The theme of heterogeneity persists within the various PPARs and across species. For example, long-term PPARα agonism in rodents leads to the development of liver cancer. Interestingly, PPARα is expressed at lower levels in human liver relative to rodent liver, and as such, PPARα agonism does not lead to liver cancer in humans [143, 145]. PPARβ/δ displays tissue-wide distribution. One of its functions is to reduce oxidative stress, such as in breast cancer [146]. However, it is expressed ubiquitously and has been shown to be involved in many cancer types, particularly in cancers under hypoxic environments, such as breast, colon, lung, and ovarian cancers, as well as chronic lymphocytic leukemia [143]. Its precise role remains controversial, but it appears that PPARβ/δ may play a role as a lipid-activated mediator of an anti-inflammatory response. Like PPARβ/δ, mystery surrounds PPARγ. Although it may be coded for by four mRNAs (*PPARG1* through *PPARG4*), PPARγ1 and PPARγ2 are responsible for most PPARγ physiological actions [143]. PPARγ1 mRNA is found ubiquitously, whereas PPARγ2 mRNA is restricted to adipocytes [147]. Some, but not all, PPARγ agonists induce apoptosis in cancer cells and have

also been reported to induce terminal differentiation. Targets of PPARγ include many genes involved in the cell cycle and apoptosis in tumors, such as p53 and PTEN. The increasing characterization of PPARγ as a biomarker in cancer led some investigators to speculate that it may be utilized in screens [148]. Together, the PPARs constitute an area of research that may prove critical in our understanding of tumor development and treatment.

The many aspects of peroxisomal signaling further convey the diversity of lipid signaling across many different types of cancers. Abnormalities within the peroxisomes themselves or within PPARs can alter the efficacy of the critical lipid signaling that cancer cells rely on. Further research, particularly in the form of genomic analyses, will be useful in harnessing this heterogeneity for personalized medicine approaches.

7 Conclusion

Therapeutic strategies targeting lipid metabolism are now in various stages of clinical development, and one approach worth highlighting is the "repurposing" of drugs from cardiology [149]. As emphasized, we urge caution based on the significance of heterogeneity in cancer lipid metabolism as we translate basic science into clinical applications. Drug combinations have become a cornerstone against refractory and heterogeneous tumors, so the question now is *how* to combine treatment options for maximum safety and efficacy [150–152]. Going forward, systems biology and bioinformatics will likely become essential tools for integrating various levels of -omic data [153, 154]. Dissecting the spatial and temporal heterogeneity of lipid metabolism with these tools will likely accelerate the tailoring of clinical care according to patient-specific signatures, as envisioned by precision medicine (Fig. 1).

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References

1. Ma, X., et al. (2016). Identification and quantitation of lipid C=C location isomers: A shotgun lipidomics approach enabled by photochemical reaction. *Proceedings of the National Academy of Sciences, 113*(10), 2573–2578.
2. Shevchenko, A., & Simons, K. (2010). Lipidomics: Coming to grips with lipid diversity. *Nature Reviews Molecular Cell Biology, 11*, 593.
3. Yang, K., & Han, X. (2016). Lipidomics: Techniques, applications, and outcomes related to biomedical sciences. *Trends in Biochemical Sciences, 41*(11), 954–969.
4. DeBerardinis, R. J., & Chandel, N. S. (2016). Fundamentals of cancer metabolism. *Science Advances, 2*(5), e1600200.
5. Beloribi-Djefaflia, S., Vasseur, S., & Guillaumond, F. (2016). Lipid metabolic reprogramming in cancer cells. *Oncogene, 5*, e189.
6. Zalba, S., & ten Hagen, T. L. M. (2017). Cell membrane modulation as adjuvant in cancer therapy. *Cancer Treatment Reviews, 52*, 48–57.
7. Rysman, E., et al. (2010). De novo lipogenesis protects cancer cells from free radicals and chemotherapeutics by promoting membrane lipid saturation. *Cancer Research, 70*(20), 8117–8126.
8. Jeon, S.-M., Chandel, N. S., & Hay, N. (2012). AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. *Nature, 485*, 661.
9. Ayala, A., et al. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014*, 31.
10. Keckesova, Z., et al. (2017). LACTB is a tumour suppressor that modulates lipid metabolism and cell state. *Nature, 543*, 681.
11. Wang, D., & Dubois, R. N. (2010). Eicosanoids and cancer. *Nature Reviews Cancer, 10*(3), 181–193.
12. Pascual, G., et al. (2016). Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature, 541*, 41.
13. Viswanathan, V. S., et al. (2017). Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature, 547*, 453.
14. Luo, X., et al. (2017). Emerging roles of lipid metabolism in cancer metastasis. *Molecular Cancer, 16*, 76.
15. Hendrich, A. B., & Michalak, K. (2003). Lipids as a target for drugs modulating multidrug resistance of cancer cells. *Current Drug Targets, 4*(1), 23–30.
16. Tadros, S., et al. (2017). De novo lipid synthesis facilitates gemcitabine resistance through endoplasmic reticulum stress in pancreatic cancer. *Cancer Research, 77*(20), 5503–5517.
17. Ellsworth, R. E., et al. (2017). Molecular heterogeneity in breast cancer: State of the science and

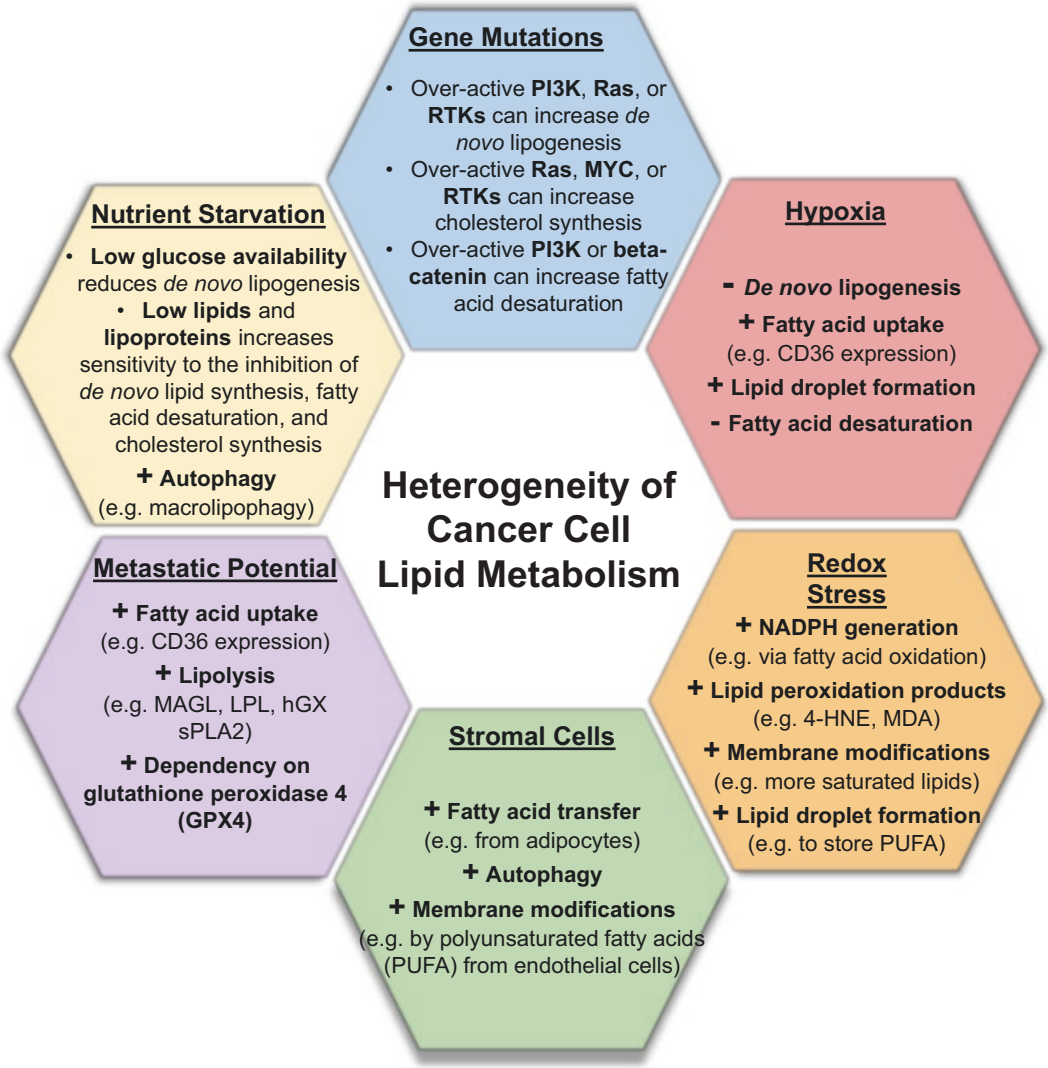

Fig. 1 Factors that can contribute to spatial and temporal heterogeneity in cancer cell lipid metabolism

implications for patient care. *Seminars in Cell & Developmental Biology, 64*, 65–72.
18. Greaves, M. (2015). Evolutionary determinants of cancer. *Cancer Discovery, 5*(8), 806–820.
19. Dang, C. V., et al. (2011). Therapeutic targeting of cancer cell metabolism. *Journal of Molecular Medicine (Berlin), 89*(3), 205–212.
20. Hirschey, M. D., et al. (2015). Dysregulated metabolism contributes to oncogenesis. *Seminars in Cancer Biology, 35*(Suppl), S129–S150.
21. Strickaert, A., et al. (2016). Cancer heterogeneity is not compatible with one unique cancer cell metabolic map. *Oncogene, 36*, 2637.
22. Nabi, K., & Le, A. (2021). The intratumoral heterogeneity of cancer metabolism. *Advances in Experimental Medicine and Biology, 1311*, https://doi.org/10.1007/978-3-030-65768-0_11
23. Antonio, M. J., Zhang, C., & Le, A. (2021). Different tumor microenvironments lead to different metabolic phenotypes. *Advances in Experimental Medicine and Biology, 1311*, https://doi.org/10.1007/978-3-030-65768-0_10
24. Catalina-Rodriguez, O., et al. (2012). The mitochondrial citrate transporter, CIC, is essential for mitochondrial homeostasis. *Oncotarget, 3*(10), 1220–1235.
25. Szutowicz, A., Kwiatkowski, J., & Angielski, S. (1979). Lipogenetic and glycolytic enzyme activities in carcinoma and nonmalignant diseases of the human breast. *British Journal of Cancer, 39*(6), 681–687.

26. Migita, T., et al. (2008). ATP citrate lyase: Activation and therapeutic implications in non-small cell lung cancer. *Cancer Research, 68*(20), 8547.
27. Yahagi, N., et al. (2005). Co-ordinate activation of lipogenic enzymes in hepatocellular carcinoma. *European Journal of Cancer, 41*(9), 1316–1322.
28. Turyn, J., et al. (2003). Increased activity of glycerol 3-phosphate dehydrogenase and other lipogenic enzymes in human bladder cancer. *Hormone and Metabolic Research, 35*(10), 565–569.
29. McGarry, J. D., Leatherman, G. F., & Foster, D. W. (1978). Carnitine palmitoyltransferase. I. The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. *Journal of Biological Chemistry, 253*(12), 4128–4136.
30. Wang, C., et al. (2015). The acetyl-CoA carboxylase enzyme: A target for cancer therapy? *Expert Review of Anticancer Therapy, 15*(6), 667–676.
31. Savage, D. B., et al. (2006). Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *Journal of Clinical Investigation, 116*(3), 817–824.
32. Milgraum, L. Z., et al. (1997). Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma. *Clinical Cancer Research, 3*(11), 2115–2120.
33. Swinnen, J. V., et al. (2000). Selective activation of the fatty acid synthesis pathway in human prostate cancer. *International Journal of Cancer, 88*(2), 176–179.
34. Nelson, M. E., et al. (2017). Inhibition of hepatic lipogenesis enhances liver tumorigenesis by increasing antioxidant defense and promoting cell survival. *Nature Communications, 8*, 14689.
35. The Cancer Genome Atlas Research Network. (2013). Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature, 499*(7456), 43–49.
36. Calvisi, D. F., et al. (2011). Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling promotes development of human hepatocellular carcinoma. *Gastroenterology, 140*(3), 1071–1083.e5.
37. Hilvo, M., et al. (2011). Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast Cancer progression. *Cancer Research, 71*(9), 3236–3245.
38. Beckers, A., et al. (2007). Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells. *Cancer Research, 67*(17), 8180–8187.
39. Jones, J. E. C., et al. (2017). Inhibition of acetyl-CoA carboxylase 1 (ACC1) and 2 (ACC2) reduces proliferation and De novo lipogenesis of EGFRvIII human glioblastoma cells. *PLoS One, 12*(1), e0169566.
40. Petrova, E., et al. (2017). Acetyl-CoA carboxylase inhibitors attenuate WNT and hedgehog signaling and suppress pancreatic tumor growth. *Oncotarget, 8*(30), 48660–48670.
41. Rios Garcia, M., et al. (2017). Acetyl-CoA carboxylase 1-dependent protein acetylation controls breast cancer metastasis and recurrence. *Cell Metabolism, 26*(6), 842–855.e5.
42. Zakikhani, M., et al. (2006). Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Research, 66*(21), 10269–10273.
43. Knowles, L. M., et al. (2008). Inhibition of fatty-acid synthase induces caspase-8-mediated tumor cell apoptosis by up-regulating DDIT4. *Journal of Biological Chemistry, 283*(46), 31378–31384.
44. Moreau, K., et al. (2006). BRCA1 affects lipid synthesis through its interaction with acetyl- CoA carboxylase. *Journal of Biological Chemistry, 281*(6), 3172–3181.
45. Chajès, V., et al. (2006). Acetyl-CoA carboxylase α is essential to breast cancer cell survival. *Cancer Research, 66*(10), 5287–5294.
46. Swinnen, J. V., Brusselmans, K., & Verhoeven, G. (2006). Increased lipogenesis in cancer cells: New players, novel targets. *Current Opinion in Clinical Nutrition and Metabolic Care, 9*(4), 358–365.
47. Alo, P. L., et al. (1996). Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. *Cancer, 77*(3), 474–482.
48. Swinnen, J. V., et al. (2002). Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. *International Journal of Cancer, 98*(1), 19–22.
49. Kridel, S. J., et al. (2004). Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. *Cancer Research, 64*(6), 2070–2075.
50. Zaytseva, Y. Y., et al. (2012). Inhibition of fatty acid synthase attenuates CD44-associated signaling and reduces metastasis in colorectal cancer. *Cancer Research, 72*(6), 1504–1517.
51. Heuer, T. S., et al. (2017). FASN inhibition and Taxane treatment combine to enhance anti-tumor efficacy in diverse Xenograft tumor models through disruption of tubulin palmitoylation and microtubule organization and FASN inhibition-mediated effects on oncogenic signaling and gene expression. *eBioMedicine, 16*, 51–62.
52. Jiang, L., et al. (2015). Metabolic reprogramming during TGFβ1-induced epithelial-to-mesenchymal transition. *Oncogene, 34*(30), 3908–3916.
53. Dean, E. J., et al. (2016). Preliminary activity in the first in human study of the first-in-class fatty acid synthase (FASN) inhibitor, TVB-2640. *Journal of Clinical Oncology, 34*(15_ suppl), 2512–2512.
54. Falkenburger, B. H., et al. (2010). Phosphoinositides: Lipid regulators of membrane proteins. *The Journal of Physiology, 588*(Pt 17), 3179–3185.
55. Samuels, Y., et al. (2004). High frequency of mutations of the PIK3CA gene in human cancers. *Science, 304*(5670), 554.

56. Samuels, Y., & Velculescu, V. E. (2004). Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle, 3*(10), 1221–1224.
57. Tennant, D. A., Duran, R. V., & Gottlieb, E. (2010). Targeting metabolic transformation for cancer therapy. *Nature Reviews Cancer, 10*(4), 267–277.
58. Ricoult, S. J. H., et al. (2016). Oncogenic PI3K and K-Ras stimulate de novo lipid synthesis through mTORC1 and SREBP. *Oncogene, 35*(10), 1250–1260.
59. Gouw, A. M., et al. (2017). Oncogene KRAS activates fatty acid synthase, resulting in specific ERK and lipid signatures associated with lung adenocarcinoma. *Proceedings of the National Academy of Sciences of the United States of America, 114*(17), 4300–4305.
60. Polivka, J., & Janku, F. (2014). Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. *Pharmacology & Therapeutics, 142*(2), 164–175.
61. Downward, J. (2003). Targeting RAS signaling pathways in cancer therapy. *Nature Reviews Cancer, 3*, 11.
62. Yang, Y.-A., et al. (2002). Activation of fatty acid synthesis during neoplastic transformation: Role of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Experimental Cell Research, 279*(1), 80–90.
63. Che, L., et al. (2017). Oncogene dependent requirement of fatty acid synthase in hepatocellular carcinoma. *Cell Cycle, 16*(6), 499–507.
64. Ventura, R., et al. (2015). Inhibition of de novo palmitate synthesis by fatty acid synthase induces apoptosis in tumor cells by remodeling cell membranes, inhibiting signaling pathways, and reprogramming gene expression. *eBioMedicine, 2*(8), 808–824.
65. Hatzivassiliou, G., et al. (2005). ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell, 8*(4), 311–321.
66. Hanai, J.-I., et al. (2012). Inhibition of lung cancer growth: ATP citrate lyase knockdown and statin treatment leads to dual blockade of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/AKT pathways. *Journal of Cellular Physiology, 227*(4), 1709–1720.
67. Svensson, R. U., et al. (2016). Inhibition of acetyl-CoA carboxylase suppresses fatty acid synthesis and tumor growth of non-small cell lung cancer in preclinical models. *Nature Medicine, 22*(10), 1108–1119.
68. Uddin, S., et al. (2010). Inhibition of fatty acid synthase suppresses c-Met receptor kinase and induces apoptosis in diffuse large B-cell lymphoma. *Molecular Cancer Therapeutics, 9*(5), 1244–1255.
69. Wieduwilt, M. J., & Moasser, M. M. (2008). The epidermal growth factor receptor family: Biology driving targeted therapeutics. *Cellular and Molecular Life Sciences: CMLS, 65*(10), 1566–1584.
70. Sierra, J. R., & Tsao, M.-S. (2011). c-MET as a potential therapeutic target and biomarker in cancer. *Therapeutic Advances in Medical Oncology, 3*(1 Suppl), S21–S35.
71. Hanai, J. I., et al. (2013). ATP citrate lyase knockdown impacts cancer stem cells in vitro. *Cell Death & Disease, 4*(6), e696.
72. Chen, Y., et al. (2016). mTOR complex-2 stimulates acetyl-CoA and de novo lipogenesis through ATP citrate lyase in HER2/PIK3CA-hyperactive breast cancer. *Oncotarget, 7*(18), 25224–25240.
73. Corominas-Faja, B., et al. (2014). Chemical inhibition of acetyl-CoA carboxylase suppresses self-renewal growth of cancer stem cells. *Oncotarget, 5*(18), 8306–8316.
74. Menendez, J. A., et al. (2004). Inhibition of fatty acid synthase (FAS) suppresses HER2/neu (erbB-2) oncogene overexpression in cancer cells. *Proceedings of the National Academy of Sciences of the United States of America, 101*(29), 10715–10720.
75. Giró-Perafita, A., et al. (2016). Preclinical evaluation of fatty acid synthase and EGFR inhibition in triple-negative breast cancer. *Clinical Cancer Research, 22*(18), 4687–4697.
76. Menendez, J. A., & Lupu, R. (2017). Fatty acid synthase regulates estrogen receptor-α signaling in breast cancer cells. *Oncogene, 6*, e299.
77. Vellaichamy, A., et al. (2010). "Topological significance" analysis of gene expression and proteomic profiles from prostate cancer cells reveals key mechanisms of androgen response. *PLoS One, 5*(6), e10936.
78. Li, J.-N., et al. (2001). Pharmacological inhibition of fatty acid synthase activity produces both cytostatic and cytotoxic effects modulated by p53. *Cancer Research, 61*(4), 1493–1499.
79. Liu, D., et al. (2016). Wnt/β-catenin signaling participates in the regulation of lipogenesis in the liver of juvenile turbot (Scophthalmus maximus L.). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 191*, 155–162.
80. Seo, M. H., et al. (2016). Exendin-4 inhibits hepatic lipogenesis by increasing β-catenin signaling. *PLoS One, 11*(12), e0166913.
81. Gelebart, P., et al. (2012). Blockade of fatty acid synthase triggers significant apoptosis in mantle cell lymphoma. *PLoS One, 7*(4), e33738.
82. Yoon, S., et al. (2007). Up-regulation of acetyl-CoA carboxylase α and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. *Journal of Biological Chemistry, 282*(36), 26122–26131.
83. Daemen, A., et al. (2015). Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors. *Proceedings of the National Academy of Sciences of the United States of America, 112*(32), E4410–E4417.
84. Xie, H., & Simon, M. C. (2017). Oxygen availability and metabolic reprogramming in cancer. *The Journal of Biological Chemistry, 292*(41), 16825–16832.

85. Bensaad, K., et al. (2014). Fatty acid uptake and lipid storage induced by HIF-1α contribute to cell growth and survival after hypoxia-reoxygenation. *Cell Reports, 9*(1), 349–365.
86. Kamphorst, J. J., et al. (2013). Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. *Proceedings of the National Academy of Sciences of the United States of America, 110*(22), 8882–8887.
87. Young, R. M., et al. (2013). Dysregulated mTORC1 renders cells critically dependent on desaturated lipids for survival under tumor-like stress. *Genes & Development, 27*(10), 1115–1131.
88. Sounni, N. E., et al. (2014). Blocking lipid synthesis overcomes tumor regrowth and metastasis after antiangiogenic therapy withdrawal. *Cell Metabolism, 20*(2), 280–294.
89. Daniëls, V. W., et al. (2014). Cancer cells differentially activate and thrive on de novo lipid synthesis pathways in a low-lipid environment. *PLoS One, 9*(9), e106913.
90. Zaidi, N., et al. (2012). ATP citrate lyase knockdown induces growth arrest and apoptosis through different cell- and environment-dependent mechanisms. *Molecular Cancer Therapeutics, 11*(9), 1925–1935.
91. Lakhter, A. J., et al. (2016). Glucose-independent acetate metabolism promotes melanoma cell survival and tumor growth. *The Journal of Biological Chemistry, 291*(42), 21869–21879.
92. Tamura, K., et al. (2009). Novel lipogenic enzyme ELOVL7 is involved in prostate cancer growth through saturated long-chain fatty acid metabolism. *Cancer Research, 69*(20), 8133–8140.
93. Jump, D. B., Torres-Gonzalez, M., & Olson, L. K. (2011). Soraphen A, an inhibitor of acetyl CoA carboxylase activity, interferes with fatty acid elongation. *Biochemical Pharmacology, 81*(5), 649–660.
94. Yang, W. S., et al. (2012). Proteomic approach reveals FKBP4 and S100A9 as potential prediction markers of therapeutic response to neoadjuvant chemotherapy in patients with breast cancer. *Journal of Proteome Research, 11*(2), 1078–1088.
95. Clendening, J. W., et al. (2010). Dysregulation of the mevalonate pathway promotes transformation. *Proceedings of the National Academy of Sciences, 107*(34), 15051–15056.
96. Platz, E. A., et al. (2006). Statin drugs and risk of advanced prostate Cancer. *Journal of the National Cancer Institute, 98*(24), 1819–1825.
97. Poynter, J. N., et al. (2005). Statins and the risk of colorectal cancer. *New England Journal of Medicine, 352*(21), 2184–2192.
98. Nielsen, S. F., Nordestgaard, B. G., & Bojesen Statin, S. E. (2012). Use and reduced cancer-related mortality. *New England Journal of Medicine, 367*(19), 1792–1802.
99. Clendening, J. W., & Penn, L. Z. (2012). Targeting tumor cell metabolism with statins. *Oncogene, 31*, 4967.
100. Campbell, M. J., et al. (2006). Breast cancer growth prevention by statins. *Cancer Research, 66*(17), 8707–8714.
101. Zhong, C., et al. (2014). HMGCR is necessary for the tumorigenicity of esophageal squamous cell carcinoma and is regulated by Myc. *Tumor Biology, 35*(5), 4123–4129.
102. Wang, X., et al. (2017). MYC-regulated mevalonate metabolism maintains brain tumor-initiating cells. *Cancer Research, 77*(18), 4947–4960.
103. Juneja, M., et al. (2017). Statin and rottlerin small-molecule inhibitors restrict colon cancer progression and metastasis via MACC1. *PLoS Biology, 15*(6), e2000784.
104. Fujiwara, D., et al. (2017). Statins induce apoptosis through inhibition of Ras signaling pathways and enhancement of Bim and p27 expression in human hematopoietic tumor cells. *Tumor Biology, 39*(10), 1010428317734947.
105. Karagkounis, G., et al. (2017). Simvastatin enhances radiation sensitivity of colorectal cancer cells. *Surgical Endoscopy, 32*(3), 1533–1539.
106. Lipkin, S. M., et al. (2010). Genetic variation in 3-hydroxy-3-methylglutaryl CoA reductase modifies the chemopreventive activity of statins for colorectal cancer. *Cancer Prevention Research, 3*(5), 597–603.
107. Menter, D. G., et al. (2011). Differential effects of pravastatin and simvastatin on the growth of tumor cells from different organ sites. *PLoS One, 6*(12), e28813.
108. Lee, Y., et al. (2017). Randomized phase II study of afatinib plus simvastatin versus afatinib alone in previously treated patients with advanced nonadenocarcinomatous non-small cell lung cancer. *Cancer Research and Treatment: Official Journal of Korean Cancer Association, 49*(4), 1001–1011.
109. Baas, J. M., et al. (2015). Safety and efficacy of the addition of simvastatin to panitumumab in previously treated KRAS mutant metastatic colorectal cancer patients. *Anti-Cancer Drugs, 26*(8), 872–877.
110. Baas, J. M., et al. (2015). Safety and efficacy of the addition of simvastatin to cetuximab in previously treated KRAS mutant metastatic colorectal cancer patients. *Investigational New Drugs, 33*(6), 1242–1247.
111. Zaidi, N., et al. (2013). Lipogenesis and lipolysis: The pathways exploited by the cancer cells to acquire fatty acids. *Progress in Lipid Research, 52*(4), 585–589.
112. Pavlova, N. N., & Thompson, C. B. (2016). The emerging hallmarks of cancer metabolism. *Cell Metabolism, 23*(1), 27–47.
113. Kuemmerle, N. B., et al. (2011). Lipoprotein lipase links dietary fat to solid tumor cell proliferation. *Molecular Cancer Therapeutics, 10*(3), 427–436.

114. van't Veer, M. B., et al. (2006). The predictive value of lipoprotein lipase for survival in chronic lymphocytic leukemia. *Haematologica, 91*(1), 56–63.
115. Hale, J. S., et al. (2014). Cancer stem cell-specific scavenger receptor CD36 drives glioblastoma progression. *Stem Cells, 32*(7), 1746–1758.
116. Nath, A., et al. (2015). Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. *Scientific Reports, 5*, 14752.
117. Guillaumond, F., et al. (2015). Cholesterol uptake disruption, in association with chemotherapy, is a promising combined metabolic therapy for pancreatic adenocarcinoma. *Proceedings of the National Academy of Sciences of the United States of America, 112*(8), 2473–2478.
118. Chaffer, C. L., & Weinberg, R. A. (2011). A perspective on cancer cell metastasis. *Science, 331*(6024), 1559–1564.
119. Hua, Y., et al. (2011). Dynamic metabolic transformation in tumor invasion and metastasis in mice with LM-8 osteosarcoma cell transplantation. *Journal of Proteome Research, 10*(8), 3513–3521.
120. Jung, Y. Y., Kim, H. M., & Koo, J. S. (2015). Expression of lipid metabolism-related proteins in metastatic breast cancer. *PLoS One, 10*(9), e0137204.
121. Nath, A., & Chan, C. (2016). Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. *Scientific Reports, 6*, 18669.
122. Uray, I. P., Liang, Y., & Hyder, S. M. (2004). Estradiol down-regulates CD36 expression in human breast cancer cells. *Cancer Letters, 207*(1), 101–107.
123. Balaban, S., et al. (2015). Obesity and cancer progression: Is there a role of fatty acid metabolism? *BioMed Research International, 2015*, 274585.
124. Schoors, S., et al. (2015). Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature, 520*(7546), 192–197.
125. McDonnell, E., et al. (2016). Lipids reprogram metabolism to become a major carbon source for histone acetylation. *Cell Reports, 17*(6), 1463–1472.
126. Padanad, M. S., et al. (2016). Fatty acid oxidation mediated by acyl-CoA Synthetase long-chain 3 is required for mutant KRAS lung tumorigenesis. *Cell Reports, 16*(6), 1614–1628.
127. Liu, Y. (2006). Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. *Prostate Cancer and Prostatic Diseases, 9*(3), 230–234.
128. Camarda, R., et al. (2016). Inhibition of fatty acid oxidation as a therapy for MYC-overexpressing triple-negative breast cancer. *Nature Medicine, 22*(4), 427–432.
129. Comerford, S. A., et al. (2014). Acetate dependence of tumors. *Cell, 159*(7), 1591–1602.
130. Mashimo, T., et al. (2014). Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. *Cell, 159*(7), 1603–1614.
131. Qu, Q., et al. (2016). Fatty acid oxidation and carnitine palmitoyltransferase I: Emerging therapeutic targets in cancer. *Cell Death & Disease, 7*(5), e2226.
132. Carrasco, P., et al. (2013). Carnitine palmitoyltransferase 1C deficiency causes motor impairment and hypoactivity. *Behavioural Brain Research, 256*, 291–297.
133. Zaugg, K., et al. (2011). Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. *Genes & Development, 25*(10), 1041–1051.
134. Wakil, S. J., & Abu-Elheiga, L. A. (2009). Fatty acid metabolism: Target for metabolic syndrome. *Journal of Lipid Research, 50*(Suppl), S138–S143.
135. Du, W., et al. (2017). HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism. *Nature Communications, 8*, 1769.
136. Huang, D., et al. (2014). HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. *Cell Reports, 8*(6), 1930–1942.
137. Fragasso, G., et al. (2009). Effects of metabolic approach in diabetic patients with coronary artery disease. *Current Pharmaceutical Design, 15*(8), 857–862.
138. Holubarsch, C. J., et al. (2007). A double-blind, randomized multicentre clinical trial to evaluate the efficacy and safety of two doses of etomoxir in comparison with placebo in patients with moderate congestive heart failure: The ERGO (etomoxir for the recovery of glucose oxidation) study. *Clinical Science, 113*(4), 205–212.
139. Lodhi, I. J., & Semenkovich, C. F. (2014). Peroxisomes: A nexus for lipid metabolism and cellular signaling. *Cell Metabolism, 19*(3), 380–392.
140. Valença, I., et al. (2015). Localization of MCT2 at peroxisomes is associated with malignant transformation in prostate cancer. *Journal of Cellular and Molecular Medicine, 19*(4), 723–733.
141. Wang, Y.-X. (2010). PPARs: Diverse regulators in energy metabolism and metabolic diseases. *Cell Research, 20*(2), 124–137.
142. Bensinger, S. J., & Tontonoz, P. (2008). Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature, 454*, 470.
143. Peters, J. M., Shah, Y. M., & Gonzalez, F. J. (2012). The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nature Reviews Cancer, 12*(3), 181–195.
144. Yousefi, B., et al. (2016). Peroxisome proliferator-activated receptors and their ligands in cancer drug resistance: Opportunity or challenge. *Anti-Cancer Agents in Medicinal Chemistry, 16*(12), 1541–1548.
145. Holden, P. R., & Tugwood, J. D. (1999). Peroxisome proliferator-activated receptor alpha: Role in rodent liver cancer and species differences. *Journal of Molecular Endocrinology, 22*(1), 1–8.
146. Wang, X., et al. (2016). PPAR-delta promotes survival of breast cancer cells in harsh metabolic conditions. *Oncogene, 5*(6), e232.

147. Vidal-Puig, A. J., et al. (1997). Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *Journal of Clinical Investigation, 99*(10), 2416–2422.
148. Robbins, G. T., & Nie, D. (2012). PPAR gamma, bioactive lipids, and cancer progression. *Frontiers in Bioscience: A Journal and Virtual Library, 17*, 1816–1834.
149. Corbet, C., & Feron, O. (2017). Cancer cell metabolism and mitochondria: Nutrient plasticity for TCA cycle fueling. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1868*(1), 7–15.
150. Elgogary, A., et al. (2016). Combination therapy with BPTES nanoparticles and metformin targets the metabolic heterogeneity of pancreatic cancer. *Proceedings of the National Academy of Sciences of the United States of America, 113*(36), E5328–E5336.
151. Bayat Mokhtari, R., et al. (2017). Combination therapy in combating cancer. *Oncotarget, 8*(23), 38022–38043.
152. Zhao, B., Hemann, M. T., & Lauffenburger, D. A. (2014). Intratumor heterogeneity alters most effective drugs in designed combinations. *Proceedings of the National Academy of Sciences of the United States of America, 111*(29), 10773–10778.
153. Benfeitas, R., et al. (2017). New challenges to study heterogeneity in cancer redox metabolism. *Frontiers in Cell and Development Biology, 5*, 65.
154. Agren, R., et al. (2012). Reconstruction of genome-scale active metabolic networks for 69 human cell types and 16 cancer types using INIT. *PLoS Computational Biology, 8*(5), e1002518.