

Fatty Acid Synthesis in Prostate Cancer: Vulnerability or Epiphenomenon?

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ABSTRACT

Tumor metabolism supports the energetic and biosynthetic needs of rapidly proliferating cancer cells and modifies intra- and intercellular signaling to enhance cancer cell invasion, metastasis, and immune evasion. Prostate cancer exhibits unique metabolism with high rates of *de novo* fatty acid synthesis driven by activation of the androgen receptor (AR). Increasing evidence suggests that activation of this pathway is functionally important

to promote prostate cancer aggressiveness. However, the mechanisms by which fatty acid synthesis are beneficial to prostate cancer have not been well defined. In this review, we summarize evidence indicating that fatty acid synthesis drives progression of prostate cancer. We also explore explanations for this phenomenon and discuss future directions for targeting this pathway for patient benefit.

Introduction

Cancer cell metabolism has been recognized to be important for cancer progression for decades. Some of the oldest and most effective cancer therapies are antimetabolites such as 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), cytarabine, gemcitabine, and methotrexate (1). Yet we are learning that the traditional idea that cancer cell metabolic programs are optimized for maximal biosynthesis for proliferation is overly simplistic. Such conclusions are based primarily on studies of cancer cell lines that have high proliferative rates *in vitro* that do not accurately model the growth rate and complexity of human tumors, and particularly prostate cancer. Recent studies indicate that metabolism also regulates intra- and intercellular signaling enhancing overall tumor fitness not only for growth, but also for invasion, metastasis, and evasion of the immune system (2–4). As such, cell metabolism is not simply a supporting process of tumorigenesis, but rather, is critical to the progression of cancer that leads to patient death.

A metabolic feature of many cancers is the ability to generate *de novo* fatty acids (5, 6). Historically *de novo* fatty acid synthesis was thought to be restricted to adipose tissue, liver, and the lactating mammary gland (7). In these organs, this pathway functions to store energy in the form of lipids under circumstances of nutrient surplus. These lipids, along with dietary lipids, are circulated, taken up, and used by many other cell types in mammals. The abundance of circulating lipids was thought to make *de novo* fatty acid synthesis by other cell types unnecessary. However, beyond the pathologic setting of tumorigenesis, *de novo* fatty acid synthesis is also induced and important for function of many normal cell types, such as pluripotent stem cells (8), and many types of immune cells, including T and B lymphocytes (9, 10), macrophages (11), and dendritic cells (12). Therefore, the ability to synthesize

fatty acids seems to be more generally beneficial for cellular function, in spite of the perceived rarity of lipid deprivation *in vivo*.

Prostate adenocarcinoma is different from other cancer types because it is exquisitely dependent on signaling through the androgen receptor (AR). Androgen deprivation therapy (ADT) is arguably the most effective systemic therapy targeting one pathway for any cancer type with a response rate of about 90% when synthesis of androgens is blocked from both the testes and adrenal glands (13, 14). When prostate cancer recurs after ADT (i.e., castration-resistant prostate cancer, CRPC), it generally remains dependent on signaling through AR and does so despite low serum androgens through AR overexpression, amplification, mutation, production of ligand-independent variants, and reprogramming of the AR cistrome (15–20).

While AR can regulate hundreds of genes, a key function of AR may be regulation of cell metabolism. Indeed, testosterone is known to alter metabolism across many tissue types including skeletal muscle, cardiac muscle, and adipose tissue (21, 22). In normal prostate epithelial cells, AR drives unique metabolic flux to promote secretion of citrate and polyamines into prostatic secretions, which support sperm survival and function in the female reproductive tract (23, 24). When the prostate epithelium becomes malignant, and even after development of castration resistance, AR continues to dictate metabolic flux, now supporting forward flux through the tricarboxylic acid (TCA) cycle and driving citrate toward *de novo* fatty acid synthesis in lieu of secretion (25, 26). In this review, we assess the evidence that high rates of *de novo* fatty acid synthesis, driven by AR activation, occurs in and is important to progression of prostate cancer, followed by a discussion of therapeutic implications.

Prostate Cancer May Engage in High Rates of Fatty Acid Synthesis

Fatty acids generated within the cell are derived from the TCA cycle intermediate citrate or from acetate (Fig. 1). Citrate is transported out of the mitochondria via the tricarboxylate transport protein or citrate transporter protein (CTP) into the cytosol where it can be cleaved by ATP citrate lyase (ACLY) to generate acetyl-CoA. Acetate can be converted to acetyl-CoA by ligation with CoA by acetyl-CoA synthetase (ACSS; ref. 27). Cytosolic acetyl-CoA is subsequently carboxylated by the key regulatory enzyme acetyl-CoA carboxylase (ACC) to generate malonyl-CoA, which can be combined with acetyl-CoA to generate the 16-carbon saturated fatty acid palmitate by the

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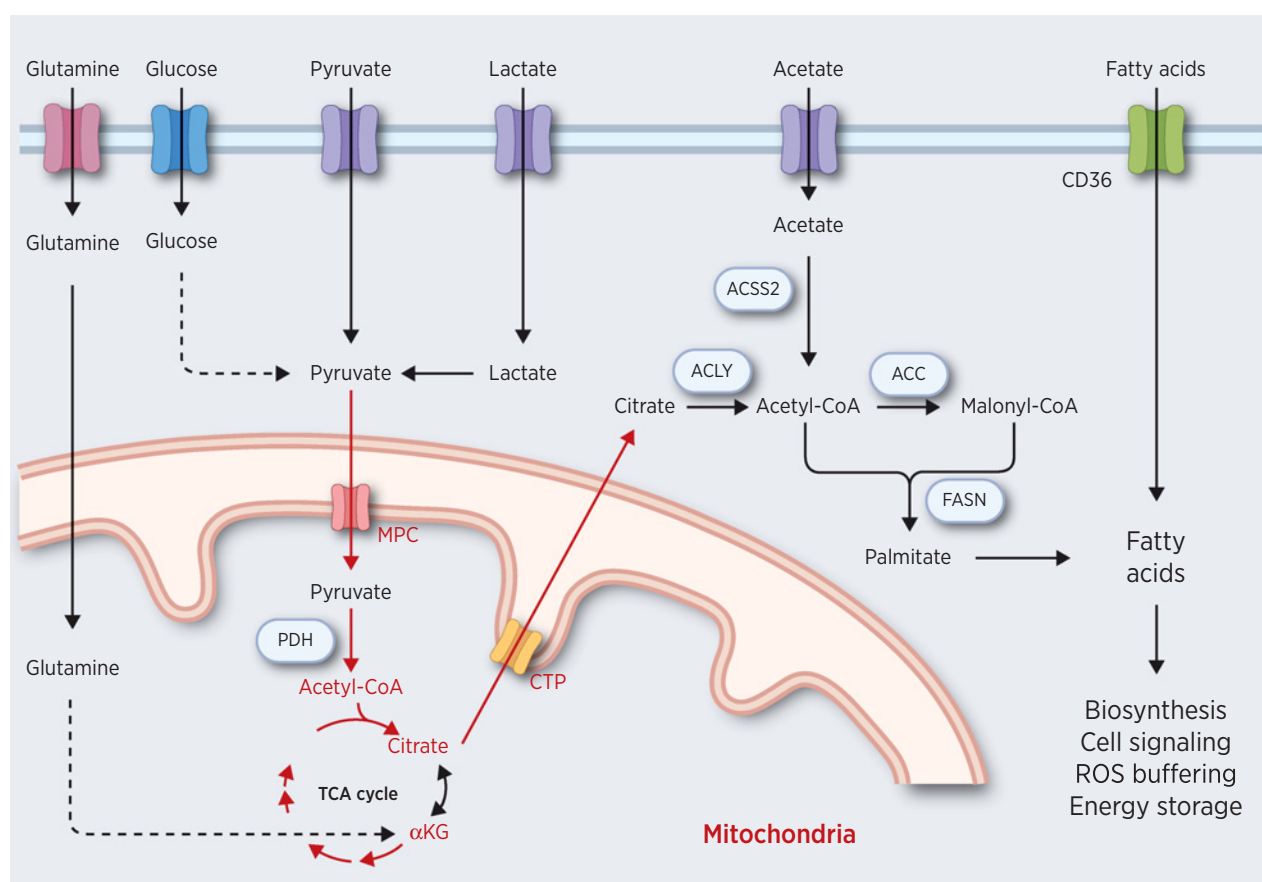


Figure 1.

Simplified schematic of generation of intracellular fatty acids. Fatty acids can be synthesized *de novo* or taken up from the tumor microenvironment. *De novo* fatty acid synthesis originates from cytosolic acetyl-CoA, which can be carboxylated by acetyl-CoA carboxylase (ACC) to generate malonyl-CoA. Malonyl-CoA can be combined with acetyl-CoA by FASN to generate palmitate. Palmitate can subsequently be elongated by elongases and/or desaturated by desaturases to generate a wide variety of fatty acids. These fatty acids can be used for biosynthesis of membranes, modification of intra- and intercellular signaling, ROS buffering, and energy storage. Created with BioRender.com

multi-enzyme protein, fatty acid synthase (FASN). Palmitate can subsequently be elongated by elongases and/or desaturated by desaturases to generate a wide variety of fatty acids. These fatty acids can subsequently be used to generate membrane molecules such as glycolipids and phospholipids, signaling molecules such as diacylglycerol (DAG) and phosphatidylinositol-3,4,5-triphosphate (PIP3), and energy storage molecules such as triacylglycerides (TAG).

A comparison of gene expression of 32 cancer types included in The Cancer Genome Atlas (TCGA) PanCan 2018 normalized analysis (28, 29) indicates that compared with other types of cancer, primary prostate cancer exhibits high expression of key proteins in the fatty acid synthesis pathway: CTP, ACLY, ACC, and FASN (Fig. 2A and B). While high expression of these proteins could be a remnant from a high rate of fatty acid synthesis in the tissue of origin (for example as may be the case in hepatocellular carcinoma), a comparison of gene expression in normal prostate compared with prostate cancer (30) suggests ACLY, ACC, and FASN are markedly higher in prostate cancer (Fig. 2C). FASN expression is undetectable by immunohistochemistry in benign prostate and nearly uniformly positive across prostatic intraepithelial neoplasia (PIN) and invasive carcinomas, and with particularly high expression in metastatic tumors (31–33). Moreover, metabolomic studies indicate that some fatty acids, including palmitate (16:0), laurate (12:0), myristate

(14:0), linoleate (18:2n6), and eicosanoate (20:1n9 or 11) are higher in primary prostate cancer compared with benign prostate, and may be higher still in metastatic prostate cancer (34–36). Although linoleate is considered an essential polyunsaturated fatty acid (PUFA) that must be taken up by the cell, the remainder of these fatty acids can be synthesized *de novo*. Conversely, quantity of the fatty acid precursor citrate is reduced in high versus low Gleason score primary prostate cancer, which may indicate high utilization (37, 38). Finally, noninvasive molecular imaging techniques using positron emission tomography (PET) also support the concept that prostate cancer engages in *de novo* fatty acid synthesis. While glucose uptake is generally low in prostate cancer (as assessed by ^{18}F -fluorodeoxyglucose PET), acetate uptake is higher (as assessed by ^{11}C -acetate PET) and is predictive of biochemical relapse after prostatectomy (39, 40). Acetate uptake seems to be used for *de novo* fatty acid synthesis because it is diminished by inhibitors of FASN (41, 42).

While these data suggest that *de novo* fatty acid synthesis occurs in human prostate cancer, and with increasing rate with disease progression (Fig. 2D), this could be assessed more definitively using isotope tracing followed by assessment of labeling patterns by mass spectrometry in biopsy samples of patients, as has been performed in patients with renal cell carcinoma (43). Moreover, given that cellular

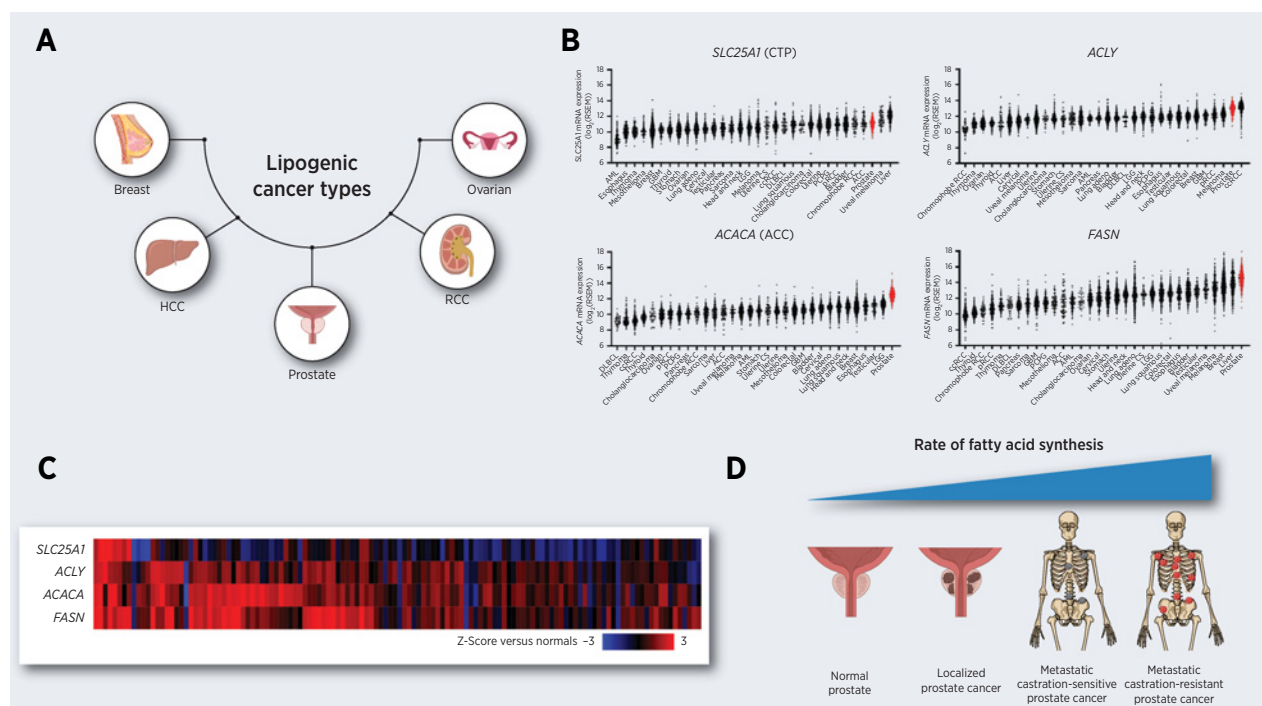


Figure 2.

Prostate cancer may engage in high rates of fatty acid synthesis. **A**, Several types of solid tumors may engage in *de novo* fatty acid synthesis including prostate cancer, HCC, hepatocellular carcinoma; RCC, renal cell carcinoma. **B**, Expression of genes in the fatty acid synthesis pathway across cancer types in the TCGA PanCan 2018 normalized analysis ($N = 10,071$), ordered by median expression, with prostate cancer in red (28, 29). AML, acute myeloid leukemia; GBM, glioblastoma; Adeno, adenocarcinoma; LGG, low grade glioma; CS, carcinosarcoma; ccRCC, clear cell renal cell carcinoma; DLBCL, diffuse large B cell lymphoma; PCPG, pheochromocytoma/paraganglioma; RCC, renal cell carcinoma; ACC, adenoid cystic carcinoma. **C**, Expression of genes in the fatty acid synthesis pathway in prostate cancer compared with normal prostate ($N = 150$) per publicly available data from Taylor and colleagues (30). **D**, Rate of fatty acid synthesis appears to increase with prostate cancer progression from normal prostate to metastatic castration-resistant prostate cancer.

metabolic flux is shaped by both cell-intrinsic factors and the microenvironment (44), future studies should consider how prostate cancer cell rates of fatty acid synthesis are altered depending on composition of surrounding cell types (i.e., anatomic location of the metastasis) and metabolite and oxygen availability. These studies are critically important not only to better define the pathophysiology of prostate cancer, but also to identify biomarkers of high rates of fatty acid synthesis that may predict clinical response to inhibitors of this pathway.

Fatty Acid Synthesis May Drive Prostate Cancer Development and Progression

Evidence that prostate cancer may engage in high rates of fatty acid synthesis begs the question of whether activation of this pathway is important or incidental (i.e., an epiphenomenon) to prostate cancer development and progression. The former is suggested by the positive correlation between levels of fatty acid synthesis enzymes and products and prostate cancer disease stage. In fact, FASN expression level predicts seminal vesicle invasion or lymph node metastases, independent of Gleason score, in primary prostate cancer (45). Among patients with PTEN loss, high FASN expression was associated with shorter overall survival (46).

To begin to understand the functional significance of fatty acid synthesis in prostate cancer, multiple groups have investigated pharmacologic and genetic modulation of enzymes in this pathway in

models of prostate cancer. Studies dating back over 20 years showed that pharmacologic inhibition of FASN using orlistat, cerulenin, or C75, as well as knock-down of FASN expression using RNAi, slowed proliferation of prostate cancer cell lines (41, 47, 48). More recently, the FASN inhibitor IPI-9119 similarly reduced proliferation of CRPC cell lines and organoids, an effect that could be rescued by addition of exogenous palmitate, and growth of mouse xenograft tumors (49). Mice with prostate-specific deletion of both *Pten* and *FASN* were found to have less extensive areas of PIN and reactive stroma compared with mice with prostate-specific deletion of *Pten* alone (46). Conversely, overexpression of FASN increased proliferation of AR-positive CRPC cell lines and was sufficient to induce invasive carcinoma in AR-positive immortalized human prostate epithelial cells (AR-iPrEC), suggesting FASN can act as a prostate cancer oncogene in the presence of AR (50).

Inhibition of FASN not only reduces intracellular *de novo* fatty acids, but also leads to accumulation of malonyl-CoA, which can inhibit fatty acid oxidation through CPT1 inhibition (51). However, inhibition of other enzymes involved *de novo* fatty acid synthesis that do not lead to elevated malonyl-CoA levels also inhibit prostate cancer growth. Inhibition of the rate-limiting enzyme ACC by RNAi-mediated silencing or by sorafenin A reduced proliferation of CRPC cell lines, which could be rescued by addition of palmitate to the media (52, 53). Moreover, genetic knock-down of the fatty acid elongases ELOVL5 (54) or ELOVL7 (26) inhibits growth of CRPC xenograft tumors and human tumor explants. Interestingly, ELOVL5

knock-down also reduced metastasis of mouse orthotopic prostate cancer, and *in vitro* growth could be rescued by supplementation with cis-vaccenic acid, the fatty acid product of ELOVL5 (54). This study suggests that *de novo* fatty acid synthesis may be important for metastasis in addition to proliferation.

Fatty acid synthesis is dependent on ample supply of TCA cycle-derived citrate. Cytosolic pyruvate is a key precursor of citrate, which can be transported into mitochondria via the mitochondrial pyruvate carrier (MPC), converted to acetyl-CoA by the pyruvate dehydrogenase complex (PDC), and subsequently combined with oxaloacetate to generate citrate. Disruption of MPC (55) or PDC (56) activity inhibited lipogenesis and growth of prostate cancer in murine models. Remarkably, *in vitro* growth inhibition due to genetic knockdown of *Pdha1*, a subunit of PDC, could be rescued by supplementation with exogenous fatty acids, providing additional evidence that prostate cancer cell-intrinsic production of fatty acids is required for proliferation (56). Citrate can also be generated from glutamine through both oxidative metabolism and reductive carboxylation (57, 58). Genetic deletion of mitochondrial aconitase, *Aco2*, reduced generation of citrate from glutamine through both pathways, resulted in diminished lipogenesis, and reduced prostate cancer growth (59).

Regulation of Fatty Acid Synthesis in Prostate Cancer

Fatty acid synthesis in prostate cancer appears to be coordinated by many factors, including AR, sterol regulatory-element binding proteins (SREBPs), PTEN/PI3K/Akt, c-Myc, and AMP-activated protein kinase (AMPK; ref. 60). Exposure of CRPC cell lines to androgens leads to upregulation and increased activity of ACLY, ACC, FASN, and malic enzyme, as well as accumulation of *de novo* neutral lipids (61–63). Conversely, AR activation represses expression of DECRL1, reducing fatty acid oxidation (64). AR may coordinate this lipogenic program through stimulation of SREBPs (62), which regulate expression of genes involved in fatty acid and cholesterol synthesis (65). Expression of SREBPs increases through stages of prostate cancer progression (66). SREBP hyperactivity due to high MAPK activation increased the abundance of intracellular saturated and monounsaturated fatty acyl chains and development of metastases in a murine model of prostate cancer (67). This study and others have shown that the inhibitor of SREBP, fatostatin, reduces prostate cancer proliferation, invasion, and metastasis (67, 68). SREBP activity is also stimulated by Akt (69) and c-Myc (70). In models of prostate cancer, Akt activation and c-Myc expression increase fatty acid synthesis (71, 72). Moreover, SREBP activity is regulated in part by nuclear PDC generation of acetyl-CoA, which can modify histone acetylation and transcriptional activation and may coordinate substrate availability with transcriptional programs of lipogenesis (56).

Negative regulation of lipogenesis can be coordinated by AMPK. Under circumstances of energetic stress often due to nutrient deprivation, the cell is programmed to activate catabolic pathways and inactivate anabolic metabolism to produce energy needed for survival. This program is orchestrated by AMPK, which is activated by a high ratio of AMP to ATP. AMPK activation inhibits fatty acid synthesis by phosphorylating and inactivating ACC and suppressing SREBP1c function to reduce FASN and ACC expression (73, 74). AMPK activation by AICAR or rosiglitazone was shown to inhibit fatty acid synthesis and proliferation of prostate cancer cells (75). The drug MT 63–78 directly activates AMPK and was also shown to reduce growth of preclinical models of prostate cancer with exogenous palmitate

partially rescuing the growth defect (76). However AMPK activation is also known to be adaptive for tumor cells under stress, as its inhibition of fatty acid synthesis was shown to conserve NADPH to compensate for reduced pentose phosphate pathway activity under glucose starvation (77). Therefore, the role of AMPK activation in prostate cancer progression is likely complex (78).

Benefit of Fatty Acid Synthesis for Prostate Cancer Progression

We can consider how *de novo* fatty acid synthesis might be beneficial for prostate cancer progression by considering what it produces and what it consumes. In other words, activation of this pathway may be beneficial by producing a growth-stimulatory substance and/or eliminating a growth-inhibitory substance.

Certainly a possibility is that increased quantities of intracellular fatty acids are beneficial. This is supported by experiments that show that (i) supplementation of fatty acids can rescue growth inhibition due to inhibition of fatty acid synthesis (49, 54, 56) and (ii) inhibition of lipid uptake also inhibits prostate cancer growth (79, 80). Indeed the dogma of *de novo* fatty acid synthesis in cancer is that it is a critical source of lipids that serve as biosynthetic building blocks for cell and organelle membrane formation for rapid proliferation. Yet this explanation feels unsatisfactory in prostate cancer, which may have high rates of fatty acid synthesis but slow proliferation compared with other cancer types. Alternatively, *de novo* fatty acids could function as intracellular signaling molecules to propagate growth, invasion, and metastasis. For example, several phosphatidylinositols (PI), which are critical second messengers in tumorigenic signaling cascades, were found to be more abundant in prostate cancer compared with benign prostate using high-resolution matrix-assisted laser desorption/ionization imaging mass spectrometry (HR-MALDI-IMS) of primary prostate cancers (81). Fatty acids can also modulate cell signaling through post-translational modifications of proteins such as palmitoylation (82). In prostate cancer, overexpression of FASN may enhance palmitoylation of WNT-1 and stabilization of β -catenin (83), as well as palmitoylation of the GTPase RhoU and cell migration (84). Finally, *de novo* production of fatty acids may protect prostate cancer cells from oxidative damage by limiting the degree of phospholipid polyunsaturation and reducing lipid peroxidation (85).

An alternate or additional possibility is that *de novo* fatty acids regulate intercellular signaling in the tumor microenvironment. Normal prostate cells are known to secrete extracellular vesicles (EV), termed prostasomes, that are composed of lipid bilayers that contain proteases and immunosuppressive agents and regulate spermatozoa motility, invasion, and immune tolerance within the female reproductive tract (86). Prostate cancer similarly secretes EVs, which some hypothesize function similarly to increase invasion and immune tolerance of prostate cancer (87), and production of EVs may increase demand for fatty acids for EV membrane synthesis. Beyond supporting production of EVs, cancer cell *de novo* fatty acid synthesis may increase the concentration of lipids in the tumor microenvironment, either by reducing cancer cell uptake of lipids or by cancer cell secretion of lipids. A tumor microenvironment with high lipid content may support cancer immune tolerance, as immunosuppressive subtypes of immune cells, including regulatory T cells (Treg), myeloid-derived suppressor cells, and M2 macrophages, engage in higher rates of fatty acid oxidation than effector immune subtypes (88–91). These cells express high levels of CD36 that allow for uptake of lipids from the environment, which is required for suppressive function (91, 92). A recent

study showed increased lipids in the prostate cancer tumor micro-environment due to reduced stromal cell lipid uptake following *CAVIN1* knockdown increased M2 macrophage infiltration and enhanced prostate cancer cell migration and invasion (93). The effect of prostate cancer *de novo* fatty acid synthesis on tumor immunity is underexplored.

Beyond production of fatty acids, fatty acid synthesis may be beneficial for prostate cancer progression by consuming a growth-inhibitory substance. Hyperactive fatty acid synthesis, particularly by high activity of FASN, has the potential to deplete upstream substrates citrate and acetyl-CoA. Inhibition of FASN increased citrate levels, which consequently decreased reductive carboxylation by IDH1 and intramitochondrial NADPH levels necessary to buffer reactive oxygen species (ROS) for tumorigenesis (94). Yet NADPH is compartmentalized such that high rates of fatty acid synthesis could theoretically have differing effects on mitochondrial and cytosolic levels of NADPH (95), with cytosolic levels decreasing due to high utilization by FASN. A possibility is that the increased ratio of cytosolic NADP⁺/NADPH could promote growth-enhancing metabolism such as ROS signaling and/or oxidative pentose phosphate pathway (PPP) flux for nucleotide synthesis. In fact, the first enzyme in the PPP, glucose-6-phosphate dehydrogenase (G6PD), consumes NADP⁺ and has high expression and activity in prostate cancer, which may have prognostic significance (96, 97).

Given that cholesterol synthesis is under similar regulation as fatty acid synthesis, it should be considered that accelerated fatty acid synthesis could be a bystander effect of a selective advantage provided by heightened cholesterol synthesis. Beyond the role of cholesterol in membrane synthesis, they may be used for steroid synthesis including perhaps production of intratumoral androgens that might promote castration resistance (98, 99). Moreover, a tumor microenvironment high in cholesterol was shown to induce CD8⁺ T-cell exhaustion and tumor immune tolerance (100). Yet this hypothesis would not explain why selective inhibition of fatty acid synthesis would inhibit growth.

Targeting Fatty Acid Synthesis as Treatment for Patients with Prostate Cancer

Inhibition of fatty acid synthesis has been proposed as a viable therapeutic strategy for treatment of cancer because activation of this pathway seems to be restricted to a limited number of normal tissues in adults, potentially creating an acceptable therapeutic index (101). Fatty acid synthesis can be inhibited directly by inhibiting the enzymes in this pathway or, perhaps indirectly, by activating AMPK.

The development of agents that directly inhibit fatty acid synthesis has historically focused on inhibiting the multifunction enzyme FASN (102), and more recently, the rate-limiting enzyme ACC. FASN is an attractive target because, although mice with global deletion of FASN are not viable and appear to die prior to implantation (103), conditional deletion of FASN in adult animals in the prostate and other organs tends to be tolerated with the exception of inactivation within the colonic epithelium, which resulted in death of 20% of animals (104). Among the first FASN inhibitors to be studied were cerulenin and C75. Cerulenin is a mycotoxin produced by the fungus *Cephalosporium caerulens* that inhibits FASN by binding to the active site cysteine of β -ketoacyl-acyl carrier protein (ACP) of the FASN complex (105). C75 is a synthetic inhibitor of FASN that structurally lacks the reactive epoxide present on cerulenin, which enhances chemical stability (106). Both agents inhibit cancer cell growth *in vitro* and in

mouse models (106–109), however they were also found to rapidly and dramatically induce weight loss in mice due to build-up of the FASN substrate malonyl-CoA that mimicked the fed state and inhibited feeding (110). Moreover, while high levels of malonyl-CoA typically inhibit fatty acid oxidation through inhibition of carnitine palmitoyl-transferase I (CPT1) to avoid a futile cycle of concurrent fatty acid synthesis and fatty acid oxidation (51), C75 was found to compete with malonyl-CoA to stimulate CPT1, paradoxically increasing fatty acid oxidation despite high malonyl-CoA, and further enhancing the reduction of adipose tissue and fatty liver beyond simple fasting (111). Therefore enthusiasm for C75 as treatment for cancer waned due to concerns for exacerbation of cachexia in this patient population, despite lack of this side-effect in mouse models of prostate cancer (108). Other agents including the green tea polyphenol epigallocatechin-3-gallate (EGCG), other naturally occurring flavonoids such as luteolin, quercetin, and kaempferol, the antibiotic triclosan, IPI-9119, and TVB-2640 appear to inhibit prostate cancer growth by inhibiting FASN, but only TVB-2640, has been studied in human trials (101).

TVB-2640 is an oral, reversible inhibitor of the β -ketoacyl reductase domain of the FASN enzyme complex being developed by Sagimet Biosciences. It has been tested in a phase I clinical trial for patients with advanced solid tumors as monotherapy or in combination with a taxane (112). This study suggested TVB-2640 engaged the target and was safe, with the dose-limiting toxicities being skin and ocular effects. This agent has also been tested in a phase I clinical trial for patients with obesity, in which it was found to reduce hepatic *de novo* lipogenesis as assessed by acetate isotope tracing and decrease intrahepatic triacylglycerols (113). Results from a phase II study of TVB-2640 in combination with the VEGF inhibitor bevacizumab for patients with glioblastoma has been reported in abstract form at the European Society for Medical Oncology (ESMO) virtual conference 2020 and suggested this regimen was well tolerated and improved progression-free and overall survival compared with historical controls (114). Clinical trials are ongoing testing safety and efficacy of this agent for patients with KRAS-mutated non-small cell lung cancer (NCT03808558), resectable colon cancer (NCT02980029), and HER2-positive advanced breast cancer (NCT03179904). To our knowledge, there are no ongoing trials testing TVB-2640 in patients with prostate cancer.

An alternate method of directly inhibiting fatty acid synthesis is through inhibition of ACC, which is the rate-limiting step. This strategy is different from inhibition of FASN, because it may lead to decreased levels of malonyl-CoA and therefore may stimulate fatty acid oxidation. While the macrocyclic myxobacterial natural product soraphen A, which inhibits ACC, has been a useful research tool (53), its poor pharmacokinetic properties limit its clinical utility. More recently, a series of potent and specific ACC inhibitors, including ND-630, ND-646, and ND-654, have been identified that prevent dimerization of both isozymes, ACC1 (cytosolic) and ACC2 (mitochondrial), to inhibit their enzymatic activity (115). In an open-label prospective randomized phase II clinical trial, ND-630 (also called GS-0976 or firsocostat) was shown to reduce hepatic *de novo* lipogenesis and hepatic steatosis in patients with nonalcoholic steatohepatitis (116). Notably this agent led to an asymptomatic increase serum triglycerides (>500 mg/dL) in some patients. ND-654 is modified for enhanced hepatic uptake and was shown to reduce development of hepatocellular carcinoma in rat models (117). ND-646 is broadly distributed and was shown to reduce development of non-small cell lung cancer and be well tolerated in mouse models (118). To our knowledge, there are no ongoing clinical trials testing ACC inhibitors as treatment for cancer.

An alternative strategy to inhibit fatty acid synthesis may be activation of AMPK, which can potently inhibit ACC. Metformin is an agent that is already approved by the Food and Drug Administration for use in humans that can lead to activation of AMPK and inactivation of fatty acid synthesis due to inhibition of mitochondrial complex I (119–121). Retrospective studies have suggested that, among diabetic patients with prostate cancer, those on metformin have better prostate cancer outcomes than those not on metformin (122, 123). Yet prospective studies in patients with prostate cancer have been disappointing to date – the TAXOMET trial, a randomized phase II study of docetaxel and metformin versus docetaxel and placebo, suggested no benefit by the addition of metformin to docetaxel (124), and the MetAb-Pro trial, a single arm trial of metformin and abiraterone for patients progressing on abiraterone, suggested no benefit from metformin in this setting (125). These trials included limited correlative studies so the effect of metformin on prostate cancer cell metabolism in patients remains unclear, however inadequate potency and transport-mediated accumulation may limit efficacy. Recently a novel agent IAC-010759 was described to be a highly potent and selective small-molecule inhibitor of mitochondrial complex I that leads to AMPK activation that can inhibit malignant cell growth in models of glioblastoma and acute myeloid leukemia (126). This agent was well tolerated in animal models, and initial reported results from the phase I clinical trial suggested good tolerance in humans with the most common side-effect being raised lactate without acidosis (127). The phase I trial included three patients with CRPC and reported one patient with heavily pretreated disease who exhibited a RECIST partial response and resolution of CRPC-related pain. Complex I inhibition has many effects on metabolism of cancer cells so it is unclear to what extent inhibition of fatty acid synthesis may contribute to efficacy of this agent.

Finally, recent studies suggest there may be therapeutic opportunity in targeting downstream handling of fatty acids in advanced prostate cancer, which is likely carefully coordinated to minimize maladaptive effects of fatty acid accumulation such as lipotoxicity and susceptibility to ferroptosis. For example, CRPC appears to increase activity of DECR1, an interesting potential therapeutic target, which facilitates oxidation of PUFAs and reduce susceptibility to ferroptosis (64, 128).

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Conclusions

While there is ample evidence that *de novo* fatty acid synthesis is important to progression of prostate cancer in preclinical models, the best evidence will come from testing of inhibitors of this pathway in humans. Prime candidate agents to test include TVB-2640 and firsocostat, which have already been shown to be safe in humans. Clinical trial designs should consider assessment of predictive biomarkers, inclusion of correlative studies to define mechanisms of sensitivity and resistance, and testing of rational combination therapies. Biomarkers that indicate high rates of fatty acid synthesis, including high tumor uptake of ¹¹C-acetate by PET, loss of *Pten*, and high expression of c-Myc, may predict response to inhibitors of this pathway. Correlative studies should measure change in both cancer cell flux of *de novo* fatty acid synthesis, as well as total quantity and composition of intracellular lipids to determine relative compensation by fatty acid uptake. Moreover, a clinical trial in patients with metastatic prostate cancer offers an opportunity to learn about tumor immunometabolism. Given that *de novo* fatty acid synthesis is important for function of many effector immune subtypes, its inhibition may be immunosuppressive. Ultimately, this may have no effect on efficacy of these agents, as the immune system appears to be highly tolerized to metastatic prostate cancer at baseline. Alternatively, relative depletion of fatty acids in the tumor microenvironment may inhibit fatty acid oxidation and function of suppressive immune cell subtypes. The assessment of this effect, direct effects on cancer cell fitness, and ultimately patient survival in a clinical trial of fatty acid synthesis inhibition will determine whether *de novo* fatty acid synthesis is a vulnerability or epiphenomenon in advanced prostate cancer.

Authors' Disclosures

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