Fatty Acid Synthase and Cancer: New Application of an Old Pathway

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Abstract

Fatty acid synthase (FAS), the sole mammalian enzyme capable of *de novo* fatty acid synthesis, is highly expressed in most human carcinomas. FAS is associated with poor prognosis in breast and prostate cancer, is elaborated into the blood of cancer patients, and its inhibition is selectively cytotoxic to human cancer cells. Thus, FAS and fatty acid metabolism in cancer has become a focus for the potential diagnosis and treatment of cancer. (Cancer Res 2006; 66(12): 5977-80)

Introduction

Altered metabolism of human cancer cells has been recognized since the 1920s, with the observation of increased anaerobic glycolysis in cancer cells by Otto Warburg (1). More recently, increased expression of fatty acid synthase (FAS) has emerged as a phenotype common to most human carcinomas (2). FAS expression is an indicator of poor prognosis in breast and prostate cancer, is found elevated in the blood of cancer patients, and its inhibition is selectively cytotoxic to human cancer cells. Thus, exploration of the role of FAS and fatty acid metabolism in cancer has become a focus for the potential diagnosis and treatment of cancer.

FAS is the sole protein in the human genome capable of the reductive *de novo* synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and nicotinamide adenine dinucleotide phosphate (NADPH; ref. 3). Although FAS is the key biosynthetic enzyme in the fatty acid synthesis pathway, it is acetyl-CoA carboxylase (ACC) that carboxylates acetyl-CoA to malonyl-CoA, which acts as the pace-setting enzyme of fatty acid synthesis (Fig. 1; ref. 4). Metabolically, fatty acid synthesis is an anabolic pathway consuming 14 ATP and 7 NADPH per fatty acid synthesized.

FAS is active in cancer cells, predominantly producing the 16-carbon saturated fatty acid palmitate, the identical product of FAS in liver and other lipogenic tissues. The fate of the palmitate, however, differs substantially between cancer cells and lipogenic tissues. In liver and adipose tissue, fatty acid synthesis occurs when energy is surfeit as a means to store excess calories from carbohydrate as triglyceride. Importantly, the high steady-state levels of malonyl-CoA during lipogenesis inhibit carnitine palmitoyltransferase-1 (CPT-1), the rate-limiting enzyme of mitochondrial fatty acid oxidation, shunting fatty acids away from oxidation and toward storage as triglycerides (4). During starvation, FAS expression and activity is rapidly down-regulated, malonyl-CoA levels decrease, and fatty acid oxidation ensues enabling survival. In contrast to liver and adipose tissue, most human cancers do not store significant amounts of triglyceride. Endogenously synthesized fatty acids in cancer cells are esterified predominantly to phospholipids, not triglyceride (5). Moreover, fatty acid synthesis in cancer cells is transcriptionally regulated either hormonally or through oncogene signaling via kinase pathways, not by diet as occurs in lipogenic tissues.

In addition to differences in pathway regulation and end-product utilization, the consequences of FAS inhibition differs widely between transformed and normal cells. FAS inhibition using cerulenin or C75 rapidly induces apoptosis in human cancer cells both *in vitro* and *in vivo*, implying the reliance of cancer cell survival on FAS activity (6–9). In contrast, treatment of leptin-deficient mice, or diet-induced obese mice with FAS inhibitors, substantially decreased fatty liver and adipocyte mass without hepatocellular injury or fat necrosis (10). Thus, FAS expression and fatty acid synthesis likely subsume discrete functions in cancer and lipogenic tissues. In this review, we will focus on the proposed mechanisms of FAS up-regulation in cancer and the selective apoptosis of cancer cells following FAS inhibition.

FAS Expression in Cancer

FAS regulation in cancer was first explored during the 1980s in human breast cancer cells possessing functioning estrogen and progesterone receptors. Treatment with progestins led to a marked increase in FAS expression and activity, analogous to FAS expression in the human breast (11). Similarly, androgens also up-regulated FAS expression in both breast and prostate cancer cell lines (12, 13). Surprisingly, following androgen ablation of prostate cancer, FAS expression initially decreased, only to return at higher levels following the transition to androgen independence (14, 15). The return of FAS expression following androgen ablation implied the existence of novel pathways driving FAS expression in cancer unrelated to sex steroids or nutrition.

Nutritional control of FAS expression in liver and adipose tissue is accomplished through transcriptional induction coordinately regulated by insulin, glucagon, glucocorticoids, and thyroid hormone (16). In contrast, both the mitogen-activated protein (MAP) kinase and phosphatidylinositol-3-kinase (PI3K) pathways are likely candidates for regulating FAS expression in cancer through the sterol regulatory element binding protein 1-c (SREBP-1c; refs. 17, 18). Inhibitors of MAP kinase and PI3K down-regulated SREBP-1 levels and decreased transcription from the FAS promoter, reducing FAS expression and fatty acid synthesis in both MCF7 and HCT116 human colon cancer cells. H-*ras* transformation of MCF-10 human breast epithelial cells increased FAS expression, fatty acid synthesis, and sensitivity to FAS inhibition which was abrogated by MAP or PI3K inhibition, or by deletion of the major SREBP binding site from the FAS promoter. Analysis of clinical breast cancer tissues for FAS and SREBP1-c mRNA found evidence of coordinate regulation further supporting the role of SREBP-1c in FAS regulation in cancer (17). In the

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Note: Under a licensing agreement between FASgen and the Johns Hopkins University, F.P. Kuhajda is entitled to a share of the royalties received by the University on sales of products described in this article. F.P. Kuhajda owns FASgen stock, which is subject to certain restrictions under University policy. The Johns Hopkins University, in accordance with its conflict of interest policies, is managing the terms of this arrangement.

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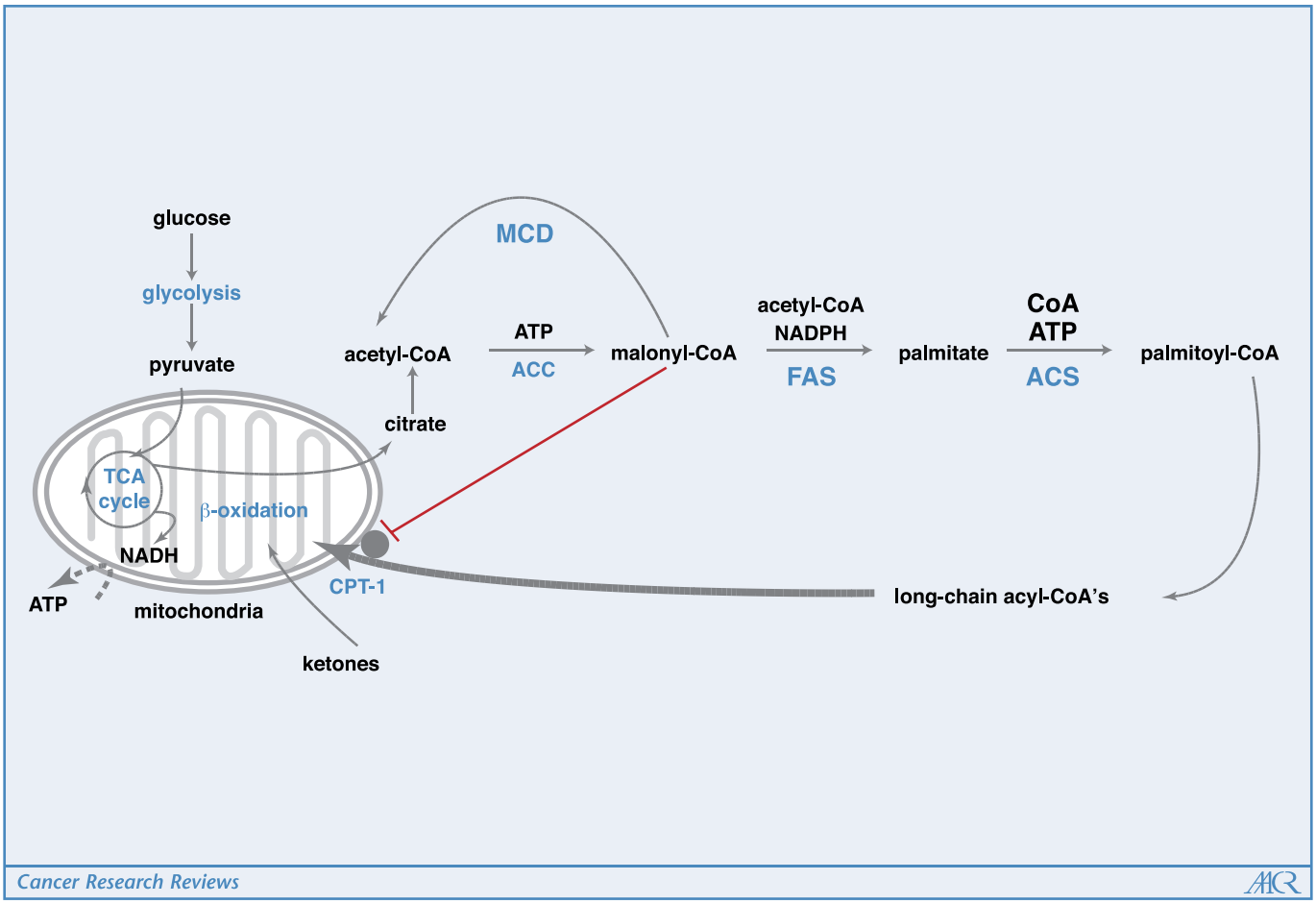

Figure 1. The fatty acid synthesis pathway. Excess glucose destined for fatty acid synthesis exits the mitochondria as citrate. Citrate lyase metabolizes citrate to acetyl-CoA, which is carboxylated to malonyl-CoA by ACC. FAS undergoes the reductive synthesis of palmitate using one acetyl-CoA, seven malonyl-CoA, and seven NADPH. Malonyl-CoA, in addition to its role as a substrate for FAS, inhibits CPT-1, preventing the oxidation of the newly synthesized palmitoyl-CoA. Although subsuming different purposes, this pathway functions in both cancers and lipogenic tissue such as liver.

phosphatase and tensin homologue deleted on chromosome 10 (PTEN)–null LNCaP human prostate cancer cells, the PI3K inhibitor LY294002 reduced the high levels of FAS expression (18). Reintroduction of PTEN also reduced FAS expression but subsequent transfection of constitutively active Akt1/protein kinase B-α restored FAS expression levels, suggesting a role for the PI3K signaling pathway in prostate cancer (18). These data were later supported by finding a positive relationship between FAS and pAkt expression in clinical samples of prostate cancer (19), and the inverse relationship of PTEN and FAS expression in clinical prostate cancer (20). HER2/*neu* signaling has also been implicated as a cause of increased FAS expression in breast cancer (9, 21), perhaps functioning through the PI3K pathway (22). Thus, the transcriptional control of FAS expression and lipogenesis in cancers mediated by MAP and PI3K pathways differ substantially from nutritional control in liver and adipose tissue.

Elevated FAS levels have also been identified in the blood of patients with breast, prostate, colon, and ovarian cancers compared with normal subjects using ELISA (23). In breast cancer, FAS levels increased with tumor stage (24), and were independent of CA27.29 levels (25). Further studies will be needed to determine the role of serum FAS levels in the detection, prognosis, or monitoring of human cancers.

FAS and Cancer Prognosis

Because fatty acid synthesis expends energy, FAS expression might confer some survival or growth advantage to human cancer. Otherwise, the futile synthesis of fatty acids would likely disadvantage the survival of a FAS expressing clone. This hypothesis has been supported particularly in breast and prostate cancer, where clinical studies found an association between FAS expression and cancer prognosis. In patients with stage I breast cancer, high levels of FAS expression connote a 4-fold increased risk of death from the disease (1). More recently, FAS expression has been associated with HER2 expression in aggressive breast cancers (26). In prostate cancer, FAS expression is associated with a 4-fold risk of disease recurrence (1) and higher Gleason grade (19). Recently, prostate cancer was studied for both FAS and PTEN expression. Again, positive FAS expression alone resulted in a 4.45-fold risk of death from disease, but when combined with negative PTEN expression, the risk of death increased to 11.52-fold (20). FAS expression has also been found to connote poor prognosis in stage I non–small cell lung cancer (27, 28), malignant melanoma (29, 30), and soft-tissue sarcomas (31). These data suggest that FAS expression and activity confer a growth or survival advantage in human cancers.

FAS as a Drug Target for Cancer

High levels of FAS expression, and its association with tumor prognosis, led to the exploration of FAS as a drug target for cancer therapy. Initially, cerulenin, a natural product inhibitor of FAS, was cytotoxic against a variety of human cancer cell lines *in vitro* and the OVCAR3 human ovarian cancer xenograft (reviewed in ref. 1). These findings led us to develop C75, a small molecule inhibitor of type 1 mammalian FAS (32). More chemically stable than cerulenin, C75 showed significant antitumor effects against human cancer cell lines *in vitro* (6), and against human breast (6), prostate (15), mesothelioma (33), and ovarian cancer xenografts (8).

Although C75 provided the initial *in vivo* evidence of tumor growth reduction following FAS inhibition, C75 also induced substantial weight loss that constituted its dose-limiting toxicity in mice (6). Consequently, we began to explore the mechanism of C75-induced weight loss with the goal of developing cytotoxic FAS inhibitors that do not incur substantial weight loss. C75 causes weight loss via the induction of anorexia while simultaneously increasing fatty acid oxidation. C75 reduces food intake by blocking the production of the anorexigenic hypothalamic neuropeptide-Y (10). C75 also increases fatty acid oxidation and directly stimulates CPT-1 activity, the enzyme responsible for the regulation of mitochondrial fatty acid oxidation (34). Although the nuances of these mechanisms remain under investigation, they raised the possibility that increased fatty acid oxidation, not FAS inhibition, was the mechanism of C75 cytotoxicity. Further pharmacologic studies of fatty acid oxidation in cancer (7), and short interfering RNA studies of FAS (35) have since established that FAS is the target enzyme responsible for cancer cell cytotoxicity. Moreover, based on these mechanistic studies, we have now developed cytotoxic FAS inhibitors that do not incur weight loss *in vivo*.[1] The recent 4.5 Å resolution X-ray crystallographic map of porcine FAS will further help guide the development of novel mammalian FAS inhibitors (36).

Why inhibition of fatty acid synthesis kills cancer cells remains an area of active investigation. Initial studies explored the relationship between pharmacologic inhibition of fatty acid synthesis and cytotoxicity. Inhibition of fatty acid synthesis through FAS inhibition induced apoptosis whereas ACC inhibition did not, and actually protected cancer cells from FAS inhibition (6, 7). These studies suggested that accumulation of the substrate malonyl-CoA, not depletion of the end-product fatty acids, was likely triggering cancer cell death.

The activity of a number of pathways modulate FAS cytotoxicity. FAS inhibition has been shown to be more effective in initiating apoptosis in cells with nonfunctioning p53 protein, whereas cells with intact p53 function tend to exhibit a cytostatic response (37, 38). HER2/*neu* overexpression has also been linked to FAS-induced cytotoxicity (9, 22). Although these observations have identified molecules and pathways that may amplify or impede FAS inhibition–induced cytotoxicity, the mechanism linking FAS inhibition to apoptosis remains elusive.

In a recent study of human ovarian cancer cells, FAS and pAkt expression were coordinately regulated, suggesting a potential mechanism for FAS-induced apoptosis. Treatment of human ovarian cancer cells harboring constitutively active Akt (pAkt) with the PI3K inhibitor, LY294002, abolished pAkt activity and potentiated apoptosis induced by FAS inhibitors, cerulenin or C75. These data suggested that constitutive activation of Akt protects against FAS inhibitor-induced cell death. Furthermore, inhibition of FAS activity resulted in the down-regulation of pAkt, which preceded the induction of apoptosis both *in vitro* or *in vivo* (8). These findings supported a model in which Akt activation regulates FAS expression, at least in part, whereas FAS activity modulates Akt activation. Future studies of FAS inhibition in cancer cells will likely explore pathways that modulate apoptosis, and continue to search for the biochemical link between FAS enzyme inhibition and cancer cell death.

Because FAS expression has been identified in premalignant lesions of the breast, prostate, and colon (1), FAS inhibition has lately been studied in animal models of chemoprevention. C75 significantly reduced mammary cancer development in the *neu* transgenic mouse model. Analysis of mammary tissue following 10 weeks of C75 treatment revealed a significant delay in mammary maturation as manifested by a reduction of the number and caliber of mammary ducts and budding epithelial structures. Apoptotic changes were increased, DNA synthesis was decreased, and the expressions of FAS, neu, Akt, pAkt, and p21(waf1) were all decreased when compared with vehicle controls, and the changes were restricted to the mammary epithelial cells (39). In the TRAMP mouse prostate cancer model, high FAS expression and activity was evident at 12 weeks of age and further increased with age, tumor progression, and in metastatic lesions. FAS pathway inhibition resulted in a dose-dependent reduction in cell survival and decreased enzyme activity in these models (40). Finally, in a rat mammary chemical carcinogenesis model using methylnitrosourea, FAS inhibition with triclosan exhibited a significant reduction in tumor development and inhibition of mammary tumor fatty acid synthesis (41). These data suggest that FAS inhibition may hold promise as a strategy for cancer chemoprevention.

FAS and the fatty acid synthesis pathway provide a number of avenues of future exploration applicable to the diagnosis, prognosis, treatment, and prevention of human cancer.

[1] F.P. Kuhajda and C.A. Townsend, unpublished observations.

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