# Chapter 7
# Fatty Acid Synthase Activity in Tumor Cells

**Joy L. Little and Steven J. Kridel**

**Abstract** While normal tissues are tightly regulated by nutrition and a carefully balanced system of glycolysis and fatty acid synthesis, tumor cells are under significant evolutionary pressure to bypass many of the checks and balances afforded normally. Cancer cells have high energy expenditure from heightened proliferation and metabolism and often show increased lipogenesis. Fatty acid synthase (FASN), the enzyme responsible for catalyzing the ultimate steps of fatty acid synthesis in cells, is expressed at high levels in tumor cells and is mostly absent in corresponding normal cells. Because of the unique expression profile of FASN, there is considerable interest not only in understanding its contribution to tumor cell growth and proliferation, but also in developing inhibitors that target FASN specifically as an anti-tumor modality. Pharmacological blockade of FASN activity has identified a pleiotropic role for FASN in mediating aspects of proliferation, growth and survival. As a result, a clearer understanding of the role of FASN in tumor cells has been developed.

**Keywords** Cancer · fatty acid synthase · lipogenesis

**Abbreviations** FASN, fatty acid synthase ACC, acetyl-CoA-carboxylase ACL, ATP-citrate lyase NADPH, nicotinamide adenine dinucleotide phosphate MAT, malonyl acetyl transferases KS, ketoacyl synthase KR, β-ketoacyl reductase DH, β-hydroxyacyl dehydratase ER, enoyl reductase TE, thioesterase ACP, acyl carrier protein VLCFA, very long chain fatty acids ELOVL, elongation of very long chain fatty acids SCD1, stearoyl-CoA desaturase-1 AMPK, AMP-activated kinase ME, malic enzyme FASKOL, liver-specific deletion of FAS PPARα, Peroxisome Proliferator-Activating Receptor alpha HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA SREBP, sterol response element binding protein S1P, site-one protease S2P, site-two

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protease RIPCre, Cre-recombinase under the control of rat insulin 2 promoter CPT1, carnitine palmityl transferase 1 MCD, malonyl-CoA desaturase SCAP, SREBP cleavage activating protein NF-Y, nuclear factor Y SP1, stimulatory protein 1 RNAi, RNA interference PI3K, phosphatidylinositol-3 kinase KGF, keratinocyte growth factor EGF, epidermal growth factor JNK, cJun N-terminal kinase RTK, receptor tyrosine kinase AR, androgen receptor PR, progesterone receptor USP2a, ubiquitin-specific protease 2a EGCG, epigallocatechin-3-gallate TOFA, 5-(tetradecyloxy)-2-furoic acid FDA, food and drug administration.

## 7.1 Fatty Acid Synthesis

### *7.1.1 The FASN Enzyme*

One of the metabolic hallmarks of a tumor cell is increased lipogenesis (Kuhajda, 2006; Swinnen et al., 2006). In fact, in many instances the vast majority of fatty acids in tumors are synthesized *de novo* (Ookhtens et al., 1984). In mammalian cells, fatty acid synthase (FASN) is the central enzyme of long chain fatty acid synthesis. FASN is a multifunctional polypeptide that is comprised of seven separate functional domains (Fig. 7.1A). The individual domains of FASN work in concert to catalyze thirty-two different reactions to synthesize the sixteen carbon fatty acid palmitate, using acetyl-CoA and malonyl-CoA as substrates and nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor. The fatty acid synthesis reaction mechanism can be separated into three functional groupings: (1) to bind and condense the substrates, (2) to reduce the intermediates and (3) to release the final saturated long chain fatty acid palmitate (Fig. 7.1B). The malonyl acetyl transferase (MAT) domain binds malonyl-CoA and acetyl-CoA, while the ketoacyl synthase (KS) domain acts to condense the acyl chain (Fig. 7.1B). This β-ketoacyl moiety is then reduced in steps by the β-ketoacyl reductase (KR), β-hydroxyacyl dehydratase (DH), and enoyl reductase (ER) domains to a saturated acyl intermediate. This derivative can then be elongated by repeating the reactions catalyzed by the five previous enzyme activities for seven cycles until the thioesterase (TE) domain cleaves the final product, the sixteen carbon fatty acid palmitate. Throughout the entire synthesis of palmitate, the acyl carrier protein (ACP) acts as a coenzyme to bind intermediates by a 4'-phosphopantetheine group (Fig. 7.1B). In total, approximately 30 intermediates are involved in the process, but it is the high specificity of the TE domain for a 16 carbon fatty acid, as well as the MAT specificity for malonyl-CoA, that are responsible for preventing leakage of intermediates (Wakil, 1989). The overall FASN reaction is as follows:

$$\begin{aligned}&\text{Acetyl-CoA} + 7\text{Malonyl-CoA} + 14\text{NADPH} + 14\text{H}^+ \rightarrow \\ &\text{Palmitic-acid} + 7\text{CO}_2 + 8\text{CoA} + 14\text{NADP}^+ + 6\text{H}_2\text{O}\end{aligned}$$

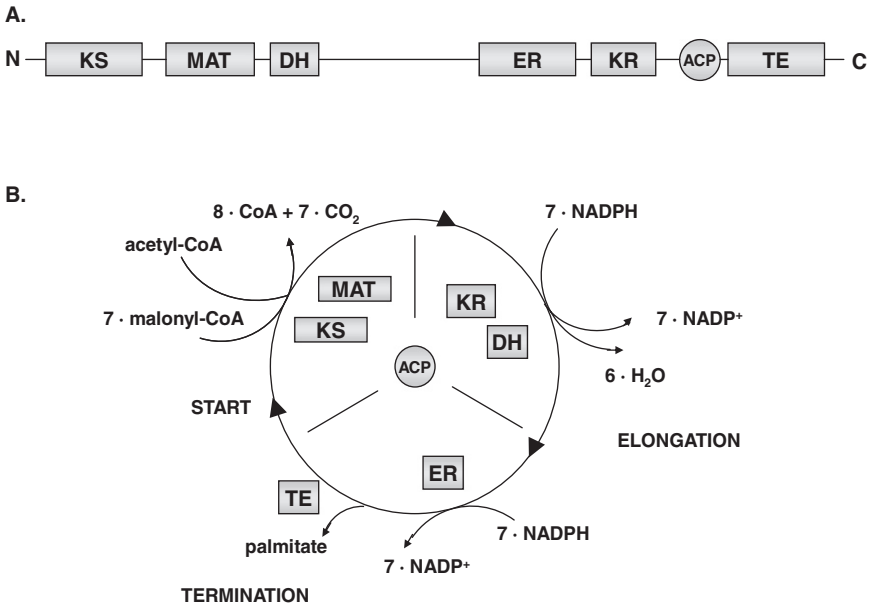


**Fig. 7.1** The FASN Enzyme. **A**. The FASN polypeptide comprises seven functional domains: the ketoacyl synthase (KS), malonyl acetyl transferase (MAT), β-hydroxyacyl dehydratase (DH), enoyl reductase (ER), β-ketoacyl reductase (KR), the acyl carrier protein (ACP), and thioesterase (TE) domains. **B**. The FASN reaction mechanism. The MAT domain of the enzyme binds malonyl-CoA and acetyl-CoA, while the KS domain acts to condense the growing acyl chain. The resulting β-ketoacyl moiety is then reduced in steps by the KR, DH, and ER to a saturated acyl intermediate. This process is repeated in seven cycles, after which, the TE domain releases the sixteen carbon fatty acid palmitate

The structure of FASN has yet to be definitively characterized, as there are two distinct models (Smith, 2006). Early complementation studies suggest that FASN functions as a homodimer in head-to-tail conformation with two simultaneous reactions beginning in one subunit and finishing in the other (Wakil, 1989; Smith et al., 2003; Rangan et al., 1998; Rangan et al., 2001). However a more recent crystal structure analysis of porcine FASN challenges this historical model. The 4.5 Å structure reveals FASN as an intertwined dimer in a conformation resembling an "X" with one central core region with two arms and two legs (Maier et al., 2006). However, at this lower resolution, the definitive placement of the flexible TE domain and ACP is not possible. It is also unclear whether the body of the FASN complex can be identified as two distinct monomers. In this model, the KS domain is near the bottom of the central core of the complex and two MAT domains are in the "legs" of the X shape. The DH domains are located in the top half of the central region just under the ER domains. Adjacent to the ER domains are the KR domains that comprise the "arms" of this X complex. The study equates the reaction

pockets of this structure as having "double hot dog" folds but observes asymmetry of the two sides of the reaction chambers that may reveal hinge regions that allow different conformations of the FASN complex (Maier, et al., 2006; Smith, 2006).

### *7.1.2 Other Players in the Fatty Acid Synthesis Pathway*

While FASN is the central enzyme of fatty acid synthesis, other enzymes and pathways upstream of FASN are required to generate and supply substrates. Glucose enters the cell and is converted through glycolysis to pyruvate which is then shuttled into the mitochondria to enter the citric acid cycle. Citrate is shuttled out of the mitochondria, where ATP-citrate lyase (ACL) catalyzes the conversion of citrate to oxaloacetate and acetyl-CoA. Acetyl-CoA Carboxylase (ACC) catalyzes the conversion of acetyl-CoA to malonyl-CoA in the rate limiting and first committed step of lipogenesis. Unlike FASN, which is primarily regulated transcriptionally, ACC is negatively regulated by post-translational phosphorylation at serine 79 by AMP-activated kinase (AMPK). Energy deficiency stimulates AMPK to regulate energy consumption of cells, specifically by regulating ACC among other enzymes. Fatty acid synthesis requires NADPH, which is provided through the hexose monophosphate shunt and malic enzyme (ME) to donate electrons (Wakil et al., 1983). Recent findings also suggest that glutamine metabolism can generate sufficient NADPH in glycolytic tumor cells, as well as act as a carbon source for fatty acid synthesis (Deberardinis et al., 2007).

After fatty acid synthesis, downstream enzymes can further modify palmitate for various cellular functions. In the endoplasmic reticulum, the 16 carbon fatty acid can be modified to fatty acids with eighteen or more carbons known as very long chain fatty acids (VLCFA), such as stearate (18:0) by a family of elongase enzymes called elongation of very long chain fatty acids (ELOVL1-6) (Jakobsson et al., 2006). Palmitate and stearate can also be desaturated by stearoyl-CoA desaturase-1 (SCD1) at the cis-9 carbon to palmitoleate (16:1) and oleate (18:1), respectively (Sampath and Ntambi, 2005).

## 7.2 FASN Expression

### *7.2.1 FASN Expression in Normal Cells*

In normal tissue, FASN is expressed and active in cells that have a high lipid metabolism, such as liver and adipose tissues, to generate triglycerides in response to excess caloric intake (Jayakumar et al., 1995; Volpe and Marasa,

1975; Wakil et al., 1983). FASN is also expressed in a niche-specific manner in specialized tissues such as lactating mammary glands (Kusakabe et al., 2000; Thompson and Smith, 1985) cycling endometrium (Pizer et al., 1997; Kusakabe et al., 2000), and various other cell types including type II alveolar cells to produce lung surfactant (Buechler and Rhoades, 1980; Kusakabe et al., 2000), brain cells (Kusakabe et al., 2000; Jayakumar et al., 1995), and seminal vesicles to produce seminal fluid (Kusakabe et al., 2000). FASN is only weakly detectable, if at all, in other rapidly dividing normal tissues such as the intestinal epithelium, stomach epithelium, and hematopoietic cells in adults and is not detectable in most other adult tissues (Kusakabe et al., 2000).

Despite the low expression profile in most adult tissues, FASN is critical for developing embryos and is highly expressed in proliferative fetal cells (Kusakabe et al., 2000). The importance of FASN in development is underscored by the fact that mice with homozygous deletions of the *FASN* gene display an embryonic lethal phenotype (Chirala et al., 2003). *FASN* $^{-/-}$ mice die before implantation around embryonic day 3.5, most likely because developing embryos are unable to acquire enough fatty acids from the mother for adequate membrane biogenesis. The importance of FASN during development is further highlighted by the fact that the majority of heterozygotes are also resorbed after implantation. Those that survive do not live long beyond birth, indicating that one *FASN* allele is usually insufficient for embryogenesis, implantation, and developing tissues (Chirala et al., 2003). The importance of the fatty acid synthesis pathway in development is further supported by the demonstration that deletion of *ACC1* in mice also results in an embryonic lethal phenotype (Abu-Elheiga et al., 2005).

Mice harboring tissue-specific deletions of *FASN* have been generated to facilitate understanding of the role of FASN in normal tissue. To date *FASN* has been deleted in liver, β-cells, and hypothalamus (Chakravarthy et al., 2005, 2007). To knock out *FASN* in the liver, mice with a "floxed" *FASN* allele were crossed with mice harboring an allele of Cre driven by a rat albumin promoter. Although this liver-specific deletion of *FASN* (FASKOL) leaves animals viable without severe physiological effects, it is not without consequence. When FASKOL mice are fed a diet containing zero fat or are fasted for prolonged periods, they develop symptoms similar to those seen in mice engineered to lack Peroxisome Proliferator-Activating Receptor alpha (PPARα) (Kersten et al., 1999). Both *PPARα* knockout and FASKOL mice become hypoglycemic, develop steatosis (fatty liver) that correlates with reduced serum and liver cholesterol, reduced expression of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, decreased cholesterol biosynthesis activity, and elevated sterol response element binding protein 2 (SREBP-2) expression. While the hypoglycemia and fatty liver may be reversed with dietary fat, all effects including cholesterol biosynthesis, HMG-CoA reductase and SREBP2 levels, as well as cholesterol levels in the serum and liver are rescued by administration of a PPARα agonist. This reveals distinct levels of metabolic regulation between *de novo* and dietary fat and indicates that products downstream of FASN activity regulate

cholesterol, glucose, and fatty-acid homeostasis in the liver through activation of PPARα (Chakravarthy et al., 2005). Interestingly, mice with a liver-specific knockout of *ACC1* are still able to undergo fatty acid synthesis, but this discrepancy can be attributed to compensatory production of malonyl-CoA by the ACC2 isoform (Harada et al., 2007).

To determine whether FASN plays a role in pancreatic β-cell function, a knockout of *FASN* was generated. Crossing floxed *FASN* mice with mice harboring Cre under the control of rat insulin 2 promoter (RIPCre) causes specific deletion of *FASN* in pancreatic β-islet cells, as well as the hypothalamus, a region of the brain known for controlling motivational states, such as feeding. The resulting *FASN* knockout (FASKO) mice exhibit reduced feeding behavior and are highly active, even while maintained on a high fat diet (Chakravarthy et al., 2007). This correlates with studies showing the small molecule FASN inhibitor C75 acts in the hypothalamus to stimulate fatty acid oxidation via carnitine palmityl transferase 1 (CPT1) and induces a reversible anorexic phenotype (see Section 7.4.2). Interestingly, the β-cells lacking FASN are unaffected as loss of FASN does not alter insulin or glucose levels during glucose tolerance testing or stimulation either *in vivo* or *in vitro*. Therefore, the fasting phenotype of FASKO mice appears to be solely attributable to the effects on the hypothalamus. As a matter of fact, this observation is in agreement with a recent study showing FASN is not required for normal insulin secretion of β-cells *in vitro* (Joseph et al., 2007). Intracerebroventricular injection of FASKO mice with a small molecule drug Wy14,643 to activate PPARα restores feeding and weight gain, indicating that FASN controls PPARα activation in the hypothalamus. Pharmacological activation of PPARα in these mice also restores expression of CPT-1 and malonyl-CoA desaturase (MCD) that control cellular levels of malonyl-CoA by controlling the rate of transfer of fatty acids into the mitochondria for β-oxidation and malonyl-CoA stability, respectively (Chakravarthy et al., 2007). These studies elucidate the importance of FASN in energy homeostasis and provide a mechanism through which FASN can regulate its effects.

### *7.2.2 FASN Expression in Tumor Cells*

As discussed above, FASN has historically been studied in relation to normal physiology and as a central mediator of energy balance. In the last few decades, however, it has become clear that FASN is associated with tumor development. Accordingly, high FASN expression has been identified in many tumor types (Kuhajda, 2000, 2006). Haptoglobin-related protein (Hpr) was demonstrated to correlate with breast cancer stage, prognosis, as well as recurrence and patient survival (Kuhajda, et al., 1989a,b). Shortly after this observation, Hpr, or oncogenic antigen (OA-519) protein was identified as FASN (Kuhajda et al.,

1994). Since these discoveries, FASN upregulation has been demonstrated in every type of solid tumor. An initial retrospective study showed FASN expression correlated with staining of the proliferation marker MIB-1 to predict survival of breast cancer patients (Jensen et al., 1995). Subsequent studies confirmed the association of FASN with breast cancer recurrence, as well as shorter overall and disease-free survival in early breast cancer patients (Alo et al., 1996, 1999b). Breast cancer is not the only tumor type with elevated FASN levels. FASN expression is associated with prostate cancer prognosis, progression, and stage (Shurbaji, et al., 1992, 1996; Epstein et al., 1995). As a matter of fact, FASN is upregulated in androgen-independent prostate tumors and expression correlates with disease stage, as the highest levels of FASN expression are in androgen independent metastases (Pizer et al., 2001; Rossi et al., 2003). FASN expression correlates with poor prognosis, advanced progression, and/or decreased survival in a number of other cancers of different origins including: ovarian (Gansler et al., 1997; Alo et al., 2000), melanoma (Innocenzi et al., 2003; Kapur et al., 2005), nephroblastoma (Wilms tumor) (Camassei et al., 2003b), retinoblastoma (Camassei et al., 2003a), bladder (Visca et al., 2003), pancreas (Alo et al., 2007), soft tissue sarcoma (Takahiro et al., 2003), non-small cell lung cancer (Visca et al., 2004), endometrium (Sebastiani et al., 2004), and Paget's disease of the vulva (Alo et al., 2005). While FASN expression correlates with decreased survival and/or poor prognosis in a large number of tumor types, there are tumor types that show elevated FASN expression but no correlation with patient survival or disease stage (Rashid et al., 1997; Nemoto et al., 2001; Silva et al., 2008). In addition, there are several tumor types that show increased FASN expression, but correlation with disease progression or patient survival has not been investigated or published at this time. These tumors include hyperplastic parathyroid (Alo et al., 1999a), stomach carcinoma (Kusakabe et al., 2002), mesothelioma (Gabrielson et al., 2001), glioma (Zhao et al., 2006), and hepatocellular carcinoma (Yahagi et al., 2005).

Increased FASN expression in tumors is an early, common event (Swinnen et al., 2002; Myers et al., 2001) and its correlation with reduced survival and increased recurrence rationalizes the potential for anti-FASN tumor therapeutics (Kuhajda, 2000, 2006; Kridel et al., 2007). As evidence that lipogenesis as a whole is important in cancer, many of the enzymes upstream of FASN show altered expression patterns in human tumor cells, as well. For instance, ACL is overexpressed in cancer cells of breast and bladder (Szutowicz et al., 1979; Turyn et al., 2003). ACC is overexpressed in breast and prostate cancer cells (Milgraum et al., 1997; Swinnen et al., 2000b, 2006; Heemers et al., 2003). Interestingly, the tumor suppressor breast cancer susceptibility gene 1 (BRCA1) can bind the phosphorylated inactive ACC to prevent re-activation (Moreau et al., 2006). In addition, squamous cell carcinomas of the lung show lower immunohistochemical staining of phosphorylated inactive ACC than adenocarcinoma with poor prognosis (Conde et al., 2007). The strong

functional correlation between upstream mediators of fatty acid synthesis and cancer underscores the importance of this pathway in tumor biology.

## 7.3 FASN Regulation

### *7.3.1 FASN Regulation in Normal Cells*

In nonmalignant tissues, FASN expression is primarily regulated at the transcriptional level (Fig. 7.2A) (Hillgartner et al., 1995). There is a single *FASN* gene and the signals in normal cells that stimulate *FASN* transcription are numerous but strictly defined (Amy et al., 1990). Transcription of *FASN* is stimulated by dietary carbohydrate, glucose, insulin, amino acids, sterols and cyclic-AMP through specific response elements (Paulauskis and Sul, 1988; Rufo et al., 2001; Foufelle et al., 1992; Moustaid et al., 1994; Wang and Sul, 1998; Wakil et al., 1983; Rangan et al., 1996; Wakil, 1989). Hormones such as the thyroid hormone triiodothyronine (T3) (Moustaid and Sul, 1991), progesterone (Lacasa et al., 2001), androgen (Heemers et al., 2003) and adrenal glucocorticoids (Volpe and Marasa, 1975) can also upregulate FASN in liver and adipose tissues. *FASN* transcription is mediated by multiple transcription factors.

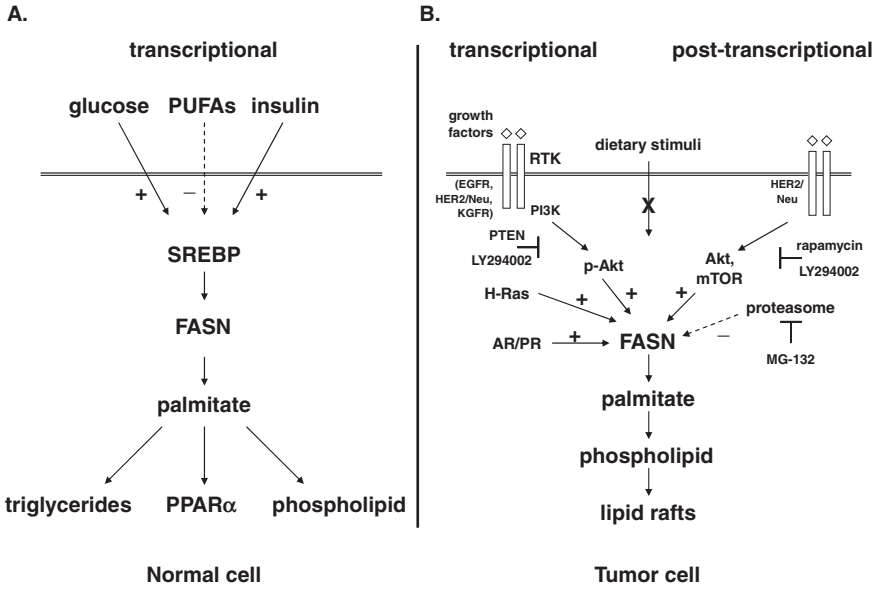


**Fig. 7.2** Regulation of FASN Expression in Normal and Tumor Cells. **A.** In normal cells (hepatocytes and adipocytes) FASN expression is primarily regulated through transcriptional mechanisms. **B.** In tumor cells, FASN expression is regulated by transcriptional and non-transcriptional mechanisms via multiple pathways

Upstream stimulatory factors (USFs) are required for insulin mediation of FASN expression, but other factors such as nuclear factor Y (NF-Y) and stimulatory protein 1 (SP1) can also play a role in *FASN* transcription (Teran-Garcia et al., 2007; Bennett et al., 1995). However, the vast majority of FASN-regulatory signals act through a family of transcription factors known as sterol response element binding proteins (SREBPs) that control lipid homeostasis and bind to various elements in the *FASN* promoter. There are three SREBP family members: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a and SREBP-1c have been most widely linked to regulation of lipogenic gene transcription, while SREBP-2 is most linked to cholesterol metabolism. The SREBPs exist as endoplasmic reticulum membrane bound precursors that are activated after proteolytic processing by site-one and site-two proteases (S1P, S2P). When sterol levels are low, S1P cleaves the SREBP molecule to release the N terminal portion from the endoplasmic reticulum (Sakai et al., 1998). SREBP then binds to the SREBP cleavage activating protein (SCAP) and is translocated to the Golgi where S2P further processes the molecule so that the transcription factor is activated. The processed SREBP then translocates to the nucleus to bind specific E box motifs and sterol response elements (SREs) (Magana and Osborne, 1996). There is evidence that dietary factors stimulate the expression of FASN in a manner mediated through signaling pathways such as the phosphoinositide-3 kinase (PI3K) pathway. For instance, nonmalignant 3T3-L1 adipocytes regulate insulin-mediated FASN expression through Akt in a manner independent of both mitogen activated protein kinase (MAPK) and P70 S6 kinase, but dependent on SREBPs (Wang and Sul, 1998; Porstmann et al., 2005).

Expression of FASN is tightly controlled so that transcription does not continue unabated under typical circumstances. Polyunsaturated fatty acids (PUFAs) (Xu et al., 1999; Moon et al., 2002; Jump et al., 1994), sterols (Adams et al., 2004; Bennett et al., 1995), and leptin (Fukuda et al., 1999) all act to repress FASN transcription and do so by specifically down-regulating SREBP-1 in hepatocytes (Worgall et al., 1998; Teran-Garcia et al., 2007). This highly complex organization of checks and balances for FASN expression is necessary to supply the cell with essential *de novo* fatty acids for cellular function and growth (Fig. 7.2A). Just as importantly, controls keep the cell from continuing unnecessary lipogenesis.

### *7.3.2 FASN Regulation in Tumor Cells*

While FASN expression is tightly controlled through dietary and hormonal stimuli in nonmalignant cells, tumor cells ignore these restrictions and increase FASN beyond typical levels (Fig. 7.2B). In fact, an early study of orthotopic hepatomas revealed that while low-fat, high-fat, and high-cholesterol diets all affected rates of fatty acid synthesis in the normal liver, the rates of hepatoma

fatty acid synthesis were unchanged (Sabine et al., 1967). It has since been discovered that deregulation of upstream signals drive FASN expression in a manner that is largely transcriptional in tumors (Fig. 7.2B) (Swinnen et al., 2006).

Overexpression of FASN in tumor cells is induced at the transcriptional level by receptor tyrosine kinase (RTK)-stimulation of Ras and Akt (Fig. 7.2B). Keratinocyte growth factor (KGF) can induce the Akt- and cJun N-terminal kinase (JNK)-dependent expression of FASN in pulmonary cancer cells (Chang et al., 2005). Epidermal growth factor (EGF) has also been shown to increase FASN in prostate cancer cells (Swinnen et al., 2000a).

In addition to growth factor signaling, activation of the RTK HER2/Neu is linked with *FASN* expression in tumor cells. HER2/Neu upregulates PI3K-dependent *FASN* transcription in breast cancer cells (Kumar-Sinha et al., 2003; Yoon et al., 2007). Interestingly, blocking HER2/Neu with Herceptin decreases FASN expression (Kumar-Sinha et al., 2003). In fact, there appears to be a crosstalk between these pathways, as inhibition of FASN activity leads to the downregulation of HER2/NEU (Menendez et al., 2004). While HER2/Neu is primarily associated with breast cancer progression, HER2/Neu and FASN expression correlate in squamous cell carcinomas of the tongue, as well (Silva et al., 2008). Surprisingly, HER2/Neu can also regulate FASN expression in prostate cancer cells (Yeh et al., 1999). These data suggest there is a coordinate regulation of activated HER2/Neu and FASN upregulation in tumor cells.

Downstream of RTK signaling, the PI3K/Akt pathway has been shown to upregulate FASN. Loss of PTEN is a frequent transformation event in cancer, that leads to a gain of function in Akt signaling (Mulholland et al., 2006; Blanco-Aparicio et al., 2007). In prostate cancer cells, this signaling cascade drives androgen receptor (AR)-mediated oncogenic transcription and progression to metastatic disease (Wang et al., 2003; Mulholland et al., 2006). The PTEN-null LNCaP tumor cell line has high levels of FASN. Reintroducing PTEN or using the PI3K inhibitor LY294002 can decrease FASN expression, whereas introducing constitutively active Akt can restore FASN expression (Van de Sande et al., 2002). The connection between FASN expression and PI3K activity is further observed in prostate carcinoma samples with high Gleason scores, where high FASN expression correlates with phosphorylated Akt that is localized to the nucleus (Van de Sande et al., 2005). Moreover, a crosstalk between these pathways has been identified. In ovarian cancer cell lines, phosphorylated Akt correlates with and drives FASN expression. Conversely, inhibiting FASN results in decreased Akt phosphorylation (Wang et al., 2005). These data suggest that PI3K signaling through Akt is an important mediator of *FASN* transcription in tumor cells.

In addition to RTK-driven stimulation of Akt, there is evidence that the small GTP-ase protein Ras can influence FASN expression in tumors. Constitutively active H-ras induces increased PI3K and MAPK-dependent FASN expression in MCF-10A cells (Yang et al., 2002). Consistent with this notion, the expression of activated K-ras correlates with FASN expression in human

colorectal cancer samples (Ogino, et al., 2006, 2007). Altogether, these data suggest that RTK signaling, Ras, and PI3K-Akt pathways can drive transcriptional up-regulation of FASN expression in tumor cells (Fig. 7.2B).

Not surprisingly, hormones are another common factor driving FAS expression in tumor cells (Fig. 7.2B). Progestins stimulate FASN expression in breast cancer cells (Chalbos et al., 1987; Lacasa et al., 2001; Menendez et al., 2005a). Consistent with this finding, increased FASN expression in endometrial carcinoma correlates with expression of both estrogen and progesterone receptors (PR) (Pizer et al., 1998b). In prostate cancer, FASN expression can be regulated by androgens in prostate cancer through upregulation of transcription factors such as S14 and SREBPs (Swinnen et al., 1997a,b; Heemers et al., 2000, 2001). In addition, HER2/Neu can drive activation of AR in prostate cancer cells to increase MAPK-dependent induction of FASN in the absence of androgen (Yeh et al., 1999).

While the main mechanism of FASN overexpression in tumors is through transcriptional upregulation, there is also evidence that FASN is regulated by post-transcriptional mechanisms (Fig. 7.2B). For instance, HER2/Neu driven expression of both FASN and ACC can be regulated at the translational level through Akt, PI3K, and mTOR-dependent mechanisms (Yoon et al., 2007). FASN stabilization is tightly linked with the de-ubiquitinating enzyme ubiquitin-specific protease 2a (USP2A) in prostate cancer cells. USP2A is androgen regulated and is not only upregulated similarly to FASN, but actually interacts with FASN to enhance FASN stability (Graner et al., 2004). Treating prostate tumor cells with the proteasome inhibitor MG-132, also increases FASN expression, further supporting evidence that FASN is regulated by the proteasome (Graner et al., 2004). Interestingly, yeast studies provided early evidence of FASN regulation by proteasomal degradation (Egner et al., 1993). It is also worth mentioning that FASN can also be upregulated in cancer cells by *FASN* gene amplification (Shah et al., 2006). The fact that numerous mechanisms act to increase FASN expression in tumor cells highlights the importance of FASN in tumor progression.

### *7.3.3 Palmitate Utilization in Normal and Tumor Cells*

Upregulation of FASN activity causes the increased production of fatty acids, particularly palmitate. While the mechanisms that drive FASN expression are different in tumors as compared to normal cells, the utilization of its products differs, as well. Fatty acids are used for a variety of cellular functions. In nonmalignant adipose and hepatic tissue, palmitate is incorporated into triglycerides for secretion and storage to be ultimately used as an energy source through β-oxidation (Thupari et al., 2002). Fatty acids such as palmitate can also comprise a regulatory pool that activates energy mediators such as PPARα in the liver and hypothalamus (Chakravarthy, et al., 2005, 2007). In addition,

key signaling molecules, such as Ras and Hedgehog, can be palmitoylated to target these proteins to cellular membranes (Resh, 2006). So far, a link between protein palmitoylation and FASN activity has not been established though. In development, fatty acids can segregate into phospholipids to create cellular membranes (Chirala et al., 2003). Similarly, tumor FASN-derived palmitate segregates into phospholipid microdomains known as lipid rafts (Fig. 7.2B) (Swinnen et al., 2003). Lipid rafts are involved in a number of key biological functions including signal transduction, polarization, trafficking, and migration (Freeman et al., 2005, 2007). Considering that palmitate can ultimately be used for a number of cellular processes, including being elongated and desaturated for subsequent events, it is apparent that FASN occupies an important niche in tumor cells.

## 7.4 Inhibiting FASN Activity

### *7.4.1 Small Molecule Inhibitors of FASN*

Because of the unique expression of FASN in tumors, much emphasis has been put toward the development of pharmacological agents that inhibit FASN activity and, therefore, inhibit tumor growth and progression. Historically, a *Cephalosporium caerulens* mycotoxin metabolite known as cerulenin [(2S, 3R)-2,3-epoxy-4-oxo-7,10-dodecadienoylamide] has been the primary FASN inhibitor used in biological studies. Cerulenin covalently binds the β-ketoacyl synthase domain in FASN that is responsible for binding and condensing the substrates (Funabashi et al., 1989). More recently, C75 was formulated as a synthetic analog of cerulenin due to instability and poor systemic availability of cerulenin (Kuhajda et al., 2000). C93 is the newest generation of C75 analogues (Zhou et al., 2007). Both C75 and C93 target the β-ketoacyl synthase activity of FASN (Kuhajda et al., 2000; Zhou et al., 2007). Recently, orlistat (Xenical©), a FDA-approved drug for obesity that targets gastrointestinal lipases, was described as a novel inhibitor of FASN thioesterase activity (Kridel et al., 2004). There also exists a growing body of literature showing that various natural products such as the green tea polyphenolic component epigallocatechin-3-gallate (EGCG) can inhibit FASN activity (Tian, 2006).

### *7.4.2 Effects* In Vivo

To date, all small molecule inhibitors of FASN have demonstrated ability to block tumor growth *in vivo*. Cerulenin greatly increases survival and delays progression of ovarian cancer xenografts without significantly affecting fatty acid synthesis in the liver (Pizer et al., 1996b). C75 reduces growth of several tumor xenograft models, including prostate, breast, ovarian and mesothelioma

(Pizer et al., 2000, 2001; Wang et al., 2005; Gabrielson et al., 2001). C93 and C75 both reduce ovarian and lung cancer xenograft growth (Zhou et al., 2007; Orita et al., 2007). The novel FASN inhibitor orlistat has also been shown to inhibit prostate tumor xenograft growth (Kridel et al., 2004). FASN inhibitors also work in genetic models of tumorigenesis, including the *Neu-N* murine mammary transgenic model (Hennigar et al., 1998; Pflug et al., 2003; Alli et al., 2005). While FASN inhibitors are not typically given orally due to poor bioavailability, recent work shows that C93 can work *in vivo* after oral administration (Orita et al., 2007). Surprisingly, cerulenin, C75 and related compounds induce a reversible anorexic phenotype that is associated with β-oxidation in the hypothalamus. This phenotype is mimicked in mice with *FASN* deleted in the hypothalamus (see Section 7.2.1) (Loftus et al., 2000; Thupari et al., 2004; Tu et al., 2005; Orita et al., 2007; Chakravarthy et al., 2007). Interestingly, the anorexic effect of FASN inhibitors has been overcome with newer generation drugs like C93 that can reduce tumor growth with no anorexic effect (Orita et al., 2007). The discrepancy between the knockout studies and pharmacological findings has yet to be explained.

### *7.4.3 Cell Cycle Effects* In Vitro

To determine the cellular consequences of FASN inhibition, numerous studies have focused on the *in vitro* anti-tumor effects of these inhibitors. Many studies have linked FASN inhibitors with cell cycle and growth arrest (Fig. 7.3). Cerulenin acts *in vitro* to inhibit fatty acid synthesis-mediated growth of breast carcinoma cells that can be rescued with palmitate (Kuhajda et al., 1994). Cerulenin induces a block at the G2/M cell cycle checkpoint in an androgen-independent

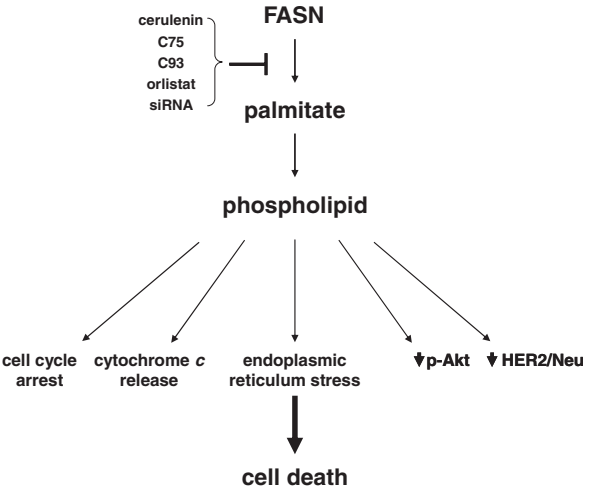


**Fig. 7.3** Inhibiting FASN in Tumor Cells. Several small molecule drugs can inhibit FASN activity. Blockade of FASN activity leads to a reduction in lipogenesis and phospholipid content in tumor cells. Inhibiting FASN also induces cycle arrest, cytochrome *c* release, and endoplasmic reticulum stress. In addition, FASN inhibitors can reduce the activation and expression of Akt and HER2/Neu

prostate cancer cell line that correlates with an induction of cyclin-dependent kinase inhibitors p21 and p27 (Furuya et al., 1997). However, glioma cells accumulate in S phase after cerulenin treatment (Zhao et al., 2006). Different hepatocellular carcinoma cell lines treated with C75 undergo either G1 or G2 cell cycle arrest independent of p53 status (Gao et al., 2006). In melanoma A-375 cells, cerulenin induces accumulation of cells in S phase, while C75 induces accumulation of G2/M phase cells (Ho et al., 2007). RKO colorectal cancer cells treated with either cerulenin or C75 show a transient accumulation of cells in S and G2/M phases, but accumulation in G1 and G2/M phases later (Li et al., 2001). Both cerulenin and C75 induce S phase arrest and inhibit DNA replication in breast, colorectal, and promyelocytic leukemia cancer cells (Pizer et al., 1998a). Orlistat induces cell cycle arrest by downregulating Skp2, a deubiquinating enzyme, leading to decreased turnover of p27/kip1, therefore blocking prostate tumor cells from entering S phase (Knowles et al., 2004). Orlistat has also been shown to induce an accumulation of breast cancer cells in S phase (Menendez et al., 2005b). Use of RNA interference (RNAi) to mediate knockdown of both the FASN and ACC$\alpha$ genes induces a decrease in S phase cells, further supporting the role of fatty acid synthesis in progression to or in S phase (Brusselmans et al., 2005). The data show there is little consensus on the phase that tumor cells arrest growth after inhibition of FASN in various tumor cells, which may be attributed to different tumor cell types. It is likely that a lack of *de novo* fatty acid synthesis in tumor cells impacts phospholipid synthesis required for proper DNA synthesis and cell cycle progression (Jackowski, 1994).

### *7.4.4 Cell Signaling Effects*

The effects of FASN inhibitors are also mediated through key tumor signaling pathways. For example, it has been demonstrated that pharmacological inhibition of FASN activity results in reduced Akt activation in multiple tumor cell lines (Fig. 7.3) (Wang et al., 2005; Liu et al., 2006). As mentioned previously, it has been demonstrated that PI3K and Akt can drive FASN expression in tumor cells (Fig. 7.2B) (Van de Sande et al., 2002; Wang et al., 2005). The demonstration that reduced FASN activity negatively affects Akt activation identifies a feedback between the two pathways. Not surprisingly, inhibiting the PI3K pathway synergizes with cell death induced by genetic and pharmacological inhibition of *FASN* (Bandyopadhyay et al., 2005; Wang et al., 2005; Liu et al., 2006).

In addition to the PI3K pathway, HER2/Neu has also been linked with FASN expression in breast and prostate cancer cells (Kumar-Sinha et al., 2003; Yoon et al., 2007; Yeh et al., 1999). Inhibiting FASN with cerulenin and C75 reduces expression of Her2/neu expression in breast cancer cell lines (Fig. 7.3) (Menendez et al., 2004; Kumar-Sinha et al., 2003). Additionally, inhibiting Her2/Neu with Herceptin synergizes with FASN inhibitors to induce

cell death (Menendez et al., 2004). Altogether, these data indicate that the very pathways that drive FASN expression in malignant cells are also affected when FASN activity is blocked. Moreover, tumor cell killing can be potentiated when FASN inhibitors are combined with inhibitors of these signaling pathways. The reason for this crosstalk has not been clearly defined, but it is tempting to speculate that inhibition of FASN activity directly impacts on lipid raft function, which results in reduced kinase signaling.

### *7.4.5* In Vitro *Tumor Cell Death*

In addition to cell cycle arrest, all FASN inhibitors induce cell death in tumor cells (Pizer et al., 1996a, 1998a; Kridel et al., 2004; Zhou et al., 2007). Cerulenin induces breast and prostate cancer cell death that correlates with DNA fragmentation and morphology characteristic of apoptosis (Pizer, et al., 1996a, 2000; Furuya, et al., 1997). The mitochondria have also been linked to facilitation of cell death induced by cerulenin. For instance, the pro-apoptotic mitochondrial factor Bax is induced in cells treated with cerulenin. (Heiligtag et al., 2002). This correlation between cerulenin and the mitochondrial pathway of apoptosis is further supported by the induction of cytochrome *c* release (Fig. 7.3) (Heiligtag et al., 2002). FASN inhibition has been linked to p53 status of tumor cells, but whether p53 plays any role in FASN-expressing cells is unclear, as FASN is expressed in tumors independent of p53 status. FASN is strongly and significantly associated with p53 expression in hyperplastic parathyroids (Alo et al., 1999a). In various cancer cells, blocking p 53 activity with a dominant negative construct potentiates FASN inhibitor-induced cell death (Li et al., 2001). Conversely, others have reported that FASN inhibitors work equally well in tumors independent of p53 status (Heiligtag et al., 2002).

Cell death induced by FASN inhibitors could be a result of the cell lacking fatty acid for membrane biogenesis. Inhibiting FASN and ACC reduces incorporation of fatty acid into membrane phospholipids, which occurs in the endoplasmic reticulum (Zhou et al., 2003). Inhibiting FASN incorporation into phospholipids corresponds to a decrease in cell volume and other morphological changes ultimately leading to apoptosis (De Schrijver et al., 2003). Inhibiting FASN with small molecules (cerulenin, C75, orlistat), or with siRNAs induces endoplasmic reticulum stress and activation of the unfolded protein response (UPR) (Little et al., 2007). The UPR is able to induce cell death if homeostasis is not restored and, therefore, FASN inhibitors may be inducing cell death that is mediated by the UPR (Fig. 7.3) (Little et al., 2007).

When FASN is inhibited malonyl-CoA accumulates (Pizer et al., 2000). One hypothesis for the mechanism of FASN inhibitor-induced cell death is attributed to this accumulation of malonyl-CoA and, potentially, its interaction with CPT-1, the enzyme responsible for transferring fatty acids into the

mitochondria for oxidation. Malonyl-CoA acts as a natural inhibitor of CPT-1 activity to prevent fatty acids being simultaneously synthesized and then oxidized (McGarry et al., 1983). Driving this hypothesis is a study showing that co-treating breast or ovarian cancer cells with the ACC inhibitor 5-(tetradecyloxy)-2-furoic acid (TOFA) partially rescues cell death induced by FASN inhibitors C75 and cerulenin (Pizer et al., 2000; Zhou et al., 2003). However, C75 alone can increase CPT-1 activity and directly compete with malonyl-CoA (Thupari et al., 2002; Yang et al., 2005). Therefore, it is important to note that MCF-7 cells co-treated with C75 and the CPT-1 inhibitor etomoxir show no effect on C75-induced cell death (Zhou et al., 2003). Hence, malonyl-CoA accumulation, not CPT-1 activation, is mediating death induced by FASN inhibitors (Fig. 7.3). In addition, siRNA-mediated knockdown of FASN induces accumulation of ceramide and malonyl-CoA that leads to inhibition of CPT-1 and induction of apoptotic genes *BNIP3*, *TRAIL*, and *DAPK2* (Bandyopadhyay et al., 2006).

Upstream lipogenesis mediators ACL and ACC are also important in maintaining tumor cell survival. RNAi-mediated knockdown or chemical inhibition of ACL in human tumor cells decreases proliferation and induces cell death *in vitro* and limits tumor growth by stimulating differentiation of tumor cells *in vivo* (Hatzivassiliou et al., 2005). ACL inhibition can also can impair Akt-mediated tumorigenesis and induce tumor cell death (Bauer et al., 2005). In addition, silencing ACC using RNAi induces apoptosis in breast and prostate cancer cells (Brusselmans et al., 2005; Chajes et al., 2006). Chemical inhibition of ACC can also induce tumor cell death (Beckers et al., 2007). While the effects of FASN inhibitors on tumor cells are clearly pleiotropic, and in some cases maybe even specific to the tumor type, it is evident that many of the effects can ultimately be tied to decreases in de novo synthesized fatty acids which can be extended to phospholipid synthesis. Whatever the mechanisms may be, the data clearly suggest that FASN occupies an important regulatory position in tumor cells to facilitate the processes that lead to tumor cell proliferation and survival.

## 7.5 Concluding Remarks

In summary, FASN is upregulated in multiple tumor types and correlates with poor patient prognosis and reduced survival. Correspondingly, a body of literature has demonstrated a requirement of FASN activity for tumor cell viability. Phospholipids synthesized from FASN-derived palmitate are important for cell cycle progression, lipid raft signaling, and endoplasmic reticulum homeostasis, all of which contribute to tumor cell survival, thereby, underscoring the importance of FASN. These findings signify a central role for fatty acid synthesis in critical cellular processes. In addition, tumor cells have developed feedback mechanisms to mediate crosstalk between FASN and signaling

pathways like PI3-kinase and Her2/Neu. The discovery and development of pharmacological agents that block FASN activity suggest that FASN can be targeted for anti-tumor therapy. So far, anti-FASN drugs have successfully inhibited tumor growth in several tumor models with minimal side effects. Therefore, FASN represents a highly tractable anti-tumor target with significant clinical potential.

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