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Progress in the development of fatty acid synthase inhibitors as anticancer targets

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ABSTRACT

Fatty acid synthase (E.C. 2.3.1.85; FASN) is a multifunctional enzyme system that catalyzes the formation of fatty acids from acetyl-CoA, malonyl-CoA, and NADPH and plays a central role in lipid biosynthesis. Two classes of FASN exist: FASN I in animals and fungi, and FASN II in plants and prokaryotes. Animal FASN I is a homodimeric protein found in the cytosol of lipogenic tissues such as the liver and brain. Many human carcinomas exhibit elevated levels of FASN I, though the benefit to cancer cells is still unclear. Inhibition of FASN I selectively effects apoptosis in cancer cells, and the role of FASN I in chemotherapy is a growing area of research with the use of natural products and small molecule inhibitors.

The de novo synthesis of the fatty acid palmitate from acetyl-CoA, malonyl-CoA, and NADPH is catalyzed by the multifunctional fatty acid synthase (E.C. 2.3.1.85; FASN) system.[1] Palmitate is a 16 carbon saturated fatty acid that is a major component of cell membranes and human breast milk, and is incorporated into triglycerides for energy storage. Palmitate is also a substrate in the palmitoylation of membrane proteins and a precursor in the synthesis of complex lipids such as glycerophospholipids and cholesterol.[1a] FASN is therefore a critical component in biosynthetic pathways.

Though FASN is ubiquitous among living organisms, it exists in two different forms, denoted FASN I or II.[2] FASN II is present in prokaryotes and plants, in which each enzymatic function is found on a discrete peptide.[3] Conversely, FASN I in fungi and animals exists as a large multifunctional complex, either in a $\alpha_6\beta_6$ configuration (fungi) or as a homodimer (animals). Until recently, the two identical subunits of FASN I were thought to adopt an antiparallel, H-shaped arrangement. Advances in crystallography have revealed that animal FASN I is an X-shaped homodimer in which the parallel subunits form dimeric structures at three domains along each peptide.[4]

The de novo synthesis of palmitate by FASN I utilizes CO_2, in the form of acetyl-CoA and malonyl-CoA, from carbohydrate or amino acid catabolism pathways.[1b] Malonyl-CoA is first formed from cytosolic acetyl-CoA by acetyl-CoA carboxylase in an ATP-dependent synthetase reaction. This preparatory step is the rate-limiting step of lipid biosynthesis. The overall equation for the synthesis of palmitate by FASN is as follows:

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

The single acetyl-CoA acts as a primer and each malonyl-CoA molecule supplies two carbons in a series of sequential elongations of the carbon chain. The six enzymes of FASN-I carry out this process: β-ketoacyl synthase (KS), malonyl/acetyl transferase (MAT), dehydrase (DH), enoyl reductase (ER), β-ketoacyl reductase (KR), and thioesterase (TE).[2] A prosthetic group called acyl carrier protein (ACP) forms thioester bonds with all acyl intermediates through its 4′-phosphopantetheine moiety.[3]

In the priming reaction of fatty acid synthesis, MAT transfers an acetyl group from CoA to ACP, which in turn transfers it to the active site of KS. Each malonyl group is also transferred to ACP by MAT; the order of these transfers by the same enzyme is random and rapid because it is not predominantly specific for either substrate.[2] Next, KS couples the condensation of malonyl-ACP and acetyl-KS with the exergonic decarboxylation of the malonyl group. The result is acetoacetyl-ACP, which is reduced to butyryl-ACP by DH, ER, and KR. Butyryl-ACP is then transferred to the active site of KS, where it undergoes condensation with another malonyl group. This cycle of condensation and reduction (Fig. 1) repeats until palmitate is released by TE.[3]

Variation among organisms of substrate specificities contribute to deviations from the accepted reaction sequence of palmitate synthesis. For example, acetoacetyl-CoA can be used as a primer

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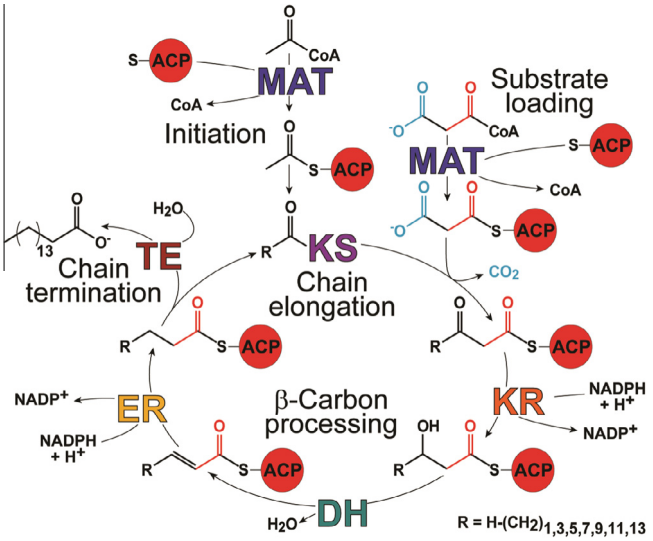

Figure 1. Palmitate synthesis from acetyl-CoA and malonyl-CoA by the enzymes of FASN I. Reprinted with permission from *Nat. Struct. Mol. Biol.* **2009**, *16*, 190.

by human FASN I in place of acetyl-CoA.[5] Human FASN I also does not yield 100% palmitate but rather 90% palmitate, 4% myristate, and 6% stearate as its final products, which is similar to rat FASN but significantly different from chicken FASN. The final product of FASN is dependent on termination by TE, which has low specificity for substrates with fewer than 16 carbon atoms; therefore, the ratio of palmitate to other termination byproducts relies on the unique specificity of TE in a given organism.[3]

Human FASN I is a 540 kDa homodimeric protein with 2511 amino acid residues.[2] Each peptide is transcribed from a single mRNA and contains seven functional domains with a non-catalytic core sequence (Fig. 2) that provides structural stability. The linear arrangement of enzymatic domains has been determined with proteolytic techniques.[1a]

The inability to obtain FASN I crystals for X-ray crystallography was a major obstacle to the elucidation of the quaternary structure of the homodimer until the past ten years.[1a,6] Attempts to describe the structure of FASN I were made with small angle neutron scattering, electron cryomicroscopy, and X-ray solution scattering and the predominant model for FASN I was an antiparallel, H-shaped arrangement.[3] When the crystal structure of mammalian FASN was obtained in 2008, it was revealed that the two 260 kDa subunits are in a parallel X-shaped arrangement (Fig. 3) with three dimeric sections between the KS, ER, and DH domains of each peptide.[4,6] This structure has a high degree of conformational flexibility which allows the transfer of substrate within the enzyme complex during elongation.[2]

The homodimer of animal FASN is the active conformation. Most enzyme domains on isolated monomeric subunits retain function, but KS activity is greatly impaired and palmitate synthesis does not occur in the monomer.[2] Interestingly, studies with 'knockout' FASN in which the dimer consists of one functional FASN protein and one modified, non-functional FASN subunit show palmitate synthesis proceeds in the dimer with only a single complement of each catalytic activity. This suggests structural, rather than chemical, reasons for the homodimeric conformation.

Animal FASN I is primarily expressed in the cytosol of healthy liver, brain, adipose, lung, and lactating mammary gland cells where lipogenesis is an important process.[5] Many human carcinomas also exhibit high levels of FASN, though its benefit to cancer cells has not been determined.[7] Inhibition of the FASN enzyme has been found to cause selective apoptosis of cancer cells.[8] Knowledge of the mechanism by which FASN inhibition causes apoptosis will be instrumental in the development of chemotherapeutic agents.

FASN is overexpressed in several human carcinomas such as breast,[9] colon,[10] esophageal,[11] lung,[12] melanoma,[13] ovarian,[14] pancreatic,[15] prostate,[16] and stomach[17] cancers. Regulation of FASN in cancer cells is markedly different than in healthy cells.[18] Whereas in liver and adipose cells FASN is transcriptionally regulated by nutritional factors via insulin, glucagon, glucocorticoids, and thyroid hormone, FASN is regulated by the transcription factor sterol regulatory element binding protein 1-c (SREBP-1c) in cancer cells.[8] FASN I present in carcinomas is also unaffected by exogenous levels of fatty acids.[7]

The incentive for increased levels of FASN I in neoplasms is yet unknown. Fatty acid synthesis is energy-intensive, and palmitate is not utilized differently in cancer cells.[7] Additionally, palmitate is produced in excess of cell requirements and secreted, indicating that the end product is not the reason for upregulation of FASN. The proposed link between high levels of glycolysis in cancer and lipogenesis has also been refuted. The biosynthetic activity of FASN I is not suspected as the impetus for its overexpression in carcinomas, though its specific role remains a topic of research.

Inhibition of FASN I is known to selectively target cancer cells for apoptosis.[19] Several postulated apoptic mechanisms of FASN inhibition include disturbance of membrane function, inhibition of DNA replication, inhibition of anti-apoptic proteins, and accumulation of the substrate malonyl-Co-A (Fig. 4). Studies have shown that it is the accumulation of malonyl-CoA, the FASN substrate, and not the depletion of palmitic acid, the final product of fatty acid synthesis, is the cause of death of cancer cells.[20]

Though the potential apoptic pathways of FASN inhibition are currently still under investigation, anti-cancer agents that target FASN catalytic domains are already known (Fig. 5).[8,21] Natural products serve as an inspiration for drug leads.[22]

Cerulenin is a metabolite produced from the fungus *Cephalosporum caerulens* that inhibits sterol and fatty acid biosynthesis (Fig. 5).[23] Cerulenin binds specifically and irreversibly with the epoxy group to the β-ketoacyl synthase (KS) domain of FASN, thus preventing the condensation reaction between the elongating fatty acid and successive acetyl or malonyl residues. Though it is a potent FASN inhibitor, it is chemically unstable and exhibits toxic side effects. Cerulenin was active against breast, melanoma, ocular, and ovarian cancer lines (Table 1, entries 1–4).

Synthetic analogs such as C75 lacking the reactive epoxy group have been designed to have fewer side effects.[24] C75 binds irreversibly to KS, ER, and TE as a competitive inhibitor.[25] Both cerulenin and C75 treatment induces rapid and profound weight loss,

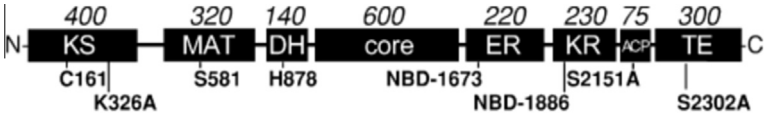

Figure 2. Generalized linear domain map of animal FASN I. The approximate number of residues in each domain is noted. Reprinted with permission from *Prog. Lipid Res.* **2003**, *42*, 289.