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Original Article

Inhibition of de novo Palmitate Synthesis by Fatty Acid Synthase Induces Apoptosis in Tumor Cells by Remodeling Cell Membranes, Inhibiting Signaling Pathways, and Reprogramming Gene Expression

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ABSTRACT

Inhibition of de novo palmitate synthesis via fatty acid synthase (FASN) inhibition provides an unproven approach to cancer therapy with a strong biological rationale. FASN expression increases with tumor progression and associates with chemoresistance, tumor metastasis, and diminished patient survival in numerous tumor types. TVB-3166, an orally-available, reversible, potent, and selective FASN inhibitor induces apoptosis, inhibits anchorage-independent cell growth under lipid-rich conditions, and inhibits in-vivo xenograft tumor growth. Dose-dependent effects are observed between 20–200 nM TVB-3166, which agrees with the IC_{50} in biochemical FASN and cellular palmitate synthesis assays. Mechanistic studies show that FASN inhibition disrupts lipid raft architecture, inhibits biological pathways such as lipid biosynthesis, PI3K–AKT–mTOR and β-catenin signal transduction, and inhibits expression of oncogenic effectors such as c-Myc; effects that are tumor-cell specific. Our results demonstrate that FASN inhibition has anti-tumor activities in biologically diverse preclinical tumor models and provide mechanistic and pharmacologic evidence that FASN inhibition presents a promising therapeutic strategy for treating a variety of cancers, including those expressing mutant K-Ras, ErbB2, c-Met, and PTEN. The reported findings inform ongoing studies to link mechanisms of action with defined tumor types and advance the discovery of biomarkers supporting development of FASN inhibitors as cancer therapeutics.

Research in context: Fatty acid synthase (FASN) is a vital enzyme in tumor cell biology; the over-expression of FASN is associated with diminished patient prognosis and resistance to many cancer therapies. Our data demonstrate that selective and potent FASN inhibition with TVB-3166 leads to selective death of tumor cells, without significant effect on normal cells, and inhibits in vivo xenograft tumor growth at well-tolerated doses. Candidate biomarkers for selecting tumors highly sensitive to FASN inhibition are identified. These preclinical data provide mechanistic and pharmacologic evidence that FASN inhibition presents a promising therapeutic strategy for treating a variety of cancers.

1. Introduction

Fatty acid synthase (FASN) is a homodimeric and multi-functional enzyme that catalyzes the biosynthesis of palmitate in a NADPH-dependent reaction (Maier et al., 2006). Normal cells in adult tissue ubiquitously express low to moderate levels of FASN; however, these cells, which primarily import lipids from the extracellular milieu, do not have a strict requirement for FASN activity. This is demonstrated in a variety of mouse models with tissue-specific knockout of FASN expression that are characterized by the absence of an effect under non-stress conditions (Chirala et al., 2003; Shearn et al., 2014). In contrast, tumor cells have an increased requirement for lipids in functions such as membrane biosynthesis, protein modification, and as signaling molecules. Consequently, tumor cells are more dependent on de novo palmitate synthesis catalyzed by FASN than normal cells (Menendez and Lupu, 2007; Flavin et al., 2010). Accordingly, FASN is overexpressed in many solid and hematopoietic tumors, including breast, ovarian, prostate, colon, lung, and pancreatic (Ueda et al., 2010; Shah et al., 2006; Zaytseva et al., 2012; Witkiewicz et al., 2008; Sebastiani et al., 2006). Moreover, FASN tumor expression is increased in a stage-

Abbreviations[1]: NADPH, nicotinamide adenine dinucleotide phosphate; HUVEC, human umbilical vein endothelial cells; NSCLC, non-small-cell lung cancer; CRC, colorectal cancer; TGI, tumor growth inhibition; MEM, minimal essential media; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; LC–MS, liquid chromatography–mass spectrometry; PBS, phosphate buffered saline; FITC, fluorescein isothiocyanate.

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[1] Gene symbols and common abbreviations such as DNA and RNA are not defined.

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dependent manner that is associated with diminished patient survival (Ueda et al., 2010; Tao et al., 2013; Nguyen et al., 2010; Notarnicola et al., 2012; Witkiewicz et al., 2008; Zaytseva et al., 2012). This expression–prognosis relationship suggests that FASN plays an important role in affecting tumor cell biology and therapeutic response across a wide range of cancer types.

Alteration of energy and macromolecular biosynthetic metabolism in tumor cells compared to non-tumor cells is well established and known as the Warburg effect, in recognition of Otto Warburg's hypothesis that extended from his observation that ascites tumor cells convert the majority of their glucose carbon to lactose in oxygen-rich environments (Ward and Thompson, 2012). Tumor cell survival, growth, and proliferation demand increased energy in the form of NADPH and increased macromolecular biosynthesis of DNA, RNA, protein, and lipids. Reprogramming of tumor cell mitochondrial metabolism to support these requirements occurs directly through growth factor signaling and the PI3K–AKT–mTOR pathway. AKT activation drives both glycolytic metabolism of glucose and mitochondrial metabolism that generates acetyl-CoA, the biosynthetic precursor of fatty acids, cholesterol, and isoprenoid synthesis. As a critical aspect of tumor cell metabolic reprogramming, mTORC1 complex activation occurs via AKT signal transduction. A central component of the mTORC1 cell growth program is stimulation of de novo lipogenesis via regulation of SREBP-mediated FASN expression (Shackelford and Shaw, 2009; Lupu and Menendez, 2006). In the synthesis of fatty acids, FASN consumes NADPH, acetyl-CoA, and malonyl-CoA. The consumption of these substrates as well as the production of fatty acids contributes to the sustained altered metabolic state that tumor cells require for growth and survival.

Palmitate and additional fatty acids derived from its function in diverse, vital biological processes. Fatty acids serve as precursors for synthesis of cellular lipids, as lipid bilayer constituents that affect membrane fluidity and architecture, and as substrates for post-translational protein modification that affect protein localization and activity. Palmitate affects membrane architecture at specialized plasma membrane microdomains known as lipid rafts. Lipid rafts are localized regions that contain high concentrations of lipids such as palmitate, cholesterol, and sphingosine, and also are rich in lipid-modified membrane-associated proteins that function in receiving, localizing, and transmitting cell growth signals (Simons and Sampaio, 2011; Staubach and Hanisch, 2011). Depletion of palmitate and other cellular lipids is expected to cause reorganization of membrane architecture and disruption of lipid raft domains. Growth factor and intracellular signal transduction require intricate membrane-associated protein–protein interactions that are dependent upon lipid raft architecture and protein lipidation. These lipid rafts facilitate the co-localization of proteins that must associate to form functional signaling complexes, and thereby regulate the efficiency of signal transduction as rafts increase and decrease in number and size. By disruption of membrane structure, FASN inhibition may disable signal transduction networks and biological processes required for cell growth, proliferation, and response to cellular stress. Activation of these pathways is a hallmark of cancer, and enables FASN inhibition to affect multiple points within a tumor cell that can produce anti-tumor activity.

FASN activity is intimately linked to receptor tyrosine kinase (RTK), PI3K–AKT–mTOR and MAPK signaling pathways, and activation of these pathways is a hallmark of aggressively growing tumor cells. Activation of the PI3K–AKT–mTOR pathway is among the most frequent aberrations in human cancers, and occurs through numerous different genetic lesions (Vivanco and Sawyers, 2002). The PI3K–AKT–mTOR pathway controls many biological processes that include glucose uptake and metabolism, protein synthesis, cell growth, and cell survival (Hollander et al., 2011). FASN gene expression is activated downstream of the PI3K–AKT–mTOR signal transduction pathway in response to cell metabolism and growth signals, and is driven by SREBP-1, ZBTB7A, and p53 family transcription factors (Van de Sande et al., 2005; Choi et al., 2008). Increased FASN activity promotes the tumorigenic capacity of cells via multiple mechanisms that include supporting enhanced macromolecular biosynthesis and glucose metabolism, cell growth and survival signal transduction, cellular stress response, and resistance to chemotherapeutic agents. In tumor cells, the connection between signal transduction pathways and FASN often becomes inextricably linked. Tumors with activated RTKs such as ERBB2 provide an example; whereby, the ERBB2–PI3K–AKT–FASN signaling axis results in continued stimulation of ErbB2 activity (Grunt et al., 2009). The interdependence enables tumor cells of this type to be killed with either ErbB2 or FASN inhibitors.

FASN inhibition using siRNAs and small molecules with varied biochemical mechanisms and selectivity profiles have been shown to inhibit Akt phosphorylation, induce tumor cell apoptosis, sensitize chemotherapy-resistant tumor cells to drug activity, and inhibit mouse xenograft tumor growth (Chuang et al., 2011; Kant et al., 2012; Kridel, 2004; Puig et al., 2008, 2009; Tomek et al., 2011). These activities of FASN inhibition have been reported in different tumor cell types that overexpress FASN, including, breast, ovary, prostate, and colorectal tumors. Despite the compelling support for FASN as an oncology therapeutic target, to date no compounds have progressed into clinical studies. Some compounds previously described in the literature suffered from significant pharmaceutical liabilities, including off-target activities such as stimulation of fatty acid oxidation that leads to significant and rapid weight loss in animal model studies and confounds interpretation of study results (Liu et al., 2010; Menendez and Lupu, 2007; Flavin et al., 2010). In vitro studies have shown that inhibition of Akt phosphorylation and induction of tumor cell apoptosis occur when FASN inhibition is uncoupled from CPT1 stimulation (Puig et al., 2008); thus suggesting that selective FASN inhibition can achieve the desired tumoricidal effects without inducing the rapid weight loss associated with activation of fatty acid oxidation. These and other observations have spurred the discovery and development of 'next generation' FASN inhibitors with optimized pharmacological properties and in vivo tolerability.

We report studies that characterize the anti-tumor activity of TVB-3166, a highly selective, potent, reversible, and oral FASN inhibitor discovered and developed by 3-V Biosciences. Using in vitro and in vivo models of human cancer we find that FASN inhibition has multiple mechanisms of action that can operate in specific types of tumors to cause tumor cell apoptosis. These mechanisms include inhibition of signal transduction through the PI3K–AKT–mTOR and β-catenin pathways that regulate tumor cell growth and survival. Our studies provide insights into how these pathways are affected by FASN inhibition and guide the discovery of biomarkers to select tumors with the greatest susceptibility to the tumoricidal effects of FASN inhibition. We also demonstrate that oral dosing of TVB-3166 modulates the target enzyme in vivo, is well tolerated, and inhibits mouse xenograft tumor growth in a dose-dependent manner.

2. Materials and Methods

2.1. Cell Lines and Antibodies

The cell lines used were obtained from ATCC and ECACC, except for OVCAR-8 cells (Biotox Sciences) The following antibodies were used: FASN (3180; Cell Signaling), pAKT-S473 (4060; Cell Signaling), pRPS6-S240 and 244 (2215; Cell Signaling), PARP-cleaved (9541, Cell Signaling), β-catenin (9582, Cell Signaling), pβ-catenin-S675 (4176, Cell Signaling), LRP6 (2560, Cell Signaling) pLRP6-S1490 (2568, Cell Signaling), c-Myc (5605, Cell Signaling), α-tubulin (2125; Cell Signaling), N-Ras (sc-519, Santa Cruz Biotechnology), and cholera-toxin subunit-B (227040; EMD Millipore).

2.2. Palmitate Synthesis Assays

Cells were seeded into 96-well culture plates at a density of 30,000 cells per well. After an overnight incubation, media were