Journal of Cell Signaling
ISSN: 2576-1471

Heuer, J Cell Signal 2016, 1:3
DOI: 10.4172/2576-1471.1000124

Review Open Access

De Novo Palmitate Synthesis Supports Oncogenic Signalling and Tumor Growth Through Diverse Mechanisms: Implications for FASN-Targeted Therapeutics

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Received date: July 13, 2016; **Accepted date:** July 28, 2016; **Published date:** August 2, 2016

Abstract

Palmitate, the enzymatic product of fatty acid synthase (FASN), provides a substrate for the synthesis of long- and short-chain fatty acids. Many recent studies have expanded our knowledge about the roles palmitate and lipid synthesis play in tumor cell biology beyond supporting energy metabolism and membrane building [1,2]. The recent article by Ventura and colleagues [3] described cell biology and pharmacology studies using a novel, selective small molecule FASN inhibitor, TVB-3166. They demonstrated that FASN inhibition disrupts oncogenic signalling and tumor growth in xenograft models through inhibition of pathways that include Wnt/beta-catenin and expression of *c-Myc*: potent oncogenes historically recalcitrant to direct pharmacological inhibition. Discussed here is how these findings advance our mechanistic understanding of the diverse biological roles of palmitate and its integration into various signalling pathways driving tumor cell proliferation and survival. These insights highlight the promising potential of selective, potent FASN inhibitors as a novel therapeutic strategy for cancer and other illnesses.

Keywords: Palmitate; Cell growth; Tumor; Proteins; Oncogenic

Introduction

Constitutive or inappropriate activation of biological signalling pathways regulating cell growth, proliferation, and survival is a well-recognized hallmark of cancer initiation and progression [1-5]. Deregulation and reprogramming of cellular metabolism has been recognized as an additional hallmark [5,6]. The role that certain lipid species and, especially, lipids derived from de novo palmitate synthesis play in both activation and repression of oncogenic signalling recently has come into sharper focus following many advances in tools for lipid analyses and tumor biology [1,7-17]. Palmitate and palmitate-derived lipids are known to serve vital roles in modulating signal transduction and protein function that contribute to maintaining the proper balance of cell growth, proliferation, survival, and apoptosis necessary for optimal human health. Genetic alterations, such as those associated with human cancer, and/or lifestyle choices that include diet and nutrition can sway cellular lipid biology to an imbalanced disease-promoting state [1,18-21].

Ventura et al. described mechanism-based effects on tumor cell biology and *in vivo* xenograft tumor growth that occurred in response to inhibition of FASN in tumor cells from a variety of tissues and genetic backgrounds. Profound reprogramming of gene expression and inhibition of signal transduction occurred following treatment of tumor cells with TVB-3166, a selective, reversible, and orally bioavailable small molecule FASN inhibitor (cellular palmitate synthesis IC50=42 nM) (Figure 1). Evidence for plasma membrane-associated lipid raft micro-domain remodelling and altered localization of signalling proteins such as NRas supported a pharmacological mechanism of action that involves impairment of the role palmitate and likely other lipid species provide for normal membrane architecture, membrane-protein interactions and associated signal transduction activity. Canonical oncology-associated pathways were inhibited following TVB-3166 treatment of lung, colon, ovarian, prostate, and pancreatic tumor cell lines. Effected pathways include Akt/mTor, Wnt/beta-catenin, and *c-Myc* expression. A separate recent study by Benjamin et al. linked *in vitro* sensitivity to FASN inhibition in several tumor cell lines with diacyl glycerol levels and PKC signalling [12], which can stimulate expression of NF-κB pathway-regulated gene expression [22], and further illustrates the variety of signalling pathways that can be modulated by FASN activity. The ability to inhibit Wnt signalling and *c-Myc* expression suggests an opportunity to block activity of a pathway that is associated with initiation, progression and cancer cell stemness in breast, colon, ovary, lung, and other tumor types, and that has evaded many attempts at pharmacological intervention. Recent Wnt pathway therapies that show signs of clinical activity include frizzled receptor antibodies in Phase 1 clinical development [23]. The studies discussed here and reported in EBIOM by Ventura et al. provide a rationale for targeting Wnt/beta-catenin and Myc by FASN inhibition.

TVB 3166 interfered with beta-catenin pathway signalling as measured by TCF reporter activity and Western blot analysis of pathway proteins in lung and colon tumor cell lines. The effects observed included significantly decreased *c-Myc* protein expression. Myc is firmly implicated in many aspects of tumor cell biology including metabolism and regulation of growth, proliferation, and survival [24-27]. Wnt and Myc pathway impairment by FASN inhibition occurred in the context of additional effects that included mislocalization of lipid-modified oncogenic signalling proteins such as NRas, inhibition of Akt/mTor signalling, and extensive gene expression reprogramming.

How might FASN inhibition result in the blockade of Wnt signalling and *c-Myc* expression? Multiple possible mechanisms are supported by existing data from several studies including inhibition of Wnt palmitoylation [28-30] and disruption of membrane protein localization and organization [3], e.g. frizzled and/or LRP proteins. Blocking beta-catenin entry into the nuclear compartment by FASN

Citation: Heuer TS (2016) De Novo Palmitate Synthesis Supports Oncogenic Signalling and Tumor Growth Through Diverse Mechanisms: Implications for FASN-Targeted Therapeutics. J Cell Signal 1: 124. doi: 10.4172/2576-1471.1000124

inhibition, where it affects the expression of a multitude of genes including *c-Myc*, provides an additional possible mechanism.

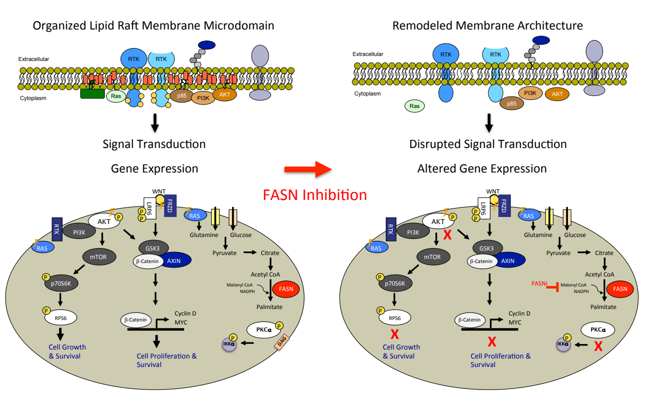

Figure 1: FASN inhibition blocks palmitate synthesis to cause a reprogramming of cellular metabolism and disrupted signal transduction. Modified from Ventura et al. [3].

The known inventory of palmitoylated protein is most certainly incomplete, but even so, hundreds of proteins are known to undergo post-translational palmitate modification [31,32] an event that impacts protein localization and function [13,33-35]. The list of known proteins includes several well-known oncogenes including Wnt, HRas, NRas, KRas4A, EGFR and Akt. Many additional proteins are acetylated with a variety of lipid-associated moieties including GPI, farnesyl, acetate, and myristate. Thus, it is clear that post-translational acylation provides a vital mechanism to regulate cell growth, proliferation, and survival, and accordingly, some of the enzymes that catalyze synthesis and transfer of lipid and acyl groups deserve serious consideration as potential targets for the discovery and development of cancer therapeutics, for example, palmitoyl transferases [3]. Farnesyl transferase inhibitors have not demonstrated clinical efficacy due to the ability of geranylation to substitute for the farnesyl group on proteins such as Ras [36,37]; however, palmitoyl transferases are not expected to suffer from this mechanism of resistance. A challenge to the development of palmitoyl transferase inhibitors is the 23 different substrate-specific enzymes that constitute this target class. Depalmitoylating enzymes, of which there is a much smaller number, present another potential target class. More work remains to be done characterizing the effect of FASN inhibition, including the investigation of many additional tumor models that vary in their dependency on Wnt and Myc activity; however, the data available at this time suggest promising possibilities for treating Wnt- and Myc-driven tumors with selective FASN inhibitors.

In the setting of multiple mechanisms of action that originate from FASN inhibition, what are the driving effects that determines tumor cell sensitivity and potential pharmacological efficacy? In addition to Wnt and Myc pathways, data reported by Ventura et al. provide a rationale for targeting Ras-activated tumors with FASN inhibition: they investigated a panel of 30 lung tumor cell lines and reported a correlation between RAS-activation and FASN sensitivity. Notably, this correlation was not evident in colon tumor-derived cell lines; many of these cell lines were highly sensitive to FASN inhibition but sensitivity did not associate with RAS mutation status. Interestingly, KRAS activation in NSCLC has been associated with suppression of non-canonical Wnt signalling, which has the ultimate effect of activating canonical Wnt/beta-catenin signalling [38]. This relationship between KRAS and Wnt signalling raises the intriguing possibility that the observed in vitro sensitivity of KRAS-mutant NSCLC cell lines is connected to Wnt and possibly Myc pathway activation. If this indeed is the underlying explanation, might it account for the difference observed between lung and colon tumors with respect to KRAS mutation associating with FASN inhibition sensitivity? Following from these data, subtypes of colon, breast, or ovarian tumors with activated Wnt signalling may represent enriched populations highly responsive to FASN inhibition.

In vivo xenograft tumor growth inhibition was observed in a variety of tumors, including KRAS-mutant and -wildtype non-small-cell lung, ovarian, and pancreatic tumor types, albeit the magnitude of xenograft tumor growth inhibition was typically 50% and infrequently as much as 87%. Results that highlight a long-standing question around FASN as a possible cancer therapeutic target: In the face of FASN inhibition can tumor cells acquire needed lipids via rewiring dietary and metabolic mechanisms that will obviate possible anti-tumor effects of FASN inhibition? An accurate and meaningful answer to this question requires further investigation, including clinical trials with selective, pharmacologically optimized FASN inhibitors such as TVB-2640; currently in late Phase 1 clinical development [39]. Evidence that diet does not replace saturated and monounsaturated lipids was observed in pharmacodynamic analysis following once-daily oral dosing of TVB-2640; significantly decreased levels ($p<0.0001$) of saturated and monounsaturated triglycerides were found in sebum collected from the skin of patients without any dietary restrictions. In the patients analysed, this effect was sustained for the duration of TVB-2640 administration that in some instances exceeded 100-200 days and was associated with good tolerability of the drug. This supports a working model that the addiction to de novo synthesized palmitate displayed by tumor cells compared to non-tumor cells derives from specific lipid-associated signalling and energetic requirements unique to tumor cell biology.

Do tumor cells, at least certain subtypes with definable features, rely on de novo synthesized saturated fatty acids for vital growth, proliferation, and survival functions? Currently available data support the concept that tumor cells require what can be thought of as privileged pools of fatty acids and lipids that can be satisfied only through FASN-mediated de novo palmitate synthesis [40]. The requirement for these privileged pools and the detailed function that they provide remains to be established unequivocally; this is an area deserving active investigation. Data from studies with this objective may inform whether the FASN inhibition-mediated effects on protein palmitoylation, diacylglycerol levels, and signalling pathway activity is a direct, proximal consequence of blocking palmitate synthesis or are perhaps secondary to metabolic reprogramming that occurs in response to decreased de novo palmitate synthesis. Loss of protein palmitoylation seems more likely to result directly from inhibition of palmitate synthesis and perhaps utilizes privileged cellular sources of palmitate; whereas, diacylglycerol levels may be dependent on both palmitate synthesis and metabolic reprogramming. Additional questions for future work that arise from the Ventura et al. data and the thoughts discussed above include: (1) would high levels of anti-tumor effect be observed in tumor models highly dependent on Wnt, *Myc*, Ras, or PKC, (2) would diet-effect studies inform the impact of dietary lipids on the anti-tumor effect of FASN inhibition, and (3) is the best use of FASN inhibition as an anti-cancer therapy in combination with other targeted or chemotherapeutic agents? FASN inhibition combined with targeted or chemotherapeutic agents might increase efficacy by restricting the capacity of tumor cells to adapt to the effects of FASN inhibition and/or by enhancing the activity of a targeted or chemotherapeutic agent administered in combination. In closing, the data reported by Ventura et al. advance our understanding of the many roles in tumor cell biology that FASN-synthesized palmitate is required for or supports. The data from this report highlight questions and areas where further study is warranted. Importantly, TVB-3166 and the highly related FASN inhibitor TVB-2640 (in Phase 1 clinical development) provide tools to address the remaining questions and illuminate the role of FASN-targeted therapeutics in the treatment of cancer and other serious human illness such as NASH and other metabolic disorders, viral infections, and disorders of the nervous system.

Acknowledgements

Thank you to George Kemble and Doug Buckley at 3-V Biosciences for critical reading of this article and helpful suggestions.

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