Commentary

Loss of tumor suppressive properties of lipid metabolism enzyme CPT2 in ovarian carcinoma: Comment on "CPT2 down-regulation promotes tumor growth and metastasis through inducing ROS/NFκB pathway in ovarian cancer" by Zhang et al.

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ABSTRACT

Lipid metabolism is an essential process in cancer growth and progression. It is highly relevant in tumors with an adipocyte-rich microenvironment, such as ovarian carcinoma (OC). Carnitine palmitoyltransferase 2 (CPT2) is a key enzyme in fatty acid oxidation (FAO) that functions as a tumor suppressor in OC. Downregulation of CPT2 is reportedly associated with poor prognosis of OC patients. At the cellular level, low CPT2 translates into reduced NADPH level and unopposed reactive-oxygen species (ROS)/nuclear factor kappa B (NFκB) signaling which are paralleled by induction of mesenchymal mediators, invasion and metastasis. While strategies to propagate the tumor suppressive properties of CPT2 have yet to be developed, a comprehensive approach of co-assessment and co-targeting of CPT2 and its family member CPT1, or/and other key FAO players with FAO-specific inhibitors or/and less specific inhibitors (e.g. targeting NFκB, STAT3) is worth pursuing to improve understanding of the metabolic aspects of OC and develop a lipid metabolism-centered therapeutic strategy that can benefit OC patients.

Lipid metabolism is an essential process in cancer growth and progression. Fatty acid synthesis provides structural and signaling molecules in the tumor and tumor microenvironment. Conversely, lipid biosynthesis is regulated by downstream signaling through various oncogenes and tumor suppressors [1]. Intra-abdominal tumors with a high frequency of omental metastases, such as ovarian cancer (OC), have become an important area of investigation revealing a role of adipocytes in promoting cancer lipid metabolism within the tumor microenvironment [2]. At the intracellular level, abnormal expression of key metabolic enzymes in fatty acid intake, synthesis, oxidation, and storage in OC cells has been associated with tumor-promoting properties [3]. Furthermore, for many of these enzymes, deregulation at the gene or/and protein level has been associated with prognostic implications in women with OC [3]. One such example is carnitine palmitoyltransferase (CPT), which catalyzes the decomposition of long-chain fatty acids and β-oxidation, and consists of two subtypes, CPT1 and CPT2 [3].

The CPT1A isozyme is of particular interest in OC as it is highly expressed in most OC cell lines and primary ovarian serous carcinomas [4]. High mRNA expression in The Cancer Genome Atlas (TCGA) OC database was associated with shorter overall survival (OS) of these patients [4]. Mechanistically, an oncogenic role of CPT1 was supported by knock-down experiments in OC cell lines and xenografts leading to cell cycle arrest via p21 induction, mediated by phosphorylation-dependent activation of the FOXO transcription factors [4].

Little was known until recently about the role of CPT2 in OC progression. In their work, Zhang et al. [5] shed light into this area. First,

they found a significantly lower expression of CPT2 transcript and protein in OC cells and human-derived tumors compared to non-malignant ovarian cells and paired peritumor normal tissues, respectively [5]. Low immunohistochemical (IHC) expression of CPT2 in primary serous OC tumors translated in a significantly shorter OS of corresponding patients compared to those with high expression [5]. Tumor heterogeneity and small number of patients-tumors might explain why no significant correlation was found between FIGO stage, grade and CPT2 IHC expression (lower versus higher score derived from percentage and intensity, compared to median). Larger prospective studies focusing on one particular subtype (e.g. serous) or stratifying their analyses according to different subtypes may be more informative in understanding whether CPT2 expression might actually serve as a prognostic tool and whether it is more relevant in serous OC or/and other OC subtypes.

To elucidate the mechanisms underlying this favorable effect of CPT2 expression on prognosis of patients with serous OC, Zhang et al. [5] performed functional experiments involving forced CPT2 overexpression via cloning vector and knockdown via small interference RNA (siRNA), respectively, in OC cell lines and mouse xenografts. CPT2 overexpression resulted in reduced cell proliferation, migration and invasion, induction of G1/G0 cell cycle arrest, and increased apoptosis *in vitro*, as well as attenuated tumor growth and fewer metastases *in vivo*. These findings were in parallel with induction of epithelial markers (E-cadherin, ZO-1) and downregulation of mesenchymal markers (N-cadherin, vimentin) suggesting inhibition of epithelial-mesenchymal (EMT) transition. CPT2

knockdown had the opposite effects, increasing cell viability, migration and invasion.

The metabolic aspects of CPT2 functional experiments were also studied, yielding interesting findings. Because fatty acid oxidation (FAO) is inherently associated with production of NADPH, which can counteract the effects of reactive oxygen species (ROS), the intracellular NADPH level, radioactivity from FAO, and fluorescent activity from ROS production were measured. CPT2 overexpression promoted FAO and increased NADPH while reducing ROS in OC cells. Among the most important pathways downstream ROS signaling, including AKT, NFκB, ERK1/2 and HIF-1α, reduced phosphorylation-dependent activation of NFκB p65 was observed, supporting the notion that CPT2 represses ROS/NFκB signaling pathway in OC through increasing FAO-mediated NADPH production. The tumor-suppressive effects of CPT2 on growth and metastasis were reversible after exogenously induced conditions of increased ROS (H_2O_2) in CPT2-overexpressing cells, and were amplified by NAC in OC cells harboring CPT2 knockdown [5].

This work by Zhang et al. [5] represents the first step towards a better understanding of the role of CPT2 and FAO in general, in OC biology. Inevitably, several implications and new questions are posed, for example what drives the expression of CPT2 and what is the net effect in the presence of aberrations in both CPT1 and CPT2 which play apparently distinct and likely opposing roles. A recent study by another group demonstrated that deletion of the tumor suppressor gene NKX2-8 which controls transcriptional repression of both CPT1 and CPT2 leads to epigenetic reprogramming of FAO in OC cells in an adipose microenvironment favoring the development of platinum-resistance which is associated with poor prognosis in these patients [6].

Enhancing the expression of CPT2 might represent a therapeutic approach to propagate its tumor suppressive properties and thereby inhibit tumor growth and prevent or delay metastasis in OC. However, it remains elusive how this could be effected pharmacologically. Treatment with CPT1 inhibitors, a class of drugs under clinical development e.g. perhexiline, might represent a more realistic strategy. Perhexiline seems to obviate NKX2-8 deletion-induced chemoresistance and was proposed as a potential tailored treatment for patients with NKX2-8-deleted OC. Another CPT1 inhibitor, etomoxir led to significant inhibition of tumor progression *in vivo* in a patient-derived xenograft model of high-grade serous OC [7]. It is important to understand that only by assessing both CPT1 and CPT2 concurrently in a comprehensive way including their methylation, mutational, transcriptional, translational and post-translational status in future studies will we be in a better position to delineate their true roles and identify the best approach for targeting them.

In a broader perspective, inhibition of other FAO enzymes may represent a less specific, targeted approach that should be tested with respect to its effects on CPT1 and CPT2. For example, the fatty acid synthase (FASN) inhibitors triclosan (antibacterial) and orlistat (obesity drug) could be repurposed for that use, given their selective *in vitro* cytotoxicity against cancer cells compared to non-malignant fibroblasts [8]. Another FASN inhibitor, TVB-2640, is in advanced, phase 2 setting of investigation in KRAS-mutant non-small cell lung cancer (NCT03808558) and in HER2-positive breast cancer combined with paclitaxel and transtuzumab (NCT03179904).

Because lipid metabolism alterations may promote and maintain cancer stem cell properties and *vice versa*, the first-in-class stemness inhibitor targeting STAT3, napabucasin, was tested in chemoresistant OC cells and induced resensitization to cisplatin and tumor growth inhibition in an immunodeficient mouse model [9]. The drug was recently reported to prolong OS compared to placebo in heavily pretreated patients with refractory colorectal cancer selected for phosphorylated STAT3 in their tumors [10]. Whether this could be applicable in OC remains to be studied. Finally, an implication from the study of Zhang et al. [5] is the potential of NFκB inhibitors or antagonists, several of which are in clinical use for various indications (e.g. bortezomib, sorafenib, sunitinib). If the involvement of activated NFκB as a direct player in OC tumors with downregulated CPT2 is confirmed, blockade of this pathway might be particularly effective in this subset of patients with unopposed ROS/NFκB signaling.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] F. Rohrig, A. Schulze, The multifaceted roles of fatty acid synthesis in cancer, Nat. Rev. Cancer 16 (11) (2016) 732–749.

[2] K.M. Nieman, H.A. Kenny, C.V. Penicka, A. Ladanyi, R. Buell-Gutbrod, M.R. Zillhardt, I.L. Romero, M.S. Carey, G.B. Mills, G.S. Hotamisligil, et al., Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth, Nat. Med. 17 (11) (2011) 1498–1503.

[3] Z. Ji, Y. Shen, X. Feng, Y. Kong, Y. Shao, J. Meng, X. Zhang, G. Yang, Deregulation of lipid metabolism: the critical factors in ovarian cancer, Front. Oncol. 10 (2020) 593017.

[4] H. Shao, E.M. Mohamed, G.G. Xu, M. Waters, K. Jing, Y. Ma, Y. Zhang, S. Spiegel, M.O. Idowu, X. Fang, Carnitine palmitoyltransferase 1A functions to repress FoxO transcription factors to allow cell cycle progression in ovarian cancer, Oncotarget 7 (4) (2016) 3832–3846.

[5] X. Zhang, Z. Zhang, S. Liu, J. Li, L. Wu, X. Lv, J. Xu, B. Chen, S. Zhao, H. Yang, CPT2 down-regulation promotes tumor growth and metastasis through inducing ROS/NFκB pathway in ovarian cancer, Transl. Oncol. 14 (4) (2021) 101023.

[6] J. Zhu, G. Wu, L. Song, L. Cao, Z. Tan, M. Tang, Z. Li, D. Shi, S. Zhang, J. Li, NKX2-8 deletion-induced reprogramming of fatty acid metabolism confers chemoresistance in epithelial ovarian cancer, EBioMedicine 43 (2019) 238–252.

[7] B.T. Sawyer, L. Qamar, T.M. Yamamoto, A. McMellen, Z.L. Watson, J.K. Richer, K. Behbakht, I.R. Schlaepfer, B.G. Bitler, Targeting fatty acid oxidation to promote anoikis and inhibit ovarian cancer progression, Mol. Cancer Res. 18 (7) (2020) 1088–1098.

[8] M.C. Sadowski, R.H. Pouwer, J.H. Gunter, A.A. Lubik, R.J. Quinn, C.C. Nelson, The fatty acid synthase inhibitor triclosan: repurposing an anti-microbial agent for targeting prostate cancer, Oncotarget 5 (19) (2014) 9362–9381.

[9] S. Schmidtova, L.C.J. Dorssers, K. Kalavska, A.J.M. Gillis, J.W. Oosterhuis, H. Stoop, S. Miklikova, Z. Kozovska, M. Burikova, K. Gercakova, et al., Napabucasin overcomes cisplatin resistance in ovarian germ cell tumor-derived cell line by inhibiting cancer stemness, Cancer Cell Int 20 (2020) 364.

[10] D.J. Jonker, L. Nott, T. Yoshino, S. Gill, J. Shapiro, A. Ohtsu, J. Zalcberg, M.M. Vickers, A.C. Wei, Y. Gao, et al., Napabucasin versus placebo in refractory advanced colorectal cancer: a randomised phase 3 trial, Lancet Gastroenterol. Hepatol. 3 (4) (2018) 263–270.