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INSIGHTS

Stopping the fat: Repurposing an antidepressant for cancer treatment

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In this issue of *JEM*, Chu and An et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20221316) describe the role of the tricyclic antidepressant nortriptyline in inhibition of fatty acid uptake. Nortriptyline promotes cell acidification and suppresses macropinocytosis, providing a link between fatty acid uptake and tumor progression.

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While cancer cells show an increased rate of glucose uptake for energy production through aerobic glycolysis, normal cells favor mitochondrial oxidative phosphorylation. Oncogenic transformation causes tumors to reprogram many metabolic pathways, including glucose transport, glutaminolysis, the electron transport chain (ETC), the pentose phosphate pathway, and lipogenesis in order to fulfill their unique energy requirements. In highly proliferative cancer cells, dysregulation of the TCA cycle often coincides with upregulated lipogenesis; however, compounds targeting the lipogenesis pathway have shown limited efficacy in tumor models (Peck and Schulze, 2016).

There are several metabolic reactions that precede de novo fatty acid synthesis, which occurs in the cytosol. First, glucose is metabolized through glycolysis to produce pyruvate (see figure), which is imported into the mitochondrion and converted into acetyl-CoA and later citrate by citrate synthase. Citrate may then proceed through the TCA cycle or be removed from the mitochondrion by the citrate carrier. In the cytosol, citrate can be converted back to oxaloacetate and acetyl-CoA. Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) are two key rate-limiting enzymes in de novo fatty acid synthesis (Mashima et al., 2009). ACC catalyzes carboxylation of cytosolic acetyl-CoA into malonyl-CoA, the first committed step in the synthesis of fatty acids. FASN then converts malonyl-CoA into long-chain fatty acids, such as 16-carbon palmitate. Fatty acid elongation, starting with stearate (18:0), is mainly performed by membrane-bound enzymes in the endoplasmic reticulum. β-oxidation of fatty acids in the mitochondria generates nicotinamide adenine dinucleotide and flavin adenine dinucleotide, co-enzymes used in the ETC, and acetyl-CoA, which enters the TCA cycle. Using these metabolic pathways, cells have the ability to produce their own fatty acids, and in the case of cancer cells, use these nutrients to drive sustained proliferation, biomass production, and, ultimately, tumor growth.

Due to the harsh nutrient-deprived conditions of the tumor microenvironment, cancer cells have evolved alternative pathways for nutrient acquisition. During metabolic stress, exogenous fatty acid uptake is a mechanism that tumors depend on to sustain their rapid growth. In cells, these exogenous fatty acids are transported across the plasma membrane by specialized transporters that include CD36, fatty acid transport proteins (FATPs), also known as the solute carrier protein 27 family, and plasma membrane fatty acid–binding proteins (FABPpm). Interestingly, these transporters display increased gene and protein expression in tumors (Hoy et al., 2021) and play a key role in tumor-stroma metabolic crosstalk within the microenvironment.

Insights from Deepika Bhullar and Cosimo Commisso.

Macropinocytosis is a highly conserved endocytic nutrient-scavenging pathway by which extracellular fluid, and its contents like serum albumin and necrotic cell debris, are internalized and delivered to lysosomes (Lambies and Commisso, 2022). Several key modulators of the actin cytoskeleton including small GTPases such as Rac, Cdc42, Arf6, Rab5, and the WAVE complex, p21-activated kinase, and phosphoinositide signaling regulators such as phosphoinositide 3-kinase and phospholipase C, can control macropinocytosis (Egami et al., 2014; Recouvreux and Commisso, 2017; Zhang and Commisso, 2019). Metabolite analyses in tumor cells have shown that amino acids derived from macropinocytic uptake of extracellular proteins are incorporated into several metabolic pathways, including glutamine anaplerosis/oxidation, acetyl-CoA metabolism, reductive carboxylation, and

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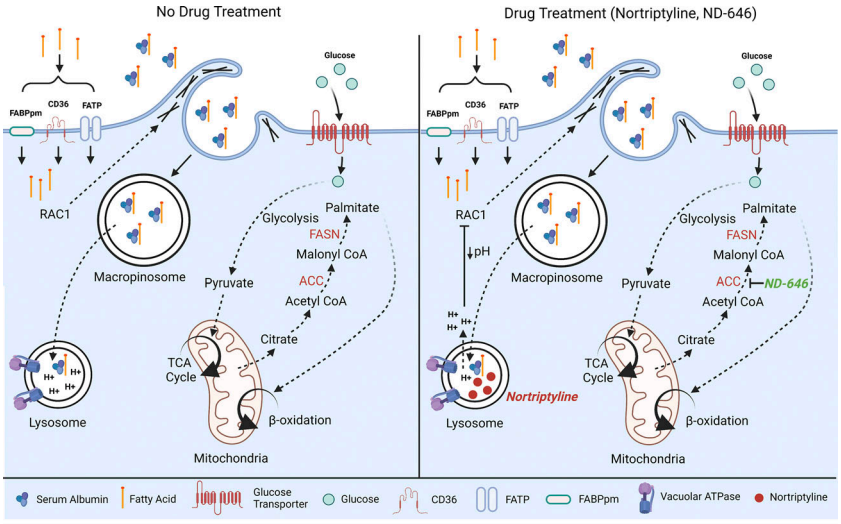

Schematic representation of macropinocytosis and fatty acid metabolism in untreated cells and cells co-treated with nortriptyline and ND-646. Figure was generated using BioRender.

serine/glycine cycling (Commisso et al., 2013).

In this issue, Chu and An et al. (2022) demonstrate a link between the antidepressant nortriptyline and tumor growth, showing that there might be potential for nortriptyline to be repurposed for the treatment of tumors and metabolic diseases. Specifically, they report that nortriptyline blocks fatty acid uptake by indirectly repressing macropinocytosis, which has been previously linked to metabolic stress and nutrient starvation (Lee et al., 2019). The mechanism underlying the effects of nortriptyline on macropinocytosis seems to involve acidification of the cytosol via the release of protons from lysosomes. It has been previously demonstrated that an inability to maintain the pH of acidic organelles can lead to the disruption of cytosolic pH homeostasis (Galenkamp et al., 2020; Liu et al., 2018). This can lead to the suppression of macropinocytosis, which accounts for fatty acid uptake, since this endocytic pathway is sensitive to changes in intracellular pH (Koivusalo et al., 2010). Importantly, this paper shows that nortriptyline alone, or in combination with ND-646, a selective ACC1/2 inhibitor, significantly repressed breast and cervical cancer growth, potentially providing a therapeutic link to its ability to control the cellular level of fatty acids. Intriguingly, the study deciphers that blockage of fatty acid synthesis through ACC1/2 depletion significantly promotes macropinocytosis-mediated fatty acid uptake, which could antagonize the suppressive effects of fatty acid synthesis inhibitors; therefore, the authors conclude that simultaneously blocking both ACC1/2 and macropinocytosis using the relevant inhibitors, such as ND-646 and nortriptyline, respectively, exerted more suppressive effects to control cellular fatty acid levels.

The anti-tumor activity of nortriptyline was attributed to its effects on cytosolic pH. A reversed pH gradient is a common characteristic of cancer cells, which show increased intracellular pH (pHi) and a decreased extracellular pH relative to normal cells. Activity of several H^+ transporters are increased in cancer cells including H^+-ATPases and Na^+/H^+ exchangers (NHEs), which actively transport H^+ ions against the gradient. Recent evidence suggests that, selectively in cancer cells, organellar sequestration of protons serves as a homeostatic mechanism of maintaining pHi (Galenkamp et al., 2020; Liu et al., 2018). In this way, acidic organelles act as "proton sinks," allowing tumor cells to maintain their reversed pH gradient (Galenkamp and Commisso, 2021). Nortriptyline seems to dysregulate pHi by causing H^+ release from lysosomes. The study by Chu and An et al. further supports the notion that the lysosome is an important part of the oncogenic reprogramming cascades that serves to regulate cancer cell pH homeostasis. Whether this activity is specific to the lysosome remains to be further interrogated.

Chu and An et al. (2022) determine that breast and colorectal carcinoma cancer cell lines have active fatty acid uptake processes in the presence of ND-646. However, blocking CD36 and FATPs did not effectively potentiate ND-646–mediated killing of cells, indicating that fatty acid uptake through mechanisms other than direct transport might be at play. Several ACC1/2 double knockout (DKO) cell lines were used to screen a library of 1,560 FDA-approved drugs. Of the nine drugs identified that significantly killed DKO cells alone or in combination with ND-646, nortriptyline, a tricyclic antidepressant, synergistically killed cells in combination with ND-646 across cell lines. Tracking fatty acid uptake using 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY)-C16, a fluorescent palmitate analog, showed that nortriptyline significantly reduced fatty acid uptake both in vitro and in vivo. The lack of synergy between ND-646 and fatty acid transport inhibitors led the authors to examine whether macropinocytic delivery was a possible route for the uptake of the fatty acids. This was indeed the case; however, the effects of nortriptyline on macropinocytosis suppression were indirectly occurring through effects on the lysosome. One possibility is that since nortriptyline can be protonated to ammonium salts in acidic environments, it could be accumulating in, and thus alkalinize, organelles such as lysosomes.

Sublethal lysosomal membrane permeabilization induced by alkalinization can be detected as lysosomal puncta of LGALS3/galectin-3. Nortriptyline-treated cells showed induction of EGFP-LGALS3 puncta formation, increased lysosomal pH, and reduced pHi similar to two other drugs, chloroquine and bafilomycin, which both affect lysosome pH. Nortriptyline also showed enrichment in lysosomes but not in mitochondria. Inhibition of NHEs via treatment with 5-(N-ethyl-N-isopropyl)-amiloride, a compound that selectively inhibits macropinocytosis by inducing submembranous acidification (Koivusalo et al., 2010), blocked fatty acid uptake and potentiated the cytotoxic effects of ND-646 on

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cancer cells. A potent vacuolar H^+-ATPase inhibitor, Bafilomycin A1, also inhibited lysosomal acidification, induced EGFP-LGALS3 puncta, enhanced lysosomal pH, decreased cellular pH, and suppressed BODIPY-C16 uptake. Thus, the mechanism of action of nortriptyline was attributed to lysosome-mediated cellular acidification, which indirectly inhibited fatty acid uptake through the blockade of macropinocytosis. Further supporting this model, genetic suppression of Rac1, a small GTPase required for actin dynamics and macropinocytic induction, in MDA-MB-231 cells significantly prevented the uptake of both tetramethylrhodamine-labeled high-molecular-mass dextran, a macropinosome marker, and BODIPY-C16, and increased the cytotoxic effects of ND-646 on the cancer cells. Hence, nortriptyline and ND-646 together synergistically reduced overall levels of cellular fatty acids and suppressed tumor growth. Importantly, this combination had minimal organismal toxicity as determined by the lack of an effect on the body weight of the mice. In addition, nortriptyline and ND-646 synergistically suppressed lipogenesis and hepatic steatosis in obese mice.

These observations linking nortriptyline treatment in cancer to decreased growth potential may represent a new therapeutic opportunity. Nortriptyline is mainly used as an antidepressant and is under clinical trials to treat depression in Parkinson's disease patients (Schrag et al., 2022). Among other effects, nortriptyline is thought to inhibit the reuptake of serotonin and norepinephrine by the presynaptic neuronal membrane, thereby increasing the concentration of those neurotransmitters in the synapse (Merwar et al., 2022). On a mechanistic level, whether the role of nortriptyline as an antidepressant is in any way related to the effects observed in the context of cancer remains to be determined. In closing, Chu and An et al. (2022) have made a noteworthy contribution to the field of cancer research, linking the antidepressant nortriptyline to macropinocytosis, cancer metabolism, and tumor progression.

Disclosures: The authors declare no competing interests exist.

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