

Even Cancer Cells Watch Their Cholesterol!

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Deregulated cell proliferation is an established feature of cancer, and altered tumor metabolism has witnessed renewed interest over the past decade, including the study of how cancer cells rewire metabolic pathways to renew energy sources and “building blocks” that sustain cell division. Microenvironmental oxygen, glucose, and glutamine are regarded as principal nutrients fueling tumor growth. However, hostile tumor microenvironments render O₂/nutrient supplies chronically insufficient for increased proliferation rates, forcing cancer cells to develop strategies for opportunistic modes of nutrient acquisition. Recent work shows that cancer cells overcome this nutrient scarcity by scavenging other substrates, such as proteins and lipids, or utilizing adaptive metabolic pathways. As such, reprogramming lipid metabolism plays important roles in providing energy, macromolecules for membrane synthesis, and lipid-mediated signaling during cancer progression. In this review, we highlight more recently appreciated roles for lipids, particularly cholesterol and its derivatives, in cancer cell metabolism within intrinsically harsh tumor microenvironments.

Nutrient scavenging and catabolism are indispensable for sustaining cellular growth by generation of energy and biomass. Because tumor microenvironments are exposed to metabolic challenges, cancer cells have increased demand for nutrients, such as glucose, glutamine, and other amino acids, to support survival and macromolecules biosynthesis. Additional evidence suggests that lipid metabolism is also altered in rapidly proliferating cells to mediate energy and membrane production (DeBerardinis and Thompson, 2012; Santos and Schulze, 2012; Swinnen et al., 2006) and homeostasis of multiple biological processes, such as steroid hormone, vitamin, bile acid, and eicosanoid generation. Mammalian cell requirements for lipids are supplied by both diet and endogenous synthetic pathways. As shown by our laboratory and others, most cancer cells exhibit altered lipid metabolism, where they become dependent on exogenous lipids given that their growth requirements are not fully met by anabolic pathways (Ackerman and Simon, 2014; Ackerman et al., 2018; Kamphorst et al., 2013; Qiu et al., 2015; Young et al., 2013) (Figure 1). Therefore, a comprehensive understanding of how cancer cells assess their lipid resources to fulfill metabolic demands of high rates of proliferation could potentiate novel anti-tumor therapeutic strategies. Triglycerides (TGs) and cholesterol are two forms of lipid, or “fat,” necessary for cell viability. Triglycerides harboring three chains of high-energy fatty acids (FAs) provide much of the energy required for tissue functionality, and phospholipids needed for organelle and plasma membranes have been extensively reviewed elsewhere (Ackerman and Simon, 2014; Beloribi-Djeafaflia et al., 2016; Currie et al., 2013). However, while cholesterol-based metabolic reprogramming has received less attention, it is increasingly recognized as an important aspect of tumor metabolism. Cholesterol availability is critical for maintaining cellular homeostasis, key cellular structures (e.g., membranes; Ikonen, 2008), and essential hormones, including estrogens, progesterone, vitamins (e.g., vitamin D), and steroids (Payne and Hales, 2004).

In this review, we summarize the most recent findings on cholesterol, and its derivatives in cancer, and their implications for anti-tumor immunity and gut microbiota.

Obesity and Cancer

The World Health Organization (WHO) estimates that more than 1 billion adults are overweight and ≥ 300 million are considered obese. Obesity is defined by a BMI ≥ 30 . In the United States, 16% of children aged 2–19 years and $\sim 40\%$ of the adult population are classified as obese, making this condition a serious public health threat. Clinical evidence demonstrates that obesity is associated with increased prevalence of certain malignancies, such as liver, gallbladder, colon, or kidney (Wolin et al., 2010). According to The Lancet Public Health (Sung et al., 2019), evidence for >10 obesity-related cancers (i.e., colorectal, liver, kidney, or pancreatic tumors) has been established in the United States between 1995 and 2014. Moreover, obesity-fueled cancers appear earlier and are on the rise in young adults.

Diet is a principal determinant of body composition and plays an important role in obesity. For example, those carrying excess abdominal fat have higher risk of both neoplastic and cardiovascular diseases (Despres, 2007). Obese individuals display elevated levels of circulating free fatty acids (FFAs), TGs, and glucose as well as insulin resistance, resulting in increased pancreatic insulin production. Global increases in high-fat diets, such as the Western diet, correspond to rapidly expanding obese and diabetic populations across the world. This lifestyle is considered an increasingly important contributor to malignancy. Obesity, high BMI, metabolic syndrome, alcoholism, and hypercholesterolemia represent risk factors for different cancers (Boffetta and Hashibe, 2006; Calle et al., 2003; Jagers et al., 2009), whereas regular exercise appears to be protective (Kerr et al., 2017). In addition to FAs, cholesterol is an important dietary component and present in dairy products, meat, eggs, cheese, and a large number of processed foods.



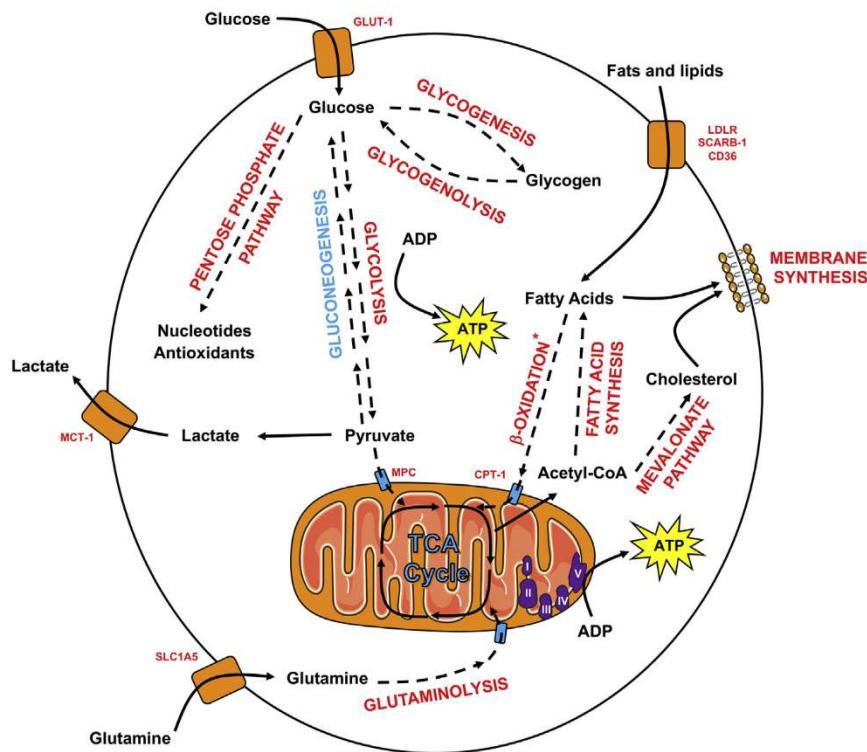


Figure 1. Overview of Major Metabolic Pathways Altered in Cancer

This figure highlights up- (red) and downregulated (blue) metabolic pathways in cancer cells. Glucose and glutamine are common precursors of fatty acids and cholesterol through increased glycolysis and/or glutaminolysis. Of note, β -oxidation (*) processes usually occur inside the mitochondria. CD36, cluster of differentiation 36; CPT-1, carnitine palmitoyltransferase 1; GLUT-1, glucose transporter 1; MCT-1, monocarboxylate transporter 1; MPC, mitochondrial pyruvate carrier; SCARB-1, scavenger receptor B1; SLC1A5, solute carrier family 1 (neutral amino acid transporter) member 5.

Ultra-processed food and cholesterol intake has been correlated to increased chances of developing stomach, lung, kidney, breast, and colon/rectal tumors (Hu et al., 2012). Several mechanisms promoting deregulation of cholesterol and its metabolites have now been proposed to explain possible influences of cholesterol on carcinogenesis and disease progression (see below).

Altered Lipid Metabolism in Cancer

Neoplasia is fundamentally a disorder of cell growth, proliferation, and motility necessitating a deeper understanding of adaptive remodeling of key players in anabolism and bioenergetics. Although solid and liquid tumors are hugely diverse in type and etiology, malignant cells frequently share attributes of metabolic abnormalities that allow them to accumulate intermediates used for survival and division, even when stressed in nutrient- and O_2 -limiting conditions (Xie and Simon, 2017). Energy metabolic reprogramming is recognized as an emerging hallmark of cancer (Pavlova and Thompson, 2016). Most non-cancerous human cells use circulating lipids for the synthesis of structural compounds, like FAs, sphingolipids, phospholipids, cholesterol, and isoprenoids. However, malignant cells exhibit enhanced *de novo* FA synthesis, as an important energy source via β -oxidation, or conversion to TGs for storage or phospholipids for membrane production (Currie et al., 2013; Xie et al., 2018). Our recent work also shows that lipid droplet TGs contribute to overall lipid homeostasis in kidney cancer cells, particularly under hypoxia (Ackerman et al., 2018; Qiu et al., 2015). It is therefore unsurprising that lipid metabolism, in particular FA synthesis and oxidation, has been recognized as another important meta-

bolic aberration required for carcinogenesis and extensively reviewed (Ackerman and Simon, 2014; Carracedo et al., 2013; Long et al., 2018; Röhrig and Schulze, 2016).

Cholesterol Metabolism Overview

Cholesterol is an essential neutral lipid needed for membrane integrity and fluidity (Cooper and Hausman, 2013). As such, cholesterol can be imported from extracellular environments or synthesized *de novo* from acetyl-coenzyme A (acetyl-CoA) via the activity of >20 enzymes catalyzing complex reactions in the mevalonate pathway (Grundy, 1983) and requires oxygen consumption (Figure 2). Therefore, aspects of the tumor microenvironment, particularly hypoxia, impact cholesterol biosynthesis through hypoxia inducible factor-1 (HIF-1)-dependent mechanisms (DeBose-Boyd, 2008; Robichon and Dugail, 2007), and HIF-1-independent mechanisms are likely to be important as well. Of note, in addition to cholesterol, the mevalonate pathway contributes to the synthesis of other sterols and isoprenoids, which are essential for tumor growth (Bathaie et al., 2017). For instance, isopentenyl pyrophosphate, farnesyl pyrophosphate, or geranylgeranyl pyrophosphate is involved in the production of a variety of metabolites contributing to tumor formation, progression, and inflammation, such as dolichol, heme-A, isopentenyl tRNA, or ubiquinone (Gruenbacher and Thurnher, 2017; Waller et al., 2019). Isoprenoids resulting from the mevalonate pathway are also critical for protein prenylation, which confers Ras and Rho proteins their oncogenic properties (Bathaie et al., 2017; Waller et al., 2019).

In addition to being an essential plasma membrane structural component, cholesterol also serves as a precursor for steroid hormone, bile acids, and specific vitamins, such as vitamin D. Because of its importance and potential toxicity, cholesterol homeostasis is tightly controlled. Circulating cholesterol levels are balanced by intracellular synthesis, uptake, and efflux of excess molecules from peripheral tissues. A master regulator of mevalonate pathway gene expression, sterol-regulatory-element-binding protein 2 (SREBP-2) is key to maintaining cholesterol homeostasis (Ilkonen, 2008) and synthesized as an inactive precursor in the endoplasmic reticulum (Brown and

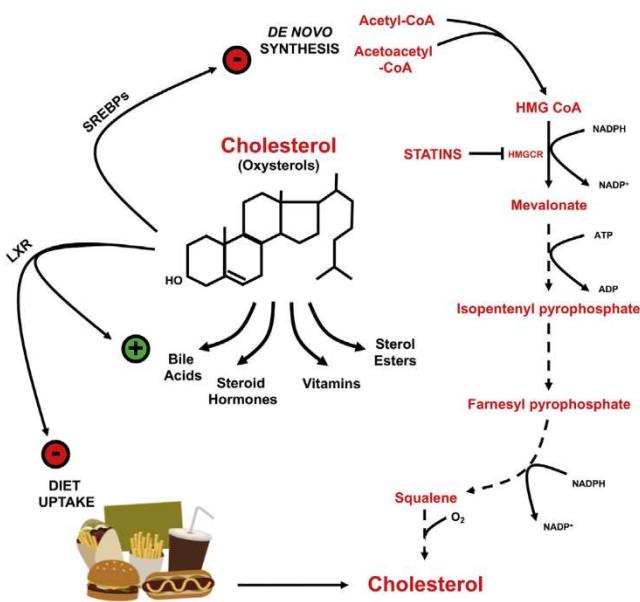


Figure 2. Cholesterol De Novo Synthesis and Derivative Molecules
 This represents a simplified cholesterol biosynthetic pathway, also called the mevalonate pathway, and major cholesterol derivatives (oxysterols, bile acids, steroid hormones, and vitamins). High cholesterol and oxysterol content inhibits (-) *de novo* synthesis through SREBP inactivation and activates LXRs, which stimulates (+) bile acid production and inhibits (-) cholesterol uptake. LXR, liver X receptor; NADP/NADPH, nicotinamide adenine dinucleotide phosphate; SREBPs, sterol-regulatory-element-binding proteins.

(Goldstein, 1997; Horton et al., 2002). Low endoplasmic reticulum cholesterol levels lead to SREBP-2 translocation to the Golgi, where it is cleaved into an active mature form, transits to the nucleus, and binds sterol regulatory elements (SREs), activating genes involved in cholesterol uptake and synthesis (Radhakrishnan et al., 2008).

To reduce high ATP consumption by *de novo* cholesterol biosynthesis (mainly in the liver), cells express receptors that mediate processing and delivery of dietary blood cholesterol, free cholesterol, and cholesterol esters (CEs) carried by lipoprotein particles. Because fats are insoluble in water, lipoproteins are indispensable for systemic lipid circulation and classified in five major groups according to density and size: high-density lipoprotein (HDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), very-low-density lipoprotein (VLDL), and ultra-low-density lipoprotein (ULDL). HDL and LDL appear to be related to cancer (Cedó et al., 2019). LDL, also known as “bad” cholesterol, whose accumulation correlates with increased risk of heart disease and stroke, acts as cholesterol carriers from the liver to other organs. Cholesterol and CE delivery occurs through a receptor-mediated mechanism implicating the LDL receptor (LDLR) (Figure 3). HDL, or “good” cholesterol, allows the body, particularly hepatocytes, to remove cholesterol excess. HDL formation and cholesterol removal from cells are key processes that prevent toxic intracellular cholesterol accumulation. Interestingly, LDL and HDL particles differ in their binding to cell surfaces (Neculai et al., 2013; Rigotti et al., 2003). The principle receptor for HDL particles is scavenger receptor B-1

(SR-B1 or SCARB-1), which allows reciprocal loading and unloading of HDL particles through formation of plasma membrane channels.

In addition to SREBP-2 regulation, cholesterol levels are also influenced by other transcription factors. High cholesterol levels activate liver X receptors (LXRs), resulting in cholesterol synthesis inhibition, activation of cholesterol efflux via increased expression of ATP-binding cassette (ABC) transporters, and reduced uptake (Figures 2 and 3). LXRs exist as two isoforms (α [NR1H3] and β [NR1H2]) that form heterodimers with retinoid X receptors (RXRs) and are ligand-activated. Several reviews thoroughly highlight the role of LXRs as master regulators of cholesterol and FA homeostasis (synthesis, catabolism, import, efflux, etc.), primarily by modulating SREBP function (Bovenga et al., 2015; Silvente-Poirot et al., 2018; Ulven et al., 2005). LXRs also have the ability to control inflammatory responses through alteration of membrane lipid composition. Cholesterol is not always beneficial; excess intracellular cholesterol is highly toxic, and levels must be controlled to maintain viability. Cellular cholesterol overabundance reduces membrane fluidity, disrupts lipid raft signaling, and generates damaging oxidative molecules, like oxysterols. These events ultimately result in cell death, and altered cholesterol balance contributes to atherosclerosis, Alzheimer’s and Parkinson’s diseases, and cancer.

Cancers Where *De Novo* Cholesterol Biosynthesis Matters

Several mechanisms promoting deregulation of cholesterol homeostasis stimulate cancer initiation and progression. Therefore, targeting cholesterol production and the mevalonate pathway represents a promising therapeutic option. Multiple enzymes involved in this pathway are deregulated in cancer cells (Mullen et al., 2016; Bathaei et al., 2017), and the rate-limiting step is controlled by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), an enzyme already targeted by statins (e.g., lovastatin, atorvastatin, and simvastatin). Systemically, HMGCR inhibition leads to decreased LDL plasma levels (Ness et al., 1996). Of note, statin consumption is associated with lowered risk of melanoma, non-Hodgkin’s lymphoma, and endometrial and breast cancers (Cardwell et al., 2014; Nielsen et al., 2012). Statins can also present carcinogenic properties in other tumors, such as breast and nonmelanoma skin cancers (Ravnskov et al., 2015). However, this is somewhat controversial, clearly highlighting a need for a deeper understanding of the overall impact of statins and cholesterol on cancer. Indeed, the use of statins will most likely impact other pathways branching off the mevalonate pathway in cancer cells. As mentioned above, various metabolites contributing to tumor formation and growth, such as isoprenoids, dolichol, or ubiquinone, rely on the mevalonate pathway (Gruenbacher and Thurnher, 2017; Waller et al., 2019), and teasing out the exact mechanisms by which statins act in cancer cells will require further studies. Other recent work suggests that targeting additional mevalonate pathway enzymes, such as lanosterol synthase, through the small molecule MI-2 (a menin inhibitor) disrupts cholesterol homeostasis and induces diffuse intrinsic pontine glioma cell death (Phillips et al., 2019).

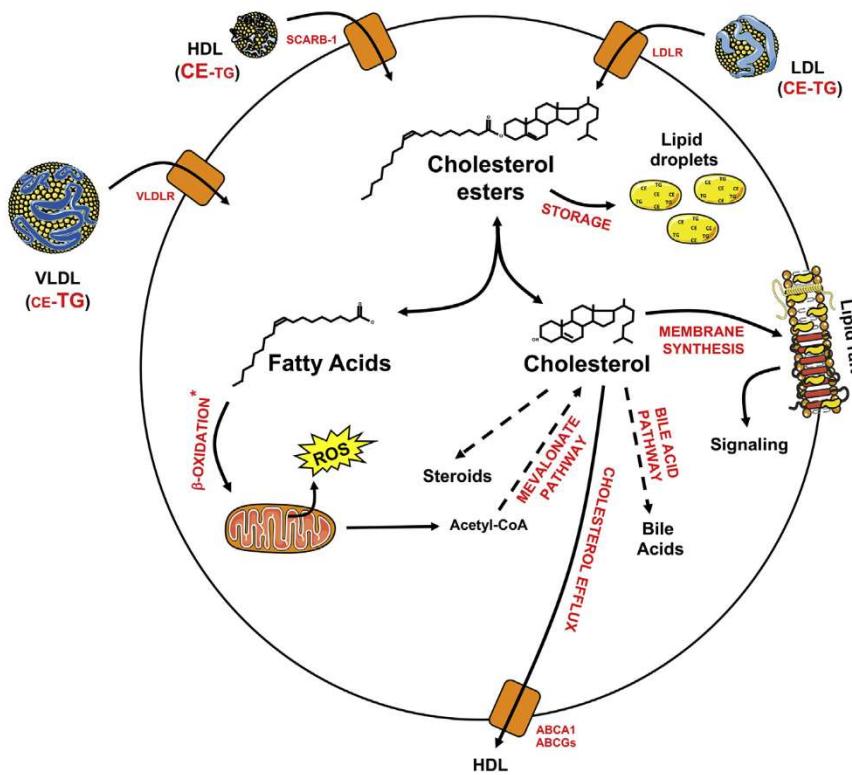


Figure 3. Potential Roles of Cellular Cholesterol

Cancer cells rely on *de novo* synthesis for their cholesterol and cholesterol ester (free cholesterol bound to fatty acids) pools but can also take up exogenous free cholesterol and cholesterol esters through HDL (CE enriched and TG low), LDL (CE and TG enriched), and VLDL (CE low and TG enriched) lipoproteins to meet their cholesterol requirements. Cholesterol can then be used for membrane synthesis, lipid raft signaling, or steroid synthesis. When in excess (and to avoid free cholesterol toxicity), cholesterol is locally stored within lipid droplets in a cholesterol ester form. It can also be directly exported or converted into bile acid first and then excreted. Cholesterol-bound fatty acids are broken down to produce energy through mitochondrial β -oxidation. Of note, the β -oxidation (*) process usually occurs inside the mitochondria. ABCA-1, ATP-binding cassette subfamily A member 1; ABCGs, ATP-binding cassette subfamily G; CE, cholesterol ester; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; ROS, reactive oxygen species; SCARB-1, scavenger receptor B1; TG, triglyceride; VLDL, very-low-density lipoprotein; VLDLR, very-low-density lipoprotein receptor.

Interestingly, intracellular cholesterol levels may account for more cancer burden than systemic serum cholesterol (Freed-Pastor et al., 2012; Sorrentino et al., 2014), suggesting that tumorigenesis is triggered or supported by altered cholesterol homeostasis. Additionally, cholesterol biosynthesis is intricately connected to oncogenic and tumor suppressor factors. For example, the mevalonate pathway is under the control of one of the most frequently mutated genes, *TP53* (Kandoth et al., 2013). *p53* appears to be a tumor suppressor in part by blocking SREBP activation. Both missense mutant (Freed-Pastor et al., 2012) and wild-type *p53* protein regulate the mevalonate pathway in opposite directions (Moon et al., 2019). In addition to *p53*, the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) axis alters metabolism to meet enhanced tumor cell demand for cholesterol (Dong et al., 2014; Yue et al., 2014). Constitutive activation of PI3K/AKT signaling increases intracellular cholesterol levels by SREBP-1 activation leading to *de novo* cholesterol biosynthesis and LDLR expression that enhance exogenous cholesterol import (Guo et al., 2011; Porstmann et al., 2008). Conversely, a feedback loop can occur via lysosomal cholesterol that activates mTORC1 and cell proliferation through a NPC1-SLC38A9 axis (Castellano et al., 2017). Finally, cholesterol also plays an important role in AKT-mediated signal transduction in cancer cells by modulating lipid raft biogenesis (Calay et al., 2010; Mollinedo and Gajate, 2015). In summary, cancer cells survive and proliferate by maintaining *de novo* cholesterol biosynthetic processes. Enhanced delineation of these will identify prognostic and predictive markers for mevalonate pathway inhibitor use in different cancer types.

Cancers Where Cholesterol Uptake Matters

To sustain whole-body cholesterol homeostasis and reduce elevated ATP consumption via *de novo* cholesterol biosynthesis, some cancer cells alter the expression of mevalonate pathway enzymes and deregulate cholesterol influx and/or efflux genes, such as *VLDLR*, *LDLR*, *SREBP-1*, and *ABCA1*. These changes render cancer cells auxotrophs for cholesterol. Among the transporters involved in cholesterol uptake, *LDLR* is often significantly elevated in breast cancer or glioblastoma (GBM) (Gallagher et al., 2017; Villa et al., 2016). Malignant cells promote *LDLR*, *VLDLR*, or *SCARB-1* receptor expression through both SREBP activation and inactivation of the LXR-IDOL axis to maintain cholesterol and redox balance (Wu et al., 2019; Zelcer et al., 2009). GBM cells exhibit constitutive EGFRvIII/PI3K signaling, which activates SREBPs and results in unrestrained *LDLR* expression (Guo et al., 2011). This auxotrophy for extracellular cholesterol can explain cancer cell resistance to HMGCR inhibitors such as statins. Moreover, in non-cancerous cells, excess cholesterol is used to synthesize oxysterols, which act as endogenous ligands to suppress *LDLR* uptake and promote cholesterol efflux (Repa et al., 2000; Venkateswaran et al., 2000). Recent evidence also points to the vulnerability of GBM to LXR agonists (Villa et al., 2016). LXR agonists selectively kill GBM cells by suppressing *LDLR* expression and upregulating *ABCA1*-dependent efflux.

In addition to *LDL/LDLR*'s role in tumorigenesis, several epidemiologic studies have shown a positive correlation between elevated HDL and cancer risk (Boyd and McGuire, 1990; Cruz et al., 2013; Muntoni et al., 2009). HDL is an important carrier of cholesterol and CEs and can function as a signaling molecule. Activation of these signaling pathways is dependent on HDL

binding to SCARB-1, resulting in transformation, cell migration, and elevated signal transduction responsible for cellular proliferation and tumor formation, such as PI3K/AKT or mitogen-activated protein kinase (MAPK) (Danilo et al., 2013; Xu et al., 2018). Another oncogenic adaptation occurs in anaplastic large cell lymphoma (ALCL) tumors, where cholesterol auxotrophy, due to a lack of squalene monooxygenase (SQLE) expression, results in accumulation of the upstream metabolite squalene. Moreover, squalene protects cancer cells from ferroptotic cell death, providing a growth advantage under conditions of oxidative stress generated by high proliferation rates (Garcia-Bermudez et al., 2019). Interestingly, squalene can be deleterious in small cell lung cancer (SCLC). These cells use lipid droplets as a storage compartment for squalene to avoid its toxic accumulation and growth inhibitory effects (Mahoney et al., 2019). Taken together, these recent papers highlight cancers that are selectively auxotrophic for cholesterol and that cholesterol dependency could be used as a potential therapeutic target.

Cholesterol Derivatives, Oxysterols, and Bile Acids: The “Dark Matter”

As stated above, cholesterol is critical to membrane fluidity and structure, signal transduction, and energy storage. However, due to their inability to dispose of cholesterol through direct catalytic reactions, mammalian cells have developed enzymatic reactions that modify its steroid backbone by addition of hydroxyl groups and shortening of conjugated side chains. These reactions generate oxysterols and ultimately bile acids, which are smaller and significantly more H₂O soluble than cholesterol. Bile acids are excreted by the liver into the intestine, and if not reclaimed through intestinal absorption, excess cholesterol is eliminated. Interestingly, cholesterol is the only precursor of oxysterols, bile acids, and steroid hormones, which also act as specific ligands for numerous nuclear hormone receptors. Interactions of cholesterol derivatives and nuclear receptors have previously been thoroughly reviewed (Ma and Nelson, 2019; Silvente-Poirot et al., 2018), and these allow cells (particularly cancer cells) to regulate numerous genes involved in proliferation, metastasis, metabolism, and/or survival.

Oxysterols as Cholesterol-Oxidized Byproducts

Oxysterols are oxidized forms of cholesterol found in low to very low concentrations in the human body (van Reyk et al., 2006). Cholesterol oxygenation usually occurs on the aliphatic side chain or steroid backbone and directly results from enzymatic activity or auto-oxidation in the presence of reactive oxygen species (ROS). Side-chain oxidation generates 22-hydroxycholesterol (22-HC), 24-HC, 25-HC, and 27-HC, and oxidation occurring on the backbone generates 7 α /β-hydroxycholesterol (7 α -HC/7 β -HC), 7-ketocholesterol (7-KC) and 5,6 α /β-epoxycholesterol (5,6 α -EC/5,6 β -EC). Oxysterols can also be generated indirectly through lipid peroxidation (Yoshida et al., 2013). Oxysterols, like cholesterol, have a role in membrane composition and fluidity. They have also been described as “influencers” of signaling pathways in human pathologies, including cancer, and contribute to the activity of certain transcriptional regulators (Silvente-Poirot et al., 2018). Intriguingly, these effects involve some degree of cell specificity. We will focus below on 22-HC,

24-HC, 25-HC, and 27-HC, which are oxysterols oxidized on the side chain of the cholesterol molecule known to have anti- and pro-tumor roles.

22-Hydroxycholesterol

22-HC is an intermediate in pregnenolone biosynthesis from cholesterol. 22-HC, 22(R)-hydroxycholesterol, and not the enantiomer 22(S)-hydroxycholesterol, is a high-affinity LXR ligand that induces ABCA1 expression, leading to cellular cholesterol efflux. 22-HC has neuroprotective effects, such as avoiding neuronal beta-amyloid-induced cell death. Relatively few studies have examined the effect of 22-HC on cancer. In cholangiocarcinoma, where the expression of cyclooxygenase-2 (COX-2) participates in biliary tract carcinogenesis, 22-HC induces COX-2 expression in a p38-dependent fashion (Yoon et al., 2004). This suggests that 22-HC contributes to cholangiocarcinoma formation. In a pluripotent human embryonic carcinoma cell line (NTera-2), 22-HC treatment induces cell differentiation, subsequently impairing proliferation (Yao et al., 2007). This anti-proliferative effect has also been reported for other cancer cell lines, such as breast, ovarian, hepatic, or prostate cancer (PC) cell lines, and seems to be mediated through cell-cycle arrest and activation of LXR signaling (Chuu and Lin, 2010). More recently, 22-HC treatment appears to exert negative feedback on steroid biosynthesis. Although the mechanism of action remains unclear, ROS production is involved, increasing p38 and CREB phosphorylation and expression of antioxidant enzymes (Chen et al., 2017).

24-Hydroxycholesterol

24-HC is also called “cerebrosterol”, as it is the major oxysterol found in the central nervous system (CNS) and has the ability to easily cross the blood-brain barrier. 24-HC results from CYP46A1 action and is catabolized by CYP39A1. 24-HC elimination from the brain contributes to CNS cholesterol homeostasis and is transferred to the liver for conversion into bile. Like most oxysterols, 24-HC also activates LXRs. Early studies reported that 24-HC accumulation is highly toxic for neuroblastoma cells (SH-SY5Y). Although 24-HC increases tyrosine hydroxylase accumulation, this toxicity is induced by lipid droplet accumulation, ROS, and intracellular calcium buildup, leading to apoptotic and necroptotic processes (Kölsch et al., 1999; Rantham Prabhakara et al., 2008; Yamanaka et al., 2014). More recently, the concentration of oxysterols, particularly 24-HC and 27-HC, was determined to be responsible for these intracellular effects. Interestingly, low 24-HC levels are protective against staurosporine, a potent apoptotic agent, but higher 24-HC concentrations have the opposite effect, promoting death in staurosporine-treated SH-SY5Y cells (Emanuelsson and Norlin, 2012). Extrapolation of these findings to normal neuronal physiology suggests that oxysterols, particularly 24-HC, could be important mediators of neurodegenerative diseases such as Alzheimer’s or Parkinson’s. In Jurkat T cell lymphoma cells, 24-HC induces apoptosis through a mechanism involving 24-HC esters and lipid droplet accumulation (Yamanaka et al., 2014). Here, genetic and pharmacologic inhibition of acyl-CoA cholesterol acyl-transferase (ACAT1) decreases 24-HC ester levels, lipid droplet formation, and cell death. 24-HC also influences angiogenesis of pancreatic neuroendocrine tumors (PNETs), where it recruits proangiogenic neutrophils contributing to a tumor “angiogenic

switch" (Soncini et al., 2016). Finally, recent evidence suggests that oxysterols and 24-HC could be used as potential biomarkers to assess the efficacy of tamoxifen treatment in breast cancer patients (Dalenc et al., 2017).

25-Hydroxycholesterol

25-HC, catabolized by CYP7B1, is a side-chain oxysterol that regulates cholesterol biosynthesis through inhibition of SREBPs. Interestingly, 25-HC affects the immune system and impacts atherosclerotic processes; LXR activation by 25-HC suppresses cholesterol homeostasis in T cells (Bah et al., 2017). It has also been recently reported in CD4⁺ T cells that 25-HC contributes to the regulation of interleukin-10 (IL-10) expression, and subsequently control anti-inflammatory response involved in chronic inflammatory diseases (Perucha et al., 2019). In B cells and monocytes, 25-HC impairs immunoglobulin production and differentiation into macrophages, respectively (Bauman et al., 2009). 25-HC may also play a role in tumorigenesis. More specifically, 25-HC promotes lung, gastric, brain, and breast cancer cell migration and invasion (Chen et al., 2017; Eibinger et al., 2013; Lappano et al., 2011; Wang et al., 2019). Mechanistically, 25-HC induces the recruitment of pro-tumor monocytes through activation of the G protein-coupled receptor 183, also known as EBI2, in GBM (Eibinger et al., 2013). 25-HC does not seem to affect cell proliferation or survival in gastric and lung adenocarcinomas but primarily enhances cancer cell migratory capacities through activation of Toll-like receptor 2 (TLR2)/nuclear factor- κ B (NF- κ B) pathways and LXR/IL-1B signaling (Chen et al., 2017; Wang et al., 2019).

27-Hydroxycholesterol

27-HC is an endogenous oxysterol and one of the most abundant oxysterols in human plasma (van Reyk et al., 2006). Its production results from the activity of sterol 27-hydroxylase (CYP27A1) and is catabolized by CYP7B1. Its conventional name is (25R)-cholest-5-ene-3 β ,26-diol or 26-HC. Similar to 24-HC, 27-HC is also considered a cerebrosterol and has the ability to easily cross the blood-brain barrier. Through its activities as a selective endogenous estrogen receptor (ER) modulator or LXR agonist, 27-HC has several biological roles associated with Alzheimer's, metabolic, heart, and neoplastic diseases, particularly breast cancer. 27-HC levels in blood samples of >500 invasive breast cancer cases reveal that 27-HC is not correlated with the overall risk of developing breast cancer. However, menopausal status seems to affect 27-HC-related risk, as higher circulating 27-HC is associated with lower breast cancer risk in postmenopausal women (Lu et al., 2019). Despite this report, hypercholesterolemia and 27-HC have previously been shown to be associated with decreased response of ER-positive breast cancers to hormonal therapies. 27-HC promotes ER-positive breast cancer cell proliferation and increases metastatic potential by favoring an epithelial-to-mesenchymal transition (EMT) in an LXR-dependent manner (Nelson et al., 2013; Raza et al., 2015; Torres et al., 2011; Wu et al., 2013).

A high-fat diet, cholesterol, CYP27A1, and 27-HC contribute to cancer cell metastasis through modulation of the immune system. Genetic or pharmacological CYP27A1 inhibition blocks metastatic processes, confirming the ability of 27-HC to increase number and activity of pro-tumor neutrophils and $\gamma\delta$ -T cells, resulting in decreased cytotoxic CD8⁺ T cell populations (Baek

et al., 2017). Additional reports confirm the role of 27-HC and CYP27A1 as potential therapeutic targets in other types of cancer. In endometrial cancer (EC), 27-HC enhances EC epithelial cell proliferation in a mechanism involving activation of ER, but not LXRs (Gibson et al., 2018). In an elegant mouse model of hypercholesterolemia and liver-specific CYP27A1 deletion, melanoma cells rely on hepatocyte-produced 27-HC for their growth. 27-HC released by hepatocytes activates the ER to sustain AKT/MAPK signaling in melanoma cells (Tian et al., 2018). 27-HC is also involved in lung adenocarcinoma metastatic process (Zhang et al., 2019). 27-HC promotes the macrophage-to-osteoclast differentiation by enhancing STAT3/c-Fos/nuclear factor of activated T cell (NFAT) interaction, therefore, favoring a microenvironment for lung cancer metastases.

In colorectal carcinoma (CRC), PC, and bladder cancer, the role of 27-HC is not as clear. High CYP27A1 expression resulting in increased levels of 27-HC, particularly in advanced stages of CRC, is associated with poor prognosis. In correlation with this, high CYP7B1 expression is associated with a better prognosis (Rossin et al., 2019; Swan et al., 2016). Mechanistically, on one hand, a high 27-HC concentration appears to increase AKT phosphorylation and the production of several chemokines, such as IL-6/8, vascular endothelial growth factor (VEGF), MCP-1, and matrix metalloproteinases (MMPs) from CRC cells. On the other hand, treatment of CRC cells with 27-HC impairs cell proliferation, independently of LXR or ER pathways, through decreased AKT signaling (Wans et al., 2018). 27-HC stimulates proliferation of PC cells through activation of nuclear hormone receptors, ERs and androgen receptor, contributing to chemoresistance (Raza et al., 2016, 2017). However, CYP27A1 levels are lower in PC compared to normal tissue and associated with shorter disease-free survival. Interestingly, CYP27A1 re-expression or 27-HC treatment alters tumor growth *in vitro* and *in vivo*, potentially through dysregulation of cholesterol homeostasis (Alfaqih et al., 2017). CYP27A1 is an important sensor of cholesterol levels in bladder cancer. Similar to PC cells, CYP27A1 overexpression leads to decreased intracellular cholesterol levels and cell growth defects via an LXR-dependent mechanism (Liang et al., 2019). LXRs stimulate the export of intracellular cholesterol through ABCA1/G1 exporters and simultaneously decrease import by negatively regulating the LDLR. As mentioned above, 27-HC is among the most abundant oxysterols found in human plasma, and its role in cancer is not clearly established. Depending on the cancer type, CYP27A1 and 27-HC have completely opposite roles. These discrepancies clearly imply that more studies are required to precisely assess the role of 27-HC in tumorigenesis.

Cholesterol Catabolism Generates Bile Acids

The principle way for humans to combat cholesterol or oxysterol overload is to transport them to the liver, where they are converted into bile acids and excreted into the digestive system. Bile acids-salts are cholesterol derivatives and key signaling molecules that play important roles as emulsifiers and lipid absorbers during digestion processes (Chiang, 2013; Russell, 2003). Bile formation is tightly regulated and requires 17 different enzymes, starting with the rate-limiting cholesterol 7 α -hydroxylase (CYP7A1) (Ge et al., 2019). After several other

hydroxylations, primary bile salts, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), are formed and excreted into the gallbladder for ultimate release into the gastrointestinal tract. Bile acids not directly reabsorbed can be further processed into secondary bile salts via the activity of gut microbiota. Colonic anaerobic bacteria deconjugate primary bile acids, converting them into deoxycholate (DCA), ursodeoxycholate (UDCA), or lithocholate (LCA) (Ramírez-Pérez et al., 2017; Singh et al., 2019). Finally, these secondary bile acids are either reabsorbed or excreted in the feces.

Bile acids are key signaling molecules, with signaling functions through nuclear receptors LXR α s (see above) and farnesoid X receptor (FXRs), and have already been reviewed previously (Chiang, 2013; Zhou and Hylemon, 2014). However, in addition to their role in cholesterol catabolism and fat solubilization, bile acids have been implicated in cancer progression, particularly in esophagus, stomach, gallbladder, and bile duct cancer (cholangiocarcinoma). Oxysterols converted into bile acids directly contribute to esophagus and bile duct cancer through increased COX-2 expression (Hashimoto, 2014; Moon et al., 2016). In pancreas cancer cells, DCA and CDCA treatment increases COX-2 and prostaglandin E2 (PGE2), enhancing inflammation (Tucker et al., 2004).

Bile acids are also suspected to cause mitochondrial dysfunction, leading to ROS accumulation in colon cells (Ignacio Barrasa et al., 2011). Of note, bile acid exposure, particularly DCA, triggers activation of epidermal growth factor receptor (EGFR), MAPK, NF- κ B, and protein kinase C (PKC) signaling pathways in normal colon and esophageal epithelium and colon cancer cells (Ignacio Barrasa et al., 2011; Naran et al., 2011; Wu et al., 2018). However, prolonged bile acid exposure induces apoptotic cell death via mitochondrial dysfunction, ROS release, and cytochrome c activation (Ignacio Barrasa et al., 2011; Lee et al., 2017; Liang et al., 2012). These mechanisms contribute to colorectal tumorigenesis. Similar to pro- and anti-tumor effects of oxysterols, bile acids also exhibit dual functions. For instance, in leukemic (HL-60 and THP-1), pancreatic (PANC-1 and Mia PaCa2), and gastric cancer (SNU-216 and MKN45) cells, DCA and CDCA impair cell proliferation and invasive capacities through various mechanisms involving modulation of PKC, Snail, and MMP expression (Pyo et al., 2015; Wu et al., 2003; Zimber et al., 2000). Finally, bile acids can impact prostate and breast cancer cell metabolism by downregulating the master metabolic transcription regulator HIF-1 (Phelan et al., 2016).

Cholesterol: A Key Molecule in Immune Microenvironments

Cholesterol is important in maintaining cell membrane stiffness and fluidity, and a cell type specifically relying the most on motility and membrane-membrane interactions with other cells are immune cells. In addition to cancer cells, immune cells (particularly T cells) are highly proliferative, requiring biomass expansion; it is therefore not surprising that T cells rely on cholesterol availability. For instance, naive T cells treated with statins exhibit cell-cycle progression defects and begin differentiating (Chakrabarti and Engleman, 1991). Deregulated metabolic pathways in cancer metabolism, such as glycolysis and lipid metabolism, have been implicated in T cell activation and

proliferation as well (Wang and Green, 2012). Similar to the growing evidence for a role of cholesterol in loss of T cell effector functions, recent work demonstrates that after homing to cholesterol-rich tumors, T cells are more prone to exhaustion due to increased cholesterol uptake and accumulation (Ma et al., 2019). Moreover, T cell exhaustion is induced by endoplasmic reticulum stress and increased expression of immune checkpoints.

Cholesterol has also recently been involved in tumor-associated macrophage (TAM) polarization, where factors such as hyaluronic acid promote macrophage cholesterol efflux. This is also associated with increased IL-4 signaling and inhibition of interferon- γ (IFN- γ) transcriptional programs, resulting in TAM reprogramming and tumor progression (Goossens et al., 2019). The tumor-associated microenvironment drives phenotypes of tumor-infiltrating immune cells, switching them from an anti-tumor to pro-tumor state. Due to the fact that cancer cells rely on cholesterol uptake from their microenvironment, it is not surprising to observe how immune cells participate to such cholesterol-dependent phenotypes. However, a key question arising from these studies is how the availability of cholesterol regulates intracellular pathways leading to the expression of distinct subsets of genes.

Gut Microbiota: Key Players in Cancer and Cancer Metabolism

Over the past century, new human behaviors in terms of increased consumption of processed foods rich in sugar and fat and decreased intake of grains, fruits, and vegetables rich in fiber have appeared. The human gut microbiome regroups 10^{14} resident microorganisms, including bacteria, viruses, fungi, and protozoa (Gill et al., 2006). Vitamins and essential amino acid synthesis, as well as generation of important metabolic byproducts from dietary breakdown, are among the prime benefits that gut microbiota provide to their host and are absolutely essential for health. Moreover, diet plays a significant role in shaping the microbiome, mediating microbial shifts that can lead to metabolic disorders and cancer (David et al., 2014; Owyang and Wu, 2014). The production of cholesterol-derived metabolites, such as coprostanol and secondary bile acids present in peripheral tissues, reveals a key role for gut microbiota in reducing and eliminating cholesterol (Antharam et al., 2016; Pyo et al., 2015; Wu et al., 2003; Zimber et al., 2000). Dysregulation of this gut-flora-mediated cholesterol homeostasis could be a risk factor for tumors relying mainly on cholesterol turnover. For example, increasing levels of DCA, known to mediate DNA damage, are associated with a higher risk of colon and liver cancer (Milovic et al., 2002; Yoshimoto et al., 2013). Even excessive fermentable fiber diets result in cholestasis and hepatocellular carcinoma (HCC) in mice, suggesting that enriching foods with fiber to manipulate microbiota should be approached with great caution (Singh et al., 2018). Another report reveals that carbohydrate-rich diets increase the abundance of short-chain FAs (SCFAs), such as butyrate, through microbial fermentation. Butyrate accumulation induces aberrant proliferation and transformation of colonic epithelial cells, ultimately resulting in polyp formation and colorectal cancer in some cases (Belcheva et al., 2014). Taken together, these findings illustrate that dietary variation

might affect gut microbial composition, opening up new possibilities for controlling diet and/or targeting gut microbiota as a strategy for various metabolic diseases that include cancer.

Concluding Remarks

Over the last decade, cancer cell metabolism and the metabolic reprogramming that cancer cells experience have been appreciated as essential to sustain tumor cell growth, survival, and motility. A potential shift away from oxidative phosphorylation to glycolysis (for some cancers), increased glucose and glutamine uptake, and enhanced FA and cholesterol synthesis and/or import may provide opportunities for optimized therapeutic intervention. For instance, targeting glucose and/or glutamine metabolism, in conjunction with restricting the supply of FAs or cholesterol, may enhance chemotherapy effectiveness (Akins et al., 2018; Bajpai and Shanmugam, 2018). It should also be noted that cancer metabolism and metabolites are tightly connected to oncogenic signaling networks and the tumor microenvironment (hypoxia, immune system, stroma, etc.). Therefore, defining the exact interactions between oncogenic, metabolic, and signaling pathways and tumor microenvironments could open new combinatorial therapeutic windows. Additionally, lipid biology is intrinsically complex, making cancer cell lipid homeostasis even more complicated. While obesity and high-fat or high-cholesterol diets (i.e., Western diets) are linked to cardiovascular diseases (Fung et al., 2001), mounting evidence also connects these behaviors to increased cancer risks. Although the underlying mechanisms are not clear, cholesterol metabolism, exacerbated oxidative stress, cholesterol oxidation, and the formation of oxysterols could be important contributors to these observations. Large-scale studies assessing cholesterol, oxysterols, and bile acids are necessary to fully understand the involvement of these metabolites in mechanisms underlying cancer formation and progression. Finally, similarities exist between cancer-associated metabolic alterations and other pathologies, particularly neurodegenerative disease such as Alzheimer's, Parkinson's, or Huntington's disease (Cai et al., 2012). Increased evidence correlates oxysterols to the onset and progression of metabolic neurodegenerative diseases, suggesting that findings in cancer cell metabolism and their therapeutic applications could be highly relevant to other pathological settings.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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