THE ROLE OF LIPOPROTEINS IN THE MICROENVIRONMENT OF HORMONE-DEPENDENT CANCERS

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Abstract

The tumor microenvironment (TME) is an attractive target to develop novel strategies for hormone-dependent cancers. Several molecules in the TME can favor tumor development and progression, including lipoproteins. Lipoproteins are taken up by cancer cells providing them with cholesterol and fatty acids. Cholesterol regulates cell signaling and it is converted into a series of bioactive metabolites, including hormones. The conflicting results of epidemiological and interventional studies suggest that the local availability of lipoproteins in the TME is more relevant for cancer biology than their circulating levels. Thus, reducing lipoprotein uptake and stimulating cell cholesterol efflux to high density lipoproteins (HDL) can represent a novel adjuvant strategy for cancer management. HDL-like particles can also act as drug delivery systems for tumor targeting.

Relevance of the microenvironment in tumor development and progression

Hormones can drive the development and the progression of malignancies at multiple sites, including adrenal, thyroid, parathyroid, pancreatic, prostate and reproductive tissues. This review is mainly focused on breast and prostate cancers (BC and PCa), which are the leading cause of cancer-related death in women and men, respectively. They are classified as hormone-dependent cancers due to the key role of steroid sexual hormones in tumor initiation and progression (see Box 1). Both types of cancer are characterized by an effective response to hormone-deprivation therapies; however, therapeutic options for locally advanced or metastatic tumors are limited and poorly effective [1,2].

In the attempt to identify the mechanisms responsible for tumor progression and to find novel therapeutic targets, the tumor microenvironment (TME) has gained increased attention. The TME is as a complex, acidic and hypoxic environment, with cancer cells and non-transformed stromal cells of different origins immersed in the extracellular matrix (ECM), that evolves during tumor progression [3]. Cellular components include endothelial cells and pericytes, fibroblasts, adipocytes, resident and infiltrating immune cells, while the ECM, which provides a structural support for these surrounding cells, is mainly composed by collagen, elastin, fibronectin, laminin and proteoglycans (PGs). The role of the different stromal cells in tumor development and progression is complex and still debated, as recently reviewed by Hanahan and Mittal [4,5]. Interestingly, TME can modulate malignant progression in multiple ways and TME signature can predict disease outcome and therapeutic response, independent from cancer cell features, as in the case of BC [6,7].

Several molecules in the TME can favor the development and progression of hormone-dependent cancers. It is well established the role of pro-oxidant and pro-inflammatory molecules, which can reach the TME from the systemic circulation or can be locally produced by stromal cells [8]. In addition, sources of cholesterol and fatty acids are present in the TME; these molecules can promote cancer cell proliferation through several mechanisms beyond their well-known structural and energetic role in cell physiology (see Box 2).

Cholesterol and its metabolites in cancer cells

The role of cholesterol in the context of cancer cell proliferation has been recently revised. In the past, cholesterol need was ascribed to its structural role in cell membranes, while now it is recognized as a key player in the regulation of cellular function (Figure 1). Its presence in cell membranes is able to modulate the activation of transmembrane receptors [9]. In addition, it

can be converted into a wide series of biologically active metabolites, such as **oxysterols** and isoprenoids [10]. Finally, cholesterol acts as a building block for the local synthesis of hormones. Consequently, cholesterol demand is higher in hormone-dependent cancers compared to other tumor types [11].

Cholesterol can be directly synthetized by cancer cells or it can be taken up by lipoproteins (see box 3 and box 4), which are present in extracellular fluids (Figure 1). Indeed, the receptor-mediated endocytosis of apoB-containing lipoproteins and, depending on the cholesterol gradient, the SR-BI-facilitated cholesterol influx from HDL, are relevant sources of cholesterol in the TME [12]. On the contrary, HDL could help in reducing cholesterol content of cancer cells acting as acceptors of cholesterol efflux [13]. To deal with their need of cholesterol, cancer cells are generally characterized by an increased expression of SREBP-regulated genes, which support cholesterol synthesis and uptake, and by a concomitant inhibition of LXR-regulated genes, including ABC transporters [14,15] (Figure 2). Cancer cell uptake of lipoproteins in the TME is promoted by **hypoxia**; indeed, in several cancer cell lines, hypoxia was shown to promote the expression of many lipoprotein receptors, as LDL-R, LRP1, VLDL-R and SR-BI [16] (see Box 3). In addition, the acidic environment increases the affinity of LDL for PGs, which favors LDL uptake by endocytosis [16]. Furthermore, PG-bound LDL are entrapped in the extracellular space, where they can undergo oxidation that increases LDL affinity to scavenger receptors and triggers pro-inflammatory and pro-oxidant cascades [17].

The relevance of cholesterol for proliferating cancer cells is supported by three main findings. First, cholesterol accumulates in cancer cells due to the upregulation of its synthesis and uptake [18-21] (Figure 2). Since unesterified cholesterol is toxic, it is quickly esterified in the cytosol by ACAT and stored in **lipid droplets** (LDs), which aberrantly accumulate in tumor cells [22]. Second, the inhibition of the SREBP pathway exerts antiproliferative and antimetastatic effects [20]. Third, when cholesterol is rapidly removed from cell membranes, as by methyl-β-cyclodextrin (βMCD, an acceptor of cholesterol through passive diffusion), cell survival is impaired [23-25].

Structural and functional role of cholesterol in the cell membrane

Cholesterol regulates the fluidity of the cell membrane and its distribution is not homogeneous; lipid rafts are sub-domains of the membrane particularly enriched in

cholesterol along with glycosphingolipids and sphingomyelin [9]. Lipid rafts have a key role in signal transduction, since many types of proteins are recruited to these domains, as glycosylphosphatidylinositol-anchored proteins, prenylated and acylated proteins, and transmembrane receptors [26]. Lipid rafts act as concentrating platforms for receptors activated by ligand binding; the activation leads to rafts' clustering, which favors the interaction among the various members of a signaling complex by close proximity [26]. Interestingly, many receptors for growth factors (as epidermal growth factors, insulin-like growth factor 1 and vascular endothelial growth factor) are located in lipid rafts and an enrichment in cholesterol was shown to increase the activation of downstream PI3K/Akt and ERK1/2 pathways, likely supporting cancer cell proliferation and migration. On the contrary, when cholesterol is removed from lipid rafts (as by βMCD or HDL), receptors are internalized and signaling events blunted [24,25].

Intra-tumor formation of cholesterol metabolites

The local production of dihydrotestosterone and 17β-estradiol from cholesterol by 5α-reductases and aromatases could explain the progression of PCa and BC even with the very low levels of circulating hormones achieved during deprivation therapies. Locally produced hormones can activate their cognate receptors, supporting cancer cell proliferation [27,28]; accordingly, 5α-reductase and aromatase inhibitors represent a therapeutic strategy for the management of hormone-dependent cancers [29]. Likewise, intra-tumor hormone synthesis from cholesterol is upregulated in other endocrine cancers, as adrenocortical and ovarian carcinomas [30,31].

Intracellular cholesterol metabolism can also lead to the generation of a wide spectrum of oxysterols through the action of the **cytochrome P450** family enzymes (CYPs). Interestingly, compared to parental non-tumor cells, PCa and BC cells could present with an altered expression of CYPs, leading to a different pattern of cholesterol metabolites; consequently, some cholesterol metabolites are overproduced in cancer cells, while other are generated in lower amounts [32]. Oxysterols are endogenous ligands of LXRs and are generally believed to exert anti-proliferative actions; LXR activation, besides regulating cholesterol homeostasis, triggers anti-inflammatory pathways in cancer and stromal cells [10,33]. For example, LXR agonists were shown to induce the release of anti-inflammatory interferon γ from

macrophages and T cells [34]. In addition, LXRs can increase the expression of sulfotransferases, leading to steroid inactivation [35]. The activity of oxysterols on cancer cells extends beyond LXR activation. Indeed, 7-ketocholesterol (as unsaturated fatty acids) was shown to act as ligand of the so-called **antiestrogen binding site** (AEBS); 5,6α- and 5,6β-epoxycholesterol and their condensation product with histamine, dendrogenin A, also displayed high AEBS affinity [36]. On the contrary, some oxysterols could contribute to tumor growth, as 27-hydroxycholesterol. Indeed, it was shown to specifically increase the growth of ER-positive BC cancer cells by acting as an ER agonist [32]. Interestingly, 27-hydroxycholesterol accumulation has been described in ER-positive BC due to the downregulation of CYP7B1, blunting the transcription of many LXR-regulated genes and in particular of ABCA1 [32]. Contrasting results were obtained for 27-hydroxycholesterol on PCa cells [37,38].

Fatty acid metabolism by cancer and immune cells in the TME can also lead to the generation of bioactive molecules, such as eicosanoids [39]. These molecules are produced from arachidonic acid and other polyunsaturated fatty acids by the action of lipoxygenases and cyclooxygenases. Their role in cancer is still in debate since they were variably associated with cancer cell proliferation; indeed, while some eicosanoids may act as tumor suppressors (as omega-3 fatty acids and resolvins), others were shown to promote the survival and the proliferation of cancer cells [39]. In particular, prostaglandin E2 levels are elevated in several human malignancies, including BC, PCa and ovarian cancer, and were associated with poor prognosis and resistance to chemotherapy and radiotherapy [40,41]. Thromboxane A2 (TXA2) is also increasingly implicated in cancer progression, especially in triple negative BC. The binding of TXA2 to its receptor (thromboxane receptor, TP) enhances BC cell migration and invasion by triggering Rho activation [29]. Through the same mechanism, TP activation induced cytoskeletal reorganization of PCa cells [42]. On the contrary, resolvins may help to lower the risk of developing cancer [43]. These bioactive lipids, derived from the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, are key players in the resolution of inflammation. In multiple tumor types, resolvins were shown to reduce tumor growth, neo-angiogenesis, metastatization and to revert the deactivation of natural killer (NK) cells [44].

Lipid metabolites for protein modification

Along the mevalonate pathway, isoprenoids (farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate) are produced, which are needed for prenylation, a post-translational modification of proteins. Prenylation provides the target protein with a hydrophobic C terminus, which guides its localization within the cell, for example to the lipid rafts, favoring protein-protein interactions [45]. Many prenylated proteins are involved in cell signaling, as small GTP binding proteins. Among these, the oncogene **RAS** is the most studied and a reduction of isoprenoid production, as by farnesyltransferase inhibitors, is under investigation with promising results in aggressive BC [46].

Fatty acids are also involved in protein modification, as some proteins undergo myristoylation or palmitoylation [47,48]. Again, many of the target proteins are crucial components of signaling pathways and the modification with fatty acids promotes their binding to the membrane for proper localization and function. Well-characterized targets include many oncogene products, such as the tyrosine kinase Src [47,48].

Lipoproteins as pharmacological targets?

Epidemiologic evidence

Several retrospective studies investigated the relationship between plasma levels of lipids/lipoproteins and the incidence of different cancer types. To date, conflicting results were obtained, since positive, negative or no relationships were found [49,50]. In the REduction by DUtasteride of prostate Cancer Events (REDUCE) trial, total cholesterol was positively associated with an increased risk of high-grade PCa (5% higher for 10 mg/dl), but not with low-grade one. HDL-C levels were also positively associated with overall risk of PCa (8% higher for 10 mg/dl) [51]. However, in the same trial, men with high HDL-C displayed reduced prostate inflammation [52]. On the contrary, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, while the positive association between total cholesterol and the risk of advanced PCa was confirmed, HDL-C was negatively associated with PCa risk [53]. The association between plasma lipids and the risk of cancer was also investigated in prospective studies. Large meta-analyses excluded the association between plasma lipids and the risk of PCa and ovarian cancer, but HDL-C was negatively associated with BC risk [54-56].

Mendelian randomization studies were performed to address the causal link between plasma lipids and PCa or BC risk. No association of plasma lipids with PCa risk was found, with a weak evidence that higher LDL-C and triglyceride levels increased aggressive PCa risk

[57]. On the contrary, genetically raised LDL-C levels were associated with higher risk of BC, especially of the ER-positive type [58]. The same relationship was found for elevated HDL-C, but it should be noted that only the effect of CETP variants was assessed, and inhibiting CETP is debated for the possible accumulation of dysfunctional HDL [59]. The role of dietary lipids on cancer risk is also debated, since a lipid-rich diet could be indicative of an unhealthy lifestyle. In addition, a significant association between dietary cholesterol intake and the risk of BC was detected only with very high intakes [60]. Another approach to address the relationship between plasma lipids and the incidence of cancer is to assess the association with **statins**. In two recent meta-analysis, statins were shown to have a neutral effect on both PCa and BC incidence [61,62]. Consistently, the Cholesterol Treatment Trialists' Collaboration showed no evidence of any effect of reducing cholesterol with long-term statin therapy on cancer incidence or mortality [63]. To explain the inconsistency between studies, the type of cancer and the time at which lipids were assessed could act as confounding factors. The stage of the disease and the consequent therapies can affect the levels of circulating lipids; plasma lipids, especially HDL-C levels, are reduced in several cancer patients during active disease, as in those having breast, ovarian, prostate, colon and pancreatic carcinomas [64]. The large HDL2 subclass is particularly reduced [65].The predictive power of low HDL-C and apoA-I levels was so strong that apoA-I was included in the screening for ovarian cancer with other traditional biomarkers [66]. The mechanisms beyond HDL-C reduction are not fully understood. Since the liver and the intestine are responsible for the synthesis of apoA-I and its lipidation by ABCA1, malignancies at these organs can directly impaired HDL biogenesis. As shown in many inflammatory states, pro-inflammatory cytokines inhibit hepatic apoA-I expression and, thus, the cancer-related sustained inflammation can repress HDL biogenesis by the same mechanism [67]. Finally, the hepatic expression of several proteins involved in lipoprotein metabolism can be modulated by inflammation and cancer, including LCAT; thus, HDL-C reduction could be part of a more complex alteration of lipoprotein metabolism induced by cancer [67].

The association between plasma lipids and the recurrence of cancer has been firmly established. Plasma levels of cholesterol are positively associated with a higher recurrence rate of BC and statin use was associated with extended recurrence free survival time [68]. This protective effect was confirmed in women with hormone receptor-positive early-stage breast cancer taking statins during adjuvant endocrine therapy [69]. Plasma levels of HDL-C

are inversely related to the prognosis; in a recent meta-analysis including both retrospective and prospective studies, patients with higher HDL-C had 37% reduced risk of death and 35% reduced risk of disease relapse compared to patients with lower HDL-C [70].

Targeting apoB-containing lipoproteins

The use of hyperlipidemic mice (*apoE*$^{-/-}$ model) fed a high fat/high cholesterol diet (HFHC) can help to address the impact of increased plasma levels of apoB-containing lipoproteins on the development of hormone-dependent cancers. The injection of non-metastatic Met-1 and metastatic Mvt-1 mammary cancer cells in *apoE*$^{-/-}$ mice resulted in larger tumors and in a greater number of lung metastases compared to wild-type mice [71]. Interestingly, the graft with BC cell lines representative of triple-negative or HER2-enriched tumors in hyperlipidemic mice resulted in reduced tumor growth when the LDL-R was silenced [72]. Consistently, LDL increased the proliferation and migration of ER-negative BC cells but not of ER-positive ones, which also accumulated less CEs than ER-negative cells [73]. These data suggest that ER-negative BC may be more sensitive to cell cholesterol-lowering strategies. Regarding PCa, mice fed a HFHC diet developed larger tumors after injection of androgen-dependent LNCaP cells compared to mice under chow diet [24]. In addition, the HFHC diet accelerated tumor incidence and burden compared to chow diet in the TRAMP mouse, a model of PCa [74]. These data are supported by several *in vitro* studies. VLDL, but not LDL, increased the formation of BC cell mammospheres, an estimate of stem cell/early progenitor activity, and cell resistance to radiotherapy, likely due to a modulation of intracellular cholesterol content [75]. Consistently, the treatment of aggressive and metastatic BC cells with statins was associated with an increased sensitivity to radiation therapy [75]. The *in vivo* relevance of these findings was supported by the shorter recurrence-free period in patients with elevated VLDL-C levels, which suggests a systemic effect of statins through the reduction of circulating apoB-containing lipoproteins [75]. Oxidized LDL (oxLDL), but not native LDL, increased the proliferation of ovarian cancer cells and decreased their sensitivity to cisplatin, an effect prevented by statins and by LXR activation [76]. OxLDL could act through the binding with its lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which stimulates the expression of pro-adhesive, pro-inflammatory and proangiogenic factors in vascular endothelial cells and macrophages. LOX-1 upregulation was reported in several different types of cancer, including PCa. Here, its activation by oxLDL promoted epithelial to

mesenchymal transition and increased tumor growth in nude mice [77]. In BC cell lines, LOX-1 inhibition blunted inflammatory and hypoxic responses [78]. Finally, apoB-containing lipoproteins and adipocytes could deliver free fatty acids to cancer cells by the action of locally expressed LPL; BCs are generally positive for LPL and its expression increased cell proliferation [79]. Moreover, adipocytes can directly provide cancer cells with fatty acids. Indeed, cancer cells can stimulate TG lipolysis by the adipose triglyceride lipase/hormone-sensitive lipase axis in adipocytes [80]. Adipocytes were shown to support the proliferation and invasiveness of highly differentiated and hormone-dependent cancers more than of poorly differentiated ones [81].

Recently, the use of statins in the management of cancer has been questioned. Statins are mainly taken up by the liver, with little amounts reaching other organs and tissues. In addition, the inhibition of cholesterol synthesis by statins induces a SREBP-mediated increase of LDL-R expression. Thus, statins could have positive systemic effects on tumors by reducing plasma levels of LDL-C but could increase LDL-R-mediated cholesterol uptake by cancer cells. On this line, Caro-Maldonado et al. recently showed that statins increased PCa aggressiveness *in vivo* [82]. On the contrary, the silencing of LDL-R gave promising results in pancreatic adenocarcinoma, in HER2-enriched and triple negative BC cells [72,83]. Similar results were obtained when ACAT was inhibited to reduce intracellular CE stores in LDs, as in PCa, BC and adrenocortical carcinoma [22,73,84-86].

Among the apoB-containing lipoproteins, Lp(a) could exert a peculiar role on cancer biology. Indeed, it was shown to inhibit angiogenesis, thus causing delayed tumor growth and metastasis formation in models of lung and colorectal cancers; the effect was ascribed to the presence of repeated kringles in the apo(a) structure, a typical feature of angiostatin-related proteins [87].

Targeting the HDL system

Animal studies provided proofs of a causal link between HDL and cancer risk. When HDL-C levels were increased by genetic manipulation (as in the human apoA-I transgenic mice) or by direct infusion of apoA-I, the growth of lung, melanoma or ovarian cancer cells after xenograft was reduced compared to control animals [88]. Consistently, in apoA-I$^{-/-}$ mice, in which HDL-C levels are dramatically reduced, tumor development increased compared to wild-type animals

[88]. More interestingly, apoA-I infusion after the development of the tumor mass resulted in tumor shrinkage [88].

In vitro studies provided some mechanistic insights. When BC cells were incubated with HDL, the formation of mammospheres was inhibited and their sensitivity to radiation therapy increased, an effect due to a reduced cholesterol content [75]. Whether this reduction was ascribed to a depletion of cell membrane content or of intracellular CE stores in LDs was not addressed. It could be speculated that, given the continual cycle of hydrolysis and esterification of CE in LDs, the removal of unesterified cholesterol from the cell membrane promoted by HDL could shift the balance towards CE hydrolysis with a consequent LD depletion, as shown in macrophages [89]. In PCa cell lines, HDL blunted basal and H_2O_2-induced oxidative stress and reduced ROS-induced proliferation, with a role for both the protein and phospholipid components [90].

On the contrary, some studies showed a SR-BI-dependent increased growth of cancer cells incubated with HDL [91,92]. In this context, large CE-enriched HDL could represent a better substrate for SR-BI-mediated cholesterol influx supporting cancer cell growth. It has to be pointed out that HDL could become dysfunctional in several pathologic conditions, including inflammatory states, metabolic diseases and cancer, due to modifications of their protein and lipid cargo [64,93]. Consistently, the proliferation of the BC cell line MCF-7 was induced by HDL isolated from type 2 diabetic patients, but not by HDL from healthy controls. Only HDL from diabetic patients were able to promote BC metastasis by increasing the adhesion of cancer cells to endothelial cells [94,95].

Two approaches can be used to raise circulating HDL. The first is to improve HDL biogenesis or to limit their catabolism. To date, no drug specifically increases HDL-C levels. Fibrates and niacin have multiple effects on lipid metabolism including the increase of HDL biogenesis, but their role in cancer management has not been investigated. CETP inhibitors specifically increase HDL-C, but their development is hampered by the lack of a clear cardiovascular benefit, together with the possibility to accumulate dysfunctional HDL [59]. The second approach is to infuse reconstituted HDL (rHDL), discoidal particles made of phospholipids and apoA-I (or synthetic apoA-I mimetic peptides) that were shown to retain the atheroprotective activities of HDL [96]. Reconstituted HDL have been already tested in multiple *in vivo* and *in vitro* tumor models [97]. Among the underlying mechanisms, anti-inflammatory activities, inhibition of angiogenesis, abrogation of growth factor-induced proliferation, migration and

invasion were described [97]. Multiple positive effects of rHDL on TME cellular composition were also shown, as a reduced content of myeloid-derived suppressor cells and the accumulation of M1 macrophages and cytotoxic CD8+ T cells [88]. Some of these effects were detected with direct apoA-I or apoA-I mimetic peptide administration, suggesting an anti-tumor activity of HDL apolipoprotein components [88]; however, it cannot be fully excluded that apoA-I or its peptides could rapidly acquire phospholipids and cholesterol in the circulation, thus generating HDL-like particles.

HDL-like particles for drug delivery

Reconstituted HDL are also under development as drug carriers for tumor targeting. They accumulate within the tumor mass by unselective mechanisms (as nanoparticles do through the leaky vasculature) and by the specific interaction with SR-BI. This receptor is highly expressed in multiple cancers and allows cytosolic delivery of the payload bypassing the endolysosomal route [98]. Unlike exogenous nanoparticles, rHDL containing apoA-I are non-immunogenic and have long circulation half-life. In addition, the small diameter (<14 nm) allows a deeper penetration of rHDL into the tumor mass if compared to nanoparticles, which is crucial for the treatment of solid tumors [99].

Several types of molecules can be incorporated into rHDL as small molecule drugs, small interfering RNAs, photothermal agents or fluorescent dyes for imaging [98]. The composition and the particle size/shape of rHDL can be tailored according to the type of molecule to be carried and the tumor to be targeted. For example, spherical rHDL were used for the encapsulation of highly hydrophobic drugs into the particle core: by this way paclitaxel and valrubicin were successfully delivered to PCa, triple-negative BC or ovarian cancer cells in vitro and in vivo [100-102].

Concluding remarks and future perspectives

Targeting the lipoprotein system is becoming an attractive approach to develop novel therapeutic strategies for the management of hormone-dependent cancers. The conflicting results of epidemiological and interventional studies suggest that the availability of lipoproteins in the TME is likely more relevant than their circulating levels. In this context, statins use seems not a promising strategy due to their preferential hepatic distribution and upregulation of the LDL-R expression. On the contrary, targeted repression of the LDL-R in

cancer cells, as by genetic silencing, already generated positive results in different types of cancer. Another approach is to increase the efficiency of the HDL system in promoting lipid removal, as by the infusion of rHDL to rise the endogenous HDL pool. This approach has some advantages. Reconstituted HDL are already under clinical development for patients with acute coronary syndrome [96]. Their protein and lipid composition can be manipulated to optimize their function. In addition, rHDL can be used as carriers of other bioactive molecules, ranging from nucleic acids to drugs [98].

Overall, targeting lipid metabolism in the TME should be considered as an adjuvant strategy to increase cancer cell sensitivity to classical therapeutic agents.

Figure legends

Figure 1. Cholesterol metabolism and function in cancer cells.

Cholesterol can be synthetized from acetyl-CoA through the mevalonate pathway or it can be taken up from lipoproteins in the tumor microenvironment. Indeed, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and their remnants can undergo endocytosis mediated by the LDL-receptor family. On the contrary, high density lipoproteins (HDL) promote cholesterol efflux from cancer cells through their interaction with ATP-binding cassette transporters A1 and G1 (ABCA1, ABCG1) or with the scavenger receptor type BI (SR-BI). Since SR-BI mediates a facilitated diffusion of cholesterol according to concentration gradient, it could also favor lipid influx. Cholesterol is a key component of lipid rafts, and it can be converted to hormones and oxysterols. Created with *BioRender*.

Figure 2. Lipid homeostasis in cancer cells.

Cellular homeostasis of lipids is regulated by sterol regulatory element-binding proteins (SREBPs) and liver X receptors (LXRs), according to the metabolic needs of the cell. In cancer cells, the balance between SREBPs and LXRs is lost, with an hyperactivation of SREBPs and LXR inhibition. Consequently, cholesterol and fatty acid synthesis is upregulated, as the uptake of lipoproteins; on the contrary, cholesterol efflux is blunted. Created with *BioRender*.

Box 1. Incidence and classification of breast and prostate cancers

Breast cancer is the most common cancer in women and the second most common cancer overall. Age-standardized incidence rate in Europe, North America and Australia is >70 cases per 100.000 women [103]. BC is highly heterogeneous and it is currently classified according to histopathological features and gene expression profiling into luminal A, luminal B, HER2-enriched and basal-like (commonly referred to as triple-negative) (Table 1) [104]. This classification could predict the clinical outcome and the response to therapeutic interventions. Luminal BCs, which are positive to estrogen and/or progesterone receptors, will likely respond to hormone therapy; HER2-targeted treatments are indicated in HER2-enriched BC, while conventional chemotherapy is used for basal-like BC [1].

Table 1. Classification of BC

	ER/PR	HER2	Ki67
Luminal A	+	-	<14%
Luminal B	+	-	>14%
HER2-enriched	-	+	
Basal-like	-	-	

ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor type II receptor; Ki67, nuclear protein used as a proliferation marker.

Prostate cancer is the most common cancer in men and the fourth most common cancer overall. Age-standardized incidence rate in Europe, North America and Australia is >60 cases per 100.000 men [103]. The morphological classification of PCa has been significantly modified in the last decade [105]. The new grading system into 5 categories is based on the relative prevalence of (i) well-formed glands, (ii) poorly formed, fused or cribriform glands, (iii) lack of gland formation or necrosis. This classification is integrated with prostate-specific antigen levels and with the following information: (i) localization and size of the tumor mass, (ii) whether the cancer has spread to nearby lymph nodes, (iii) whether the cancer has metastasized. The final result is PCa staging from I to IV, which guides the selection of treatment approach among active surveillance, prostatectomy, radiotherapy, hormone-deprivation therapy and chemotherapy for hormone-refractory tumors [2].

Box 2. Energetic metabolism of cancer cells

Normal cells rely on fatty acids oxidation (FAO) and mitochondrial oxidative phosphorylation (OXPHOS) for acetyl-CoA and ATP production. Cancer cells rewire their energetic machinery from OXPHOS to anaerobic **glycolysis**, especially in the hypoxic core of solid tumors; glycolysis occurs even in the presence of oxygen (the Warburg effect), generating large amounts of lactate that contributes to TME acidity [106]. Altered tumor cell metabolism affects TME cells; in particular, the release of lactate and TME acidification inhibit dendritic and T cell activation, thus favoring tumor immune escape [107]. Interestingly, ATP is still produced in the mitochondria of tumor cells and mitochondrial tricarboxylic acid cycle intermediates are needed as precursors for macromolecule synthesis, as citrate for lipid synthesis and oxaloacetate for nucleotide synthesis [106]. In this context, FAO is the main source of acetyl-CoA for mitochondrial OXPHOS.

Interestingly, low rates of glycolysis were found in PCa and, indeed, PCa cells were shown to have a dominant uptake of fatty acids over glucose [108]. In addition, stromal cells and, most interestingly, **cancer stem cells** still rely on OXPHOS for energy production. The latter are now widely considered having a strong metastatic potential and resistance to radiotherapy and chemotherapy [109].

Box 3. Structure and metabolism of lipoproteins

Lipoproteins carry lipids in the circulation; they are composed by a core of hydrophobic triglycerides (TG) and cholesteryl esters (CE), surrounded by a double layer of phospholipids, unesterified cholesterol and apolipoproteins. According to their density and apolipoprotein composition, they are classified into chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL), which have distinct roles in lipid metabolism [110].

Chylomicrons are secreted by intestinal epithelial cells and transport dietary lipids to the liver. They are the largest and less dense lipoproteins, because their lipid cargo is mainly composed by TG. Their main protein component is apoB-48. After TG hydrolysis by lipoprotein lipase (LPL) and the release of free fatty acids to peripheral tissues, the liver takes up chylomicron remnants through the interaction of apoE with members of the LDL receptor family (LDL-R and the LDL-related receptor protein, LRP-1).

VLDL are secreted by the liver and are enriched in TG; their main protein component is apoB-100. After TG hydrolysis by LPL, generated remnants are converted into LDL, whose core is mainly composed by CE. LDL deliver cholesterol to peripheral tissues by the interaction of apoB-100 with LDL-R. Circulating LDL can be taken up by the liver through the same mechanism. The liver also secretes lipoprotein (a) (Lp(a)), which is a LDL-like particle with one molecule of apo(a) covalently bound to apoB. Apo(a) is structurally similar to plasminogen, with multiple copies of kringle domains, but lacks the fibrinolytic activity; thus, by competing with plasminogen, apo(a) could exert a potential pro-thrombotic effect [111].
The biogenesis of HDL is more complex [112]. Their main protein component, apoA-I, is secreted by the liver and by the intestine and is rapidly lipidated by the interaction with the ABC transporter A1 (ABCA1) and the formation of discoidal nascent HDL. Further uptake of cholesterol and phospholipids and cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT) leads to the formation of mature spherical HDL. In addition, HDL can be generated from the dissociation of lipids and apoA-I during the remodeling of apoB-containing lipoproteins. The main role of HDL is to deliver cholesterol to the liver. The first step of this process is the efflux of cholesterol and phospholipids from cell membranes to HDL or apoA-I [13]. Cholesterol efflux can (i) occur by passive diffusion, (ii) be actively promoted by ABCA1 and ABCG1, or (iii) occur by diffusion facilitated by the scavenger receptor type BI (SR-BI). Thus, according to the gradient concentration between cell membranes and HDL, SR-BI can mediate cholesterol efflux or influx. Once cholesterol is esterified by LCAT, HDL can directly deliver CE to the liver through hepatic SR-BI, which mediates the selective uptake of CE without HDL endocytosis. However, in humans the majority of CE is indirectly routed to the liver by the action of CE transfer protein (CETP), which mediates the exchange of CE for TG between HDL and apoB-containing lipoproteins. CE are then taken up by the liver through the endocytosis of apoB-containing lipoproteins by LDL-R and LRP-1.

Box 4. Effects of lipoproteins on cell metabolism: insights from cardiovascular diseases

Most of the available evidence on the role of lipoproteins in cell metabolism comes from the field of vascular biology and **atherosclerosis**. Lipoproteins can be divided into pro-atherogenic (apoB-containing lipoproteins, i.e. VLDL, LDL, Lp(a) and TG-rich remnants) and anti-atherogenic ones (HDL). ApoB-containing lipoproteins promote the development of

atherosclerosis because they mediate the transport of lipids from the liver to peripheral tissues, including the arterial wall [113]. On the contrary, HDL are the main acceptors of cholesterol from peripheral tissues and mediate its transport to the liver for excretion, among the so-called “reverse cholesterol transport” [13]. While systemic levels of lipids can widely vary in different physiologic and pathologic conditions, their cell content is tightly regulated [114,115]. Cells can synthesize cholesterol and fatty acids or lipids can be taken up from circulating lipoproteins. However, excess cholesterol is toxic for the cells and, consequently, it is removed through efflux towards extracellular acceptors as HDL, or it is esterified by acyl-coA:cholesterol acyltransferase (ACAT) and stored in cytosolic lipid droplets (LDs); the same happens to fatty acids, which are stored in LDs as TG. The cellular sensors of cholesterol and fatty acid content, that regulate the pathways described above, are the **sterol regulatory element binding proteins** (SREBPs) and the **liver X receptors** (LXRs) [114,115]. When cells need cholesterol and/or fatty acids, SREPBs are activated, promoting lipid synthesis and uptake, while LXRs are inhibited. On the contrary, when intracellular levels of lipids are increased, SREBPs are switched off and LXRs activated. LXRs favor lipid efflux by increasing the expression of the ABC transporters, and they decrease lipoprotein uptake through the downregulation of the LDL-R (see box 3) [116,117]. However, scavenger receptors, as the cluster of differentiation 36 (CD36), can mediate an unregulated uptake of lipoproteins and, consequently, an excessive lipid accumulation in macrophages within the arterial wall [118]. The role of lipoproteins is complex and not limited to the in/out transport of lipids. ApoB-containing lipoproteins, including TG-rich remnants, exert pro-inflammatory activities on different cell types, especially after oxidation [17]. On the contrary, HDL were shown to exert a series of anti-inflammatory and antioxidant activities on several cells involved in the atherosclerotic process [119]. In addition, lipoproteins, especially HDL, can act as carriers of several bioactive molecules, including miRNAs [120].

Glossary

Antiestrogen binding site (AEBS): It has been identified as a microsomal high-affinity binding site for the estrogen antagonist tamoxifen, distinct from the estrogen receptor. It does not bind estrogens. AEBS is composed by the 3β-hydroxysterol-Δ^8-Δ^7-isomerase (D8D7I) and the 7-dehydrocholesterol reductase (DHCR7) enzymes, which are involved in cholesterol synthesis.

Atherosclerosis: Pathologic process characterized by the accumulation of lipids and cells in the arterial wall, causing a progressive narrowing of the arterial lumen.

Cancer stem cells: Cancer cells expressing markers of hematopoietic stem cells, which are able to initiate tumors *in vivo*. Their presence was shown in all solid tumors.

Cytochrome P450 enzymes (CYPs): A family of oxidative enzymes involved in the synthesis and metabolism of various molecules within the cells. They are essential for the metabolism and excretion of xenobiotics. The liver is enriched in CYPs, but they are expressed throughout the body.

Glycolysis: A sequence of reactions that converts glucose into pyruvate.

Hypoxia: A condition in which local oxygen supply is insufficient for cell metabolic requirements. The TME becomes hypoxic due to the fast proliferation of cancer cells that is not supported by an adequate formation of novel vessels. When the tumor mass grows, oxygen delivered by blood is quickly consumed by cancer cells that are closest to the vessels, thus hampering its diffusion into the tumor mass. Consequently, most solid tumors display regions that are permanently or transiently in hypoxic conditions.

Lipid droplets: Intracellular stores of fatty acids and cholesterol in the form of neutral lipids. They are hydrolyzed in the cytosol or in the lysosomes to meet cell energy requirements.

Liver X receptors (LXRs): Nuclear receptors activated by oxysterols. LXRα is mainly expressed in the liver, while LXRβ is ubiquitously expressed. LXRs play a crucial role in cell metabolism since they control the transcription of genes involved in cholesterol, fatty acid and glucose homeostasis.

Mendelian randomization studies: Epidemiologic studies in which genetic variations are used to investigate the causal link between a potentially modifiable risk factor and disease outcome.

Oxysterols: Oxygenated derivatives of cholesterol produced by enzymatic or nonenzymatic peroxidation. They are intermediates or end-products of cholesterol excretion by its transformation into water-soluble bile acids. Oxysterols regulate cholesterol homeostasis and can exert potent effects on several biological processes.

RAS: Oncogene coding for small GTP binding proteins involved in the regulation of the cell proliferation and death. Members of the *RAS* family include *KRAS*, *HRAS*, and *NRAS*. *RAS* is frequently mutated in human cancers.

Statins: Lipid-lowering agents that inhibit the 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis along the mevalonate pathway.

Sterol regulatory element-binding proteins (SREBPs): Family of transcription factors consisting of two genes, SREBF1 and SREBF2, that encode for three different proteins: SREBP1a, SREBP1c and SREBP2. They regulate the transcription of genes involved in cholesterol biosynthesis and uptake, and fatty acid biosynthesis.

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Outstanding questions

The modulation of plasma levels of lipids by genetic manipulation and/or dietary approaches affected tumor development in mice. However, epidemiological and interventional studies in humans gave inconsistent results. What is the relevance of circulating versus local lipids for tumor development and progression?

The inhibition of cholesterol synthesis and lipoprotein uptake in cancer cells can affect cell viability. To this aim, statins are not promising due to their distribution and mechanism of action. The development of LXR agonists is hampered by their side effects. Other approaches, as the inhibition of the SREBP pathway or the silencing of the LDL-R are under investigation. In addition, the role of lipoprotein remnants in cancer biology has not been addressed to date. What is the optimal target for reducing cholesterol content in cancer cells?

The HDL system seems a promising target to limit cancer cell content of cholesterol and its metabolites. The development of HDL-targeted approaches should consider the role of SR-BI, which could be upregulated in cancer and promote cholesterol uptake from HDL. Could small discoidal reconstituted HDL overcome this problem?

Lipid metabolites as oxysterols and eicosanoids are produced in the TME and can affect both cancer and stromal cells; these metabolites need further dedicated investigations since different molecules seem to exert opposite effects on cancer cells. Is the pattern of oxysterol and eicosanoid production in cancer cell modifiable?

Plasma levels of apoA-I are used as a biomarker for ovarian cancer. Acute reduction of circulating HDL-C along with apoA-I is common during active disease in many cancer types. Could plasma levels of apoA-I be used as a biomarker for other types of tumor?

Trends

Tumor microenvironment (TME) can favor tumor progression and TME signature can predict disease outcome. Sources of lipids, pro-inflammatory and pro-oxidant molecules are present in the TME, including lipoproteins.

Cholesterol affects cell proliferation as a component of lipid rafts regulating cell signaling, and as building block for the local synthesis of hormones and oxysterols.

Very low- and low-density lipoproteins in the TME can provide cancer cells with lipids and exert pro-inflammatory and pro-oxidant activities.

High density lipoproteins (HDL) can promote the removal of cholesterol and its metabolites from cancer cell and blunt inflammation and oxidative stress. Boosting the HDL system by the infusion of reconstituted HDL could represent a promising approach to affect cancer cell viability.