

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

3
4 Monica Gomaraschi¹

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6 ¹Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari,
7 Università degli Studi di Milano, Milan, Italy

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12 Corresponding author:

13 Monica Gomaraschi, PhD

14 Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari,

15 Università degli Studi di Milano

16 Via Balzaretti 9, 20133 Milan (Italy)

17 monica.gomaraschi@unimi.it

18

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23 **Abstract**

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

34 **Relevance of the microenvironment in tumor development and progression**

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

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

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

61 **Cholesterol and its metabolites in cancer cells**

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

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

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

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

94 95 *Structural and functional role of cholesterol in the cell membrane*

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

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

110

111 *Intra-tumor formation of cholesterol metabolites*

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

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

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

141 Fatty acid metabolism by cancer and immune cells in the TME can also lead to the generation
142 of bioactive molecules, such as eicosanoids [39]. These molecules are produced from
143 arachidonic acid and other polyunsaturated fatty acids by the action of lipoxygenases and
144 cyclooxygenases. Their role in cancer is still in debate since they were variably associated
145 with cancer cell proliferation; indeed, while some eicosanoids may act as tumor suppressors
146 (as omega-3 fatty acids and resolvins), others were shown to promote the survival and the
147 proliferation of cancer cells [39]. In particular, prostaglandin E2 levels are elevated in several
148 human malignancies, including BC, PCa and ovarian cancer, and were associated with poor
149 prognosis and resistance to chemotherapy and radiotherapy [40,41]. Thromboxane A2
150 (TXA2) is also increasingly implicated in cancer progression, especially in triple negative BC.
151 The binding of TXA2 to its receptor (thromboxane receptor, TP) enhances BC cell migration
152 and invasion by triggering Rho activation [29]. Through the same mechanism, TP activation
153 induced cytoskeletal reorganization of PCa cells [42]. On the contrary, resolvins may help to
154 lower the risk of developing cancer [43]. These bioactive lipids, derived from the omega-3
155 fatty acids eicosapentaenoic acid and docosahexaenoic acid, are key players in the resolution
156 of inflammation. In multiple tumor types, resolvins were shown to reduce tumor growth, neo-
157 angiogenesis, metastatization and to revert the deactivation of natural killer (NK) cells [44].
158

159 *Lipid metabolites for protein modification*

160 Along the mevalonate pathway, isoprenoids (farnesyl-pyrophosphate and geranylgeranyl-
161 pyrophosphate) are produced, which are needed for prenylation, a post-translational
162 modification of proteins. Prenylation provides the target protein with a hydrophobic C
163 terminus, which guides its localization within the cell, for example to the lipid rafts, favoring
164 protein-protein interactions [45]. Many prenylated proteins are involved in cell signaling, as
165 small GTP binding proteins. Among these, the oncogene **RAS** is the most studied and a
166 reduction of isoprenoid production, as by farnesyltransferase inhibitors, is under investigation
167 with promising results in aggressive BC [46].
168 Fatty acids are also involved in protein modification, as some proteins undergo myristoylation
169 or palmitoylation [47,48]. Again, many of the target proteins are crucial components of
170 signaling pathways and the modification with fatty acids promotes their binding to the
171 membrane for proper localization and function. Well-characterized targets include many
172 oncogene products, such as the tyrosine kinase Src [47,48].

173

174 **Lipoproteins as pharmacological targets?**

175 *Epidemiologic evidence*

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

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

192 [57]. On the contrary, genetically raised LDL-C levels were associated with higher risk of BC,
193 especially of the ER-positive type [58]. The same relationship was found for elevated HDL-C,
194 but it should be noted that only the effect of CETP variants was assessed, and inhibiting
195 CETP is debated for the possible accumulation of dysfunctional HDL [59]. The role of dietary
196 lipids on cancer risk is also debated, since a lipid-rich diet could be indicative of an unhealthy
197 lifestyle. In addition, a significant association between dietary cholesterol intake and the risk
198 of BC was detected only with very high intakes [60]. Another approach to address the
199 relationship between plasma lipids and the incidence of cancer is to assess the association
200 with **statins**. In two recent meta-analysis, statins were shown to have a neutral effect on both
201 PCa and BC incidence [61,62]. Consistently, the Cholesterol Treatment Trialists'
202 Collaboration showed no evidence of any effect of reducing cholesterol with long-term statin
203 therapy on cancer incidence or mortality [63]. To explain the inconsistency between studies,
204 the type of cancer and the time at which lipids were assessed could act as confounding
205 factors. The stage of the disease and the consequent therapies can affect the levels of
206 circulating lipids; plasma lipids, especially HDL-C levels, are reduced in several cancer
207 patients during active disease, as in those having breast, ovarian, prostate, colon and
208 pancreatic carcinomas [64]. The large HDL2 subclass is particularly reduced [65]. The
209 predictive power of low HDL-C and apoA-I levels was so strong that apoA-I was included in
210 the screening for ovarian cancer with other traditional biomarkers [66]. The mechanisms
211 beyond HDL-C reduction are not fully understood. Since the liver and the intestine are
212 responsible for the synthesis of apoA-I and its lipidation by ABCA1, malignancies at these
213 organs can directly impaired HDL biogenesis. As shown in many inflammatory states, pro-
214 inflammatory cytokines inhibit hepatic apoA-I expression and, thus, the cancer-related
215 sustained inflammation can repress HDL biogenesis by the same mechanism [67]. Finally, the
216 hepatic expression of several proteins involved in lipoprotein metabolism can be modulated
217 by inflammation and cancer, including LCAT; thus, HDL-C reduction could be part of a more
218 complex alteration of lipoprotein metabolism induced by cancer [67].
219 The association between plasma lipids and the recurrence of cancer has been firmly
220 established. Plasma levels of cholesterol are positively associated with a higher recurrence
221 rate of BC and statin use was associated with extended recurrence free survival time [68].
222 This protective effect was confirmed in women with hormone receptor-positive early-stage
223 breast cancer taking statins during adjuvant endocrine therapy [69]. Plasma levels of HDL-C

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

227

228 *Targeting apoB-containing lipoproteins*

229 The use of hyperlipidemic mice (*apoE*^{-/-} model) fed a high fat/high cholesterol diet (HFHC) can
230 help to address the impact of increased plasma levels of apoB-containing lipoproteins on the
231 development of hormone-dependent cancers. The injection of non-metastatic Met-1 and
232 metastatic Mvt-1 mammary cancer cells in *apoE*^{-/-} mice resulted in larger tumors and in a
233 greater number of lung metastases compared to wild-type mice [71]. Interestingly, the graft
234 with BC cell lines representative of triple-negative or HER2-enriched tumors in hyperlipidemic
235 mice resulted in reduced tumor growth when the LDL-R was silenced [72]. Consistently, LDL
236 increased the proliferation and migration of ER-negative BC cells but not of ER-positive ones,
237 which also accumulated less CEs than ER-negative cells [73]. These data suggest that ER-
238 negative BC may be more sensitive to cell cholesterol-lowering strategies. Regarding PCa,
239 mice fed a HFHC diet developed larger tumors after injection of androgen-dependent LNCaP
240 cells compared to mice under chow diet [24]. In addition, the HFHC diet accelerated tumor
241 incidence and burden compared to chow diet in the TRAMP mouse, a model of PCa [74].
242 These data are supported by several *in vitro* studies. VLDL, but not LDL, increased the
243 formation of BC cell mammospheres, an estimate of stem cell/early progenitor activity, and
244 cell resistance to radiotherapy, likely due to a modulation of intracellular cholesterol content
245 [75]. Consistently, the treatment of aggressive and metastatic BC cells with statins was
246 associated with an increased sensitivity to radiation therapy [75]. The *in vivo* relevance of
247 these findings was supported by the shorter recurrence-free period in patients with elevated
248 VLDL-C levels, which suggests a systemic effect of statins through the reduction of circulating
249 apoB-containing lipoproteins [75]. Oxidized LDL (oxLDL), but not native LDL, increased the
250 proliferation of ovarian cancer cells and decreased their sensitivity to cisplatin, an effect
251 prevented by statins and by LXR activation [76]. OxLDL could act through the binding with its
252 lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which stimulates the
253 expression of pro-adhesive, pro-inflammatory and proangiogenic factors in vascular
254 endothelial cells and macrophages. LOX-1 upregulation was reported in several different
255 types of cancer, including PCa. Here, its activation by oxLDL promoted epithelial to

256 mesenchymal transition and increased tumor growth in nude mice [77]. In BC cell lines, LOX-
257 1 inhibition blunted inflammatory and hypoxic responses [78]. Finally, apoB-containing
258 lipoproteins and adipocytes could deliver free fatty acids to cancer cells by the action of
259 locally expressed LPL; BCs are generally positive for LPL and its expression increased cell
260 proliferation [79]. Moreover, adipocytes can directly provide cancer cells with fatty acids.
261 Indeed, cancer cells can stimulate TG lipolysis by the adipose triglyceride lipase/hormone-
262 sensitive lipase axis in adipocytes [80]. Adipocytes were shown to support the proliferation
263 and invasiveness of highly differentiated and hormone-dependent cancers more than of
264 poorly differentiated ones [81].

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

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

280

281 *Targeting the HDL system*

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

287 [88]. More interestingly, apoA-I infusion after the development of the tumor mass resulted in
288 tumor shrinkage [88].

289 *In vitro* studies provided some mechanistic insights. When BC cells were incubated with HDL,
290 the formation of mammospheres was inhibited and their sensitivity to radiation therapy
291 increased, an effect due to a reduced cholesterol content [75]. Whether this reduction was
292 ascribed to a depletion of cell membrane content or of intracellular CE stores in LDs was not
293 addressed. It could be speculated that, given the continual cycle of hydrolysis and
294 esterification of CE in LDs, the removal of unesterified cholesterol from the cell membrane
295 promoted by HDL could shift the balance towards CE hydrolysis with a consequent LD
296 depletion, as shown in macrophages [89]. In PCa cell lines, HDL blunted basal and H₂O₂-
297 induced oxidative stress and reduced ROS-induced proliferation, with a role for both the
298 protein and phospholipid components [90].

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

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

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

326

327 *HDL-like particles for drug delivery*

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

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

343

344 **Concluding remarks and future perspectives**

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

351 cancer cells, as by genetic silencing, already generated positive results in different types of
352 cancer. Another approach is to increase the efficiency of the HDL system in promoting lipid
353 removal, as by the infusion of rHDL to rise the endogenous HDL pool. This approach has
354 some advantages. Reconstituted HDL are already under clinical development for patients with
355 acute coronary syndrome [96]. Their protein and lipid composition can be manipulated to
356 optimize their function. In addition, rHDL can be used as carriers of other bioactive molecules,
357 ranging from nucleic acids to drugs [98].
358 Overall, targeting lipid metabolism in the TME should be considered as an adjuvant strategy
359 to increase cancer cell sensitivity to classical therapeutic agents.

360 Figure legends

361 Figure 1. Cholesterol metabolism and function in cancer cells.

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

371

372 Figure 2. Lipid homeostasis in cancer cells.

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

379 **Box 1. Incidence and classification of breast and prostate cancers**

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

389 Table 1. Classification of BC

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

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

392

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

404

405 **Box 2. Energetic metabolism of cancer cells**

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

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

422

423 **Box 3. Structure and metabolism of lipoproteins**

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

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

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

461

462 **Box 4. Effects of lipoproteins on cell metabolism: insights from cardiovascular** 463 **diseases**

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

468 atherosclerosis because they mediate the transport of lipids from the liver to peripheral
469 tissues, including the arterial wall [113]. On the contrary, HDL are the main acceptors of
470 cholesterol from peripheral tissues and mediate its transport to the liver for excretion, among
471 the so-called “reverse cholesterol transport” [13]. While systemic levels of lipids can widely
472 vary in different physiologic and pathologic conditions, their cell content is tightly regulated
473 [114,115]. Cells can synthesize cholesterol and fatty acids or lipids can be taken up from
474 circulating lipoproteins. However, excess cholesterol is toxic for the cells and, consequently, it
475 is removed through efflux towards extracellular acceptors as HDL, or it is esterified by acyl-
476 coA:cholesterol acyltransferase (ACAT) and stored in cytosolic lipid droplets (LDs); the same
477 happens to fatty acids, which are stored in LDs as TG. The cellular sensors of cholesterol and
478 fatty acid content, that regulate the pathways described above, are the **sterol regulatory**
479 **element binding proteins** (SREBPs) and the **liver X receptors** (LXRs) [114,115]. When
480 cells need cholesterol and/or fatty acids, SREPBs are activated, promoting lipid synthesis and
481 uptake, while LXRs are inhibited. On the contrary, when intracellular levels of lipids are
482 increased, SREBPs are switched off and LXRs activated. LXRs favor lipid efflux by increasing
483 the expression of the ABC transporters, and they decrease lipoprotein uptake through the
484 downregulation of the LDL-R (see box 3) [116,117]. However, scavenger receptors, as the
485 cluster of differentiation 36 (CD36), can mediate an unregulated uptake of lipoproteins and,
486 consequently, an excessive lipid accumulation in macrophages within the arterial wall [118].
487 The role of lipoproteins is complex and not limited to the in/out transport of lipids. ApoB-
488 containing lipoproteins, including TG-rich remnants, exert pro-inflammatory activities on
489 different cell types, especially after oxidation [17]. On the contrary, HDL were shown to exert
490 a series of anti-inflammatory and antioxidant activities on several cells involved in the
491 atherosclerotic process [119]. In addition, lipoproteins, especially HDL, can act as carriers of
492 several bioactive molecules, including miRNAs [120].

493 **Glossary**

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

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

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

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

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

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

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

516 **Liver X receptors (LXRs):** Nuclear receptors activated by oxysterols. $LXR\alpha$ is mainly
517 expressed in the liver, while $LXR\beta$ is ubiquitously expressed. LXRs play a crucial role in cell
518 metabolism since they control the transcription of genes involved in cholesterol, fatty acid and
519 glucose homeostasis.

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

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

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

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

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

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538

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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.