Role of cholesterol metabolism in the anticancer pharmacology of selective estrogen receptor modulators

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Abstract

Selective estrogen receptor modulators (SERMs) are a class of compounds that bind to estrogen receptors (ERs) and possess estrogen agonist or antagonist actions in different tissues. As such, they are widely used drugs. For instance, tamoxifen, the most prescribed SERM, is used to treat ERα-positive breast cancer. Aside from their therapeutic targets, SERMs have the capacity to broadly affect cellular cholesterol metabolism and handling, mainly through ER-independent mechanisms. Cholesterol metabolism reprogramming is crucial to meet the needs of cancer cells, and different key processes involved in cholesterol homeostasis have been associated with cancer progression. Therefore, the effects of SERMs on cholesterol homeostasis may be relevant to carcinogenesis, either by contributing to the anticancer efficacy of these compounds or, conversely, by promoting resistance to treatment. Understanding these aspects of SERMs actions could help to design more efficacious therapies. Herein we review the effects of SERMs on cellular cholesterol metabolism and handling and discuss their potential in anticancer pharmacology.

Keywords: Selective estrogen receptor modulators, cholesterol metabolism, intracellular cholesterol trafficking, cancer, cell proliferation.

Abbreviations

ACAT, acyl-coenzyme A:cholesterol acyltransferase; AEBS, anti-estrogen binding site; BC, breast cancer; CAD, cationic amphiphilic drug; CE, cholesteryl ester; DDA, dendrogenin A; EMT, epithelial to mesenchymal transition; ER, estrogen receptor; LE/L, late endosomes/lysosomes; HUVEC, human umbilical vein endothelial cells; LDLR, LDL receptor; NPC, Niemann-Pick type C; OCDO, 6-oxo-cholestan-3β,5α-diol; SERM, selective estrogen receptor modulator; TAM, tumor-associated macrophage; 27HC, 27-hydroxycholesterol.

1. Introduction

Selective estrogen receptor modulators (SERMs) are a diverse class of compounds that bind to estrogen receptors (ERs) and induce a unique receptor conformation that results in a specific effect in estrogen-responsive tissues. By interacting with ERs, SERMs exert effects that fall into a spectrum between full agonist and full antagonist activity in a tissue-specific manner. This peculiarity depends on several factors, including differential expression of the ER subtypes (α and β) and ligand-dependent receptor conformational changes that condition the interaction with coactivators and corepressors which, in turn, are differentially expressed in different tissues [1-3]. SERMs are primarily used to treat different conditions related to women's pathophysiology [3-6]. The first three SERMs approved for clinical practice were tamoxifen, toremifene and raloxifene. Tamoxifen, the pioneering SERM, is a triphenylethylene derivative used for the treatment of ERα-positive breast cancer (BC), and is the standard of care for premenopausal patients and for risk reduction in both premenopausal and postmenopausal women. Toremifene, which differs from tamoxifen only by the presence of a chlorine atom in the ethyl chain, is indicated for the treatment of advanced BC. Raloxifene, a benzothiophene derivative, is used for the treatment and prevention of osteoporosis, and in the United States, it is also used for risk reduction of BC in postmenopausal women. Approximately 75% of women diagnosed with early stage BC have ERα-positive disease. Although the importance of ERα in BC development is well established, the role of ERβ is unclear, despite its presence in mammary tumors [7,8].

Tamoxifen, the most frequently used SERM, acts as an ER antagonist in breast tissue, yet as a partial agonist in other tissues such as the endometrium and bone [3-6]. The in vivo efficacy of tamoxifen is attributed to its active metabolites, 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyl-tamoxifen (endoxifen), both being products of cytochrome P450 enzymes (CYP2D6, CYP3A and CYP2C). Endoxifen is found at much higher concentrations than 4-hydroxytamoxifen, and is thus considered the most important tamoxifen metabolite [9,10]. However, a large proportion of patients shows de novo or acquired resistance to tamoxifen treatment [6,11].

Aside from its ER-mediated effects, at pharmacologically relevant concentrations, tamoxifen displays multiple ER-independent actions that may also be involved in its anticancer effects [12,13], such as, for example, the inhibition of protein kinase C signaling [14-17]. These off-target effects may explain that 5–10% of ER-negative tumors are sensitive to tamoxifen treatment [18], and have led to clinical trials of tamoxifen for non-BC [12]. Tamoxifen and other SERMs have been shown to alter different cellular processes relevant to cholesterol homeostasis through ER-dependent or -independent mechanisms. This has an impact at the systemic level, as SERMs consistently reduce circulating concentrations of total and low-density lipoprotein (LDL) cholesterol in women, which, by analogy with the effect of estrogens, is usually attributed to ER agonism in the liver [1,5]. However, although some studies have reported an increase in high-density lipoprotein (HDL) cholesterol by SERM treatment [19-23], these drugs very often do not change this variable [1,5], and one study in tamoxifen-treated men found a decrease in HDL-cholesterol [24]. Furthermore, SERMs can increase plasma triacylglycerols [25,26], occasionally causing severe hypertriglyceridemia [27].

Cholesterol is a critical component of cell membranes, in that it is required for membrane biogenesis and intracellular signaling cascades initiated in lipid rafts. Added to its toxicity when cholesterol is in excess, its cellular concentrations are tightly controlled [28,29]. Tumor cells demand high amounts of cholesterol for proliferation, which can be satisfied by increasing

both cholesterol biosynthesis and LDL uptake through the LDL receptor (LDLR) [30-32]. Internalized LDL is degraded in late endosomes/lysosomes (LE/L) to release free cholesterol, which leaves these organelles. Moreover, proliferating cells reduce the removal [33] and increase the storage of cholesterol [34]. The expression of cholesterol biosynthesis enzymes and the LDLR is mainly controlled by sterol regulatory element-binding protein (SREBP) 2, an integral endoplasmic reticulum membrane protein that, upon cholesterol depletion, is transported, escorted by the SREBP-cleavage activating protein (SCAP), to the Golgi, where SREBP-2 is activated to subsequently stimulate the transcription of target genes [35,36]. Another isoform, SREBP-1c, activates genes for fatty acid biosynthesis, whereas SREBP-1a stimulates the biosynthesis of both cholesterol and fatty acids. When sufficient cholesterol levels are reached in the endoplasmic reticulum SCAP prevents the transport and activation of SREBP-2. Moreover, the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin complex 1 (mTORC1) axis stimulates the SREBP pathway in response to energy levels, growth factors and nutrients [36]. Excess cholesterol is esterified by acyl-coenzyme A:cholesterol acyltransferase, or sterol O-acyltransferase (ACAT/SOAT) 1. Cholesteryl esters are incorporated into cytoplasmic lipid droplets, a form of cholesterol storage until it is used following cholesterol ester hydrolysis. On the other hand, cholesterol excess is sensed by the liver X receptors (LXR) α and β, which are nuclear receptors activated by certain oxysterols, ultimately leading to increased cellular cholesterol efflux to HDL and decreased cholesterol uptake [37]. This is accomplished by stimulating the transcription of membrane cholesterol transporters, such as the ATP-binding cassette transporter (ABC) A1 and ABCG1, and that of the inducible degrader of the LDLR (IDOL), respectively. The cholesterol derivative 27-hydroxycholesterol is an LXR ligand that, interestingly, has also been recognized as an endogenous SERM and cancer promoter (see section 3.6.2).

The first indication of cholesterol involvement in cancer was the finding that prostate adenomas and other tumor tissues had increased cholesterol content compared to normal tissues [38-40]. In fact, intratumoral cholesterol synthesis, measured through squalene epoxidase (SQLE) expression in prostate biopsies, has been shown to be a marker of poor prognosis in prostate cancer [41], which is in accordance with the reprogramming of lipid metabolism, including cholesterol metabolism, in cancer cells in order to fuel rapid proliferation [30,42,43]. Innumerable epidemiological studies have been conducted to establish the relationship between cancer incidence and mortality with serum cholesterol levels, dietary cholesterol intake and treatment with hypocholesterolemic drugs, as well as experimental studies to understand the biochemical bases of that relationship. The first prospective studies arose from the suspicion that there was a correlation between mortality rates from colon cancer and coronary heart disease, with dietary cholesterol as a common etiological factor [44]. Surprisingly, it was found that men developing colon or lung cancer had initial serum cholesterol levels that were significantly lower than those non-affected [44-49]. Subsequent studies, however, concluded that low cholesterol levels were a consequence of tumour activity rather than a predisposing factor [47,50-52]. A paradigmatic case is leukemia, where serum cholesterol levels are substantially reduced [53-55]. Later prospective studies in the general population found a positive correlation between serum cholesterol levels and the incidence of prostatic cancer [56,57], but discrepant results were observed for other cancers [58]. These differences among cancer sites and the influence of sex, dietary habits and cancer stage [59-62] have made it difficult to generalize the impact of cholesterol homeostasis in cancer.

The effects of hypolipidemic drugs, mostly statins, have been extensively studied in both humans and experimental models. In this regard, it is important to distinguish between early and late stages of cancer progression. The largest retrospective studies showed no influence of

statins on the incidence of breast [63-65], prostate [66], lung [67,68] and colorectal [69] cancers. On the other hand, most studies concurred that statins reduce the recurrence and mortality from all-site cancer [70,71], particularly in breast [72,73], prostate [74], lung [75] and colorectal cancers [76], supporting that statins impact tumor progression rather than early carcinogenesis [77]. Cholesterol homeostasis is influenced by lifestyle factors, mainly diet and exercise, which are considered to be associated with cancer development [78]. Although data on dietary cholesterol intake and cancer risk are not unanimous, many studies indicate that a high cholesterol intake is linked to increased risk of several types of cancer, including BC [60,79,80]. Physical activity is inversely associated and sedentary behaviour is positively associated with an elevated risk of cancer [78].

Aside from its requirement for cell proliferation, several mechanisms have been proposed to explain the possible role of cholesterol in cancer. Among them, a role has been attributed to bile acids, which derive from cholesterol and undergo biotransformation to secondary bile acids by gut microbiota [81]. Secondary bile acids, especially deoxycholic acid, are potential causative agents of cancers of the digestive system [81,82]. On the other hand, cholesterol and cholesterol-derived oxysterols affect the immune response in the tumor microenvironment [83] (see below). Therefore, multiple connections exist between cholesterol metabolism and relevant processes in cancer progression. In this context, the effects of SERMs on cholesterol metabolism and homeostasis may be relevant, either by contributing to their anti-tumoral effects or, on the contrary, by inducing resistance to treatment. In this review, we summarize the effects of SERMs on cellular cholesterol metabolism and handling and discuss their impact, often scarcely examined, on the anticancer pharmacology of SERMs.

2. Role of cholesterol in cell proliferation and differentiation

Mammalian cells require cholesterol for cell proliferation and there is a striking dependence of cell cycle progression on cholesterol availability [84,85]. Cholesterol has the structural features to optimally support the growth of mammalian cells. These features include a 3β-hydroxyl group, a *trans* A/B ring system and a Δ^5-double bond [86]. Of note, desmosterol, the immediate precursor of cholesterol in the Bloch pathway (Fig. 1), is as suitable as cholesterol for cell proliferation [87,88], as they share these structural characteristics. It has been proposed that, for proliferation, cholesterol exerts two functions: a structural or bulk function related to membrane formation that can be satisfied by diverse sterols, and a much more specific, regulatory function, which involves cell cycle kinases [86,89-91].

Proliferating cells express both a high rate of cholesterol synthesis, with increased HMG-CoA reductase (HMGCR) activity, and elevated LDLR activity [30-32]. Indeed, cancer cells harboring mutant p53 have increased transcription of SREBP-modulated genes [92-95]. Cells with dysfunctional LDLR depend on endogenous cholesterol synthesis to satisfy their need to proliferate [96,97]. Experimentally, in cells incubated in medium free of cholesterol, inhibition of cholesterol biosynthesis unfailingly leads to cell proliferation abrogation, but the cell cycle may be affected differently depending on the cell type. Cells harboring wild-type p53 are usually arrested at the G0/G1 phase, as a consequence of the stimulation of $p21^{WAF1/CIP1}$ and $p27^{Kip1}$ and subsequent inactivation of different cyclin-dependent kinases [98-102], whereas promyelocytic HL-60 and lymphoblastic MOLT-4 cells, with null and mutant-inactive p53, respectively, do transit through S phase and accumulate at G2/M [86,103,104], showing decreased expression of cyclin B1 and Cdk1-Cyclin B activity [90,103] and are unable to complete cytokinesis [105]. Supplementation of the culture medium with LDL or free cholesterol dissolved in ethanol, but not other sterols, prevented all these effects, showing the

requirement of cholesterol for cytokinesis [86,103-105]. This is in line with the accumulation of cholesterol in the scission furrow during mitosis [106] and the activation of cholesterol biosynthesis during the G2/M phase [107,108].

HL-60 cells grown in a cholesterol-free medium have a cell cholesterol content of approximately 5–6 μg/mg of cell protein [104]. When cells are treated with inhibitors of post-lanosterol enzymes, they may complete the first division, but in the next cycle they are arrested at G2/M because the cholesterol content of daughter cells falls below the threshold level (~2.5 μg/mg cell protein) [104]. Several inhibitors may exert these effects, including SKF104976, AY9944 and SR31747; however, the more upstream the affected enzyme, the more severe the effect [104]. A similar pattern was observed in colorectal cancer cells treated with TASIN compounds, which inhibit 3β-hydroxysteroid-Δ^8,Δ^7-isomerase (D8D7I/EBP), 7-dehydrocholesterol reductase (DHCR7) and 3β-hydroxysterol-Δ^{24}-reductase (DHCR24) (Fig. 1): cell viability was reduced only when D8D7I was affected [109]. These cell proliferation findings parallel the severity of post-lanosterol congenital defects, where for a similar affectation in cholesterol availability, the symptoms depend on which step in the pathway is altered (and which sterol is accumulated): the earlier, the more severe [110-112]. As the effects of cholesterol synthesis inhibition were mitigated by LDL or free cholesterol, it is tempting to conclude that cholesterol depletion was the sole cause of cell proliferation inhibition; however, a contribution of intermediate sterols accumulation cannot be ruled out.

The effects of HMGCR inhibition have been extensively studied and the results demonstrated that, in addition to cholesterol, mevalonic acid or its non-sterol isoprenoid derivatives are also needed for cell division [113-115]. In human leukemia cells incubated in a medium free of cholesterol and mevalonic acid, the effects of statins on cell viability and cell cycle distribution greatly depend on the dose used. With supratherapeutic doses (≥ 50 μM lovastatin), which blocked both cholesterol biosynthesis and protein prenylation [103], massive apoptosis occurred and the proportion of cells accumulating in G0/G1 highly increased to the detriment of the active S and G2/M phases [101,103,116,117]. This effect was independent of p53 status as the addition of exogenous mevalonate prompted cells arrested at G1 to progress through the S phase and reach G2/M [103]. This is in accordance with the increase in protein prenylation during early-to-mid G1 [118-120]. With lower lovastatin doses, which only suppressed cholesterol biosynthesis, cells accumulated preferentially in G2/M phase, an effect that was both prevented and reversed by cholesterol supply [103]. These results demonstrate the distinct roles of mevalonate, or its non-sterol derivatives, and cholesterol in cell cycle progression, the first in G1-to-S transition and the second in mitosis completion. By contrast, inhibition of mevalonate-pyrophosphate decarboxylase (MVD), which acts a few steps downstream of HMGCR, induced DNA damage and the accumulation of cells in the S phase, indicative of replication stress, via dNTP depletion [121]. Given that replication stress is a major source of genomic instability [122,123], the possibility exists that the accumulation of mevalonic acid phosphorylated derivatives induces tumorigenesis.

With regard to SERMs, their effects on cell proliferation have been studied as both ER-modulators and as inhibitors of the cholesterol synthesis pathway. Focusing on this second action, treatment of BC MCF-7 cells with tamoxifen at doses of 1 μM or higher was shown to inhibit cell proliferation and arrest the cell cycle at G0/G1 [124]. These effects were accompanied by the accumulation of both zymostenol and 7-dehydrocholesterol. Given that cells were incubated with 5% serum, it may be that these effects on cell cycle progression were not only due to cholesterol deficiency. Indeed, the provision of zymostenol or 7-dehydrocholesterol to cells not treated with tamoxifen recapitulated the effects of the drug: namely cell proliferation inhibition and cell cycle arrest [124]. These results show that alterations of the intermediate sterols/cholesterol balance may impact cell proliferation.

The potential of cholesterol synthesis inhibition to reduce cell growth in vivo has also been demonstrated. As circulating LDL is a main source of cholesterol for cells, it is expected that highly proliferative and not sufficiently irrigated cells, such as tumor cells, would be the most affected by cholesterol biosynthesis inhibition, as has been demonstrated for different cancer xenografts in mice. Thus, statins reduced growth and induced apoptosis in many of these models [125-128]. RO 48-8071, which inhibits lanosterol synthesis, selectively suppressed growth of human prostate cancer cells, without signs of toxicity [129]. Ketoconazole, which inhibits lanosterol metabolization, retarded the growth of neuroblastoma [130], glioblastoma [131] and epidermoid carcinoma [132]. Inhibition of DHCR24 by triparanol reduced the growth of lung cancer cells [133], and TASIN-1, by inhibiting D8D7I, suppressed tumor growth of colorectal cancer cells [134].

Most tumors show abnormalities in the differentiation stage of their clonal cell constituents (anaplasia). Induction of a differentiation response in malignant cells, including leukemia cells, may have positive clinical implications, such as the loss of proliferative potential and the induction of apoptosis [135]. The earliest evidence linking cholesterol metabolism to cell differentiation comes from the 1980s. Connor et al. demonstrated that exposure of HL-60 cells to dimethyl sulfoxide, retinoic acid or hypoxanthine rapidly decreased sterol and phospholipid syntheses, long before inhibition of DNA synthesis and signs of myeloid differentiation were observed [136]. These results were in line with previous findings that showed that circulating granulocytes, in contrast to lymphocytes and monocytes, lack SQLE and, moreover, the ability to transform lanosterol into cholesterol [137,138]. In line with this, treatment of HL-60 cells with either SKF104976, a specific inhibitor of lanosterol 14α-demethylase (CYP51A1), or zaragozic acid, a potent inhibitor of squalene synthase (FDFT1), was sufficient to differentiate cells following the granulocyte lineage, similar to all-trans retinoic acid [139]. This effect was prevented by the early supply of exogenous cholesterol to cells, suggesting a link to cholesterol depletion [139]. Furthermore, treatment with low concentrations of lovastatin (< 1 μM) induced granulocytic differentiation of the acute myeloid leukemia cell lines AML-5 and NB-4, as indicated by increased expression of CD11b and CD18 [135].

Cholesterol metabolism appears to be involved in other differentiation processes. In primary avian erythroid progenitor cells, specific inhibition of oxidosqualene cyclase (OSC/LSS) arrested cell self-renewal and triggered differentiation into erythrocytes [140]. In gonads, the changes in sterols composition during both spermatogenesis and oogenesis have been recognized for years. The accumulation of the so-called meiosis activating sterols (MAS) is characteristic: follicular fluid MAS (FF-MAS) and testis MAS (T-MAS), two post-lanosterol precursor sterols in the cholesterol biosynthesis pathway [141-145] (Fig. 1). These changes are due to specific alterations in the activity of certain enzymes involved in cholesterol biosynthesis including CYP51A [145,146]. Interestingly, the supply of lanosterol or MASs induced meiosis resumption in oocytes in vitro supporting the hypothesis that MASs play a role in the sequence of proliferative and differentiation phases in oogenesis [147,148]. However, the possibility that lanosterol is also active in spermatogenesis cannot be ruled out [144]. Additionally, the accumulation of C8-9 unsaturated sterols, as achieved by inhibition of CYP51, sterol Δ^{14}-reductase (TM7SF2), or D8D7I in the post-lanosterol pathway (Fig. 1), has been demonstrated to induce the formation of oligodendrocytes from oligodendrocyte progenitor cells (OPCs) and to enhance remyelination [149,150]. The supply of different MASs was sufficient to differentiate OPCs into mature oligodendrocytes, whereas analogous sterols lacking the C8-9 double bond were ineffective [149]. It is pertinent to mention that both tamoxifen and raloxifene also induced OPC differentiation due to D8D7I inhibition [149]. Moreover, SERMs induce differentiation of MCF-7 BC cells, as indicated by the accumulation and secretion of lipids, and the expression of a milk fat globule protein [124,151,152]. These results underscore the role of intracellular sterol balance in cell fate.

Taken together, a scheme may be drawn where both stem and cancer cells have active cholesterol metabolism that allows them to proliferate, and modulation of post-lanosterol enzymes resulting in changes in the intermediate sterols/cholesterol ratio contributes to the proliferation/differentiation decision making.

3. Selective estrogen receptor modulators, cholesterol metabolism and cancer

3.1. Cholesterol biosynthesis

Cholesterol biosynthesis is considered a branch of the mevalonate pathway. A scheme of the whole pathway in mammalian cells starting from acetyl-CoA is shown in Fig. 1. The synthesis of cholesterol involves more than 30 reactions and more than 20 enzymes. In this pathway, in addition to cholesterol, a number of sterol and non-sterol isoprenoids with biological relevance are also synthesized. Worth mentioning due to their relevance in tumor growth are mevalonate-5-phosphate, which disrupts binding of DnaJ heat shock protein family (Hsp40) member A1 (DNAJA1) to misfolded proteins [93], and farnesyl- and geranylgeranyl pyrophosphate needed for protein prenylation [92,94]. The mevalonate pathway is upregulated in cancer cells through p53 mutations and alterations in different signal transduction pathways relevant in cell proliferation, all converging on SREBP [43,92,153]. Also illustrating the importance of cholesterol biosynthesis in cancer cells, the upregulation of the enzymes of this pathway was identified as a mechanism of resistance of ER-positive BC cells to estrogen deprivation [154,155]. Enzymes involved in this pathway are finely regulated at both the transcriptional and posttranscriptional levels. Among the former, the main, but not only, mechanism is mediated by SREBP-2 in response to cholesterol availability [35,36]. Moreover, the pathway is coordinated with other metabolisms, including fatty acid, glucose and energy metabolisms. As the enzyme giving rise to mevalonate, HMGCR is the rate-limiting step in the route, while OSC/LSS specifically modulates the biosynthesis of sterols. A handful of enzyme inhibitors are available, acting at different steps on the pathway (Fig. 1). Statins, which are widely used, are competitive inhibitors of HMGCR and indirectly reduce plasma cholesterol levels via LDLR upregulation.

Despite the importance of HMGCR for the survival and proliferation of cancer cells, there is controversy about the impact of tumor expression of this enzyme on the clinical outcome. Immunohistochemical studies have found either no association between HMGCR expression and BC outcome [156,157] or a significant association between high HMGCR expression and a better prognosis of breast, ovarian or colorectal cancer [158-161]. However, gene expression studies have found that high HMGCR mRNA levels negatively impact BC prognosis, especially in patients with ER-positive tumors [162,163]. The reason for this discrepancy is unclear, but it has been suggested that the favorable outcome in some immunohistochemical studies was influenced by the specificity of the antibody against HMGCR that was used [162].

It is well known that tamoxifen and other SERMs inhibit cholesterol biosynthesis [164-166], which may contribute to the decrease in serum cholesterol levels when SERMs are used therapeutically [1,5,165,166]. This effect is independent of their action on ER [167], but is due to the inhibition of enzymes involved in the post-lanosterol part of the cholesterol biosynthesis pathway. When individual sterols were measured in the sera of women treated with tamoxifen or toremifene, a substantial increase in zymostenol was detected, indicative of the inhibition of D8D7I (EBP) [166]. A milder increase in desmosterol, the immediate precursor of cholesterol through the reaction of DHCR24, was also observed with both SERMs [166]. In cultured cells, similar changes in sterol composition were observed, whose intensity varied depending on the cell type and the rate of cholesterol synthesis. In general, a decrease in the content of cholesterol and the accumulation of zymostenol were detected, in addition to other precursor sterols depending on the SERM and the dose used [149,168-171].

Mechanistically, tamoxifen and other SERMs bind with high affinity to the so-called anti-estrogen binding site (AEBS), which is a microsomal hetero-oligomeric complex including the cholesterogenic enzymes D8D7I and DHCR7 [168,172]. Given that DHCR7 can interact physically and functionally with DHCR24, the possibility exists that DHCR24 also forms part of the AEBS [173]. This is consistent with the fact that 4-OH-tamoxifen, also a high affinity AEBS ligand, caused the accumulation of mostly desmosterol [168]. It has been shown that the accumulation of sterol intermediates leads to the appearance of multilamellar bodies and eventually induce cell death and autophagy [174,175]. As further discussed in section 3.6.1, the interaction with the AEBS has been suggested to be relevant for the anti-tumoral efficacy of tamoxifen [152]. D8D7I features a binding site that accommodates multiple different ligands including tamoxifen and other cationic amphiphilic drugs (CADs), whose positively-charged amine group mimics the carbocationic sterol intermediate [176,177]. In keeping with this, toremifene inhibits D8D7I, while ospemifene, which differs from toremifene in the absence of the amino group, does not [149]. This mechanism could also explain the broad specificity exhibited by different CADs (i.e. SERMs, antipsychotics, AY9944, U18666A) at inhibiting enzymes that generate carbocationic intermediates in the respective sterol reduction reaction, such as D8D7I, DHCR7, DHCR14 and DHCR24 [178-180].

As a result of cholesterol biosynthesis inhibition by SERMs, activation of SREBP-2 and induction of SRE-containing genes are predictable [35,36,181], as observed for *HMGCR* [169,182]. This response is expected to be ineffective in terms of cholesterol formation as long as the SERM is present in the medium. In fact, stimulation of upstream enzymes expression would further increase the accumulation of SERM-targeted enzyme substrates that may have biological activities [149,174,175]. Another gene highly expressed in response to SERMs is *LDLR*, which is consistent with the increase in LDL uptake seen in cells in vitro [169,182].

3.2. LDL uptake and endolysosomal cholesterol trafficking

LDL uptake and subsequent endolysosomal degradation constitute the fundamental pathway for exogenous cholesterol supply to cells [183]. Upon its binding to the LDLR at the plasma membrane, LDL is endocytosed, dissociates from the LDLR in acidic endosomes and the receptor is recycled to the plasma membrane. LDL is then delivered to LE/L, where its cholesteryl esters are hydrolyzed by lysosomal acid lipase. The free cholesterol generated is exported from LE/L with the participation of the Niemann-Pick type C (NPC) proteins 1 and 2 and it is ultimately transported to two key destinations, the plasma membrane and the endoplasmic reticulum [184,185].

SERMs increase the expression and activity of LDLR, as found in different human cancer cells [169,182,186], primary lymphocytes [182] and rat liver [187]. This effect may contribute to their hypocholesterolemic action in patients (see section 1). In cells expressing ERα, tamoxifen and 17β-estradiol enhanced *LDLR* transcription by inducing the binding of an ERα/Sp1 complex to a Sp1-*cis*-element in the *LDLR* promoter [186]. However, we have shown that tamoxifen, toremifene, raloxifene and the tamoxifen-active metabolite endoxifen can increase LDLR expression and activity through an ER-independent mechanism: first, the effect on LDLR was observed in MOLT-4 lymphoblastic leukemia cells [169,182], which do not express ERs [188]; second, unlike SERMs, 17β-estradiol produced no effect in lymphocytes [182]; and third, the anti-estrogen ICI 182,780 did not alter the effect of SERMs [182]. The up-regulation of LDLR was associated with enhanced SREBP-induced transcriptional activity and gene expression, also affecting cholesterogenic and lipogenic genes [169,182]. SREBP activation was not just a consequence of the inhibition of cholesterol biosynthesis by SERMs, because the combined treatment with one SERM and lovastatin, a more potent cholesterol biosynthesis inhibitor, produced a synergistic increase in LDLR expression and activity, indicating the involvement of

different mechanisms of action of both drugs [169,182]. Notably, coadministration of raloxifene and simvastatin, another HMGCR inhibitor, to hypercholesterolemic postmenopausal women was superior to monotherapy with either drug in reducing LDL-cholesterol concentration [189]. We found that the SERMs interfere with endosomal cholesterol trafficking, resulting in the accumulation of LDL-derived free cholesterol in LE/L in an ER-independent manner [169,182,190]. This effect, which was observed in diverse cell lines and primary cells, resembled what occurs in fibroblasts from patients with NPC disease, caused by defective NPC1 or NPC2 proteins [191]. This blockade prevents LDL-derived cholesterol from inhibiting SREBP activation and LDLR expression, and thereby cells continue to take up LDL.

Higher tumor LDLR expression is associated with a worse prognosis in BC patients, including patients undergoing tamoxifen therapy [192,193]. Silencing the LDLR in Her2-overexpressing BC cells inhibited tumor growth in mice [192]. Glioblastomas appear to rely primarily on exogenous rather than endogenous cholesterol for growth [194,195]. In these tumors, where LDLR is overexpressed relative to normal brain tissue [194,195], a hyperactivated epidermal growth factor receptor/PI3K/AKT pathway is common, thereby promoting SREBP-1 activation and unrestrained LDLR expression [194]. However, the expression of genes for cholesterol biosynthesis was downregulated relative to normal brain, as well as that of genes for the synthesis of oxysterols that are LXR ligands [195]. Targeting the LDLR by treatment with LXR agonists induced IDOL-mediated LDLR degradation and augmented ABCA1 expression, which caused inhibition of tumor growth and increased apoptosis in mouse models [194,195]. Tumors of ALK+ anaplastic large cell lymphoma are an extreme case of cholesterol biosynthesis suppression, where the loss of SQLE renders them auxotrophic for cholesterol [196]. Therefore, inhibiting exogenous cholesterol supply, as accomplished through interference with LDL-derived cholesterol trafficking, is conceivably detrimental for tumor growth, and especially for cholesterol auxotrophic tumors.

The mechanism for cholesterol trafficking inhibition caused by tamoxifen, toremifene and raloxifene is not well defined, but it may be secondary to lysosomal trapping of these drugs. Like most SERMs, these drugs are CADs which can enter the lysosome where, by protonation of their amino group at acidic pH, lose their ability to diffuse across the membrane and are confined in this organelle [197]. In agreement with this, tamoxifen inhibits acidification of endosomes and lysosomes [198]. Lysosomal trapping is considered the major cause of CAD-induced phospholipidosis [197]. Protonated CADs disrupt the interaction between negatively charged bis(monoacylglycero)phosphate, highly enriched in intralysosomal vesicles, and positively charged phospholipases, such as acid sphingomyelinase, this being released and degraded into the lysosomal lumen. Accumulating sphingomyelin, which has high affinity for cholesterol, is known to inhibit cholesterol transport by NPC2 and, therefore, to trigger accumulation of cholesterol in lysosomes [197]. Moreover, given that several CADs are able to bind to the sterol sensing domain of NPC1, the distortion of NPC1 function by SERMs at the lysosomal membrane cannot be discarded [199-202]. Although the structural requirements for this interaction are not precisely delineated, one study with U18666A, a CAD and prototypical inhibitor of cholesterol trafficking [203], showed that correct positioning of the amino group is necessary [199].

Besides its degradative function, the lysosome is a platform for several homeostatic signaling pathways. This organelle is essential for mTORC1 signaling, which is activated at the lysosomal surface [204]. mTOR is a serine/threonine kinase that coordinates cell growth and division in response to energy levels, growth signals and nutrients, and which, when deregulated, is implicated in cancer [205,206]. Importantly, the activation of the PI3K/AKT/mTOR pathway confers treatment resistance in ER-positive BC and correlates with a poorer outcome in patients treated with endocrine therapies, including tamoxifen [6]. In fact, the PI3K/AKT/mTOR

pathway and downstream effectors can phosphorylate and activate the ER, even in the absence of estrogen [6,207]. It has been shown that cholesterol drives lysosomal recruitment and activation of mTORC1, which requires SLC38A9, a lysosomal transmembrane amino acid transporter that contains cholesterol-responsive motifs and which interacts with mTORC1 and NPC1 [208]. Moreover, the oxysterol binding protein-mediated delivery of cholesterol across endoplasmic reticulum-lysosome contacts is essential for mTORC1 activation [209]. SERMs [210] and other inhibitors of endolysosomal cholesterol trafficking [201,202,211] reduce mTORC1 signaling in endothelial cells (HUVEC). In vitro and preclinical studies have found that such compounds also inhibit angiogenesis [201,202,210,212], a process controlled by mTOR signaling and which is essential for tumor growth and metastasis [213]. The anti-angiogenic effect of tamoxifen has been demonstrated in ER-negative animal models [214]. The inhibition of both mTORC1 signaling and angiogenesis is prevented by exogenous cholesterol delivered by cholesterol/methyl-β-cyclodextrin complexes without affecting the endolysosomal accumulation of cholesterol [201,202,210]. Like the CAD-mediated cholesterol trafficking blockade, depletion of cellular cholesterol by methyl-β-cyclodextrin treatment also abrogates mTORC1 signaling, and addition of LDL or cholesterol/methyl-β-cyclodextrin restores it [208]. Therefore, depletion of membrane cholesterol by either method impedes mTORC1 activity, likely due to impairment of the functionality and/or stability of different membrane attached molecules required for signaling through the PI3K/AKT/mTORC1 pathway [205,206]. Collectively, these observations indicate that cholesterol trafficking has a critical role in cell growth and, particularly, in angiogenesis, making it a promising target against cancer [215].

The SERM-mediated restriction of cholesterol availability through inhibition of both intracellular cholesterol trafficking and biosynthesis could deeply affect tumor growth, potentially contributing to the anticancer effect of SERMs. However, the deprivation of cholesterol caused by SERMs elicits a SREBP-mediated regulatory response aimed to restore cholesterol supply [169,182]. As master regulators of lipid biosynthesis and uptake [35,36], SREBPs play a central role in lipid metabolism reprogramming in cancer cells. SREBPs are upregulated in a variety of cancers and mediate the activation of lipid metabolism by oncogenic signaling, like that through the PI3K/AKT/mTORC1 pathway, thus supporting rapid tumor growth [216]. Blocking SREBP/SCAP translocation to the Golgi or suppressing SREBP activating cleavage attenuate the growth of different cancer cells and tumors, which points to the SREBP pathway as an attractive target in cancer treatment [216]. In the face of cellular cholesterol shortage, the SREBP-mediated regulatory response might limit the effect of SERMs. Whether inhibition of the SREBP pathway combined with SERM treatment results in additional antitumor efficacy deserves further research.

3.3. Cholesterol esterification

Cholesterol esterification is a mechanism for buffering excess cellular free cholesterol, which is toxic, and stores it for use when needed. Cholesterol esterification is catalyzed by ACAT (SOAT), ACAT1 being expressed in all tissues whereas ACAT2 is localized in hepatocytes and enterocytes [217]. Accumulation of cholesteryl ester (CE) droplets is a hallmark of macrophages and smooth muscle cells in atherosclerotic lesions, and can be induced in vitro by exposing macrophages to acetyl-LDL [218,219]. ACAT resides in the endoplasmic reticulum, so that LDL-derived cholesterol must egress from lysosomes to be re-esterified. In cells from NPC patients [220,221] or treated with U18666A [222] the LDL-mediated stimulation of cholesterol esterification is diminished, concomitant with the impairment of the feedback response of LDLR activity and cholesterol biosynthesis. Similarly, tamoxifen, toremifene and raloxifene inhibit ACAT activity [182,190,223], thus suppressing the acetyl-LDL-induced increase of CE levels in macrophages [190,223]. Moreover, a direct inhibition of ACAT activity

by tamoxifen has been found in rat liver microsomal extracts, raloxifene being a weak inhibitor in this system [223].

Cholesterol esterification is deregulated in a variety of cancer cells and tumors, which show CE and lipid droplet accumulation and ACAT1 overexpression [34]. These parameters correlate inversely with survival of patients with glioblastoma [224] or pancreatic cancer [225]. In BC, markers of aggressiveness are higher in CE-rich than CE-poor tumors [226]. Consistently, inhibition of CE accumulation by abrogating ACAT1 activity or expression suppresses the development of glioblastoma, pancreatic and prostate cancer in mice [224,225,227,228]. There is a close link between CE accumulation and increased cholesterol supply, mainly through enhancing LDLR expression and LDL uptake [225-227]. The underlying mechanism in prostate and pancreatic cancer has been shown to be the loss of PTEN (phosphatase and tensin homolog) and subsequent stimulation of the PI3K/AKT/mTOR pathway, which results in SREBP activation [225,227]. ACAT1 prevents the subsequent accretion of intracellular free cholesterol and induction of endoplasmic reticulum stress and ultimately apoptosis [225,229,230]. These findings imply that targeting cholesterol esterification by inhibiting ACAT1 or cholesterol supply may be beneficial against such tumors. SERMs could be effective in this regard. Nevertheless, there is evidence that CE accumulation is lower in ER-positive than in ER-negative BC tumors [226] and cell lines [231], and that ACAT1 inhibition has less effect on the proliferation of ER-positive cells [231]. Therefore, inhibition of cholesterol esterification may have a poor contribution to the efficacy of ER-positive BC treatment with tamoxifen.

3.4. Scavenger receptor class B type I (SR-BI)

SR-BI is a membrane protein that mediates the selective delivery of HDL-CE to the liver for cholesterol excretion in the final stage of reverse cholesterol transport, and it is a key determinant of HDL metabolism [232,233]. The human homologue of rodent SR-BI was cloned first and denominated CD36 and LIMPII analogous 1 (CLA-1) [234], and was subsequently shown to exhibit a wide specificity for native and modified lipoproteins [235]. Although highly expressed in liver and steroidogenic tissues, SR-BI displays an ample cell and tissue distribution, and in peripheral tissues is able to mediate the bidirectional flux of cholesterol between cells and HDL [232,233,235,236]. Thus, SR-BI can contribute to cholesterol delivery to cells to sustain cell proliferation [237-240] and steroidogenesis [232]. Additionally, upon HDL binding and cholesterol flux, SR-BI can initiate multiple signaling pathways, such as the activation of the oncogenic kinase c-Src, for which the interaction of SR-BI with the scaffold protein PDZK1 is required [241,242].

We have found that tamoxifen or raloxifene treatment of male mice fed a western-type diet enhanced hepatic SR-BI protein expression, but had no effect on mRNA levels. This was associated with decreased serum HDL-cholesterol concentrations and accelerated HDL-CE catabolism [190]. Similar results were obtained in female fasted rats treated with the SERM acolbifene [187]. The mechanism for the increase in liver SR-BI protein expression by SERMs is unknown, but the involvement of an estrogenic effect is unlikely, because 17α-ethinyl estradiol inhibited SR-BI expression in rat liver, although stimulated its expression in steroidogenic tissues [236]. Nevertheless, SERMs may not increase SR-BI expression in women's liver, because, as mentioned above, SERMs do not reduce circulating HDL-cholesterol in treated women, but one study in tamoxifen-treated men reported a decrease in HDL-cholesterol [24]. On the other hand, tamoxifen, raloxifene and toremifene did increase SR-BI expression in human monocyte-derived macrophages [190].

SR-BI is overexpressed in a range of cancer cells and tumors, and in patients, it is associated with disease aggressiveness and poor prognosis [243-246]. Overall or event-free survival is reduced in BC [247], clear cell renal cell carcinoma [240], lung adenocarcinoma [248] and neuroblastoma [249] expressing high tumor levels of SR-BI. In prostate cancer, while SR-BI levels are associated with higher Gleason grade and poor prognosis, LDLR levels are not [250], thus highlighting the relative importance of SR-BI in certain malignancies. In a mouse xenograft model of prostate cancer, progression to castration-resistance involves the increase of SR-BI protein expression and cholesterol biosynthesis, likely fuelling de novo androgen production [251]. Aside from contributing to cholesterol supply [238,240,252], SR-BI mediates the HDL-induced activation of c-Scr and, subsequently, PI3K/AKT and mitogen activated protein kinase (MAPK) pathways, thereby stimulating proliferation, migration, invasion and protection from apoptosis in cancer cells [237-240,253]. Moreover, the interaction with SR-BI and activation of the PI3K/AKT axis mediates the angiogenic effect of HDL in hypoxia by regulating the hypoxia-inducible factor 1α pathway [254]. Remarkably, SR-BI knockdown attenuates the growth of both ER-negative and ER-positive BC cells-derived xenografts in mice [238].

The above evidence supports the notion that SR-BI potentiates cancer progression. If SERMs were able to upregulate SR-BI in cancer cells, as they do in human macrophages and rodent liver [187,190,255], this could, in principle, favor disease progression. In the case of macrophages, increased SR-BI expression could have an immunosuppressive effect. It has been described that macrophage SR-BI has an anti-inflammatory role [233,246] and, on the other hand, tumor-associated macrophages (TAMs) are correlated with cancer aggressiveness and poor prognosis [256]. Therefore, SERM-induced upregulation of macrophage SR-BI could contribute to promote treatment resistance, an issue that remains to be explored.

3.5. LXR pathway and cholesterol efflux

LXRα and LXRβ are pivotal regulators of cholesterol homeostasis that function as transcription factors by heterodimerization with the retinoid X receptor. LXRs are activated by certain cholesterol-derived oxysterols and the cholesterol precursor desmosterol in response to increased cellular sterol content [37]. As a consequence, cholesterol efflux is stimulated through the induction of ABCA1 and ABCG1 expression. ABCA1 transfers cholesterol to lipid-free apolipoprotein A-I, the major apolipoprotein component of HDL, and small HDL particles, whereas ABCG1 exports cholesterol to larger HDL particles [257]. Moreover, LXRs stimulate the expression of IDOL, an E3 ubiquitin ligase that promotes degradation of the LDLR, and inhibit cholesterol biosynthesis [37]. The most abundant LXR-activating oxysterol is 27-hydroxycholesterol, formed by cholesterol hydroxylation in mitochondria. In dividing cells, the high demand for cholesterol requires concomitant upregulation of the SREBP and downregulation of the LXR transcriptional programs [33]. Thus, LXR agonists suppress cell proliferation, whereas LXR ablation promotes it [33,258-261]. The effect of LXR activation is more pronounced in ER-positive than ER-negative BC cells and is accompanied by inhibition of ERα expression [258].

Tamoxifen, raloxifene and toremifene decrease cholesterol efflux from AcLDL-loaded macrophages to HDL and, especially, to apolipoprotein A-I. This effect is due to impairment of both the induction of ABCA1 and ABCG1 protein expression and the exit of lipoprotein-derived cholesterol from LE/L, while it is ER-independent [190]. The SERMs also decrease ABCA1 gene expression in proliferating cells, such as lymphoblastoma MOLT-4 cells [169]. Concordantly,

SERMs oppose the LDL- and AcLDL-induced transcriptional activity of LXR in hepatoma HepG2 cells and THP-1 macrophages, respectively (article in preparation). Moreover, tamoxifen or raloxifene administration in mice downregulated liver protein expression of ABCG5 and ABCG8, two LXR targets expressed in hepatocytes and enterocytes that, forming a heterodimer, in hepatocytes pump sterols to the bile duct [190]. Collectively, these results suggest that SERMs, by blocking intracellular cholesterol trafficking, impair the generation of LXR-activating oxysterols from lipoprotein cholesterol and subsequent induction of the expression of ABC transporters. Similarly, cells with defective cholesterol trafficking due to mutant NPC proteins fail to generate oxysterols in response to LDL [262]. The SERM-mediated inhibition of cholesterol biosynthesis also likely contributes to impair the supply of LXR activators.

LXRs are involved in numerous types of cancer, thus arising as potential therapeutic targets [263-265]. LXR expression is positively associated with survival in colorectal [266] and non-small-cell lung cancer [267]. Moreover, LXRβ polymorphisms influence gallbladder cancer risk [268]. Multiple evidence from mouse studies using LXR agonist administration or genetic models show that LXRs inhibit the progression of different types of cancers, including BC [194,195,259,269-274]. The tumor suppressor role of LXRs is linked to the stimulation of ABC transporters expression and IDOL-mediated LDLR degradation, thus limiting cholesterol availability for cell proliferation and survival [194,195]. Nonetheless, different mechanisms may mediate the anticancer effect of LXRs [263-265]. LXR activation disturbs the activity of the PI3K/Akt pathway, at least in part by altering membrane lipid rafts secondary to increased cholesterol efflux [271,275]. Additionally, LXR upregulates the long non-coding RNA LINC01125, which mediates the increase in PTEN, a PI3K/AKT axis inhibitor, and p53 expression [274]. On the other hand, LXRα induces the metabolic deactivation of 17β-estradiol by stimulating the hepatic expression of estrogen sulfotransferase (Est or Sult1e1), consequently attenuating estrogen-dependent BC growth in mice [276].

In line with its function as a cholesterol exporter, ABCA1 displays anticancer activity in mice [277,278], and loss-of-function mutations, including somatic mutations present in human tumors, reduce the capacity of ectopically expressed ABCA1 to inhibit the growth of colon cancer xenografts [277]. In keeping with this, decreased ABCA1 expression has been observed in several types of tumors [278-281], and ovarian cancer patients with lower ABCA1 expression have shorter progression-free survival [278]. Transcriptional induction of ABCA1 by p53 has recently been shown to be involved in p53-mediated suppression of liver tumorigenesis. ABCA1 overexpression blocked SREBP-2 activation and, thereby, the upregulation of the mevalonate pathway [95], possibly by facilitating retrograde sterol transport from the plasma membrane to the endoplasmic reticulum [282]. Collectively, the previous observations suggest that, by impairing LXR-induced expression of ABCA1 and ABCG1 and subsequent cholesterol efflux, SERMs may increase the availability of cholesterol for cell proliferation. However, the effects of SERMs on the LXR pathway are actually a consequence of the blockade of intracellular cholesterol trafficking and cholesterol biosynthesis, thereby restricting cholesterol supply, which is congruent with the SERM-mediated inhibition of cell proliferation and increase of cell death in vitro [124,174,175].

Despite the results above, the LXR pathway has also been involved in cancer progression. ABCA1 expression is elevated in triple negative BC and is associated with the histological grade [283]. High ABCA1 expression and the presence of genetic variants in tumors of colorectal cancer patients are associated with a poor prognosis, and ABCA1 overexpression confers

increased cell migration and invasive capacities through stabilization of caveolin-1 [284,285]. In androgen-independent prostate cancer cells, HDL promotes proliferation and migration by an ABCA1-dependent mechanism involving extracellular signal-regulated kinase (ERK) 1/2 and AKT [286]. With regard to ABCG1, it has been found to be overexpressed in some tumors [287,288] and to be associated with poor prognosis in patients suffering from BC or other carcinomas [288,289]. ABCG1 knockdown increased endoplasmic reticulum stress-induced apoptosis in vitro and reduced glioma growth in a mouse model of mesenchymal glioblastoma [288].

Importantly, the LXR pathway may foster tumorigenicity by dampening the immune surveillance. It has been shown that tumors release LXR oxysterol ligands that suppress the expression of CC chemokine receptor-7 on maturing dendritic cells by LXRα activation. This impairs the migration of dendritic cells to draining lymph nodes, thus suppressing the presentation of tumor antigens to T cells [290]. ABCA1 and ABCG1 had a critical role in the tumor-promoting effect of TAMs in a mouse model of metastatic ovarian cancer. These cancer cells secreted hyaluronic acid, which stimulated cholesterol efflux from TAMs and the subsequent destabilization of their membrane lipid rafts. This increased IL-4 signaling, thereby driving an immune-suppressive and tumor-promoting function [291]. Moreover, myeloid-specific ablation of ABCA1 or ABCG1 inhibits melanoma and bladder tumorigenesis in association with reduced accumulation of immune suppressive myeloid-derived suppressor cells in the tumor bed [292,293].

Therefore, different evidence suggests that in certain conditions, which may depend on the tumor microenvironment, the specific genotype or the metabolic state, inhibiting LXR activation and/or ABC transporters expression by SERMs could hinder cancer progression. This could be especially relevant for those processes and microenvironments that dampen the immune response towards tumor cells. In particular, SERMs could blunt the tumor-mediated stimulation of cholesterol efflux from TAMs, thus preventing tumor escape from immune response, an issue that merits further research.

3.6. Oxysterol metabolism

Oxysterols are oxidized forms of cholesterol, many of them acting as key effectors in cholesterol homeostasis. Oxysterols have a role in cancer pathophysiology, with disease promoting or suppressing effects through multiple mechanisms, as has been recently and extensively reviewed [43,294-296].

3.6.1. 5,6-Epoxycholesterols and their derivatives

Tamoxifen and other SERMs cause the accumulation of 5,6α- and 5,6β-epoxycholesterol in cancer cells by stimulating cholesterol peroxidation and simultaneously inhibiting cholesterol-5,6-epoxide hydrolase in an ER-independent manner [175,297]. Curiously, this enzymatic activity is carried out by the AEBS, and catalyzes the hydration of 5,6-epoxycholesterols into cholestane-3β,5α,6β-triol [298]. Such epoxycholesterols induce cell differentiation and death, although using different mechanisms. Cytotoxicity induced by 5,6β-epoxycholesterol is linked to the control of the anti-apoptotic protein Bcl-2, but the effect of 5,6α-epoxycholesterol requires its conversion to 5,6α-epoxycholesterol-3β-sulfate, catalyzed by the sulfotransferase SULT2B1b, and the subsequent modulation of LXRβ activity by this sulfation product [297]. The accumulation of 5,6-epoxycholesterols and subsequent cell death caused by SERMs and other AEBS ligands were abrogated by the antioxidant vitamin E, thus suggesting the involvement of reactive oxygen species in cholesterol epoxidation and cytotoxicity [297]. Moreover, 5,6α-

epoxycholesterol has been shown to be an LXR ligand with cell and gene context-dependent effects [299], and in BC MCF-7 cells mediates tamoxifen-induced triacylglycerol biosynthesis through the modulation of LXRβ [297].

Inhibiting the formation of cholestane-3β,5α,6β-triol by SERMs also prevents its subsequent conversion to another relevant oxysterol, 6-oxo-cholestan-3β,5α-diol (OCDO), catalyzed by 11β-hydroxysteroid-dehydrogenase-type-2 (11βHSD2) [300], the enzyme that converts cortisol into cortisone. It has been demonstrated that OCDO promotes the proliferation of BC cells by binding to the glucocorticoid receptor, and accelerates the growth of BC xenografts in mice, irrespective of their ER expression status. In BC patients, the levels of OCDO, its oxysterol precursors and 11βHSD2 were elevated in tumors compared with normal adjacent tissue, and high levels of 11βHSD2 mRNA were associated with poor prognosis [300]. In line with this, a recent study in patients with luminal BC found that high plasma levels of cholestane-3β,5α,6β-triol were associated with lower disease-free survival [301]. By contrast, however, cholestane-3β,5α,6β-triol has been shown to suppress proliferation, migration and invasion of prostate cancer cells and to delay xenograft growth in mice [302].

On the other hand, 5,6α-epoxycholesterol condensates with histamine to form dendrogenin A (DDA), which has been characterized as a tumor suppressor [303]. DDA is an AEBS ligand and potent inhibitor of cholesterol-5,6-epoxide hydrolase [304], thus also suppresses OCDO formation [300]. Additionally, DDA binds to LXRβ to stimulate the expression of Nur77, NOR1 and LC3-II, thus leading to lethal autophagy, a distinct effect not produced by canonical LXR ligands [305]. Interestingly, DDA levels are significantly decreased in breast tumors relative to normal adjacent tissues [304]. Whether tamoxifen, through the accumulation of 5,6α-epoxycholesterol, augments DDA concentration in mammary tumors is unknown. Treatment with DDA triggers BC cell re-differentiation and inhibits tumor growth of several cancers in mouse models [304,305]. Therefore, the impact of 5,6-epoxycholesterols and their derivatives on cancer is complex, with some oxysterols displaying tumor suppressing and others tumor promoting actions, suggesting that the balance between these compounds may be critical. Pending new studies, SERMs appear to favor this balance towards tumor suppressing oxysterols, which may contribute to their therapeutic efficacy.

3.6.2. 27-Hydroxycholesterol, an endogenous SERM

Cholesterol is hydroxylated to 27-hydroxycholesterol (27HC) by sterol 27-hydroxylase (CYP27A1), a mitochondrial P450 enzyme mainly expressed in liver and macrophages. This oxysterol is catabolized by 25-hydroxycholesterol 7-alpha-hydroxylase (CYP7B1), another P450 enzyme [306,307]. 27HC is the most abundant circulating oxysterol, mainly transported in LDL and HDL, its serum concentration being correlated with that of cholesterol [308,309]. It constitutes a mechanism to deliver excess sterols from peripheral tissues to the liver and is a substrate for bile acid synthesis [306,310]. However, 27HC has other biologically relevant actions. Aside from activating LXR, 27HC promotes the degradation of HMGCR [311] and inhibits SREBP-2 activation [312], further limiting the availability of intracellular cholesterol. Importantly, 27HC has been characterized as an endogenous SERM that binds to and regulates the transcriptional activity of ERα and ERβ [313,314]. 27HC induces a conformational change in ERα that is distinct from that induced by 17β-estradiol, 4-hydroxytamoxifen or the antiestrogen ICI 182,780 [314]. In models of cardiovascular disease, 27HC behaves as an ER antagonist, while in osteoblasts and cellular models of ER-positive BC it behaves as a partial ER agonist [313-316]. Although less potent, 25-hydroxycholesterol also modulates the activity of ERα and promotes breast and ovarian cancer cell proliferation [313,317].

27HC has been suggested to be a link between hypercholesterolemia and BC [307,318]. 27HC was found to stimulate the proliferation of ER-positive BC cells in vitro and increase breast tumor growth in various murine models [261,270,314]. These effects were ER-dependent, as

they were inhibited by ICI 182,780 [261,270]. CYP27A1 inhibition or genetic ablation reduced mammary tumor growth [270]. One mechanism mediating the inhibition of proliferation of ER-positive BC cells by 27HC is downregulation of p53 through the E3 ubiquitin ligase MDM2 [319]. Given that synthetic LXR ligands inhibit cell proliferation and tumor growth (see section 3.5), breast tumor promotion by 27HC suggests that its ER activating effect predominates over LXR activation. The 27HC-mediated inhibition of cholesterol supply also seems to be ineffective in this setting.

Treatment with 27HC also increased the expression of markers of epithelial to mesenchymal transition (EMT) and the number of metastatic foci in mice lungs [270,320]. However, this effect was independent of ER and involved LXR activation. In fact, the pro-metastatic role of 27HC is quite complex [318,321]. For example, 27HC increased EMT hallmarks and migratory and invasive capacities of ER-positive and ER-negative BC cells via activation of the signal transducer and activator of transcription (STAT) 3 [322]. Moreover, 27HC exerts cancer-cell extrinsic effects that contribute to metastasis, such as stimulating the function of polymorphonuclear-neutrophils and γδ-T cells [320]. 27HC might also have a role in developing resistance to estrogen deprivation. Under long-term estrogen deprivation, ER-positive BC cells increased the expression of cholesterol biosynthetic and CYP27A1 genes and the binding of ERα to regulatory elements, suggesting that endogenously generated 27HC can substitute for estrogen [154].

There is clinical evidence for a pathological role of 27HC in BC. Concentrations of this oxysterol within ER-positive breast tumors are higher than in adjacent normal tissue, and are higher in adjacent normal tissue than in breast tissue from healthy women [261]. Increased expression of CYP7B1 mRNA in tumors is associated with increased patient survival [261,270,307]. Expression of CYP27A1 protein was found to be elevated in tumor cells and macrophages of higher-grade tumors [270]. Actually, macrophages highly express CYP27A1 [270], and it is documented that TAMs are a poor prognostic factor [256]. However, later analysis showed that elevated tumoral expression of CYP27A1 mRNA is a beneficial prognostic indicator, an association only apparent in ERα-positive cases and in women aged ≤ 50 years [307]. A number of studies have reported the involvement of 27HC in other types of cancer, such as thyroid carcinoma [323] and glioblastoma [324]. However, the role of 27HC in certain cancers, including prostate cancer, is unclear, due to existing conflicting results [43,318,321]. Whatever the reason, the possibility exists that the role of 27HC in a particular cancer depends on the predominant mechanism involved, i.e. ERs, LXRs or others.

The effect of synthetic SERMs on 27HC signaling is unknown. Presumably, by inhibiting cholesterol biosynthesis and trafficking, synthetic SERMs may impair the generation of 27HC, and they may also reduce the availability of LDL-derived 27HC. Furthermore, SERMs might compete with 27HC for binding to or alter the interaction of 27HC with ERs, thus lowering ER activity. Research on this subject is warranted.

4. Concluding remarks

Cancer progression is contingent on cellular cholesterol metabolism reprogramming to meet the requirements of cancer cells. Not only do different oncogenic signals target cholesterol metabolism to ensure cholesterol availability for cell proliferation, but also cholesterol and certain oxysterols modulate different pathways critical for tumor progression, including those involved in the immune response. Many of these pathways have been proposed as therapeutic targets, and it is conceivable that restricting cholesterol supply can also improve other therapeutic interventions. SERMs display a complex pharmacology, with multiple effects exerted through either ER-dependent or independent mechanisms. In large part through the

latter mechanisms, SERMs broadly affect cellular cholesterol metabolism and handling, including cholesterol supply, storage, export and oxidation (Fig. 2), which may expand the anticancer properties of these drugs. It is noteworthy that several effects of SERMs on cholesterol homeostasis rely on the interference with endolysosomal cholesterol trafficking and, thus, restriction of exogenous cholesterol supply. Another remarkable consequence of this cholesterol traffic jam may be the abrogation of oncogenic signaling through mTORC1. Additionally, suppressing de novo biosynthesis by SERMs further limits cholesterol availability and produces the accumulation of intermediate sterols with potential regulatory actions. The simultaneous inhibition of endogenous and exogenous cholesterol supply blunts the SREBP-mediated regulatory response. The modulation of the formation of different oxysterols may also play a significant role in the therapeutic efficacy of SERMs. In particular, decreasing cholesterol availability may impair the generation of 27HC, an endogenous SERM with cancer promoting activity. However, synthetic SERMs are not exempt from potentially harmful effects. The activation of the SREBP pathway to restore cholesterol supply and the inability to activate the LXR pathway and cholesterol efflux in SERM-treated cells might eventually counterbalance the shortage of intracellular cholesterol and constitute a mechanism of treatment resistance. The SERM-induced increase in SR-BI expression may also compensate for cholesterol deprivation and, moreover, stimulate oncogenic signaling. Nevertheless, shutting off the LXR pathway is a potential mechanism to abrogate other processes crucial for cancer progression, such as those driving metastatic dissemination or tumor immune evasion. A still unaddressed but pertinent question is how SERMs affect the expression of microRNAs relevant to cholesterol homeostasis. MicroRNAs are critical post-transcriptional regulators of cholesterol metabolism [325] and, on the other hand, microRNA expression profiling has been shown to be associated with tumour development, progression and response to therapy [30,326]. Delineating the contribution of these processes to the therapeutic effect of SERMs is essential. This knowledge could be eventually exploited in the design of new therapeutic strategies.

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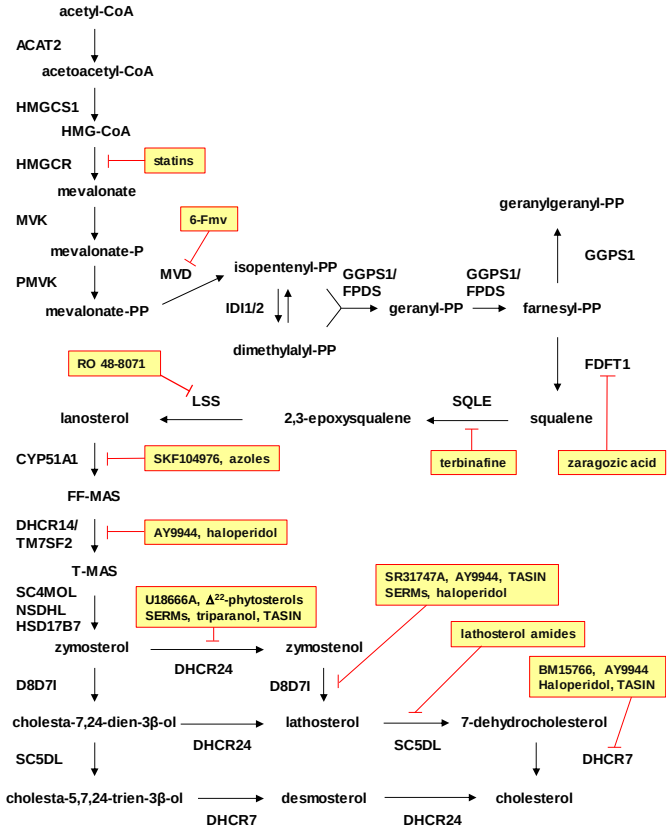
Figure legends

Figure 1. Scheme of the cholesterol biosynthesis pathway and enzymes inhibited by different molecules. Cholesterol biosynthesis from acetyl-CoA is depicted, as well as the branch to geranylgeranyl-diphosphate. The lanosterol-to-cholesterol pathway, which actually may proceed through two routes, the so-called Bloch pathway, which uses Δ^{24}-unsaturated sterols, and the Kandutsch–Russell pathway, with side-chain saturated sterols, has been simplified. The Bloch pathway has been diverted at zymosterol and cholesten-7,24-dien-3β-ol to show intermediate sterols that accumulate after treatment with inhibitors of interest. HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; FF-MAS, follicular fluid meiosis activating sterol; T-MAS, testicular meiosis activating sterol. Enzyme acronyms: ACAT2, acetyl-CoA acetyltransferase, cytosolic; HMGCS1, hydroxymethylglutaryl-CoA synthase, cytoplasmic; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MVK, mevalonate kinase; PMVK, phosphomevalonate kinase; MVD, diphosphomevalonate decarboxylase; IDI1/2, isopentenyl-diphosphate Δ-isomerase, isoforms 1 and 2; GGPS1, geranylgeranyl diphosphate synthase; FDPS, farnesyl diphosphate synthase; FDFT1, squalene synthase; SQLE, squalene monooxygenase; LSS, lanosterol synthase; CYP51A1, lanosterol 14α-demethylase; DHCR14 (LBR), Δ^{14}-sterol reductase (lamin-B receptor); TM7SF2, Δ^{14}-sterol reductase; SC4MOL, methylsterol monooxygenase 1; NSDHL, sterol-4α-carboxylate 3-dehydrogenase, decarboxylating; HSD17B7, 3-keto-steroid reductase; D8D7I (EBP), 3β-hydroxysteroid-Δ^{8},Δ^{7}-isomerase (emopamil-binding protein); DHCR24, Δ^{24}-sterol reductase; SC5DL, lathosterol oxidase; DHCR7, 7-dehydrocholesterol reductase. 6-Fmv, 6-fluoromevalonate; SERMs, selective estrogen receptor modulators; TASIN, truncated APC selective inhibitors. Double arrows indicate several reactions catalyzed by different enzymes.

Figure 2. Effects of SERMs on cellular cholesterol metabolism and homeostasis. SERMs suppress endolysosomal cholesterol (C) trafficking and, moreover, cholesterol biosynthesis through inhibition of the AEBS enzymatic complex, thus inducing the accumulation of zymostenol (Zymo) and desmosterol (Desm). Consequently, the processing of the SREBP-2 precursor (pSREBP-2) to generate nuclear SREBP-2 (nSREBP-2) remains active, which results in LDLR overexpression, whereas LXR activation and subsequent expression of ABCA1 and ABCG1 in response to LDL are blunted. Induction of cholesterol esterification and ACAT-1 activity are also abrogated, as well as mTORC1 activation on the lysosomal surface. Inhibition of the AEBS together with the generation of reactive oxygen species (ROS) causes the accumulation of 5,6-epoxycholesterols (5,6α-EC and 5,6β-EC), which cannot be successively converted to cholestane-3β,5α,6β-triol (CT) and 6-oxo-cholestan-3β,5α-diol (OCDO). Furthermore, SERMs increase SR-BI expression by an unknown mechanism. The principal effects of SERMs on the levels of the indicated sterols, proteins and mTORC1 are depicted with up or down arrows.
* Denotes ligand-mediated activation; P denotes phosphorylation; 5,6-ECS, 5,6α-epoxycholesterol-3β-sulfate; GR, glucocorticoid receptor; Hist, histamine.

Figure 1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HMG-CoA
HMGCR
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MVD
isopentenyl-PP
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GGPS1
dimethylalyl-PP
RO 48-8071
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squalene
terbinafine
zaragozic acid
CYP51A1
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FF-MAS
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SERMs, haloperidol
lathosterol amides
zymosterol
DHCR24
zymostenol
D8D7I
D8D7I
BM15766, AY9944
Haloperidol, TASIN
cholesta-7,24-dien-3β-ol
DHCR24
lathosterol
SC5DL
7-dehydrocholesterol
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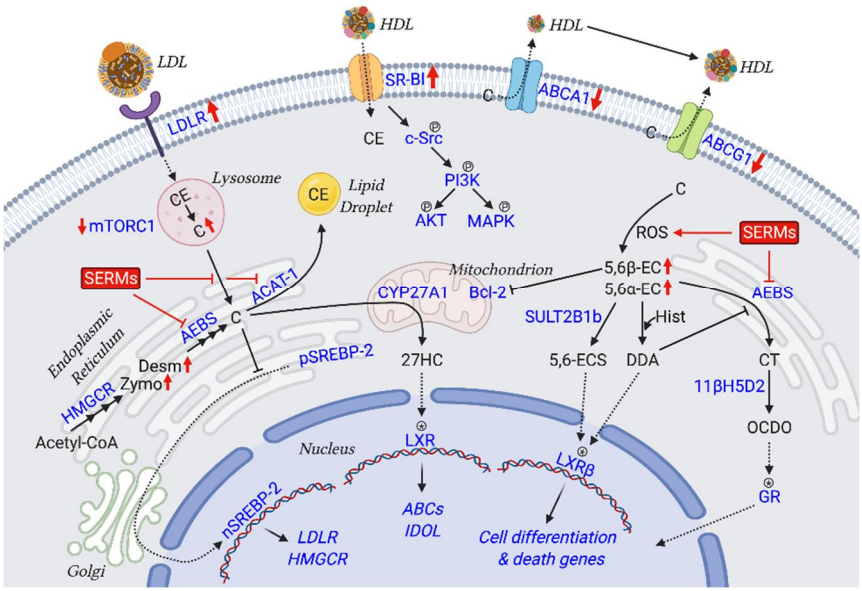
cholesta-5,7,24-trien-3β-ol
DHCR7
desmosterol
DHCR24
cholesterol

Figure 2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mTORC1
SERMs
ACAT-1
AEBS
C
ROS
SERMs
Mitochondrion
5,6β-EC
5,6α-EC
CYP27A1
Bcl-2
AEBS
SULT2B1b
Hist
Endoplasmic Reticulum
Desm
Zymo
HMGCR
Acetyl-CoA
pSREBP-2
27HC
5,6-ECS
DDA
CT
11βH5D2
OCDO
Nucleus
LXR
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LDLR
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ABCs
IDOL
Cell differentiation & death genes
Golgi