



HAL
open science

Pharmacological targeting of cholesterol biosynthesis in cancer: Sterol intermediates, oxysterols, and sterol flux rewiring

Philippe De Médina, Julio Buñay, Silia Ayadi, Sandrine Silvente-Poirot, Marc Poirot

► To cite this version:

Philippe De Médina, Julio Buñay, Silia Ayadi, Sandrine Silvente-Poirot, Marc Poirot. Pharmacological targeting of cholesterol biosynthesis in cancer: Sterol intermediates, oxysterols, and sterol flux rewiring. *Biochemical Pharmacology*, 2026, 250 (part 2), pp.118003. <10.1016/j.bcp.2026.118003>. <inserm-05614145>

HAL Id: inserm-05614145

<https://inserm.hal.science/inserm-05614145v1>

Submitted on 6 May 2026

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License



Review

Pharmacological targeting of cholesterol biosynthesis in cancer: Sterol intermediates, oxysterols, and sterol flux rewiring

Philippe de Médina^{*}, Julio Buñay, Silia Ayadi, Sandrine Silvente-Poirot^{*}, Marc Poirot^{*}

Cancer Research Center of Toulouse (CRCT), Inserm, CNRS, University of Toulouse, Toulouse, France



ARTICLE INFO

Keywords:

Cholesterol biosynthesis
Oxysterols
Sterol metabolism
Cancer metabolism
Cholesterol-5,6-epoxide hydrolase
Dendrogenin A
Oncosterone
Sterol flux rewiring
Chemical Compounds:
Cholesterol (CID5997)
desmosterol (CID954)
7-dehydrocholesterol (CID5280795)
lanosterol (CID246983)
zymosterol (CID6436305)
zymostenol (CID10115551)
lathosterol (CID441448)
squalene (CID638072)
oxidosqualene (CID12303662)
5,6-epoxycholestanol (CID12303662)
5,6 α -epoxycholestanol (CID12303662)
5,6 β -epoxycholestanol (CID12303663)
cholestane-3 β ,5 α ,6 β -triol (CID11905190)
dendrogenin A (CID49849821)
oncosterone (CID159461)
PBPE (CID71311880)
27-hydroxycholesterol (CID5283567)
27-hydroxy-oncosterone (CID171117793)
follicular fluid meiosis-activating sterol (FF-MAS) (CID11905192)
tamoxifen (CID2733526)
tesmilifene / DPPE (CID119543)

ABSTRACT

Cholesterol metabolism has emerged as an important regulator of tumor cell biology. Beyond its structural role in membranes, the cholesterol biosynthetic pathway generates sterol intermediates and oxidized derivatives able to modulate signaling pathways controlling proliferation, differentiation, and survival. Early studies mainly emphasized the requirement of the mevalonate pathway for cholesterol synthesis and for production of non-sterol isoprenoid intermediates involved in protein prenylation, but increasing evidence indicates that the biological effects of inhibiting cholesterol synthesis cannot be explained solely by cholesterol depletion. Blocking specific enzymatic steps frequently causes accumulation of sterol precursors that differ structurally from cholesterol and are highly susceptible to oxygenation under conditions of oxidative stress commonly observed in tumor cells, leading to formation of bioactive oxysterols. Among these metabolites, cholesterol-5,6-epoxides represent a central metabolic node linking sterol imbalance, lipid peroxidation, and sterol signaling. Depending on the enzymatic context, these epoxides can be converted into metabolites with tumor-promoting or tumor-suppressive properties, indicating that inhibition of cholesterol biosynthesis redistributes sterol metabolic flux rather than simply reducing cholesterol levels. Identification of sterol metabolites formed after perturbation of cholesterol synthesis remains difficult because many derivatives differ only by subtle structural modifications or exist as stereoisomers, often requiring chemical synthesis of reference compounds and dedicated analytical methods. These observations support the concept of sterol flux rewiring, in which the biological outcome of targeting cholesterol biosynthesis depends on the nature of accumulated intermediates, the oxidative environment, and the enzymatic context of the cell, with important implications for pharmacological targeting of sterol metabolism in cancer.

Abbreviations: AEBS, Antiestrogen Binding Site; AKT, Protein kinase B; CYP11A1, Cytochrome P450 family 11 subfamily A member 1; CYP51A1, Lanosterol 14 α -demethylase; ChEH, Cholesterol-5,6-epoxide hydrolase; CT, Cholestane-3 β ,5 α ,6 β -triol; DDA, Dendrogenin A; DHCR7, 7-dehydrocholesterol reductase; DHCR24, 24-dehydrocholesterol reductase; EGFR, Epidermal Growth Factor Receptor; ER, Estrogen Receptor; ERBB, Erb-B receptor tyrosine kinase family; FPP, Farnesyl pyrophosphate; FF-MAS, Follicular fluid meiosis-activating sterol; GR, Glucocorticoid Receptor; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HMGCR, HMG-CoA reductase; 11 β -HSD2, 11 β -hydroxysteroid dehydrogenase type 2; IPP, Isopentenyl pyrophosphate; LXR, Liver X Receptor; MAPK, Mitogen-activated protein kinase; MAS, Meiosis-activating sterols; NSDHL, NAD(P)-dependent steroid dehydrogenase-like, OCDO, Oncosterone; PI3K, Phosphoinositide 3-kinase; PPAR, Peroxisome proliferator-activated receptor; ROS, Reactive oxygen species; ROR, Retinoic acid receptor-related orphan receptor; SC4MOL, Sterol-C4-methyl oxidase-like; SERM, Selective estrogen receptor modulator; SLOS, Smith-Lemli-Opitz syndrome; SREBP, Sterol regulatory element-binding protein; TNBC, Triple-negative breast cancer; 5,6-EC, cholesterol-5,6-epoxides, 5,6-epoxycholestanol; 5, 6 α -EC, 5,6 α -epoxycholestanol; 5, 6 β -EC, 5,6 β -epoxycholestanol; 7-DHC, 7-dehydrocholesterol; 27-HC, 27-hydroxycholesterol; 27-OH-OCDO, 27-hydroxy-oncosterone.

^{*} Corresponding author.

E-mail addresses: philippe.de-medina@inserm.fr (P. Médina), sandrine.poirot@inserm.fr (S. Silvente-Poirot), marc.poirot@inserm.fr (M. Poirot).

<https://doi.org/10.1016/j.bcp.2026.118003>

Received 20 March 2026; Received in revised form 17 April 2026; Accepted 20 April 2026

0006-2952/© 2026 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

SR31747A (CID6439330)
 Boxidine (CID31742)

1. Introduction

Metabolic reprogramming is a defining feature of cancer cells and allows tumors to sustain proliferation, resist stress, and adapt to changes

in nutrient availability. Among the metabolic pathways that play a central role in tumor biology, the mevalonate pathway has attracted considerable attention because it provides precursors required for cholesterol synthesis, for the production of non-sterol isoprenoid

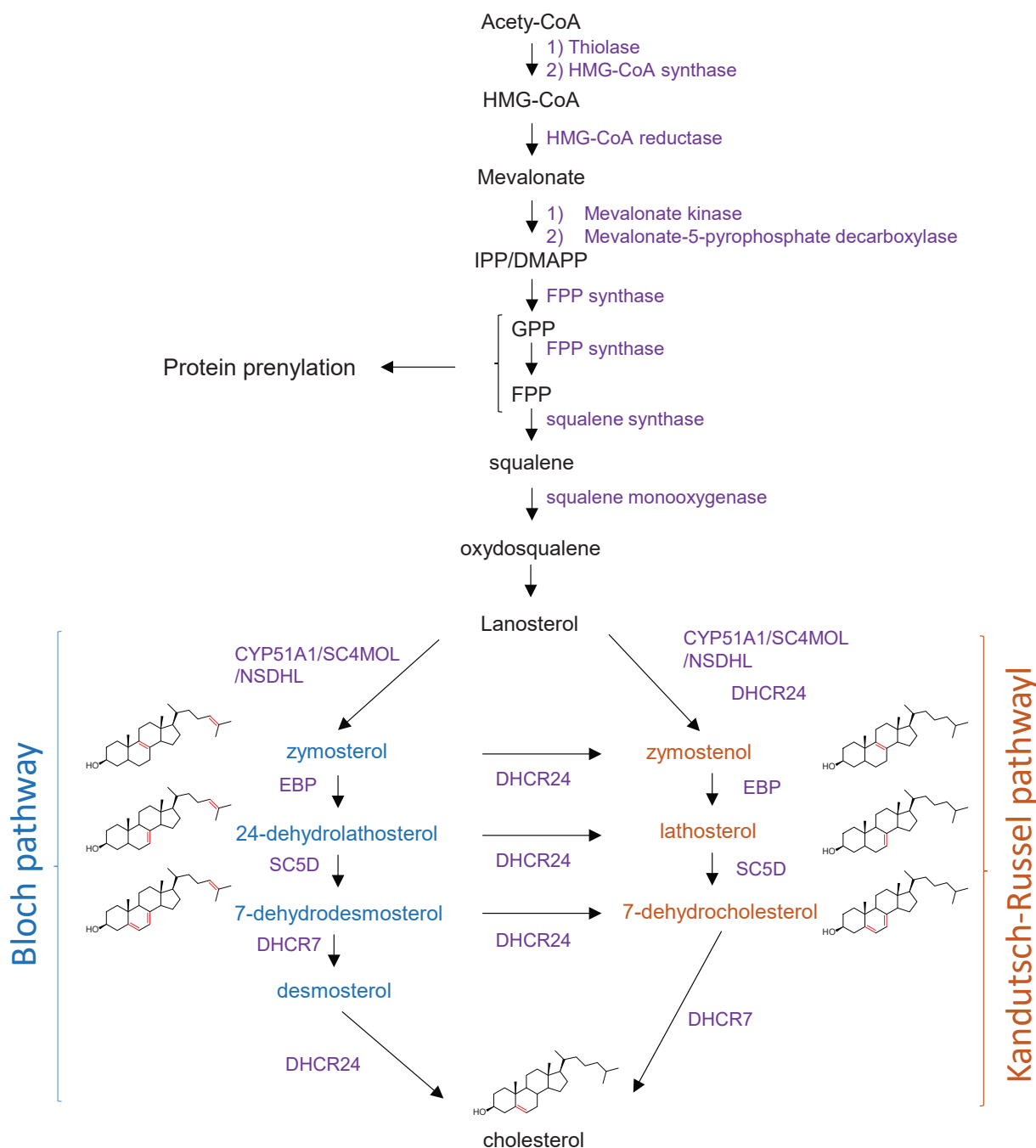


Fig. 1. Cholesterol biosynthesis pathway showing the Bloch and Kandutsch–Russell branches. Schematic representation of the cholesterol biosynthetic pathway from acetyl-CoA to cholesterol, highlighting the post-lanosterol segment and the two alternative routes leading to cholesterol formation, known as the Bloch pathway and the Kandutsch–Russell pathway. After conversion of acetyl-CoA to mevalonate through the action of thiolase, HMG-CoA synthase, and HMG-CoA reductase, isoprenoid intermediates are produced and condensed to form squalene, which is converted to lanosterol by squalene monooxygenase and oxidosqualene cyclase. From lanosterol, sterol C4-demethylation involving CYP51A1, SC4MOL, and NSDHL generates zymosterol and zymostenol intermediates, which are further processed through parallel enzymatic reactions catalyzed by EBP, SC5D, DHCR7, and DHCR24. In the Bloch pathway, desmosterol is the immediate precursor of cholesterol, whereas in the Kandutsch–Russell pathway, cholesterol is formed through lanosterol and 7-dehydrocholesterol intermediates. The pathway also produces non-sterol isoprenoid intermediates required for protein prenylation.

intermediates involved in protein prenylation, and for the generation of multiple bioactive lipids [1–3] (Fig. 1). Dysregulation of this pathway has been reported in many tumor types and is frequently associated with activation of oncogenic signaling networks including PI3K–AKT–mTOR, MYC, and mutant RAS pathways [4,5].

Early studies investigating the role of the mevalonate pathway in cancer focused primarily on the requirement for cholesterol as a structural component of cellular membranes and on the production of non-sterol isoprenoid intermediates required for protein prenylation [6,7]. These observations led to the view that inhibition of cholesterol biosynthesis would impair tumor growth mainly by limiting membrane formation or by preventing prenylation of oncogenic proteins. In addition, in hormone-dependent tumors such as prostate and breast cancers, cholesterol biosynthesis also provides substrates for intratumoral steroid hormone production, thereby contributing to endocrine signaling pathways that support tumor growth [8,9]. However, accumulating evidence indicates that this interpretation is incomplete and that the biological consequences of inhibiting cholesterol biosynthesis cannot be explained solely by cholesterol depletion [10–12].

The cholesterol biosynthetic pathway is a complex metabolic network that generates numerous sterol intermediates before the final production of cholesterol [13,14]. These intermediates differ in their chemical structure and may display distinct biological properties. Several sterol precursors, including desmosterol, methylsterols, and other post-lanosterol intermediates, have been shown to regulate nuclear receptor signaling, membrane trafficking, and receptor turnover, indicating that intermediates of the pathway may function as signaling molecules rather than passive metabolic substrates [15–17].

Both cholesterol and its biosynthetic precursors are susceptible to oxygenation, particularly under conditions of increased reactive oxygen species commonly observed in tumor cells [18,19]. Oxidative stress, mitochondrial dysfunction, and inflammatory signaling promote lipid peroxidation and favor the formation of oxidized sterols collectively referred to as oxysterols. Some oxysterols act as ligands for nuclear receptors such as liver X receptors (LXRs) [20–23] and retinoic acid receptor-related orphan receptors (RORs) [24–26], thereby linking sterol metabolism to transcriptional regulation [27–29]. Among these metabolites, 27-hydroxycholesterol (27-HC) has been shown to promote the growth of estrogen receptor–positive breast cancer, whereas the oxysterol oncosterone (OCDO) stimulates proliferation of both estrogen receptor–positive and triple-negative breast cancer cells, demonstrating a direct link between sterol metabolism and tumor progression [9,30].

Oxysterol signaling is further complicated by the existence of downstream metabolic conversions that modify biological activity. Recent work has shown that both 27-HC and OCDO can be converted into 27-hydroxy-OCDO (27-OH-OCDO), a metabolite displaying anti-proliferative properties, indicating that sterol metabolism involves branching pathways capable of generating metabolites with opposing biological effects [31].

A critical intersection between cholesterol biosynthesis, oxidative stress, and sterol signaling is provided by the formation of cholesterol 5,6-epoxides (5,6-ECs), which arise during lipid peroxidation of membrane cholesterol. These epoxides are metabolized by cholesterol-5,6-epoxide hydrolase (ChEH) [12], an enzymatic activity associated with the microsomal antiestrogen binding site (AEBS) and composed of enzymes of the cholesterol biosynthetic pathway [32,33]. Hydrolysis of 5,6-ECs generates cholestane-3 β ,5 α ,6 β -triol (CT), which can be further converted into OCDO in cells expressing 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) [30,34]. In contrast, alternative metabolic routes, including conversion of 5,6 α -EC into dandrogenin A (DDA) in normal tissues, may generate sterol metabolites with tumor-suppressive properties, illustrating that the biological outcome of sterol oxidation depends on the enzymatic context of the cell [35–39].

These observations indicate that inhibition of cholesterol biosynthesis does not simply reduce cholesterol levels but instead redistributes sterol metabolic flux, leading to the accumulation and transformation of

intermediates that can influence tumor cell behavior. This concept, referred to as sterol flux rewiring, proposes that pharmacological inhibition of cholesterol biosynthesis reshapes sterol metabolic networks and promotes the formation of bioactive sterol derivatives whose effects depend on the redox state of the cell and on the expression of enzymes involved in sterol metabolism [11].

In this review, we examine the cholesterol biosynthetic pathway as a dynamic metabolic network capable of generating bioactive sterol metabolites. We discuss the signaling functions of sterol intermediates and oxysterols, the role of oxidative sterol metabolism in cancer, and the pharmacological implications of targeting specific steps in cholesterol biosynthesis. Particular attention is given to the concept of sterol flux rewiring and to the mechanisms by which sterol intermediates and their oxidation products may contribute to tumor progression or tumor suppression.

2. Architecture of the cholesterol biosynthetic pathway

The cholesterol biosynthetic pathway is one of the most complex metabolic networks in mammalian cells. Beginning with acetyl-CoA, the pathway proceeds through more than twenty enzymatic reactions that ultimately produce cholesterol. These reactions can be divided into two major segments: the pre-lanosterol segment, which generates the isoprenoid precursor squalene and its epoxide derivative, and the post-lanosterol segment, which converts lanosterol into cholesterol through a series of demethylation, reduction, and isomerization reactions [13] (Fig. 1).

The early stages of the pathway involve the condensation of acetyl-CoA molecules to produce 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is subsequently reduced by HMG-CoA reductase to form mevalonate. This reaction represents the principal rate-limiting step of cholesterol biosynthesis and is tightly regulated at transcriptional and post-translational levels through sterol regulatory element-binding proteins (SREBPs) and sterol-dependent feedback mechanisms [2].

Mevalonate is further converted into activated isoprenoid intermediates including isopentenyl pyrophosphate, geranyl pyrophosphate, and farnesyl pyrophosphate. These molecules represent branch points in metabolism because they serve as precursors not only for sterol synthesis but also for non-sterol isoprenoids required for protein prenylation, dolichol synthesis, ubiquinone production, and other essential cellular processes [3,7].

The expression of enzymes involved in cholesterol biosynthesis and sterol metabolism is not uniform across tissues and is tightly regulated at multiple levels, including transcriptional control by sterol regulatory element-binding proteins (SREBPs) and modulation by oncogenic signaling pathways frequently altered in cancer [1,2], and tumor-dependent metabolic reprogramming may further influence the spectrum of sterol intermediates and oxysterols produced.

The conversion of farnesyl pyrophosphate into squalene marks the commitment to sterol biosynthesis. Squalene is then oxidized by squalene epoxidase to form 2,3-oxidosqualene, which undergoes cyclization catalyzed by lanosterol synthase to generate lanosterol, the first sterol intermediate of the pathway [13].

From this point onward, the pathway enters the post-lanosterol phase, during which lanosterol is progressively converted into cholesterol through a series of enzymatic reactions involving demethylation at the C4 position, reduction of double bonds, and rearrangement of sterol structures. These reactions involve multiple enzymes including sterol-C4-methyl oxidase-like protein (SC4MOL), NAD(P)-dependent sterol dehydrogenase-like protein (NSDHL), emopamil-binding protein (EBP), 7-dehydrocholesterol reductase (DHCR7), and 24-dehydrocholesterol reductase (DHCR24) [13,40] (Fig. 1).

Importantly, these enzymatic steps generate a series of sterol intermediates that differ structurally from cholesterol. Because inhibition of specific enzymes leads to the accumulation of defined intermediates, the post-lanosterol segment of the pathway represents a potential source

of bioactive sterol molecules capable of influencing membrane organization, receptor trafficking, and intracellular signaling pathways. These properties make late steps of cholesterol biosynthesis particularly relevant in the context of cancer metabolism.

3. Post-Lanosterol sterol intermediates as potential signaling molecules

Although cholesterol biosynthesis was historically viewed as a linear sequence of reactions culminating in the production of cholesterol, it is now clear that several sterol intermediates generated during the post-lanosterol segment possess biological activities that extend beyond their role as transient metabolic precursors. The conversion of lanosterol into cholesterol produces a variety of intermediates including zymosterol, zymostenol, lathosterol, 7-dehydrocholesterol, desmosterol and methylsterols such as FF-MAS, each characterized by specific structural features that influence their interactions with membranes and proteins.

One of the best-characterized examples of a bioactive sterol intermediate is desmosterol, the immediate precursor of cholesterol in the Bloch pathway. Desmosterol has been identified as an endogenous ligand of liver X receptors (LXRs), nuclear receptors that regulate genes involved in cholesterol transport, lipid metabolism, and inflammatory responses [17,41,42]. In macrophages, desmosterol accumulation activates LXR-dependent transcription while suppressing inflammatory gene expression, demonstrating that sterol intermediates generated during cholesterol biosynthesis can function as signaling molecules linking lipid metabolism to immune regulation. Desmosterol was also identified as a ROR γ modulator that controls the T(H)17 differentiation [25].

Other sterol intermediates may influence cellular physiology through mechanisms that are less well understood. Sterols retaining a $\Delta 7$ or $\Delta 8$ double bond, such as zymostenol or 7-DHC, differ from cholesterol in their effects on membrane properties and protein trafficking. Genetic studies have shown that perturbations of sterol demethylation or reduction steps can profoundly alter the trafficking and stability of growth factor receptors, including the epidermal growth factor receptor (EGFR), thereby influencing oncogenic signaling pathways [43,44].

Another important aspect of sterol intermediate biology is their susceptibility to oxygenation. Intermediates generated during cholesterol biosynthesis may contain additional double bonds and methyl groups. They may also have a double bond in a position other than the $\Delta 5$ position on the B ring of the steroid backbone. These differences have been shown to increase their sensitivity to oxidative modification [18,19]. This property becomes particularly relevant in tumor cells, which frequently experience elevated oxidative stress due to mitochondrial dysfunction, oncogenic signaling, and inflammatory processes within the tumor microenvironment. Under these conditions, sterol intermediates accumulating after inhibition of cholesterol biosynthesis may undergo oxidative reactions associated with lipid peroxidation, generating oxidized sterol derivatives whose biological functions remain incompletely characterized [18,19,45].

These observations indicate that the post-lanosterol segment of cholesterol biosynthesis should not be viewed simply as a sequence of reactions producing cholesterol, but rather as a metabolic network capable of generating sterol molecules with potential signaling properties [20,28]. Understanding the biological roles of these intermediates is therefore essential for interpreting the consequences of pharmacological interventions targeting cholesterol biosynthesis in cancer cells.

4. Oxysterol biology in cancer

Oxysterols are oxygenated derivatives of cholesterol that play important roles in the regulation of cellular signaling pathways. These molecules can be produced through enzymatic oxidation reactions mediated mainly by cytochrome P450 enzymes [46–48] or through non-

enzymatic oxidation processes driven by reactive oxygen species and lipid peroxidation [49–53]. Because oxysterols can interact with nuclear receptors, membrane proteins, and intracellular signaling pathways, they represent a key interface between lipid metabolism and gene regulation.

Several oxysterols act as ligands for nuclear receptors including liver X receptors (LXRs) [20,22,23,29] and retinoic acid receptor-related orphan receptors (RORs) [24–26,54], thereby controlling transcriptional programs involved in cholesterol homeostasis, lipid metabolism, and inflammation [27–29]. In the context of cancer, these signaling pathways may influence tumor growth both directly, through effects on tumor cells, and indirectly, through modulation of immune responses within the tumor microenvironment.

Among the oxysterols implicated in cancer progression, 27-hydroxycholesterol (27-HC), produced by CYP27A1, has been shown to stimulate the proliferation of estrogen receptor-positive breast cancer cells and to promote tumor growth *in vivo* [9]. It should be noted, however, that several *in vitro* studies investigating the effects of 27-hydroxycholesterol have employed concentrations that may exceed physiological levels observed in human tissues, and this should be taken into account when interpreting their relevance to tumor biology [8,9]. In addition to its effects on tumor cells, 27-HC can modulate immune cell function and inflammatory responses, illustrating how oxysterol signaling can influence multiple components of the tumor microenvironment [8,35,55].

Oxysterols can also arise from lipid peroxidation, a process that occurs when reactive oxygen species attack polyunsaturated lipids and other susceptible molecules within biological membranes. Although cholesterol is less prone to oxidation than polyunsaturated fatty acids, it can nonetheless undergo oxidative modification under conditions of increased oxidative stress, generating a variety of oxysterols including hydroperoxides, epoxides, and ketones [19,45,51,53,56–58]. Many of these molecules display biological activities distinct from those of cholesterol and may influence cell proliferation, differentiation, or survival.

Among the oxysterols generated during lipid peroxidation, cholesterol 5,6-epoxides (5,6-ECs) represent particularly important metabolites because they enter a specific metabolic pathway capable of producing additional bioactive sterol derivatives. These epoxides therefore occupy a central position in sterol metabolic networks linking oxidative stress, cholesterol biosynthesis, and sterol signaling pathways [35,36,39].

5. The 5,6-Epoxy metabolic node linking lipid peroxidation and sterol signaling

Oxidative modification of cholesterol is a major biochemical consequence of increased lipid peroxidation associated with sterol imbalance. Accumulation of free cholesterol or sterol precursors can perturb cellular redox homeostasis, promote the generation of reactive oxygen species, and enhance lipid peroxidation within biological membranes. Under these conditions, cholesterol present in lipid bilayers becomes susceptible to non-enzymatic oxidation, leading to the formation of several oxysterols, among which 5,6-ECs occupy a central position in sterol metabolic networks (Fig. 2).

5,6-ECs exist as two stereoisomeric forms, 5 α ,6 α -epoxycholestanol (5,6 α -EC) and 5 β ,6 β -epoxycholestanol (5,6 β -EC), generated by radical-mediated oxidation of the $\Delta 5$ double bond of cholesterol during lipid peroxidation reactions. Because cholesterol is abundant in cellular membranes, even moderate increases in oxidative stress may result in significant formation of these epoxide derivatives. Numerous studies performed in models of oxidative injury, atherosclerosis, neurodegeneration, and metabolic disorders have shown that 5,6-ECs are reliable markers of cholesterol oxidation and membrane lipid peroxidation [35,36,39].

Once formed, 5,6-ECs enter a specific metabolic pathway that connects cholesterol oxidation with steroid metabolism. A central step in

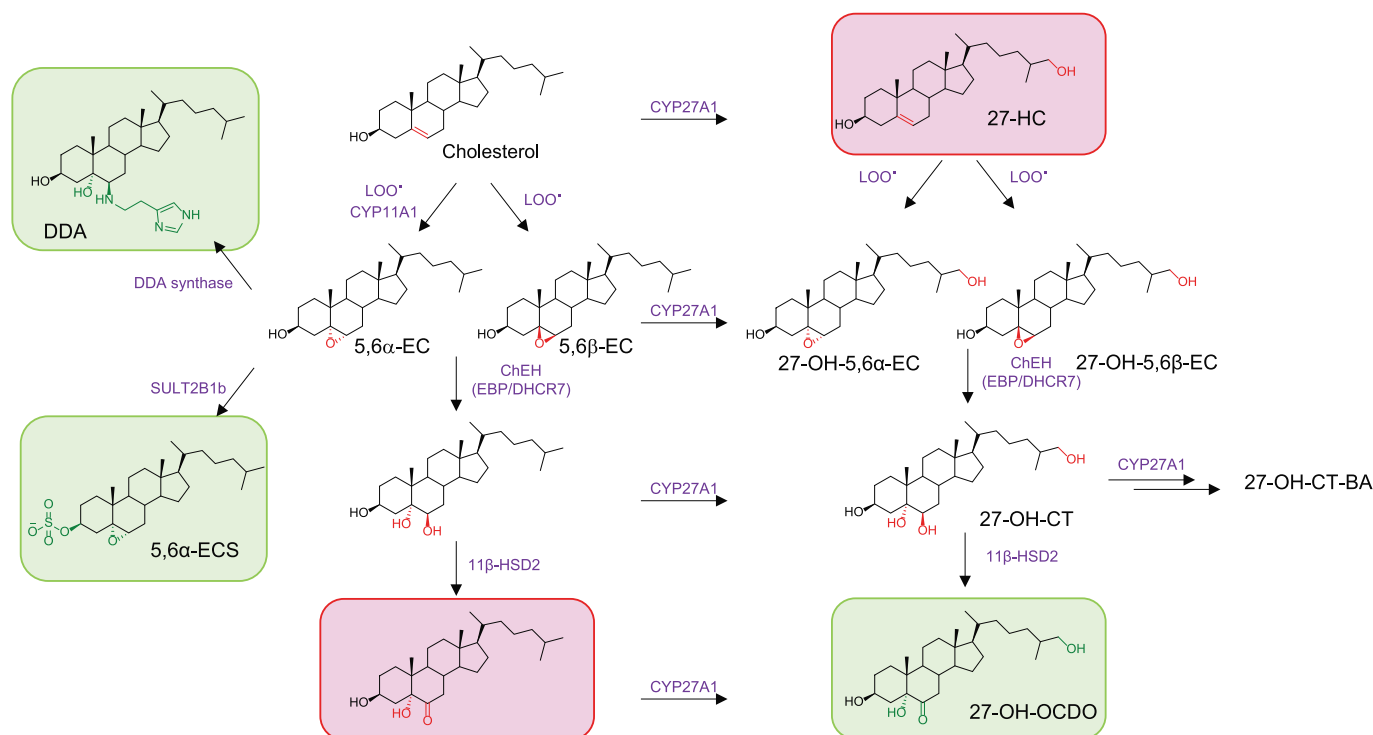


Fig. 2. Metabolism of cholesterol epoxides and formation of bioactive sterols. Metabolic pathways linking cholesterol oxidation to the formation of bioactive sterol metabolites. Cholesterol can undergo free-radical-mediated oxidation during lipid peroxidation to generate 5,6-epoxycholestanols (5,6-ECs), including the 5,6 α - and 5,6 β -EC isomers. These epoxides are hydrolyzed by cholesterol-5,6-epoxide hydrolase (ChEH), an enzymatic activity associated with the emopamil-binding protein (EBP) and 7-dehydrocholesterol reductase (DHCR7), to form cholestane-3 β ,5 α ,6 β -triol (CT). Side-chain hydroxylation by CYP27A1 generates additional derivatives including 27-hydroxycholesterol (27-HC) and 27-hydroxylated metabolites such as 27-OH-OCDO. 27-OH-CT can be metabolized further into bile acids, which are schematized as 27-OH-CT-BA. In normal tissues, 5,6 α -EC can also be converted into dendrogenin A (DDA) through a specific enzymatic activity, whereas this pathway is frequently lost in tumors. These reactions illustrate the branching network linking cholesterol oxidation to sterol signaling pathways with tumor-promoting or tumor-suppressive activities.

this pathway is the hydrolysis of the epoxide group catalyzed by cholesterol-5,6-epoxide hydrolase (ChEH), an enzymatic activity associated with the microsomal antiestrogen binding site (AEBS) [12,32,59]. Molecular studies have shown that ChEH activity results from the cooperation of two enzymes of the cholesterol biosynthetic pathway, emopamil-binding protein (EBP) and 7-dehydrocholesterol reductase (DHCR7), illustrating the intimate link between cholesterol biosynthesis and sterol oxidation pathways [12,32,59].

ChEH converts 5,6-ECs into cholestane-3 β ,5 α ,6 β -triol (CT), a metabolite that can undergo additional enzymatic transformations depending on the metabolic context of the cell. In tumor cells expressing 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), CT can be oxidized to form oncoesterone (OCDO), a sterol metabolite that promotes tumor cell proliferation and tumor growth [12,35,59]. Because OCDO formation requires prior hydrolysis of the epoxide, ChEH represents a critical control point in this pathway. Inhibition of ChEH prevents CT production and thereby blocks oncoesterone biosynthesis.

In addition to its conversion into oncoesterone, CT can also undergo further metabolic processing through CYP27A1-dependent side-chain oxidation pathways, generating 27-hydroxylated intermediates such as 27-OH-CT that are likely involved in its conversion toward bile acid derivatives, as suggested by studies in Niemann–Pick type C disease and supported by the identification of CT as a substrate of CYP27A1 [31,60,61].

The metabolic fate of 5,6-ECs depends strongly on the enzymatic environment of the cell. In normal tissues, the 5,6 α -EC can be converted into dendrogenin A (DDA) through an enzymatic conjugation with histamine. DDA is an endogenous sterol metabolite with tumor-suppressive properties [62]. In contrast, in tumors expressing both ChEH and 11 β -HSD2, 5,6-ECs may be preferentially metabolized toward OCDO

formation, favoring proliferative signaling. These observations indicate that the biological outcome of cholesterol oxidation depends not only on the level of oxidative stress but also on the balance between competing enzymatic pathways downstream of 5,6-ECs.

The 5,6-EC metabolic node therefore represents a critical intersection between cholesterol biosynthesis, lipid peroxidation, and sterol signaling. Perturbations of cholesterol metabolism that increase oxidative stress or alter sterol composition may enhance the formation of 5,6-ECs, while the expression of downstream enzymes determines whether these metabolites are converted into tumor-promoting or tumor-suppressive sterol derivatives. This concept provides a mechanistic basis for the idea that inhibition of cholesterol biosynthesis can redirect sterol metabolic flux toward pathways with distinct biological consequences.

In addition to the 5,6-ECs pathway, several other sterol-derived metabolites have been reported to regulate tumor biology, including enzymatically generated oxysterols, meiosis-activating sterols, and methylsterol intermediates identified in genetic models of cholesterol biosynthesis defects.

6. Tamoxifen, AEBS Ligands, and cholesterol epoxide metabolism

The relationship between antiestrogen pharmacology and sterol metabolism represents one of the most unexpected intersections between cancer therapeutics and lipid biochemistry. Long before the molecular identity of the microsomal antiestrogen binding site (AEBS) was elucidated, several antiestrogenic compounds were known to interact with a high-affinity binding site distinct from the classical estrogen receptor [63–66]. This binding activity, detected in microsomal fractions

of various tissues, was termed the microsomal antiestrogen binding site (AEBS) and was found to bind structurally diverse antiestrogenic molecules including tamoxifen and related diphenylmethane derivatives [67,68].

Subsequent biochemical studies demonstrated that the AEBS corresponds to ChEH, an enzymatic activity resulting from the association of two proteins of the cholesterol biosynthetic pathway, emopamil-binding protein (EBP) and 7-dehydrocholesterol reductase (DHCR7) [12,32,59]. This discovery established a direct molecular link between antiestrogen pharmacology, cholesterol biosynthesis, and sterol oxidation pathways.

Ligands of AEBS, including tamoxifen, tesmilifene (also known as DPPE), PBPE, and related compounds, inhibit ChEH activity and thereby prevent the hydrolysis of 5,6-ECs [32,38]. As a consequence, treatment with these compounds leads to accumulation of 5,6-ECs as well as several sterol intermediates of the post-lanosterol segment of cholesterol biosynthesis [15,33,38,69]. These metabolic perturbations illustrate how drugs originally developed as endocrine therapies can profoundly remodel sterol metabolic networks [35,36,39].

Importantly, AEBS ligands do not simply block a single enzymatic reaction but induce a broader reorganization of sterol metabolism that includes accumulation of biosynthetic precursors, changes in membrane sterol composition, and increased oxidative stress. In breast cancer cells, tamoxifen and related compounds induce the accumulation of sterol intermediates such as zymosterol, zymosterol, or 7-DHC, depending on the enzymatic step affected [12]. Importantly, cholesterol precursors, including 7-dehydrocholesterol, zymosterol, zymostenol, and lathosterol, display higher propagation rate constants for free-radical peroxidation than cholesterol itself, making them particularly susceptible to oxidative modification under conditions of increased reactive oxygen species [18,19].

Consistent with this mechanism, treatment with AEBS ligands increases lipid peroxidation and promotes the formation of oxidized sterols, including 5,6-ECs. Because the cellular cholesterol pool is not completely depleted under these conditions, the remaining cholesterol can undergo oxidation, leading to accumulation of 5,6-ECs even when sterol precursors are increased [38]. These observations support the concept that perturbation of cholesterol biosynthesis may enhance oxysterol formation indirectly through changes in redox homeostasis and membrane organization rather than through increased cholesterol synthesis.

ChEH inhibition has important consequences for downstream sterol metabolism. Under normal conditions, ChEH hydrolyzes 5,6-ECs to generate cholestane-3 β ,5 α ,6 β -triol (CT), which can then be converted into oncosterone (OCDO) in cells expressing 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) [30]. Because OCDO formation requires prior hydrolysis of the 5,6-ECs, inhibition of ChEH prevents CT production and thereby blocks oncosterone biosynthesis. Thus, AEBS ligands simultaneously increase 5,6-ECs levels while reducing OCDO formation.

At the same time, accumulation of 5,6-ECs in the presence of ChEH inhibition may favor alternative metabolic routes. In normal tissues, 5,6-EC can be converted into dendrogenin A (DDA) [62], an endogenous sterol metabolite displaying tumor-suppressive properties [70–76]. In tumor cells, however, DDA production is frequently lost. Although the mechanisms underlying this loss remain incompletely understood, potential explanations include reduced expression or activity of enzymes involved in DDA biosynthesis, alterations in histamine metabolism, or broader metabolic rewiring associated with tumor progression [62,75].

Tamoxifen illustrates the complexity of these mechanisms. In addition to its classical antiestrogenic activity, tamoxifen inhibits ChEH and

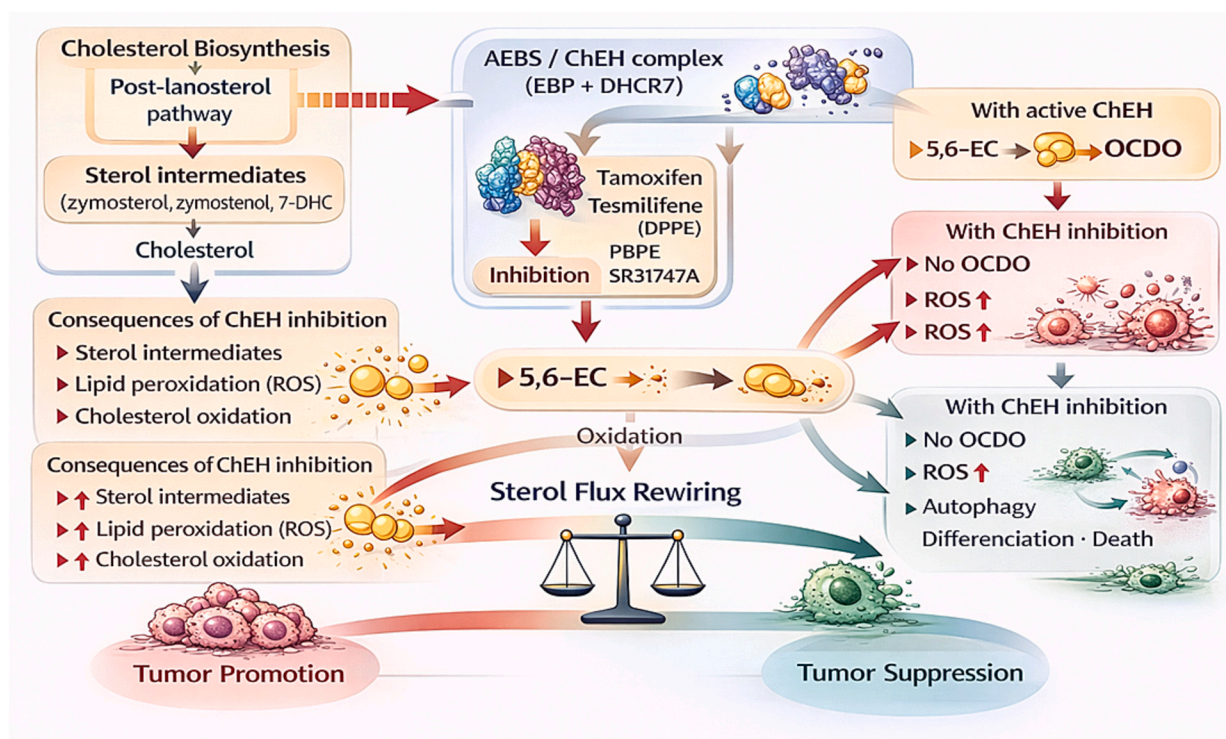


Fig. 3. AEBS / ChEH and cholesterol epoxide metabolism. Model illustrating the relationship between ligands of the microsomal antiestrogen binding site (AEBS), cholesterol-ChEH, and sterol metabolism. The AEBS corresponds to a protein complex formed by EBP and DHCR7, which catalyzes the hydrolysis of 5,6-ECs. Ligands of AEBS, including tamoxifen, tesmilifene (DPPE), PBPE, and SR31747A, inhibit ChEH activity and perturb late steps of cholesterol biosynthesis, resulting in accumulation of sterol intermediates upstream of the blocked enzymatic reactions. This accumulation is associated with increased oxidative stress, enhanced lipid peroxidation, and oxidation of cholesterol and sterol intermediates, leading to increased formation of 5,6-ECs. Because ChEH activity is required for conversion of 5,6-ECs into CT and subsequently into oncosterone (OCDO), inhibition of ChEH prevents OCDO formation while promoting alternative metabolic fates of 5,6-ECs. These changes illustrate how pharmacological inhibition of sterol metabolism induces sterol flux rewiring and alters tumor cell fate depending on the enzymatic context.

perturbs late steps of cholesterol biosynthesis, leading to sterol precursor accumulation, increased lipid peroxidation, and remodeling of sterol metabolism [77,78]. In breast cancer cells, these metabolic changes are associated with induction of autophagy, redifferentiation, and cell death, although the final biological response depends on the metabolic and redox context of the cell [15,33,38,69,73]. (Fig. 3).

These observations illustrate a general principle: pharmacological inhibition of sterol metabolism influences tumor cell behavior not only through cholesterol depletion but also through the combined effects of precursor accumulation, oxidative stress, and altered oxysterol metabolism. Within the framework of sterol flux rewiring, AEBS ligands represent a paradigm of drugs that redirect sterol metabolism toward oxidative pathways whose biological effects depend on the enzymatic context of the tumor.

7. Sterol precursor oxygenation and lipid peroxidation

Sterol intermediates generated during cholesterol biosynthesis possess structural features that increase their susceptibility to oxygenation. Unlike cholesterol, which contains a single double bond at the $\Delta 5$ position, many sterol precursors produced during late steps of the pathway display a different location of the double bond or retain additional unsaturations that facilitate radical-mediated oxidation [18,19,45,58]. This property becomes particularly relevant in tumor cells, where mitochondrial dysfunction, oncogenic signaling, and inflammatory processes frequently generate elevated levels of reactive oxygen species.

One of the most striking examples is 7-DHC, the immediate precursor of cholesterol in the Kandutsch-Russell pathway. Biochemical studies have demonstrated that the rate constant for free-radical oxidation of 7-DHC is exceptionally high, exceeding that of many polyunsaturated fatty acids [45,58]. As a consequence, accumulation of 7-DHC leads to the formation of numerous oxidized sterol derivatives with potent biological activities [79] (Fig. 4A-D).

Oxidized derivatives of 7-DHC have been implicated in the pathogenesis of Smith-Lemli-Opitz syndrome, a genetic disorder caused by mutations in *DHCR7* [80,81]. In this condition, accumulation of 7-DHC

is associated with increased lipid peroxidation, mitochondrial dysfunction, and oxidative stress, illustrating how buildup of highly oxidizable sterol intermediates can profoundly alter cellular redox homeostasis [79,82].

Other sterol intermediates generated during cholesterol biosynthesis may exhibit similar susceptibility to oxygenation. Sterols containing $\Delta 7$ or $\Delta 8$ double bonds, such as zymostenol or lathosterol, can undergo oxidative modification during lipid peroxidation reactions [18,19] (Fig. 4A-D). Experimental studies have shown that accumulation of such intermediates during pharmacological inhibition of cholesterol synthesis can increase oxidative damage to membranes and promote the formation of oxidized sterol products [18].

In tumor cells, where oxidative stress is elevated [83,84] or pharmacologically induced [38,73], accumulation of sterol intermediates may therefore create conditions favorable for the generation of oxidized sterol metabolites (Fig. 4E). These molecules may interact with nuclear receptors, membrane proteins, or redox-sensitive signaling pathways, suggesting that oxidative modification of sterol precursors could represent an additional layer of metabolic regulation linking cholesterol biosynthesis to tumor cell behavior.

Advances in lipidomics and mass spectrometry now allow the detection of numerous oxidized sterol species that were previously difficult to identify [85,86]. These technologies will be essential for determining the contribution of oxidized sterol intermediates to the biological effects observed after pharmacological or genetic inhibition of cholesterol biosynthesis.

8. SC4MOL and NSDHL: Sterol demethylation and oncogenic signaling

The removal of C4-methyl groups from lanosterol represents a critical step in the conversion of lanosterol into cholesterol and is catalyzed by a multienzyme complex involved in sterol C4-demethylation. This complex includes sterol-C4-methyl oxidase-like protein (SC4MOL) and NAD(P)-dependent steroid dehydrogenase-like protein (NSDHL), which act sequentially to convert C4-methyl sterols into demethyl intermediates that can be further processed toward cholesterol synthesis

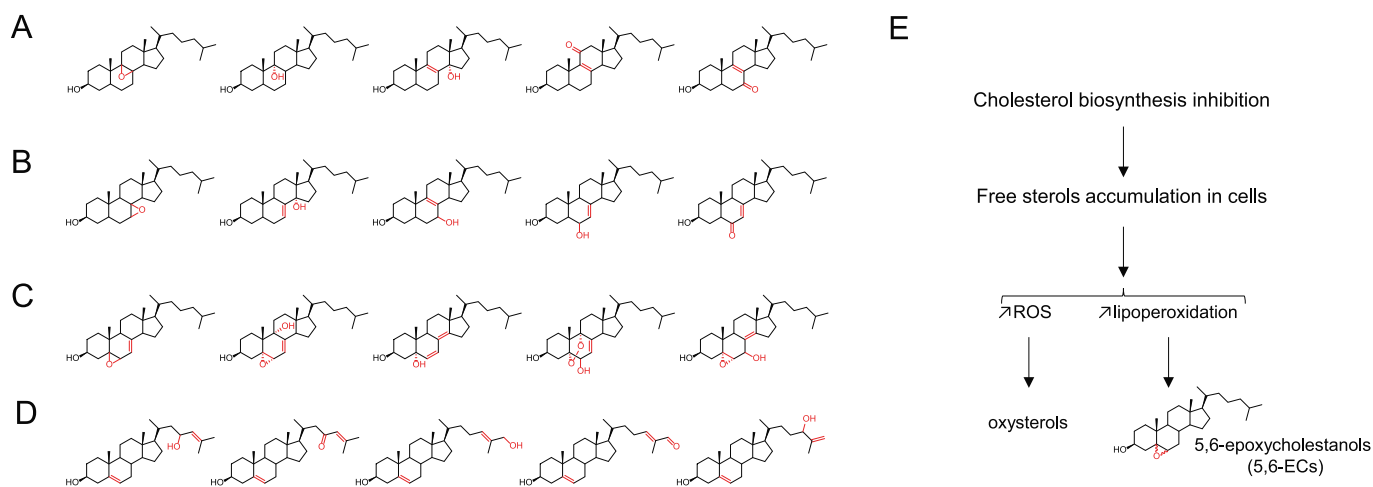


Fig. 4. Oxygenation of sterol intermediates and lipid peroxidation during inhibition of cholesterol biosynthesis. Oxidative modification of sterol intermediates generated during cholesterol biosynthesis. (A–D) Several sterol precursors produced during late steps of the pathway contain double bonds that increase their susceptibility to radical-mediated oxygenation. Examples include oxidation products derived from (A) lathosterol, (B) zymostenol, (C) 7-dehydrocholesterol (7-DHC), and (D) the unsaturated side chain of desmosterol. Because of these structural features, these intermediates can undergo oxidation during lipid peroxidation reactions, generating multiple oxysterol derivatives with potential biological activity. Among these sterols, 7-DHC is particularly prone to free-radical oxidation and readily forms numerous oxidized products that contribute to oxidative stress and membrane damage. (E) Effect of pharmacological inhibition of cholesterol biosynthesis. Blockade of cholesterogenic enzymes leads to intracellular accumulation of free sterols and sterol intermediates, which perturbs membrane organization and increases the production of reactive oxygen species (ROS). Enhanced lipid peroxidation promotes oxidation of cholesterol and sterol precursors, resulting in the formation of oxysterols, including 5,6-ECs. These oxidized sterols may act as signaling molecules capable of modulating nuclear receptors, membrane-associated pathways, and redox-sensitive signaling cascades, thereby linking sterol metabolism, oxidative stress, and tumor cell behavior.

[13].

Genetic and functional studies have demonstrated that perturbation of this demethylation step profoundly affects oncogenic signaling. In particular, inhibition or loss of SC4MOL or NSDHL alters intracellular trafficking of the epidermal growth factor receptor (EGFR) and promotes its internalization and degradation, thereby reducing downstream signaling in cancer cells [43,44]. These effects are associated with the accumulation of C4-methyl sterol intermediates, which modify membrane composition and disrupt membrane microdomains required for proper receptor recycling.

In addition to their effects on membrane organization, sterol intermediates generated upstream of the C4-demethylation step can function as signaling molecules. Gabitova and colleagues demonstrated that blockade of SC4MOL or NSDHL leads to the accumulation of specific sterol metabolites that suppress tumor growth in EGFR- and KRAS-dependent cancers [16]. These sterol intermediates were shown to activate liver X receptor (LXR)-dependent transcriptional programs, resulting in inhibition of cell proliferation and reduced tumor progression. These findings indicate that the cholesterol biosynthetic pathway regulates oncogenic signaling not only through cholesterol availability but also through the controlled production of bioactive sterol ligands.

These observations highlight that the sterol composition of cellular membranes directly influences receptor trafficking and signal transduction while sterol intermediates can regulate gene expression through nuclear receptors. Because EGFR signaling plays a central role in the proliferation of several tumor types, including melanoma, pancreatic cancer, and lung cancer, enzymes involved in sterol demethylation have emerged as potential therapeutic targets [16,87].

Thus, inhibition of SC4MOL or NSDHL does not simply reduce cholesterol synthesis but alters the balance of sterol intermediates

within the cell, leading to changes in membrane organization, nuclear receptor activation, receptor trafficking, and tumor cell proliferation. These findings identify the C4-demethylation step as a critical regulatory node linking cholesterol biosynthesis to oncogenic signaling pathways and suggest that pharmacological modulation of this step may represent a strategy to suppress tumor growth by promoting the accumulation of growth-inhibitory sterol metabolites (Fig. 5).

9. Pharmacological targeting of cholesterol Biosynthesis: Lessons from clinical failures

Attempts to target cholesterol metabolism for cancer therapy have a long history, but clinical results have often been disappointing. Retrospective analysis suggests that many failures resulted from incomplete understanding of the metabolic consequences of inhibiting cholesterol biosynthesis.

One of the earliest examples is boxidine, investigated in the early 1970 s as a potential antineoplastic agent. Clinical studies showed that boxidine markedly altered serum sterol profiles in humans [88–91]. Subsequent work showed that boxidine inhibits DHCR7, leading to accumulation of 7-DHC, a sterol highly susceptible to oxygenation [79]. The resulting oxidative stress and formation of toxic sterol derivatives likely contributed to the toxicity that limited clinical development.

Tesmilifene (DPPE, BMS-217380-01, PubChem CID: 108092) represents another instructive case. Originally developed as a chemosensitizing agent [92], tesmilifene is a selective ligand of AEBs that inhibits ChEH and perturbs late steps of cholesterol biosynthesis [32,33,67,68]. Treatment with tesmilifene induces accumulation of sterol intermediates including 7-DHC and zymosterol derivatives and promotes the formation of 5,6-ECs [33,38]. These metabolic effects were

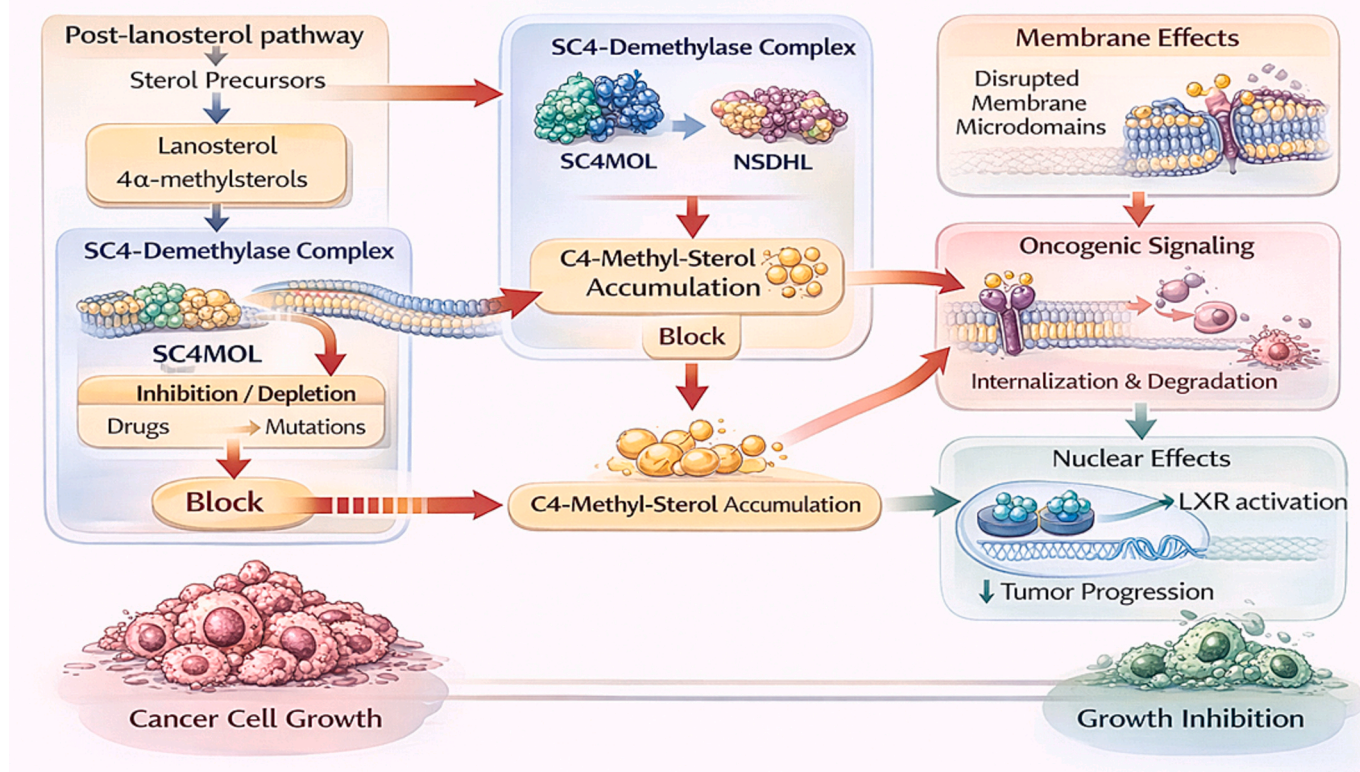


Fig. 5. Oxidative stress, sterol accumulation, and formation of oxysterols. Conceptual model showing the relationship between inhibition of cholesterol biosynthesis, intracellular sterol accumulation, and oxidative stress. Blockade of cholesterogenic enzymes leads to accumulation of free sterols and sterol intermediates in cellular membranes. Sterol accumulation perturbs membrane organization and promotes the production of reactive oxygen species (ROS), resulting in increased lipid peroxidation. Under these conditions, cholesterol and sterol intermediates become susceptible to oxygenation, leading to the formation of oxysterols, including 5,6-ECs. These oxidized sterols can participate in signaling pathways that influence tumor cell proliferation, differentiation, or cell death, linking cholesterol metabolism to redox-dependent regulation of tumor biology.

not fully appreciated during early clinical trials, which may explain the difficulty in identifying responsive patient populations.

SR31747A (PubChem CID: 6439330) provides another example of a sterol-targeting drug whose mechanism was incompletely understood during clinical development. This compound inhibits EBP, leading to zymostenol accumulation [93] and ChEH [32]. Although SR31747A showed antiproliferative activity in experimental models [93,94], clinical development was limited, possibly because the metabolic perturbations induced by the drug were not fully characterized.

These historical examples illustrate that pharmacological inhibition of cholesterol biosynthesis produces complex metabolic rearrangements characterized by accumulation of sterol intermediates and increased formation of oxidized sterols. Understanding these metabolic changes is essential for interpreting both the therapeutic and toxic effects of sterol-targeting drugs.

10. Sterol flux rewiring in Cancer: A Conceptual model

The diverse biological effects observed after inhibition of cholesterol biosynthesis suggest that this pathway should be viewed as a dynamic metabolic network rather than a simple linear route leading to cholesterol. Pharmacological or genetic inhibition of cholesterologenic enzymes redistributes metabolic flux within the sterol network, leading to accumulation and transformation of intermediates. This process can be described as sterol flux rewiring [10,11].

Inhibition of specific enzymatic steps often results in buildup of sterol intermediates upstream of the blocked reaction. These intermediates may modify membrane properties, alter receptor trafficking, modulate nuclear receptors, or undergo oxygenation reactions that generate bioactive sterols. In addition, sterol accumulation can perturb cellular redox homeostasis, increasing lipid peroxidation and promoting the formation of oxidized sterols.

Within this framework, the 5,6-ECs pathway represents a central metabolic node linking oxidative stress, sterol metabolism, and tumor signaling. Depending on the enzymatic context, 5,6-ECs may be converted into tumor-promoting metabolites such as OCDO or into tumor-suppressive sterols such as DDA. Additional downstream transformations, including conversion of 27-HC or OCDO into 27-OH-OCDO [31], further illustrate that sterol metabolism operates as a branching network rather than a linear pathway.

Upstream of cholesterol, inhibition of late steps of biosynthesis leads

to accumulation of highly oxygenation-prone intermediates, which may enhance lipid peroxidation and generate oxidized sterols with signaling properties.

Thus, the biological consequences of targeting cholesterol biosynthesis depend on multiple factors, including the nature of the accumulated intermediates, the oxidative state of the cell, and the expression of enzymes involved in sterol metabolism. The concept of sterol flux rewiring provides a unifying explanation for the diverse and sometimes paradoxical effects observed after pharmacological inhibition of cholesterologenesis (Fig. 6).

Although experimental evidence supports the existence of multiple bioactive sterol intermediates, the identity and biological functions of many of these metabolites remain incompletely characterized, and further analytical and biochemical studies will be required to validate their roles in tumor biology.

11. Outstanding questions in sterol metabolism and cancer

Despite major advances in understanding cholesterol biosynthesis and oxysterol signaling, many fundamental questions remain unresolved regarding the role of sterol metabolism in cancer biology. One of the most important issues concerns the relative contribution of cholesterol depletion versus sterol precursor accumulation in mediating the biological effects of cholesterologenic inhibitors. Although early studies assumed that inhibition of cholesterol synthesis impairs tumor growth mainly by reducing membrane cholesterol content or limiting protein prenylation, increasing evidence indicates that the accumulation of sterol intermediates generated upstream in the pathway may play an equally important role in regulating oncogenic signaling and tumor proliferation [5,16,44]. Distinguishing between these mechanisms remains a critical challenge for interpreting the effects of pharmacological interventions targeting the mevalonate and post-lanosterol pathways.

Another major unresolved question concerns the identity and biological functions of oxidized derivatives of sterol intermediates. While the oxidation products of cholesterol have been extensively studied, comparatively little is known about the oxidation products derived from intermediates such as zymosterol, lathosterol, desmosterol, or methylsterols. These intermediates are either positioning isomers of the double bond or contain additional double bonds. This increases their susceptibility to oxygenation [18]. Their accumulation may generate oxidized sterols with signaling properties distinct from those of classical

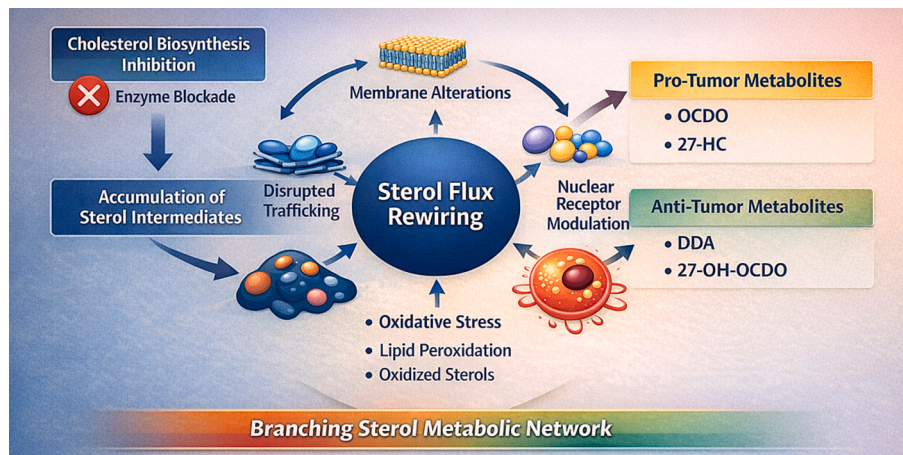


Fig. 6. Sterol flux rewiring in cancer: conceptual model. Conceptual model illustrating the concept of sterol flux rewiring following inhibition of cholesterol biosynthesis. Pharmacological or genetic blockade of specific enzymes redistributes metabolic flux within the sterol network, leading to accumulation of sterol intermediates upstream of the inhibited step. Accumulated sterols can modify membrane properties, alter receptor trafficking, and activate nuclear receptors, while increased oxidative stress promotes lipid peroxidation and formation of oxidized sterols. The 5,6-ECs pathway represents a central metabolic node linking oxidative stress to sterol signaling. Depending on the enzymatic context, 5,6-ECs may be converted into tumor-promoting metabolites such as OCDO and 27-HC, or into tumor-suppressive metabolites such as DDA and 27-OH-OCDO. These pathways illustrate that the biological effects of targeting cholesterol biosynthesis depend on the nature of accumulated intermediates, the oxidative state of the cell, and the expression of enzymes involved in sterol metabolism.

oxysterols. The development of advanced lipidomic approaches now makes it possible to detect these metabolites, but their biological functions remain largely unexplored.

The relationship between sterol metabolism and cellular redox homeostasis also remains incompletely understood. Accumulation of free cholesterol or biosynthetic precursors can perturb membrane organization, impair mitochondrial function, and promote the production of reactive oxygen species, thereby enhancing lipid peroxidation [95–97]. In tumor cells, where oxidative stress is frequently elevated, these processes may favor the formation of 5,6-ECs and other oxidized sterols generated during lipid peroxidation [45,52,84]. Because several sterol intermediates are more susceptible to oxygenation than cholesterol itself, perturbations of the cholesterol biosynthetic pathway may further enhance the production of bioactive oxysterols capable of modulating signaling pathways controlling proliferation, differentiation, or cell death [28,35,47,82,98].

Determining how oxidative stress interacts with sterol metabolism to shape tumor behavior therefore represents an important area for future investigation. Another unresolved issue concerns the regulation of downstream metabolic pathways derived from 5,6-ECs. Depending on the enzymatic context, 5,6-ECs may be converted into a tumor-promoting metabolite such as oncosterone or for 5,6 α -EC into a tumor-suppressive molecule such as DDA [34–37,72]. In addition, further oxidation of oxysterols, including conversion of 27-HC or OCDO into 27-OH-OCDO, may generate metabolites with antiproliferative properties. Understanding how these competing metabolic pathways are regulated in different tumor types will be essential for predicting the biological consequences of perturbing cholesterol biosynthesis.

Finally, the therapeutic implications of sterol flux rewiring remain largely unexplored. If the biological effects of cholesterogenic inhibitors depend on the nature of the intermediates that accumulate and on the oxidative state of the cell, then the same drug may produce very different outcomes depending on tumor type, metabolic context, and expression of sterol-metabolizing enzymes. Identifying biomarkers that reflect sterol metabolic status may therefore be required for rational development of therapies targeting cholesterol biosynthesis.

12. Future experimental strategies

Addressing these questions will require the integration of complementary experimental approaches combining lipidomics, genetics, pharmacology, redox biology, and analytical chemistry. Although modern mass spectrometry-based lipidomic techniques allow broad profiling of known sterol species, these approaches are often insufficient for the identification of novel sterol metabolites generated after pharmacological or genetic perturbation of cholesterol biosynthesis. A major analytical challenge arises from the existence of multiple stereoisomeric sterols that may co-elute during chromatographic separation. In practice, unequivocal identification often requires the synthesis of authentic standards combined with high-resolution analytical techniques, as illustrated by the characterization of metabolites such as DDA, and related sterols [31,36,62,99]. This step is essential for the unambiguous identification and quantification of sterol metabolites in biological samples. The identification of compounds such as DDA or 27-OH-OCDO illustrates this requirement, as the synthesis of these molecules was necessary before their presence in cells and tissues could be demonstrated and their biological activities characterized.

Genetic approaches provide another powerful strategy for dissecting the role of specific enzymatic steps in sterol metabolism. CRISPR-Cas9-mediated gene editing can be used to selectively inactivate enzymes involved in the post-lanosterol pathway, including SC4MOL, NSDHL, EBP, DHCR7, or DHCR24, allowing analysis of the biological consequences of accumulating defined sterol intermediates. Combining genetic perturbations with targeted analytical methods should make it possible to establish causal relationships between specific sterol metabolites and cellular phenotypes.

The development of selective pharmacological probes targeting individual enzymes of the cholesterol biosynthetic pathway will also be essential. Many currently available inhibitors affect multiple steps of sterol metabolism or produce complex metabolic changes that are difficult to interpret. Designing compounds that selectively block defined enzymatic reactions could allow controlled manipulation of sterol flux and facilitate the identification of metabolites responsible for specific biological effects. Such studies will require analytical strategies capable of distinguishing between cholesterol depletion, precursor accumulation, and formation of oxidized or otherwise transformed sterol derivatives.

Integration of sterol metabolism with redox biology represents another important direction for future research. Because several sterol intermediates are highly susceptible to oxygenation, tumors characterized by increased lipid peroxidation may generate sterol oxidation products that are difficult to predict and may exist as multiple stereoisomeric forms. Detecting these species will often require the development of dedicated analytical workflows, including synthesis of reference compounds and optimization of chromatographic separation.

Finally, studies performed in patients will be required to determine whether sterol metabolic alterations observed in experimental models are also present in human tumors. Analysis of sterol and oxysterol profiles in tumor samples may help identify metabolic signatures associated with specific cancer types or with response to therapy. However, translation to clinical material will depend on the availability of validated analytical methods capable of detecting low-abundance sterol metabolites with high structural specificity.

13. Conclusions and Perspectives

Cholesterol biosynthesis has long been regarded as an attractive therapeutic target in cancer because of its essential role in membrane biogenesis and its contribution to the production of non-sterol isoprenoid intermediates required for protein prenylation. However, the experimental and clinical observations discussed throughout this review indicate that the biological consequences of perturbing cholesterol metabolism in tumor cells cannot be explained solely by a reduction in cholesterol availability. Instead, inhibition of cholesterogenesis alters the distribution of metabolic flux within the sterol network and promotes the accumulation and transformation of multiple intermediates that may exert distinct, and sometimes opposing, biological effects. These findings support the view that the cholesterol biosynthetic pathway should be considered as a dynamic metabolic system capable of generating bioactive sterols whose signaling properties depend on the enzymatic context, the oxidative environment, and the differentiation state of the cell.

One of the central conclusions emerging from recent studies is that pharmacological or genetic inhibition of cholesterogenic enzymes frequently results in the accumulation of sterol precursors upstream of the blocked step rather than in complete depletion of cellular cholesterol. Because these intermediates differ structurally from cholesterol, their accumulation may modify membrane organization, influence receptor trafficking, or modulate transcriptional programs through interaction with nuclear receptors such as LXRs, ROR, or steroid hormone receptors. In addition, several sterol precursors contain additional double bonds that increase their susceptibility to oxygenation, thereby favoring the formation of oxidized sterols under conditions of oxidative stress. These properties provide a mechanistic basis for the concept that the biological effects of cholesterol biosynthesis inhibitors may arise not only from reduced cholesterol synthesis but also from the signaling activities of accumulated sterol intermediates and their oxidation products.

The formation of specific oxysterols represents a critical interface between sterol metabolism and cellular redox homeostasis. Tumor cells often display elevated levels of reactive oxygen species as a consequence of mitochondrial dysfunction, oncogenic signaling, and inflammatory

processes within the tumor microenvironment. Under these conditions, cholesterol and several sterol precursors are prone to oxygenation during lipid peroxidation, leading to the generation of multiple oxysterols with potent biological activities. Among these metabolites, 5,6-ECs constitute a central metabolic node because they can enter distinct enzymatic pathways that produce sterols with either tumor-promoting or tumor-suppressive properties. Hydrolysis of 5,6-EC by ChEH, an activity associated with the anti-estrogen binding site formed by EBP and DHCR7, generates CT, which can be further converted by 11 β -HSD2 into oncoesterone, a sterol metabolite that promotes proliferation and tumor growth in several experimental models. Conversely, inhibition of ChEH prevents CT formation and blocks oncoesterone biosynthesis, demonstrating that the biological outcome of cholesterol epoxide formation depends on the enzymatic context of the cell.

An alternative fate of cholesterol epoxides has been described in normal tissues, where the 5,6 α -EC can be converted into DDA through a specific enzymatic activity responsible for its conjugation with histamine. DDA displays tumor-suppressive properties, including induction of differentiation, activation of autophagy, and stimulation of antitumor immune responses, and is detected in normal tissues but is absent or strongly reduced in tumors of various origins. These observations suggest that the balance between oncoesterone production and DDA formation may represent a critical determinant of the biological effects of sterol oxidation in cancer. In addition, further metabolic transformations of oxysterols can generate derivatives with distinct activities. For example, both 27-hydroxycholesterol and oncoesterone can undergo additional hydroxylation reactions leading to the formation of 27-hydroxy-OCDO, a metabolite that displays antiproliferative properties, illustrating that sterol metabolism involves branching pathways capable of generating products with opposing effects on tumor cell growth.

The consequences of inhibiting cholesterol biosynthesis are not restricted to late oxidative steps of the pathway. Perturbation of lanosterol demethylation, for example through inhibition or genetic inactivation of enzymes such as SC4MOL or NSDHL, leads to the accumulation of methylsterols that profoundly modify membrane sterol composition and receptor trafficking. Experimental studies have shown that buildup of these intermediates promotes internalization and degradation of growth factor receptors, including EGFR, resulting in decreased PI3K-AKT signaling and reduced tumor cell proliferation. These findings indicate that post-lanosterol enzymes may represent potential therapeutic targets, although pharmacological validation of these targets remains incomplete and requires further investigation. Importantly, the accumulation of sterol intermediates in these conditions may also enhance their susceptibility to oxygenation, suggesting that redox-dependent mechanisms could contribute to the biological effects of demethylation defects.

Together, these observations support the concept of sterol flux rewiring, according to which inhibition of specific enzymatic steps in cholesterol biosynthesis redistributes metabolic flux toward the accumulation, oxidation, and transformation of sterol intermediates. In this model, the final biological outcome depends on multiple factors, including the identity of the inhibited enzyme, the nature of the sterol species that accumulate, the level of oxidative stress, and the expression of enzymes involved in sterol oxidation, reduction, and conjugation. As a consequence, inhibition of cholesterol biosynthesis may lead to tumor suppression in some contexts, while promoting tumor growth in others, depending on how sterol metabolism is redirected.

An important limitation in the current field is that the identification of sterol metabolites generated after perturbation of cholesterol biosynthesis remains technically challenging. Many sterol derivatives differ only by subtle structural modifications or exist as multiple stereoisomers, making their detection difficult without authentic reference compounds. In practice, the characterization of new sterol metabolites often requires the formulation of structural hypotheses followed by chemical synthesis of candidate molecules, which can then be used to establish targeted analytical methods. The identification of compounds

such as DDA or 27-OH-OCDO illustrates this requirement, as synthesis of these molecules was necessary before their presence in biological samples could be demonstrated and their biological activities investigated. Progress in the field will therefore depend not only on advances in lipidomics, but also on the development of analytical strategies combining organic synthesis, stereochemical characterization, and high-resolution mass spectrometry.

These considerations may help explain why several pharmacological agents targeting sterol metabolism have produced variable or unexpected results in clinical studies. Compounds affecting late steps of cholesterol biosynthesis, AEBS/ChEH activity, or sterol demethylation can induce complex metabolic perturbations that were not fully understood at the time of their development. In some cases, accumulation of specific sterol intermediates or oxysterols may contribute to therapeutic effects, whereas in other situations the same metabolic changes could favor tumor-promoting pathways or produce unwanted toxicity. A better characterization of sterol metabolic profiles in tumor cells, combined with identification of the enzymatic activities expressed in each tumor type, will therefore be essential for the rational design of therapies targeting cholesterol biosynthesis.

This suggests that future pharmacological strategies targeting cholesterol biosynthesis should not only inhibit cholesterol production, but also control the accumulation of sterol intermediates, as these metabolites may influence therapeutic efficacy and toxicity.

In conclusion, the cholesterol biosynthetic pathway should no longer be viewed as a simple linear route leading to cholesterol, but rather as a complex metabolic network capable of generating a wide spectrum of bioactive sterols. Perturbation of this network can reprogram sterol metabolism and redirect metabolic flux toward pathways that either promote or suppress tumor growth. Understanding how inhibition of cholesterol biosynthesis rewires sterol metabolic flux may provide the basis for the development of new pharmacological strategies aimed at selectively directing sterol metabolism toward tumor-suppressive pathways while avoiding the accumulation of metabolites that promote tumor progression.

Funding sources

This work was funded by an internal grant from the “Institut National de la Santé et de la Recherche Médicale”, the “Centre National de la Recherche Scientifique”, the “Université de Toulouse”, the Institut National du Cancer (PLBIO2020-028 and PLBIO2023-050), the Agence Nationale de la Recherche (DASYNT2, ANR-20-CE11-0005, DASYNT3, ANR-24-CE44-4332), the World Cancer Research Foundation (IIG-FULL_2024_015). We thank the charity associations “ELLES”, “J’y V”, Orange Care and URAFIKI JUU for their generous support.

CRedit authorship contribution statement

Philippe de Médina: Writing – review & editing, Writing – original draft. **Julio Buñay:** Writing – review & editing, Writing – original draft. **Silia Ayadi:** Writing – review & editing, Writing – original draft. **Sandrine Silvente-Poirot:** Writing – review & editing, Writing – original draft, Conceptualization. **Marc Poirot:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The team is a member of the French Network for Nutrition And

Cancer Research (NACRe network) and of the European Network for Oxysterol Research (ENOR).

References

- [1] P.J. Mullen, R. Yu, J. Longo, M.C. Archer, L.Z. Penn, The interplay between cell signalling and the mevalonate pathway in cancer, *Nat. Rev. Cancer* 16 (11) (2016) 718–731.
- [2] J.D. Horton, J.L. Goldstein, M.S. Brown, SREBPs: transcriptional mediators of lipid homeostasis, *Cold Spring Harb. Symp. Quant. Biol.* 67 (2002) 491–498.
- [3] J.L. Goldstein, M.S. Brown, Regulation of the mevalonate pathway, *Nature* 343 (6257) (1990) 425–430.
- [4] W.A. Freed-Pastor, H. Mizuno, X. Zhao, A. Langerod, S.H. Moon, R. Rodriguez-Barrueco, A. Barsotti, A. Chicas, W. Li, A. Polotskaia, M.J. Bissell, T.F. Osborne, B. Tian, S.W. Lowe, J.M. Silva, A.L. Borresen-Dale, A.J. Levine, J. Bargonetti, C. Prives, Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway, *Cell* 148 (1–2) (2012) 244–258.
- [5] J.W. Clendening, L.Z. Penn, Targeting tumor cell metabolism with statins, *Oncogene* 31 (48) (2012) 4967–4978.
- [6] S.M. Sebti, A.D. Hamilton, Farnesyltransferase and geranylgeranyltransferase I inhibitors and cancer therapy: lessons from mechanism and bench-to-bedside translational studies, *Oncogene* 19 (56) (2000) 6584–6593.
- [7] P.J. Casey, M.C. Seabra, Protein prenyltransferases, *J. Biol. Chem.* 271 (10) (1996) 5289–5292.
- [8] L. Ma, W. Cho, E.R. Nelson, Our evolving understanding of how 27-hydroxycholesterol influences cancer, *Biochem. Pharmacol.* 196 (2022) 114621.
- [9] E.R. Nelson, S.E. Wardell, J.S. Jasper, S. Park, S. Suchindran, M.K. Howe, N. J. Carver, R.V. Pillai, P.M. Sullivan, V. Sondhi, M. Umetani, J. Geradts, D. P. McDonnell, 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology, *Science* 342 (6162) (2013) 1094–1098.
- [10] O.F. Kuzu, M.A. Noory, G.P. Robertson, The role of cholesterol in cancer, *Cancer Res.* 76 (8) (2016) 2063–2070.
- [11] S. Silvente-Poirot, M. Poirot, Cancer, cholesterol and cancer, in the balance, *Science* 343 (6178) (2014) 1445–1446.
- [12] S. Silvente-Poirot, M. Poirot, Cholesterol epoxide hydrolase and cancer, *Curr. Opin. Pharmacol.* 12 (6) (2012) 696–703.
- [13] W.D. Nes, Biosynthesis of cholesterol and other sterols, *Chem. Rev.* 111 (10) (2011) 6423–6451.
- [14] K. Bloch, The biological synthesis of cholesterol, *Science* 150 (3692) (1965) 19–28.
- [15] B. Payre, P. de Medina, N. Boubekeur, L. Mhamdi, J. Bertrand-Michel, F. Terce, I. Fourquaux, D. Goudouneche, M. Record, M. Poirot, S. Silvente-Poirot, Microsomal antiestrogen-binding site ligands induce growth control and differentiation of human breast cancer cells through the modulation of cholesterol metabolism, *Mol. Cancer Ther.* 7 (12) (2008) 3707–3718.
- [16] L. Gabitova, D. Restifo, A. Gorin, K. Manocha, E. Handorf, D.H. Yang, K.Q. Cai, A. J. Klein-Szanto, D. Cunningham, L.E. Kratz, G.E. Herman, E.A. Golemis, I. Astsaturov, Endogenous sterol metabolites regulate growth of EGFR/KRAS-dependent tumors via LXR, *Cell Rep.* 12 (11) (2015) 1927–1938.
- [17] N.J. Spann, L.X. Garmire, J.G. McDonald, D.S. Myers, S.B. Milne, N. Shibata, D. Reichart, J.N. Fox, I. Shaked, D. Heudobler, C.R. Raetz, E.W. Wang, S.L. Kelly, M.C. Sullards, R.C. Murphy, A.H. Merrill Jr., H.A. Brown, E.A. Dennis, A.C. Li, K. Ley, S. Tsimikas, E. Fahy, S. Subramaniam, O. Quehenberger, D.W. Russell, C. K. Glass, Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses, *Cell* 151 (1) (2012) 138–152.
- [18] C.R. Lamberson, H. Muchalski, K.B. McDuffee, K.A. Tallman, L. Xu, N.A. Porter, Propagation rate constants for the peroxidation of sterols on the biosynthetic pathway to cholesterol, *Chem. Phys. Lipids* 207 (Pt B) (2017) 51–58.
- [19] L. Xu, N.A. Porter, Free radical oxidation of cholesterol and its precursors: Implications in cholesterol biosynthesis disorders, *Free Radic. Res.* 49 (7) (2015) 835–849.
- [20] M. Poirot, J. Buñay, S. Ayadi, S. Silvente-Poirot, P. de Medina, Liver X receptors and the hallmarks of aging: from molecular mechanisms to therapeutic opportunities, *Ageing Res. Rev.* 114 (2026) 102967.
- [21] R.M. Evans, D.J. Mangelsdorf, Nuclear receptors, RXR, and the big bang, *Cell* 157 (1) (2014) 255–266.
- [22] B.A. Janowski, M.J. Grogan, S.A. Jones, G.B. Wisely, S.A. Kliewer, E.J. Corey, D. J. Mangelsdorf, Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRBeta, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1) (1999) 266–271.
- [23] B.A. Janowski, P.J. Willy, T.R. Devi, J.R. Falck, D.J. Mangelsdorf, An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha, *Nature* 383 (6602) (1996) 728–731.
- [24] F.R. Santori, P. Huang, S.A. van de Pavert, E.F. Douglass Jr., D.J. Leaver, B. A. Haubrich, R. Keber, G. Lorbeck, T. Konijn, B.N. Rosales, D. Rozman, S. Horvat, A. Rahier, R.E. Mebius, F. Rastinejad, W.D. Nes, D.R. Littman, Identification of natural RORgamma ligands that regulate the development of lymphoid cells, *Cell Metab.* 21 (2) (2015) 286–298.
- [25] X. Hu, Y. Wang, L.Y. Hao, X. Liu, C.A. Lesch, B.M. Sanchez, J.M. Wendling, R. W. Morgan, T.D. Aicher, L.L. Carter, P.L. Toogood, G.D. Glick, Sterol metabolism controls T(H)17 differentiation by generating endogenous RORgamma agonists, *Nat. Chem. Biol.* 11 (2) (2015) 141–147.
- [26] P. Soroosh, J. Wu, X. Xue, J. Song, S.W. Sutton, M. Sablad, J. Yu, M.I. Nelen, X. Liu, G. Castro, R. Luna, S. Crawford, H. Banie, R.A. Dandridge, X. Deng, A. Bittner, C. Kuei, M. Tootoonchi, N. Rozenkrants, K. Herman, J. Gao, X.V. Yang, K. Sachen, K. Ngo, W.P. Fung-Leung, S. Nguyen, A. de Leon-Tabaldo, J. Blevitt, Y. Zhang, M. D. Cummings, T. Rao, N.S. Mani, C. Liu, M. McKinnon, M.E. Milla, A.M. Fourie, S. Sun, Oxysterols are agonist ligands of RORgamma and drive Th17 cell differentiation, *Proc. Natl. Acad. Sci. U. S. A.* 111 (33) (2014) 12163–12168.
- [27] O. Guillemot-Legris, G.G. Muccioli, The oxysterome and its receptors as pharmacological targets in inflammatory diseases, *Br. J. Pharmacol.* 179 (21) (2022) 4917–4940.
- [28] P. de Medina, S. Silvente-Poirot, M. Poirot, Oxysterols are potential physiological regulators of ageing, *Ageing Res. Rev.* 77 (2022) 101615.
- [29] V. Mutemberezi, O. Guillemot-Legris, G.G. Muccioli, Oxysterols: from cholesterol metabolites to key mediators, *Prog. Lipid Res.* 64 (2016) 152–169.
- [30] M. Voisin, P. de Medina, A. Mallinger, F. Dalenc, E. Huc-Claustre, J. Leignadier, N. Serhan, R. Soules, G. Segala, A. Mougel, E. Noguer, L. Mhamdi, E. Bacquie, L. Iuliano, C. Zerbinati, M. Lacroix-Triki, L. Chaltiel, T. Filleron, V. Cavaillès, T. Al Saati, P. Rochaix, R. Duprez-Paumier, C. Franchet, L. Ligat, F. Lopez, M. Record, M. Poirot, S. Silvente-Poirot, Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor, *Proc. Natl. Acad. Sci. U. S. A.* 114 (44) (2017) E9346–E9355.
- [31] S. Ayadi, S. Friedrichs, R. Soules, L. Pucheu, D. Lutjohann, S. Silvente-Poirot, M. Poirot, P. de Medina, 27-Hydroxylation of oncosterone by CYP27A1 switches its activity from pro-tumor to anti-tumor, *J. Lipid Res.* 64 (12) (2023) 100479.
- [32] P. de Medina, M.R. Paillasse, G. Segala, M. Poirot, S. Silvente-Poirot, Identification and pharmacological characterization of cholesterol-5,6-epoxide hydrolase as a target for tamoxifen and AEBs ligands, *Proc. Natl. Acad. Sci. U. S. A.* 107 (30) (2010) 13520–13525.
- [33] B. Kedjouar, P. de Medina, M. Oulad-Abdelghani, B. Payre, S. Silvente-Poirot, G. Favre, J.C. Faye, M. Poirot, Molecular characterization of the microsomal tamoxifen binding site, *J. Biol. Chem.* 279 (32) (2004) 34048–34061.
- [34] M. Poirot, R. Soules, A. Mallinger, F. Dalenc, S. Silvente-Poirot, Chemistry, biochemistry, metabolic fate and mechanism of action of 6-oxo-cholestan-3beta,5alpha-diol (OCDO), a tumor promoter and cholesterol metabolite, *Biochimie* 153 (2018) 139–149.
- [35] J. Buñay, S. Ayadi, C. Gressein, P. de Medina, S. Silvente-Poirot, M. Poirot, Oxysterols at the crossroads of cholesterol metabolism and cancer, *Semin. Cancer Biol.* 121 (2026) 22–32.
- [36] J. Buñay, S. Ayadi, C. Gressein, M. Boudani, M. Commerçon, P. Le, L. Pucheu, M. Record, P. de Medina, S. Silvente-Poirot, M. Poirot, 5,6-Epoxycholestanols Metabolism and Functions: defining the Epoxycholestanoid(EChA) Family, preprintAuthorea (2026), <https://doi.org/10.22541/au.176824984.47866037/v1>.
- [37] P. de Medina, K. Diallo, E. Huc-Claustre, M. Attia, R. Soules, S. Silvente-Poirot, M. Poirot, The 5,6-epoxycholesterol metabolic pathway in breast cancer: Emergence of new pharmacological targets, *Br. J. Pharmacol.* 178 (16) (2021) 3248–3260.
- [38] G. Segala, P. de Medina, L. Iuliano, C. Zerbinati, M.R. Paillasse, E. Noguer, F. Dalenc, B. Payre, V.C. Jordan, M. Record, S. Silvente-Poirot, M. Poirot, 5,6-Epoxy-cholesterols contribute to the anticancer pharmacology of tamoxifen in breast cancer cells, *Biochem. Pharmacol.* 86 (1) (2013) 175–189.
- [39] M. Poirot, S. Silvente-Poirot, Cholesterol-5,6-epoxides: chemistry, biochemistry, metabolic fate and cancer, *Biochimie* 95 (3) (2013) 622–631.
- [40] F.D. Porter, G.E. Herman, Malformation syndromes caused by disorders of cholesterol synthesis, *J. Lipid Res.* 52 (1) (2011) 6–34.
- [41] E.D. Muse, S. Yu, C.R. Edillor, J. Tao, N.J. Spann, T.G. Troutman, J.S. Seidman, A. Henke, J.T. Roland, K.A. Ozeki, B.M. Thompson, J.G. McDonald, J. Bahadorani, S. Tsimikas, T.R. Grossman, M.S. Tremblay, C.K. Glass, Cell-specific discrimination of desmosterol and desmosterol mimetics confers selective regulation of LXR and SREBP in macrophages, *Proc. Natl. Acad. Sci. U. S. A.* 115 (20) (2018) E4680–E4689.
- [42] E.J. Zerenturk, L.J. Sharpe, E. Ikonen, A.J. Brown, Desmosterol and DHCR24: unexpected new directions for a terminal step in cholesterol synthesis, *Prog. Lipid Res.* 52 (4) (2013) 666–680.
- [43] A. Sukhanova, A. Gorin, I.G. Serebriiskii, L. Gabitova, H. Zheng, D. Restifo, B. L. Egleston, D. Cunningham, T. Bagnyukova, H. Liu, A. Nikonova, G.P. Adams, Y. Zhou, D.H. Yang, R. Mehra, B. Burntess, K.Q. Cai, A. Klein-Szanto, L.E. Kratz, R. I. Kelley, L.M. Weiner, G.E. Herman, E.A. Golemis, I. Astsaturov, Targeting C4-demethylating genes in the cholesterol pathway sensitizes cancer cells to EGF receptor inhibitors via increased EGF receptor degradation, *Cancer Discov.* 3 (1) (2013) 96–111.
- [44] I. Astsaturov, V. Ratushny, A. Sukhanova, M.B. Einarson, T. Bagnyukova, Y. Zhou, K. Devarajan, J.S. Silverman, N. Tikhmyanova, N. Skobeleva, A. Pecherskaya, R. E. Nasto, C. Sharma, S.A. Jablonski, I.G. Serebriiskii, L.M. Weiner, E.A. Golemis, Synthetic lethal screen of an EGFR-centered network to improve targeted therapies, *Sci. Signal.* 3 (140) (2010) ra67.
- [45] H. Yin, L. Xu, N.A. Porter, Free radical lipid peroxidation: mechanisms and analysis, *Chem. Rev.* 111 (10) (2011) 5944–5972.
- [46] A.A. Hajjeh, W.J. Griffiths, Y. Wang, A.J. Finch, V.B. O'Donnell, The biosynthesis of enzymatically oxidized lipids, *Front Endocrinol (lausanne)* 11 (2020) 591819.
- [47] W.J. Griffiths, Y. Wang, Oxysterols as lipid mediators: their biosynthetic genes, enzymes and metabolites, *Prostaglandins Other Lipid Mediat.* 147 (2020) 106381.
- [48] D.W. Russell, Oxysterol biosynthetic enzymes, *Biochim. Biophys. Acta* 1529 (1–3) (2000) 126–135.
- [49] N.A. Porter, A perspective on free radical autoxidation: the physical organic chemistry of polyunsaturated fatty acid and sterol peroxidation, *J. Org. Chem.* 78 (8) (2013) 3511–3524.
- [50] L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, *Chem. Phys. Lipids* 164 (6) (2011) 457–468.
- [51] G.J. Schroepfer Jr., Oxysterols: modulators of cholesterol metabolism and other processes, *Physiol. Rev.* 80 (1) (2000) 661–554.

- [52] L.L. Smith, Review of progress in sterol oxidations: 1987-1995, *Lipids* 31 (5) (1996) 453–487.
- [53] L.L. Smith, *Cholesterol Autoxidation*, Springer, 1981.
- [54] K.R. Beck, S.G. Inderbinen, S. Kanagaratnam, D.V. Kratschmar, A.M. Jetten, H. Yamaguchi, A. Odermatt, 11beta-Hydroxysteroid dehydrogenases control access of 7beta,27-dihydroxycholesterol to retinoid-related orphan receptor gamma, *J. Lipid Res.* 60 (9) (2019) 1535–1546.
- [55] N. Krawczynska, Y. Wang, K. Lim, A. Das Gupta, R. Molino, A. Lenczowski, M. Abughazaleh, S.V. Bendre, Y. Fei, H. Kim, L.I. Kockaya, C.P. Schane, D. Pradeep, D. Rodriguez-Casiano, A.G. Hernandez, J. Drnevich, J. Chan, L.W. Dobrucki, M. D. Boppert, S.M. Cologna, J. Ostrander, E.R. Nelson, Neutrophils exposed to a cholesterol metabolite secrete extracellular vesicles that promote epithelial-mesenchymal transition and stemness in breast cancer cells, *Cancer Lett.* 636 (2026) 218105.
- [56] Z.A.M. Zielinski, D.A. Pratt, H-Atom abstraction vs addition: accounting for the diverse product distribution in the autooxidation of cholesterol and its esters, *J. Am. Chem. Soc.* 141 (7) (2019) 3037–3051.
- [57] Z.A. Zielinski, D.A. Pratt, Cholesterol autoxidation revisited: debunking the dogma associated with the most vilified of lipids, *J. Am. Chem. Soc.* 138 (22) (2016) 6932–6935.
- [58] D.A. Pratt, K.A. Tallman, N.A. Porter, Free radical oxidation of polyunsaturated lipids: New mechanistic insights and the development of peroxy radical clocks, *Acc. Chem. Res.* 44 (6) (2011) 458–467.
- [59] P. de Medina, S. Ayadi, K. Diallo, J. Bunay, L. Pucheu, R. Soules, M. Record, S. Brillouet, L. Vija, F. Courbon, S. Silvente-Poirot, M. Poirot, The cholesterol-5,6-epoxide hydrolase: a metabolic checkpoint in several diseases, *Adv. Exp. Med. Biol.* 1440 (2024) 149–161.
- [60] F. Mazzacava, P. Mills, K. Mills, S. Camuzeaux, P. Gissen, E.R. Nicoli, C. Wassif, D. Te Vruchte, F.D. Porter, M. Maekawa, N. Mano, T. Iida, F. Platt, P.T. Clayton, Identification of novel bile acids as biomarkers for the early diagnosis of Niemann-pick C disease, *FEBS Lett.* 590 (11) (2016) 1651–1662.
- [61] X. Jiang, R. Sidhu, L. Mydock-McGrane, F.F. Hsu, D.F. Covey, D.E. Scherrer, B. Earley, S.E. Gale, N.Y. Farhat, F.D. Porter, D.J. Dietzen, J.J. Orsini, E. Berry-Kravis, X. Zhang, J. Reunert, T. Marquardt, H. Runz, R. Giuliani, J.E. Schaffer, D. S. Ory, Development of a bile acid-based newborn screen for Niemann-pick disease type C, *Sci. Transl. Med.* 8 (337) (2016) 337ra63.
- [62] P. de Medina, M.R. Paillasse, G. Segala, M. Voisin, L. Mhamdi, F. Dalenc, M. Lacroix-Triki, T. Filleron, F. Pont, T.A. Saati, C. Morisseau, B.D. Hammock, S. Silvente-Poirot, M. Poirot, Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties, *Nat. Commun.* 4 (2013) 1840.
- [63] C.K. Watts, R.L. Sutherland, Studies on the ligand specificity and potential identity of microsomal antiestrogen-binding sites, *Mol. Pharmacol.* 31 (5) (1987) 541–551.
- [64] J.C. Faye, S. Jozan, G. Redeuilh, E.E. Baulieu, F. Bayard, Physicochemical and genetic evidence for specific antiestrogen binding sites, *Proc. Natl. Acad. Sci. U. S. A.* 80 (11) (1983) 3158–3162.
- [65] R.L. Sutherland, L.C. Murphy, M. San Foo, M.D. Green, A.M. Whybourne, Z. S. Krozowski, High-affinity anti-oestrogen binding site distinct from the oestrogen receptor, *Nature* 288 (5788) (1980) 273–275.
- [66] J.C. Faye, B. Lasserre, F. Bayard, Antiestrogen specific, high affinity saturable binding sites in rat uterine cytosol, *Biochem. Biophys. Res. Commun.* 93 (4) (1980) 1225–1231.
- [67] P. de Medina, G. Favre, M. Poirot, Multiple targeting by the antitumor drug tamoxifen: a structure-activity study, *Curr. Med. Chem. Anticancer Agents* 4 (6) (2004) 491–508.
- [68] M. Poirot, P. De Medina, F. Delarue, J.J. Perie, A. Klæbe, J.C. Faye, Synthesis, binding and structure-affinity studies of new ligands for the microsomal antiestrogen binding site (AEBS), *Bioorg. Med. Chem.* 8 (8) (2000) 2007–2016.
- [69] P. de Medina, B. Payre, N. Boubekeur, J. Bertrand-Michel, F. Terce, S. Silvente-Poirot, M. Poirot, Ligands of the antiestrogen-binding site induce active cell death and autophagy in human breast cancer cells through the modulation of cholesterol metabolism, *Cell Death Differ.* 16 (10) (2009) 1372–1384.
- [70] J. Buñay, M. Record, P. de Medina, S. Ayadi, L. Pucheu, C. Colacios, B. Segui, M. Horing, G. Liebisch, H. Martin, M. Poirot, S. Silvente-Poirot, The Phospholipid bis(monoacylglycerol)phosphate confers antitumour immunogenicity to exosomes secreted by dendrogenin A, which activates its biosynthesis in tumour cells, *J. Extracell. Vesicles* 15 (2) (2026) e70225.
- [71] M. Record, M. Attia, K. Carayon, L. Pucheu, J. Bunay, R. Soules, S. Ayadi, B. Payre, L. Perrin-Cocon, F. Bourgaill, A. Lamaziere, V. Lotteau, M. Poirot, S. Silvente-Poirot, P. de Medina, Targeting the liver X receptor with dendrogenin A differentiates tumour cells to secrete immunogenic exosome-enriched vesicles, *J. Extracell. Vesicles* 11 (4) (2022) e12211.
- [72] M. Poirot, S. Silvente-Poirot, The tumor-suppressor cholesterol metabolite, dendrogenin A, is a new class of LXR modulator activating lethal autophagy in cancers, *Biochem. Pharmacol.* 153 (2018) 75–81.
- [73] G. Segala, M. David, P. de Medina, M.C. Poirot, N. Serhan, F. Vergez, A. Mougel, E. Saland, K. Carayon, J. Leignadier, N. Caron, M. Voisin, J. Chierier, L. Ligat, F. Lopez, E. Noguier, A. Rives, B. Payre, T.A. Saati, A. Lamaziere, G. Despres, J. M. Lobaccaro, S. Baron, C. Demur, F. de Toni, C. Larrue, H. Boutzen, F. Thomas, J. E. Sarry, M. Tosolini, D. Picard, M. Record, C. Recher, M. Poirot, S. Silvente-Poirot, Dendrogenin A drives LXR to trigger lethal autophagy in cancers, *Nat. Commun.* 8 (1) (2017) 1903.
- [74] S. Silvente-Poirot, P. de Medina, M. Record, M. Poirot, From tamoxifen to dendrogenin A: the discovery of a mammalian tumor suppressor and cholesterol metabolite, *Biochimie* 130 (2016) 109–114.
- [75] M. Poirot, S. Silvente-Poirot, When cholesterol meets histamine, it gives rise to dendrogenin A: a tumour suppressor metabolite, *Biochem. Soc. Trans.* 44 (2) (2016) 631–637.
- [76] F. Dalenc, M. Poirot, S. Silvente-Poirot, Dendrogenin A: a mammalian metabolite of cholesterol with tumor suppressor and neurostimulating properties, *Curr. Med. Chem.* 22 (30) (2015) 3533–3549.
- [77] F. Khallouki, L. Hajji, S. Saber, T. Bouddine, M. Edderkaoui, M. Bourhia, N. Mir, A. Lim, A. El Midaoui, J.P. Giesy, M.A.M. Aboul-Soud, S. Silvente-Poirot, M. Poirot, An Update on tamoxifen and the chemo-preventive potential of vitamin E in breast cancer management, *J. Pers. Med.* 13 (5) (2023).
- [78] J. Leignadier, F. Dalenc, M. Poirot, S. Silvente-Poirot, Improving the efficacy of hormone therapy in breast cancer: the role of cholesterol metabolism in SERM-mediated autophagy, cell differentiation and death, *Biochem. Pharmacol.* 144 (2017) 18–28.
- [79] L. Xu, Z. Korade, N.A. Porter, Oxysterols from free radical chain oxidation of 7-dehydrocholesterol: product and mechanistic studies, *J. Am. Chem. Soc.* 132 (7) (2010) 2222–2232.
- [80] H.R. Waterham, F.A. Wijburg, R.C. Hennekam, P. Vreken, B.T. Poll-The, L. Dorland, M. Duran, P.E. Jira, J.A. Smeitink, R.A. Wevers, R.J. Wanders, Smith-Lemli-Opitz syndrome is caused by mutations in the 7-dehydrocholesterol reductase gene, *Am. J. Hum. Genet.* 63 (2) (1998) 329–338.
- [81] B.U. Fitzky, M. Witsch-Baumgartner, M. Erdel, J.N. Lee, Y.K. Paik, H. Glossmann, G. Utermann, F.F. Moebius, Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome, *Proc. Natl. Acad. Sci. U. S. A.* 95 (14) (1998) 8181–8186.
- [82] Z. Korade, L. Xu, R. Shelton, N.A. Porter, Biological activities of 7-dehydrocholesterol-derived oxysterols: implications for Smith-Lemli-Opitz syndrome, *J. Lipid Res.* 51 (11) (2010) 3259–3269.
- [83] A. Schulze, A.L. Harris, How cancer metabolism is tuned for proliferation and vulnerable to disruption, *Nature* 491 (7424) (2012) 364–373.
- [84] D. Trachootham, J. Alexandre, P. Huang, Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.* 8 (7) (2009) 579–591.
- [85] W.J. Griffiths, E. Yutuc, Y. Wang, Mass spectrometry imaging of cholesterol and oxysterols, *Adv. Exp. Med. Biol.* 1440 (2024) 73–87.
- [86] W.J. Griffiths, Y. Wang, Sterolomics in biology, biochemistry, medicine, *Trends Anal. Chem.* 120 (2019) 115280.
- [87] L. Gabitova-Cornell, A. Surumbayeva, S. Peri, J. Franco-Barraza, D. Restifo, N. Weitz, C. Ogier, A.R. Goldman, T.R. Hartman, R. Francescone, Y. Tan, E. Nicolas, N. Shah, E.A. Handorf, K.Q. Cai, A.M. O'Reilly, I. Sloma, R. Chiavarelli, R.A. Moffitt, V. Khazak, C.Y. Fang, E.A. Golemis, E. Cukierman, I. Astsaturov, Cholesterol pathway inhibition induces TGF-beta signaling to promote basal differentiation in pancreatic cancer, *Cancer Cell* 38 (4) (2020) 567–583 e11.
- [88] S. Gailani, J.F. Holland, A. Glick, Effects of oxidized on human serum sterols and neoplasms, *Clin. Pharmacol. Ther.* 13 (1) (1972) 91–96.
- [89] S. Gordon, W.P. Cekleniak, 1-(2-[4-(trifluoromethyl)-4-biphenyloxy]ethyl)pyrrolidine, A potent hypocholesterolemic agent, *J. Med. Chem.* 11 (5) (1968) 993–996.
- [90] F.L. Bach, J.C. Barclay, F. Kende, E. Cohen, Nonsteroidal hypocholesteremic agents. II. the synthesis and serum sterol lowering properties of 4-(2'-dialkylaminoalkoxy)-4'-substituted biphenyls and related compounds, *J. Med. Chem.* 11 (5) (1968) 987–993.
- [91] F.L. Bach, J.C. Barclay, E. Cohen, Nonsteroidal hypocholesteremic agents. I. the synthesis and serum sterol lowering properties of substituted 4-(2'-dialkylaminoethoxy)diphenylamines and related compounds, *J. Med. Chem.* 10 (5) (1967) 802–806.
- [92] L. Reyno, L. Seymour, D. Tu, S. Dent, K. Gelmon, B. Walley, A. Pluzanska, V. Gorbunova, A. Garin, J. Jassem, T. Pienkowski, J. Dancy, L. Pearce, M. MacNeil, S. Marlin, D. Lebow, M. Voi, K. Pritchard, M.a., National Cancer Institute of Canada Clinical Trials Group Study, phase III study of N,N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine (BMS-217380-01) combined with doxorubicin versus doxorubicin alone in metastatic/recurrent breast cancer: National Cancer Institute of Canada Clinical Trials Group Study MA.19, *J. Clin. Oncol.* 22 (2) (2004) 269–276.
- [93] C. Labit-Le Bouteiller, M.F. Jamme, M. David, S. Silve, C. Lanau, C. Dhers, C. Picard, A. Rahier, M. Taton, G. Loison, D. Caput, P. Ferrara, J. Lupker, Antiproliferative effects of SR31747A in animal cell lines are mediated by inhibition of cholesterol biosynthesis at the sterol isomerase step, *Eur. J. Biochem.* 256 (2) (1998) 342–349.
- [94] Y. Berthois, B. Bourrie, S. Galiegue, H. Vidal, P. Carayon, P.M. Martin, P. Casellas, SR31747A is a sigma receptor ligand exhibiting antitumour activity both in vitro and in vivo, *Br. J. Cancer* 88 (3) (2003) 438–446.
- [95] M. Mari, F. Caballero, A. Colell, A. Morales, J. Caballeria, A. Fernandez, C. Enrich, J.C. Fernandez-Checa, C. Garcia-Ruiz, Mitochondrial free cholesterol loading

- sensitizes to TNF- and Fas-mediated steatohepatitis, *Cell Metab.* 4 (3) (2006) 185–198.
- [96] B. Feng, P.M. Yao, Y. Li, C.M. Devlin, D. Zhang, H.P. Harding, M. Sweeney, J. X. Rong, G. Kuriakose, E.A. Fisher, A.R. Marks, D. Ron, I. Tabas, The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages, *Nat. Cell Biol.* 5 (9) (2003) 781–792.
- [97] I. Tabas, Cholesterol in health and disease, *J. Clin. Invest.* 110 (5) (2002) 583–590.
- [98] Y. Wang, E. Yutuc, W.J. Griffiths, Cholesterol metabolism pathways - are the intermediates more important than the products? *FEBS J.* 288 (12) (2021) 3727–3745.
- [99] R. Soules, F. Audouard-Combe, E. Huc-Claustre, P. de Medina, A. Rives, E. Chatelut, F. Dalenc, C. Franchet, S. Silvente-Poirot, M. Poirot, B. Allal, A fast UPLC-HILIC method for an accurate quantification of dendrogenin A in human tissues, *J. Steroid Biochem. Mol. Biol.* 194 (2019) 105447.