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27-Hydroxycholesterol, an endogenous Selective Estrogen Receptor Modulator

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Abstract

Estrogen receptors (ERs) mediate the actions of the steroidal estrogens, and are important for the regulation of several physiological and pathophysiological processes, including reproduction, bone physiology, cardiovascular physiology and breast cancer. The unique pharmacology of the ERs allows for certain ligands, such as tamoxifen, to elicit tissue- and context-specific responses, ligands now referred to as selective estrogen receptor modulators (SERMs). Recently, the cholesterol metabolite 27-hydroxycholesterol (27HC) has been defined as an endogenous SERM, with activities in atherosclerosis, osteoporosis, breast and prostate cancers, and neural degenerative diseases. Since 27HC concentrations closely mirror those of cholesterol, it is possible that 27HC mediates many of the biological effects of cholesterol. This paper provides an overview of ER pharmacology and summarizes the work to date implicating 27HC in various diseases. Wherever possible, we highlight clinical data in support of a role for 27HC in the diseases discussed.

Keywords

cholesterol; 27-hydroxycholesterol; estrogen receptor; estrogen; selective estrogen receptor modulator

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Contributors

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Conflict of interest

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Introduction

Originally characterized for their roles in female reproduction, it is now appreciated that estrogens and their cognate receptors (estrogen receptors; ERs) play important modulatory roles in several different physiological systems including development, the cardiovascular system, brain function, immune system, and bone [1]. Estrogens are chemically related compounds derived from androgen precursors but containing a defining aromatic and hydroxyl group at the 17 position (Fig. 1). 17β -estradiol is the main physiologic hormone, but it is speculated that estriol, estetrol and estrone may play important roles during pregnancy and post-menopause [2–4].

Interestingly, recent work has identified oxysterols such as 27-hydroxycholesterol (27HC) and 25-hydroxycholesterol as being able to bind and modulate the activity of ERs [5–7]. The differential effects of 27HC across tissues was reminiscent of findings from Tamoxifen, and thus has led to the classification of 27HC as an endogenous Selective Estrogen Receptor Modulator (SERM) [6, 8, 9].

The objective of this review is to summarize our current understanding of ER-pharmacology and highlight recent reports of 27HC as SERM in physiology and pathophysiology. Given 27HC levels are correlated with those of cholesterol [10], and the prevalence of hypercholesterolemia (39.7% of U.S. population) [11], it is important to consider the roles of this major cholesterol metabolite in the etiology of diseases.

1 Estrogen Receptors

Two mammalian estrogen receptors have been described: ER α and ER β . ER α , originally cloned from the MCF7 breast cancer cell line, is widely expressed throughout many tissues [12, 13]. ER β was originally cloned from rat prostate and has a distribution largely restricted to the ovary, lung and prostate, although there are reports of its activity in other cell types [13, 14]. Both ER α and ER β are members of the nuclear receptor superfamily. As such, they share the same general structure, containing an N-terminal domain, DNA-binding domain, hinge region, ligand-binding domain, and C-terminal F region. Two activation function domains (AF1 & AF2), which regulate the transcriptional activity of ERs, are located within the N-terminal domain and ligand-binding domain respectively. ER α and ER β share a high degree of sequence homology in the DNA-binding domain (more than 95% amino acid identity) and ligand-binding domain (~55% amino acid identity). However, the N-terminal domain of ER β is shorter than that of ER α , and the sequence homology between the two isoforms is only ~15% [15, 16].

Upon ligand binding, the ERs are thought to dissociate from a complex of heat-shock proteins and subsequently dimerize to form either homo or heterodimers. Through the two zinc fingers on each receptor, the ligand-bound ER-dimers then bind to DNA at specific estrogen response elements (EREs) located in the regulatory regions of target genes where they act as a hub for a large transcriptional complex including co-activators and co-repressors, ultimately resulting in altered gene transcription [16]. The ligand-bound ER-

dimers can also alter transcription indirectly by interacting with other transcription factors such as AP1, C/EBP β and Sp1 [17].

The above transcriptional regulating cascade upon ER activation is described as ‘genomic’ or ‘classical’ ER signaling. In addition, ERs can regulate different signal transduction cascades in a ‘non-genomic’ and ‘rapid’ manner. Such membrane-initiated cascades were studied utilizing one of the following two approaches: (1) Estradiol conjugated to form large dendromeric structures that prevents the ligand from entering the nucleus. (2) Mice harboring a mutant ER α that fails to bind DNA but should retain its non-genomic actions (non-classical ER knock-in, NERKI). Although both approaches suggest the potential importance of non-classical ER signaling, it should be noted that they do not always agree [18, 19], and this continues to be an emerging field. *In vitro* studies in support of non-classical ER signaling indicate that ERs can interact with and modulate the activities of MAPK, adaptor protein Shc, caveolins, c-Src protein kinase complex and the regulatory subunit of phosphoinositide-3 kinase (p85) [20, 21].

In addition to ER α and ER β , there is growing evidence that estrogens can bind to and activate the G-protein associated receptor GPER (also known as GPR30) [22–24]. Curiously, GPER knockout mice did not manifest a reproductive phenotype. However, GPER has been implicated in obesity, insulin resistance, cardiovascular dysfunction, and breast cancer progression [25]. As many of these studies have relied on the GPER knockout mouse model, the physiological relevance of this receptor in mediating the actions of estrogens remains controversial. Unless otherwise specified, the remainder of this review will focus primarily on the nuclear ERs.

2 Estrogen Receptor Ligands; Pharmacological Concept of Selective Estrogen Receptor Modulators

As described above, estrogens comprise the natural ligands for ERs, with 17 β -estradiol being a potent agonist. It was traditionally thought that the binding of ER agonists would induce a conformational change in the receptors, conferring the ability for coactivators to bind; while ER antagonists would compete for binding. However, the complex pharmacology of the ERs was first revealed with tamoxifen, originally described as an ER antagonist. Indeed, it behaved as an ER antagonist in breast cancer. Conversely, and quite the opposite of what would be expected for an ER antagonist, tamoxifen exhibited estrogenic activity in bone, protecting against bone loss in postmenopausal women [26]. Tamoxifen has also been shown to behave as an ER agonist in the uterus, promoting hypertrophy [27]. Thus, tamoxifen was behaving as both an ER agonist and an antagonist depending on the tissue context, and therefore reclassified as a Selective Estrogen Receptor Modulator (SERM). This unique pharmacology of the ERs offers a rare advantage for drug development as at least in theory it would be possible to develop ligands with tissue selective agonist and antagonist activities. SERMs that are currently on the market in USA include tamoxifen (breast cancer), raloxifene (osteoporosis), bazedoxifene (in combination with Premarin for the treatment of osteoporosis and vasomotor symptoms associated with menopause), and ospemifene (dyspareunia associated with menopause).

It is clear that the differences in binding affinity alone cannot explain the mixed agonist/antagonist effects of SERMs. However, their different affinities for the different subtypes of ERs (α and β), along with the relative different tissue expression of these subtypes, may explain some of SERMs' pharmacology. On the other hand, recent evidence suggests that binding of the receptor even by structurally related compounds results unique conformational changes, allowing it to recruit distinct sets of coactivators and/or corepressors [28, 29]. In addition, over 300 proteins have been demonstrated to have coregulatory functions associated with ERs, potentially allowing an incredibly diverse range of signaling, and therefore likely to explain why different ligand-bound ER complexes can exhibit variable transcriptional activities – even within the same cell [30]. Other described mechanisms for SERM's unique actions include different ERE/promoter sequences, engagement of different signal transduction pathways and the contribution of non-classical signaling pathways [31]. Despite the abundant data supporting the context-specific actions of synthetic SERMs, it remained unclear whether there existed an endogenous SERM, or if this aspect of ER pharmacology was purely 'anthropogenic'.

3 27-hydroxycholesterol, an endogenous SERM

The first evidence suggesting there may be an endogenous SERM came from studies of Premarin. Premarin's component steroidal estrogens had agonist/antagonist activities that differed between cells, and this phenomenon could not be explained by the differences in affinity alone [32]. In an effort to determine if mammals produce ER ligands other than the previously described estrogens, and whether there may exist an endogenous SERM, Umetani et. al. screened a library of cholesterol metabolites using Gal4-ER co-transfection assay [6]. They, along with DuSell et. al., identified several oxysterols with the ability to modulate ER activity, with 27-hydroxycholesterol (27HC) emerging with the lowest IC_{50} for ER α and ER β [5, 6]. In functional studies, it was found that 27HC behaved as an ER agonist in hepatoma, breast and colon cancer cells, but as an antagonist in vascular endothelial cells and murine models of cardiovascular disease [5, 33–37]. When bound to the ER α , 27HC induces a conformational change that is distinct from estradiol, 4-hydroxytamoxifen (active metabolite of tamoxifen), or ICI 182,780 (Fulvestrant; pure ER-antagonist) [5]. Since its identification as a SERM, 27HC has been implicated in the pathologies of several diseases including atherosclerosis, osteoporosis, breast cancer, prostate cancer and Alzheimer's Disease.

27HC is the most abundant circulating oxysterol and its serum concentration is closely correlated with plasma cholesterol concentration [10]. In healthy human subjects, 27HC concentrations range from ~0.2 – 0.9 μ M, and it is dramatically increased with hypercholesterolemia [10, 38, 39]. It is important to keep in mind that the approximate IC_{50} of 27HC for the ERs is 1 μ M [6]. 27HC is synthesized from cholesterol by CYP27A1, a mitochondrial P450 enzyme, which is primarily expressed in liver and cells of the myeloid immune lineage such as macrophages [40]. 27HC is metabolized by CYP7B1, another P450 enzyme that is expressed primarily in the brain, liver and other peripheral organs [41].

4 The importance of 27HC in bone health

It is well known that estrogens play a bone-protective role, as best evidenced by post-menopausal women being at a significantly higher risk of developing osteoporosis [29]. Interestingly, several studies have now indicated that post-menopausal women taking cholesterol lowering drugs such as statins (HMGCoA reductase inhibitors) have better bone health [42, 43]. Furthermore, elevated cholesterol is a recognized risk factor for osteoporosis in post-menopausal women [44–48]. Thus, cholesterol is strongly implicated in the pathophysiology of osteoporosis. This is also supported by observations from preclinical murine models where a high fat and high cholesterol diet, or just a high cholesterol diet resulted decreased bone mineral density (BMD) and bone quality [9, 49].

Due to 27HC's SERM qualities, it was hypothesized that 27HC may be mediating the negative effects of cholesterol on bone quality, which is supported by the following observations. Disruption of 27HC catabolism (CYP7B1^{-/-} mice), which elevates circulating 27HC concentration (~3–5 fold), also resulted decreased BMD as well as decreased other markers of bone quality (bone volume over total volume, trabecular number, trabecular thickness and cortical thickness) [8]. Direct administration of exogenous 27HC resulted in decreased BMD and poor bone quality. In addition, 27HC could induce alkaline phosphatase and stimulate osteoclast precursor migration in an ER-dependent manner [9]. Interestingly however, estradiol supplementation only partially rescued the decreases in bone quality observed in CYP7B1^{-/-} mice. Collectively, these results indicated that (1) ERs partially mediate 27HC's effects on bones, and (2) there likely exists an additional target.

In regards to the latter, previous studies described 27HC as a partial agonist of the Liver X Receptors (LXR α and LXR β) [50, 51]. LXRs are important mediators of cellular cholesterol homeostasis by promoting cholesterol efflux and inhibiting cholesterol synthesis [52]. Several oxysterols and cholesterol metabolites have been identified as LXR agonists. The LXR agonist activity of 27HC was confirmed in osteoblasts and osteoclasts [9]. Upon treatment with a pharmacologic LXR agonist, female mice were found to have decreased bone quality. In a series of experiments, it was determined that 27HC activates LXRs in osteoblasts, increasing the expression of tumor necrosis factor (TNF α) which then induces RANKL synthesis. RANKL then drives osteoclastogenesis and subsequent bone resorption. In addition, 27HC inhibits osteoblastogenesis, likely through the downregulation of the osteoblast differentiation transcription factor RUNX2. Interestingly, many of the LXR mediated actions of 27HC were attenuated by estradiol, through induction of Small Heterodimeric Partner (SHP), an orphan nuclear receptor known to inhibit the activity of LXRs [9, 29]. Thus, in addition to its direct SERM activities, 27HC also engages the LXRs; the result of this complex combinatorial pharmacology having a negative impact on bone quality.

5 The role of 27HC in atherosclerosis

Atherosclerosis, arterial thickening caused by leukocyte infiltration and the proliferation of smooth muscle resulting a fibro-fatty plaque, is the major underlying cause of cardiovascular diseases such as heart attacks and strokes. Cholesterol, in particular LDL-cholesterol, is a

driving factor of plaque formation and inflammation. Infiltrating macrophages consume LDL-cholesterol and fat deposits, differentiating into foam cells which contribute significantly to the pathophysiology of atherosclerosis. It is important to note that macrophages highly express CYP27A1, the enzyme responsible for the conversion of cholesterol to 27HC [53]. Thus, it is perhaps not surprising that 27HC concentrations can be 20-fold higher in atherosclerotic plaques [54]. In addition, the 27HC concentration increases with the severity of the lesion and the abundance of macrophages. [34, 55, 56]

Since 27HC is the most abundant circulating oxysterol, it is likely to be an important physiologic modulator of LXR activity, and thus cholesterol homeostasis. And since 27HC-induced LXR activities would result in increased reverse cholesterol transport, it has been suggested that 27HC might exhibit atheroprotective effects by stimulating cholesterol efflux through LXR activation [57]. In addition, 27HC might contribute towards the stabilization of atherosclerotic plaques. It is considered that there are two broad functional states of macrophages: M1 macrophages, which secrete pro-inflammatory and immune-stimulatory cytokines and are found predominantly in rupture-prone atherosclerosis plaques; while M2 macrophages, which induce myofibroblast activation and release immune-regulatory cytokines, mainly localize in stable plaques [58, 59]. A recent study found that 27HC promotes M2 polarization of human macrophages and exhibits anti-inflammatory effects *in vitro*, thereby potentially leading to the stabilization of atherosclerotic plaques [60].

However, despite these potential beneficial effects of 27HC, more recent studies suggest 27HC promotes atherosclerosis. It is suggested that 27HC promotes the development of atherosclerosis via following two mechanisms: (1) Attenuation of the cardio-protective effects of estrogen, and (2) promotion of inflammation. The cardio-protective effects of estrogens are mediated at least partially through modulating the production of nitric oxide (NO) by nitric oxide synthases (iNOS and eNOS), which mediates important physiological functions such as vasodilation and endothelialization after vascular injury. Umetani et al. first discovered that 27HC competes with estradiol, and inhibits estradiol-stimulated NO production [6]. Subsequently, in the setting of hypercholesterolemia (apoe^{-/-} mice), they found that elevation of 27HC by CYP7B1 deletion (apoe^{-/-}; cyp7b1^{-/-} mice) or exogenous 27HC administration resulted in increased lipid deposition and greater atherosclerotic lesions [61]. This is consistent with observations from Zurkinden et al. that under hypercholesterolemia, complete 27HC inhibition via cyp27a1 deletion (apoe^{-/-};cyp27a1^{-/-} mice) is atheroprotective [62].

In addition to its regulation of NO production, 27HC promotes inflammation. 27HC upregulation via cyp7b1 deletion resulted in increased macrophage infiltration, macrophage-endothelial cell adhesion, as well as the upregulation of pro-inflammatory genes (IL-6, MMP-9, TNFα) [61]. These activities were found to be ER-dependent. In addition, 27HC leads to the activation of the NF-κB pathway through ERα by stimulating Erk1,2 and JNK-dependent IκBα degradation. Conversely, complete 27HC inhibition via cyp27a1 deletion is associated with decreased macrophage infiltration in the aortic root [62].

It is important to note that estrogen too might also have both positive and negative effects in the cardiovascular system. In early stage of the disease, estrogen mostly produces protective

effects. However, at late stage atherosclerosis, it can promote inflammation leading to the destabilization of plaques, increased coagulation and thrombosis [37, 63, 64]. Likewise, the effects of 27HC within the cardiovascular system may carry both positive and negative consequences.

6 Impact of 27HC on the Progression of Breast and Prostate Cancer

Breast cancer is the most commonly diagnosed cancer in women, with the majority of cases being hormone (estrogen receptor) dependent [65]. In terms of risk of onset, the role of cholesterol is unclear, further complicated as to whether total, LDL or HDL cholesterol impart risk [35, 66, 67]. Likewise, retrospective analysis of people on statin therapy indicates that these drugs might have conflicting roles in terms of breast cancer onset [68]. However, for recurrence of metastatic breast cancer, the roles of cholesterol are clearer. Specifically, elevated total cholesterol was found to be associated with increased breast cancer recurrence [69]. Furthermore, several retrospective studies indicate that patients taking cholesterol lowering drugs (statins) exhibit significantly increased recurrence free survival [70–72]. In a recently published phase III double-blind trial including 8,010 postmenopausal women with early-stage, hormone receptor-positive invasive breast cancer, it was found that taking cholesterol lowering medication during endocrine therapy was associated with increased recurrence free survival time and distant recurrence-free interval [73].

In murine models, a high fat, high cholesterol diet increases breast tumor growth and metastasis [35]. In a more controlled study, it was found that elevating only dietary cholesterol was sufficient to increase tumor growth in MMTV-PyMT mice [74]. Importantly, the tumorigenic properties of a high cholesterol diet were lost in mice lacking the ability to synthesize 27HC (CYP27A1^{-/-} mice). Furthermore, the effects of a high fat diet on the growth of E0771 tumors in transgenic human APOE3 replacement mice was attenuated by treatment with statin or a small molecule inhibitor of CYP27A1 [74]. Within human tissues it was found that (1) increased CYP27A1 protein expression is associated with higher grade tumors, (2) elevated CYP7B1 mRNA expression (and thus likely decreased tumoral 27HC) is a good prognostic factor, and (3) that 27HC concentrations were higher in breast tumor tissue compared to normal adjacent or tissue obtained from healthy volunteers [74, 75]. In mice, exogenous 27HC treatment resulted increased tumor size in an ER-dependent manner. Collectively, these data strongly suggest that 27HC is responsible for mediating many of cholesterol's effects on breast cancer progression.

Subsequent work has shown that the tumor growth promoting properties of 27HC are ER-dependent as they are inhibited by co-administration of the ER antagonist Fulvestrant [74, 75]. Recent evidence indicates that tumor suppressor protein p53 might be involved in this process; 27HC induces MDM2-mediated p53 inactivation through ERs, which subsequently results cell proliferation [36]. 27HC has been found to promote breast cancer cell migration and invasion through the induction of epithelial-mesenchymal transition (EMT) via STAT-3. Two independent pathways might be involved: the classic ER α /STAT-3 in ER-positive cells, and STAT-3/MMP9 signaling in both ER-positive and ER-negative cells [76]. 27HC may also promote angiogenic properties of breast cancer cells by up-regulating vascular

endothelial growth factor (VEFG) through either a classic ER α pathway or in an ER-independent manner through reactive oxygen species (ROS) activation of STAT-3 [77]. Interestingly, 27HC was also shown to promote breast cancer metastasis, the mechanism of which likely involves LXRs [74].

In addition, recent reports indicate that 27HC might be involved in reprogramming breast cancer cells to develop resistance to aromatase inhibitor therapy. Under long term estrogen deprivation (mimicking patients undergoing aromatase inhibitor therapy), breast cancer cells undergo epigenetic reprogramming and become more invasive. During this reprogramming process, cholesterol biosynthesis, especially 27HC biosynthesis (CYP27A1 expression) is significantly upregulated. 27HC-bound ER α can be recruited to common regulatory regions of estrogen-bound ER α , and these reprogrammed cells also have a higher average ER α binding comparing to their parental cells, suggesting 27HC-ER α axis might be involved in developing resistance [78]. However, it's worth noting that it has been found that many aromatase inhibitors cross-over and also inhibit CYP27A1 [79].

Obesity and hypercholesterolemia are also risk factors for prostate cancer [80, 81]. As a major cholesterol downstream metabolite, 27HC was suspected to play a role. Raza et al. reported that 27HC stimulates the proliferation of normal prostate cells (human prostate epithelial cells: RWPE-1) and increases androgen receptor (AR) transcriptional activity, which might contribute to the onset of prostate cancer [82]. The same group also found that 27HC stimulated the proliferation of prostate cancer cells (LNCaP and PC3) via an ER β -dependent manner [83], suggesting its role in the progression of the disease. In addition, 27HC was found to block docetaxel-induced apoptosis, and therefore might play a role in drug resistance [82]. However, in contrast to the above findings, 27HC inhibited prostate cancer cell invasion [83]. Furthermore, adopting a bioinformatics approach, Alfaqih et al. found CYP27A1 is dramatically downgraded in prostate cancer [84]. More importantly, low expression of the enzyme is associated with decreased progression free survival as well as higher tumor grade, suggesting 27HC might actually be beneficial. In support of this notion are their findings that 27HC inhibits the proliferation prostate cancer cells (LNCaP and 22RV1) and colony formation. Overexpression of CYP27A1 led to slower tumor growth of 22RV1 xenografts. The growth-inhibitory effects were attributed to the ability of 27HC to inhibit sterol regulatory-element binding protein 2 (SREBP2) [84]. Regardless, more work investigating the impact of 27HC on prostate cancer progression will be required to resolve the results of these conflicting studies.

7 27HC is associated with cognitive impairment

It is generally agreed that estrogens exhibit neuroprotective effects [85–87]. Depletion of estrogen in female rodents by ovariectomy significantly increases soluble A β in brain, and promotes Alzheimer's Disease (AD)-like pathogenesis. On the other hand, estrogen prevents A β -induced neuronal death, which is mediated through ER α since agonists of ER α produce the same neuroprotective effect [87–89]. The SERM tamoxifen, has been found to protect memory from A β -induced toxicity by acting as a ER agonist while enhancing spatial and contextual memory by reducing dopamine metabolism and increasing neurotransmitter acetylcholine [90].

It's been well-established that cholesterol and cholesterol trafficking are an important risk factors for cognitive impairment and neurodegenerative diseases such as Alzheimer's Disease (AD) [91, 92]. However, the blood brain barrier is impermeable to cholesterol and thus a high cholesterol diet does not change cholesterol concentrations within the brain. On the other hand, the slightly more polar metabolite of cholesterol, 27HC, can cross the blood-brain barrier. It is of interest therefore, that rabbits on a high cholesterol diet exhibit increased 27HC in the brain, which is also associated with altered expression of ER target genes and markers of neurodegeneration [88]. In late-stage AD brains, CYP27A1 mRNA levels are significantly increased as are 27HC concentrations within the frontal and occipital cortex, and cerebrospinal fluid (CSF), suggesting 27HC might be involved in AD progression [93, 94]. CSF 27HC concentration is correlated with soluble amyloid precursor protein (sAPP) [95]. Furthermore, cholesterol-mediated memory impairment is ablated in CYP27A1^{-/-} mice, while 27HC treatment results spatial learning and memory impairment, indicating 27HC mediates at least some aspects of cholesterol-induced AD progression [96, 97].

A recent study by Brooks *et al.* on late-onset AD reported reduced ER α and elevated ER β in association with high cholesterol/27HC [88]. The same pattern of reduced ER α and elevated ER β , is also found in the hippocampus of AD patients [98]. Brooks *et al.* also reported that a reduction of post-synaptic marker PSD-95 in association with the high cholesterol/27HC/decreased ER α , indicating decreased synaptogenesis with cholesterol/27HC. Since ER α plays a modulatory role in synaptogenesis, it is possible ER α mediates cholesterol/27HC-induced disruption of synaptogenesis [88]. However, the effects of 27HC on cognition and associated diseases is an emerging field, and as such the mechanisms by which 27HC results in neuro-impairments are still unclear.

Conclusions

27HC has emerged as an endogenous SERM with implications in physiology and pathophysiology. As a SERM, 27HC behaves as both an ER agonist and antagonist, depending on the tissue context. Furthermore, 27HC is also an LXR agonist. Since the ERs and LXRs play significant roles in many different tissues, it is likely that 27HC also impacts biology in these tissues. Indeed, pre-clinical models have demonstrated that 27HC stimulates the progression of osteoporosis, atherosclerosis, breast cancer, and is implicated in both prostate cancer and Alzheimer's disease. Although their circulating concentrations are far lower than those of 27HC, other oxysterols also have the ability to modulate the activity of the ERs and LXRs [6, 7]. It is possible that under pathological conditions, these oxysterols also play important roles. Therefore, it will be important for future studies investigating either ER signaling or the effects of cholesterol to consider the likely contribution of oxysterols.

CYP27A1 is a cytochrome P450 enzyme responsible for the conversion of cholesterol to 27HC. Given the evidence in support of pathological roles of 27HC, CYP27A1 represents a logical therapeutic target. Two small molecule CYP27A1 inhibitors, GW273297X and GI268267X, have been described and demonstrated in preclinical models to decrease circulating 27HC and reduce breast tumor growth [74, 99]. Interestingly, the currently FDA

approved drugs cyclosporine and several aromatase inhibitors have also been shown to inhibit CYP27A1 [79, 99]. On the other hand, statin therapy to reduce circulating cholesterol levels might also be a viable approach. Indeed, statin administration does lead to decreased circulating 27HC concentrations [100, 101]. Excitingly, the proliferative marker, Ki67, is reduced in tumors from women placed on atorvastatin, highlighting the potential for this treatment paradigm [102]. However, HMGCoA was also upregulated with statin therapy, indicating that the local microenvironment may be compensating by increasing cholesterol biosynthesis. Thus, more direct therapies targeting CYP27A1 may be more clinically advantageous for chronic treatment. Finally, it is important to keep in mind that humans with mutations of CYP27A1 within the catalytic domain develop cerebrotendinous xanthomatosis, which is characterized by disturbed lipid handling, neuronal dysfunction and premature atherosclerosis. At this point it is unclear whether this is due to a lack of this enzyme throughout development and/or whether acute inhibition of CYP27A1 would also manifest in this disorder. Furthermore, 24(S)-hydroxycholesterol, another oxysterol, can also be metabolized by CYP27A1 [37]. Therefore, it will be important to evaluate the safety of CYP27A1 inhibition in models that accurately reflect human cholesterol biology.

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Highlights

27-hydroxycholesterol (27HC) has emerged as an endogenous selective estrogen receptor modulator (SERM).

- Elevated cholesterol is associated with several pathological conditions.
- This review discusses the clinical and preclinical data for a role of 27-hydroxycholesterol in various pathological conditions.

Figure 1. Chemical structures and biosynthesis pathways for endogenous estrogens and the endogenous selective estrogen receptor modulator (SERM) 27-hydroxycholesterol (27HC). Tamoxifen, a synthetic SERM is shown for comparison. 27HC is a primary metabolite of cholesterol, hydroxylated by the actions of CYP27A1, and is further metabolized by CYP7B1. Estrogens are formed when they are aromatized by the enzyme aromatase (CYP19) from precursor androgens.