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Re-adopting classical nuclear receptors by cholesterol metabolites

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Abstract

Since the first cloning of the human estrogen receptor (ER) α in 1986 and the subsequent cloning of human ER β , there has been extensive investigation of the role of estrogen/ER. Estrogens/ER play important roles not only in sexual development and reproduction but also in a variety of other functions in multiple tissues. Selective Estrogen Receptor Modulators (SERMs) are ER ligands that act as agonists or antagonists depending on the target genes and tissues, and until recently, only synthetic SERMs have been recognized. However, the discovery of the first endogenous SERM, 27-hydroxycholesterol (27HC), opened a new dimension of ER action in health and disease. In addition to the identification of 27HC as a SERM, oxysterols have been recently demonstrated as indirect modulators of ER through interaction with the nuclear receptor Liver X Receptor (LXR) β . In this review, the recent progress on these novel roles of oxysterols in ER modulation is summarized.

Keywords

27-hydroxycholesterol; oxysterol; estrogen receptor; SERM; LXR

1. Estrogen receptors, estrogen and SERM

Estrogen plays critical roles in reproduction, development, bone mineralization, and metabolism as well as cardiovascular, immune system and brain function. It is also an important factor in breast tumor progression. Its receptor, the estrogen receptor (ER), is a member of the nuclear hormone receptor superfamily, and it consists of the following domains: amino-terminal ligand-independent activation function-1 (AF-1), DNA binding domain, hinge region, and ligand-binding domain, which contains the carboxyl-terminal activation function 2 (AF-2). There are two subtypes of ER, ER α and ER β , and they share 96% homology at the amino acid level in the DNA binding domain and 59% homology in the ligand-binding domain in humans. ER α and ER β have distinct tissue expression patterns,

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and together with differences in their ligand affinity, the abundance of the two subtypes in cells and tissues define the physiological function of estrogen (1). The relatively large ligand-binding pockets of ERs allow many compounds other than steroid structures to bind and differentially alter ER structure (2–4). In contrast to estrogens, which have various levels of agonistic activity in all tissues, Selective Estrogen Receptor Modulators (SERMs), which are diverse ER ligands with nonsteroidal structures, act as agonists or antagonists depending on the target genes and tissues. Although the exact mechanism of the tissue-selective effects of SERMs is still incompletely understood, these ligands exert agonistic/antagonistic properties mainly based upon the amount of ER in the tissue, ligand-dependent ER conformational changes, and differential interaction with various coactivators and corepressors. Clomiphene was the first SERM used as a pituitary gonadotropin inhibitor in 1967, and tamoxifen was the first SERM to be clinically developed and marketed for the treatment of breast cancer. SERMs are classified into several groups according to their core structure, including triphenylethylenes such as tamoxifen, benzothiophenes such as raloxifene, indoles such as bazedoxifene (BZA), benzopyrans and tetrahydronaphthalenes (5). The ideal SERM for clinical use would have estrogenic effects in some tissues such as bone and the cardiovascular system, but have anti-estrogenic or neutral effects in other tissues, such as the breast and endometrium. Many classical SERMs show limited ER subtype selectivity; however, a growing number of novel ER subtype-selective ligands have been developed (6). In addition, the combination of a SERM with estrogens as a tissue-selective estrogen complex (TSEC) has been tested for the potential to achieve a more favorable clinical profile in postmenopausal women. This approach is expected to combine the desired ER agonist activities of estrogens with the tissue selectivity of a SERM. BZA paired with conjugated estrogens (CE) is the most advanced TSEC for the treatment of menopausal symptoms, and BZA/CE has shown a significant improvement of said symptoms in clinical trials (7). There is increasing evidence that in addition to its transcriptional activity in the nucleus, ER also has a nonnuclear action in the plasma membrane caveolae/lipid rafts, where it participates in kinase-mediated signaling pathways (8). In contrast to our understanding of the mechanisms of action of SERMs on the transcriptional activity of ER, the impact of SERMs on the nonnuclear action of ER still remains unclear.

2. 27HC as a novel endogenous SERM

Through screening oxysterols that exist in the human body for an effect on the transcriptional activity of ER, we discovered that certain oxysterols modulate ER activity (9). Among such oxysterols, 27-hydroxycholesterol (27HC) is the most abundant circulating oxysterol, and its plasma concentration correlates with that of total cholesterol (10). Using combinatorial peptide phage display, we found that 27HC induces a unique active conformational change in ER α (11). In addition, while 27HC shows an anti-estrogenic effect in vascular endothelial cells (ECs), it shows a pro-estrogenic effect in hepatoma and colon cancer cells (9). These findings identified 27HC as the first naturally occurring SERM and revealed that it has distinct functions in various tissues as described below.

27HC is generated and metabolized by the P450 enzymes CYP27A1 and CYP7B1, respectively (12). These enzymes are involved in the conversion of cholesterol to bile acids

in the liver. There are two (classical and alternative) pathways of cholesterol-bile acid conversion in liver; CYP27A1 is involved in both pathways, but CYP7B1 is only involved in the alternative pathway. Both enzymes are not very responsive to negative feedback by bile acids (13), and dietary manipulation, such as a high cholesterol diet, does not alter the expression of the enzymes (14). Instead, these enzymes are regulated by many factors other than cholesterol metabolism (Tables 1 and 2), suggesting that 27HC has important actions other than cholesterol metabolism.

2.1 Impact of 27HC in cardiovascular dysfunction

Estrogen and ER play critical yet complex roles in the cardiovascular system. Estrogen protects against cardiovascular complications in animal models and in human observational studies in which estrogen is administered shortly after the cease of the endogenous estrogen supply, resembling conditions such as hormone replacement therapy just after menopause or surgical ovariectomy (15–18). In contrast, estrogen does not show any beneficial effects or even shows detrimental effects with prolonged treatment or after a period of estrogen deficiency (19). It has been speculated that these factors may account for the failure of hormone replacement therapy in two large randomized studies (20,21). However, the mechanism by which estrogen fails to exert a protective effect against cardiovascular disease after a period of estrogen deficiency is still unclear. Considering that postmenopausal women have atherosclerosis with cholesterol accumulation and 27HC levels increase with cholesterol levels (22), it is plausible that increased 27HC blocks the protective effects of estrogen. Indeed, 27HC levels are greatly increased, up to millimolar levels, in the atherosclerotic lesion (10), and 27HC administration augments atherosclerotic lesion development in mice (23). Although a large portion of 27HC in circulation and tissues is esterified, hypercholesterolemia caused sufficiently increased 27HC levels in the aorta to modify ER function (9). The WHI study reported that there was no association between plasma 27HC levels and cardiovascular disease risk (24). However, the plasma 27HC levels reported in that study are far lower than previous published data from human serum (10). Therefore, whether the 27HC measurement was performed correctly or not remains unclear. In vascular ECs, estrogen promotes nitric oxide (NO) production by increased transcription (genomic action by ER) and enzymatic activity (nonnuclear action) of endothelial type NO synthase (eNOS), and increased levels of 27HC suppress the NO production induced by estrogen in ECs (9). This antagonism by the oxysterol leads to the inhibition of the protective effects of ER in the cardiovascular system (Figure 1). Therefore, the relative balance between endogenous estrogen, which decreases with age, and 27HC, the levels of which are increased by hypercholesterolemia, may be an important risk factor for cardiovascular disease. Interestingly, in addition to its effects as a competitor for estrogen binding to ER, 27HC directly modulates ER function (23). In monocytes/macrophages and ECs, 27HC upregulates proinflammatory reactions and promotes leukocyte adhesion to ECs via ER α . These recent findings collectively indicate that 27HC potentially impairs the beneficial effects of estrogen on vascular function and that it promotes atherosclerosis through unique proinflammatory processes mediated by ER α . Strategies to lower 27HC levels may complement existing approaches that target cholesterol to prevent vascular disease and also lead to a greater understanding of why hormone replacement therapy is ineffective in postmenopausal women.

2.2 Impact of 27HC in breast cancer progression

The promotion of ER-positive breast cancer by estrogens is one of the detrimental actions of estrogens. Breast cancer is the second most common malignancy in women, and ER-positive breast cancer is the most abundant type of breast cancer (25). Even in postmenopausal women, who have considerably decreased levels of endogenous estrogens, the risk of ER-positive breast cancer remains high, and endocrine-based therapies with synthetic SERMs or aromatase inhibitors are often ineffective or elicit drug resistance, even though the processes by which these therapies lose their effectiveness are mostly ER dependent (26–29). Because cholesterol is one of the known risk factors for breast cancer progression and 27HC levels increase with cholesterol levels and with age (30), the potential actions of 27HC on ER-positive breast cancer were investigated.

In MCF-7 breast cancer cells, 27HC modulates gene expression by recruiting ER α to the ER-Responsive Element (ERE)-containing region of ER target genes. 27HC also increases cell proliferation by upregulating cyclin D1 expression and increasing the number of cells entering S phase (11). We recently demonstrated that 27HC is the first non-estrogen ER ligand to be identified that stimulates ER-positive breast tumor growth in mice. Furthermore, we found that 27HC promotes breast tumor progression induced by high fat diet (HFD) in mice, which increases circulating and local 27HC levels in peripheral tissues. Importantly, inhibition of CYP27A1 is sufficient to prevent HFD-induced tumor proliferation without changing cholesterol levels, indicating that the effect is specific to 27HC elevation and is independent of total circulating cholesterol (31). Mechanistic studies further showed that 27HC can be locally produced in the breast tumor tissue, and it stimulates cell-autonomous, ER-dependent cell proliferation. In addition, in human tumor tissues, increased 27HC is correlated with reduced expression of CYP7B1, the 27HC-metabolizing enzyme, and reduced expression of CYP7B1 in tumors is associated with poorer patient survival. In cancer patients, a comparison of the lowest versus highest quartiles for tumor CYP7B1 expression revealed that low CYP7B1 expression is associated with poor overall outcome, even after adjusting for age, tumor size, nodal status and perioperative therapy. Although the disruption of CYP7B1 in mice causes elevated levels of 25HC and 27HC, 25HC has the same characteristics as 27HC as an ER action modifier (32 and unpublished data). Thus, the new finding on the role of 27HC and its regulatory enzymes in breast cancer may explain treatment failures with classical SERMs and aromatase inhibitors. Assessing 27HC levels or CYP7B1 enzyme abundance in tumors may lead to the development of a novel therapy to modulate the endogenous promotion of breast tumor progression.

2.3 Impact of 27HC in bone mineral density

In addition to its actions in the cardiovascular system and breast cancer progression, estrogen also plays an important role in the regulation of bone mineralization. Estrogen deficiency caused by menopause or surgical removal of the ovaries results in pathological bone loss, and this can be reversed by estrogen replacement (33,34). In addition, hypercholesterolemia is an independent risk factor for decreased bone mass in postmenopausal women (35,36), suggesting that 27HC is also involved in the regulation of bone mineral density. Indeed, in mouse models, 27HC administration decreases bone

mineral density, bone volume/total volume fraction, trabecular number and trabecular thickness and increases trabecular separation. In contrast, mice deficient in 27HC have increased numbers of trabeculae (37). Although exogenous estrogen treatment increases bone mineral density and trabecular number/thickness in wild-type mice, estrogen treatment failed to affect bone mineral density in mice with elevated 27HC levels. As observed in vascular cells and breast cancer cells, 27HC acts either as an agonist or antagonist of estrogen, depending on the cell types and relative estrogen amount. Furthermore, 27HC increases bone resorption through the indirect activation of osteoclast differentiation and increased monocyte recruitment to the bone surface (38). These findings indicate that 27HC likely has an adverse impact on bone mineralization and that some of the effects of 27HC may be related to its activities as a SERM. It was also suggested that some of the effects of 27HC on the bone mineral density are due to the activation of the Liver X Receptor (LXR) by 27HC (38). In mice, a high-cholesterol diet, which creates a physiological condition that increases 27HC levels, decreases bone mineral density, and the activation of LXR causes reduced osteoblast differentiation. The mechanisms by which 27HC affects bone function via ER and LXR are distinct; ER is linked to the CXCL12 signaling pathway, whereas LXR activation is linked to the TNF α -RANKL signaling pathway. Therefore, 27HC, as a SERM and a LXR ligand, affects both ER and LXR function in the bone.

3. ER modulation by oxysterols through LXR activation

In addition to its ligand-dependent function, ER is also known to be activated in the absence of its ligand binding. For example, growth factors activate the MAP kinase pathway and thereby activate ER α transcriptional activity by the phosphorylation of ER α at Ser 118 (39,40). Through the investigation of the modulation of ER action by oxysterols, we found that oxysterols also indirectly activate the nonnuclear action of ER α through the activation of another nuclear receptor, LXR, in ECs (41). LXR plays important roles mainly in lipid metabolism (42,43). The two isoforms, LXR α and LXR β , share high similarity in protein structure and in target genes; however, they show different tissue distributions. LXR α is primarily expressed in the liver, intestine, adipose tissue, and macrophages, whereas LXR β is expressed ubiquitously (43). Similar to ER, LXR activation reduces the development of atherosclerosis despite modest changes in plasma lipoprotein levels (44), suggesting that the underlying mechanism(s) may involve direct effects on vascular cell functions. Oxysterols such as 22(R)-hydroxycholesterol and 24(S)-hydroxycholesterol are major endogenous LXR ligands. Although previous reports indicate that 27HC acts as a LXR ligand in some cell types (38,45), 27HC has little, if any, activity as a LXR ligand in the cardiovascular system and in the liver, and it does not induce the expression of LXR target genes in these tissues (9,23). 24(S)-hydroxycholesterol is detected in circulation; however, unlike 27HC, the levels of 24(S)-hydroxycholesterol are not increased in atherosclerotic lesions (10). In investigating the role of LXR involved in tissue response to oxysterols, we discovered that LXR activation promotes beneficial effects on vascular ECs through a mechanism similar to that of ER (41). LXR β is the dominant isoform of LXR in vascular ECs, and activation of LXR β , but not LXR α , promotes EC migration that requires ER α signaling to eNOS. We found that LXR β directly interacts with ER α in the plasma membrane caveolae/lipid rafts, where ER exerts non-nuclear actions. LXR β activation increases its direct binding to the

ligand-binding domain of ER α and initiates an extranuclear signaling cascade that requires ER α Ser118 phosphorylation by PI3K/Akt. Although it has been reported that ER α can be activated in the absence of ligand binding by the phosphorylation of ER α , this is the first report that ER α phosphorylation is required for the nonnuclear action of ER α . These studies demonstrate that LXR β has a nonnuclear function in EC plasma membrane caveolae/lipid rafts that entails crosstalk with ER α , which promotes NO production and maintains endothelial monolayer integrity (Figure 1). The knowledge that the interaction between LXR β and ER α is required for the maintenance of endothelial monolayer integrity has potential implications in cardiovascular health and disease. Disruption of the endothelial monolayer plays an integral role in the initiation of atherosclerosis (46). Because LXR β has multiple cellular targets and a wide range of actions in vivo, it will be critical to determine how the endothelial-specific actions of LXR β play a role in protection against atherosclerosis. The potential of this role was demonstrated by previous findings using isoform-specific LXR-deficient mice, which showed that whereas LXR α has a role in limiting atherosclerosis in the context of hypercholesterolemia, LXR β activation can reduce atherosclerosis in the absence of LXR α (47,48). In addition, the activation of LXR α causes several adverse effects, such as hepatic steatosis via LXR α action in liver (49). Our findings further support the concept that pharmacologic activation of LXR β through its activation of ER α may be a promising new therapy against cardiovascular diseases. Further investigation of the roles of the LXR β -ER α interaction in other biological function is warranted.

4. Conclusion and future directions

Oxysterols have been previously recognized as intermediates in the conversion of cholesterol to bile acids to excrete excess cholesterol. However, it is now clear that they have important functions in regulating ER action in various tissues. Investigating how 27HC functionally interacts with other SERMs or aromatase inhibitors in therapies for ER-positive breast cancer will be important to understand the possible mechanism by which breast cancer gains resistance against such treatments during the therapy. Furthermore, investigating the role of 27HC in the ER-mediated function in tissues other than the cardiovascular system, breast cancer, and bone may provide additional information on oxysterol biology. One of the primary interests will be the role of 27HC in brain function, in which there is growing evidence of the importance of this oxysterol (50,51). In addition, mutations in CYP27A1, the 27HC-generating enzyme, and CYP7B1, the 27HC-metabolizing enzyme, alter the levels of cholesterol and 27HC in the brain and cause neurological dysfunction (51–56). Such studies also have the potential to lead to the development of a novel therapeutic approach to diseases related to metabolic dysfunction in the brain. It remains possible that oxysterols affect nuclear receptor function in different ways, and further investigation is warranted to clarify the role of oxysterols in human physiology.

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Highlights

1. 27HC was the first identified endogenous SERM.
2. 27HC inhibits estrogen's cardioprotective effects and stimulates inflammation in vasculature.
3. 27HC is estrogenic in breast cancer, and its tissue levels increase with disease stage in patients.
4. 27HC negatively regulates bone mineralization through effects on ER and LXR.
5. LXR stimulates the nonnuclear action of ER α and exerts vascular protection.

Figure 1.

Regulation of vascular function by oxysterols, LXR and ER. In normal conditions of low cholesterol/27HC, oxysterols or LXR ligands bind to LXR β . LXR activation stimulates the nonnuclear action of ER α , thereby increasing NO release and promoting recovery from vascular injury and vascular dilation. In hypercholesterolemia, which includes high 27HC levels, 27HC inhibits ER action and suppresses the vasoprotective effects mediated by ER. Modified from ref. 9.

Table 1

Factors studied with the respect to the regulation of CYP27A1 expression

Factors	↑/↓	Tissue (in vivo)/ Cell line	Species	References
Estrogen	↑	LNCaP	Human	(57)
Estrogen	↓	Hep G2, RWPE-1	Human	(57)
Estrogen	→	Liver	Mouse	(58)
Testosterone	↑	Hep G2	Human	(57)
Testosterone	↓	RWPE-1	Human	(57)
Testosterone	→	LNCaP	Human	(57)
Thyroid hormone	↑	Liver	Mouse	(59)
Thyroid hormone	↓	Hep G2	Human	(60)
Glucocorticoid	↑	Huh7, HepG2	Human	(60,61)
Glucocorticoid	↑	Liver	Rat	(62)
Vitamin D3	↑	RWPE-1, RWPE2-W99, 3T3-L1	Human	(63,64)
GH	↑	Hep G2	Human	(60)
IGF-1	↑	Hep G2	Human	(60)
TNF α	↓	HepG2	Human	(65)
TNF α	→	THP-1, HAEC	Human	(66)
IL-1 β	↓	Liver	Hamster	(65)
IL-1 β	↓	HepG2	Human	(65)
IL-1 β	→	THP-1, HAEC	Human	(66)
LPS	↓	Liver	Hamster, mouse	(65)
LPS	↓	HepG2	Human	(65)
LPS	→	BM-derived macrophage	Mouse	(67)
IFN γ	↓	THP-1, HAEC	Human	(66)
PMA	↓	Hep G2	Human	(60)
Bile acids	↓	Huh7, HepG2, HEK293	Human	(61,68,69)
Bile acids	→	Hepatocyte	Human	(70)
Bile acids	↓	Liver	Rat	(61,68,69)
Bile acids	→	Liver	Human, rat	(71–73)
PPAR γ	↑	THP-1, monocyte-derived macrophage	Human	(74,75)
RAR	↑	monocyte-derived macrophage	Human	(75,76)
LXR	↑	Astrocyte	Rat	(77)
LXR	→	THP-1, monocyte-derived macrophage, microglia	Human, rat	(74,77)
RXR	↑	THP-1, monocyte-derived macrophage	Human	(74,75)
Atherosclerotic lesion	↑	Aorta, carotid artery	Human	(75,78,79)
High cholesterol diet feeding	→	Liver	Mouse	(14)
Monocyte to macrophage differentiation	↑	Monocyte	Human	(75,80,81)
Aging	→	Liver	Swine	(82)

GH, growth hormone; IGF-1, insulin-like growth factor-1; TNF α , tumor necrosis factor α ; IL-1 β , interleukin-1 β ; LPS, lipopolysaccharide; IFN γ , interferon γ ; PMA, phorbol 12-myristate 13-acetate; PPAR γ , peroxisome proliferator-activated receptor γ ; RAR, retinoic acid receptor; RXR, retinoid X receptor

Table 2

Factors studied with the respect to the regulation of CYP7B1 mRNA expression

Factors	↑/↓	Tissue/ Cell line	Species	References
Estrogen	↑	Liver	Mouse	(58,83,84)
Estrogen	↑	HepG2	Human	(58,83)
Estrogen	↓	LnCaP, astrocyte	Human, rat	(85,86)
Testosterone	↑	Liver	Mouse	(87)
Testosterone	↓	Prostate	Canine	(85,88)
Testosterone	↓	LnCaP, HEK293	Human	(85,88)
TNF α	↑	fibroblast-like synoviocyte	Human	(89,90)
IL-1 β	↑	fibroblast-like synoviocyte	Human	(89,90)
IL-17	↑	fibroblast-like synoviocyte	Human	(89)
LPS	↑	Monocyte-derived macrophage	Human	(81)
LPS	→	BM-derived macrophage	Mouse	(67)
LPS	↓	Liver	Mouse	(65)
Hepatic insulin resistance	↓	Liver	Mouse	(91)
Insulin	→*	Liver	Hamster	(92)
High fat diet feeding	↓	Liver	Mouse	(91)
High fat diet feeding	→	Liver	Mouse	(93,94)
High sucrose diet feeding	↓	Liver	Mouse	(95)
Bile acids	→	Hepatocyte	Human	(70)
Bile acids	→	Liver	Human	(73)
FXR	↓	Liver	Mouse	(91,96)
PPAR α	↓	Liver	Mouse	(84)
PPAR α	↓	HepG2	Human	(84)
LXR	↓	Liver	Mouse	(97)
LXR	↓	Hepatocyte	Human	(98)
PXR	↓	Liver	Mouse	(96)
ROR α	↑	Liver	Mouse	(98)
ROR α	↑	HepG2, hepatocyte	Human, mouse	(98)
SREBP	↓	McA-RH777	Rat	(99)
NASH	↑	Liver	Human	(100)
Aging	↑	Liver	Swine	(82)
Aging	↓*	Kidney	Swine	(82)

FXR, farnesoid X receptor; ROR, retinoid-related orphan receptor; SREBP, sterol regulatory element-binding protein; NASH, nonalcoholic steatohepatitis.

* Enzymatic activity