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Liver X Receptors and their Agonists: Targeting for Cholesterol Homeostasis and Cardiovascular Diseases

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Abstract
Liver X receptors α (LXRα) and β (LXRβ) are essential for protection against cardiovascular diseases. LXRs are members of the nuclear receptor superfamily of DNA-binding transcription factors and act as sensors of cholesterol homeostasis. In this review, we introduce LXRs and briefly describe the roles of LXRs in reverse cholesterol transport and trans-intestinal cholesterol efflux. We discuss LXR agonists and the downstream genes of LXRs that are involved in the regulation of cholesterol transport. In addition, we describe the cardioprotective effects of LXRs against atherosclerosis, myocardial ischemia/reperfusion injury, diabetic cardiomyopathy, and myocardial hypertrophy. Finally, we expand our discussion to the actions of LXRs in atherosclerosis and suggest several potential research avenues that may be of interest to clinicians and basic scientists. The information included herein may be useful for the design of future experimental research studies and may advance the investigation of LXRs as therapeutic targets.

Introduction
Liver X receptors (LXRs) are members of the nuclear receptor superfamily of DNA-binding transcription factors and are sensors of cholesterol homeostasis. The LXR subfamily contains two isoforms, α and β (Ulven et al., 2005), which are regulated by the binding of physiological ligands (e.g., oxysterols and desmosterol) (Peet et al., 1998a; Yang et al., 2006), and synthetic LXR agonists (e.g., GW3965 and T0901317) (Harasiuk et al., 2015; Kappus et al., 2014). Cardiovascular diseases are the most common causes of mortality and morbidity worldwide, and emerging evidence shows that LXRs play cardioprotective roles in maintaining cholesterol homeostasis (Hong and Tontonoz, 2014), suppressing inflammation (Kappus et al., 2014), decreasing oxidation and apoptosis (He et al., 2014a), and suppressing insulin resistance (He et al., 2014b) and hypertrophy (Kuipers et al., 2010a).

The focus of this review is to summarize the latest progress regarding the protective effects of LXRs in cholesterol homeostasis and cardiovascular diseases. First, we briefly discuss the background information of LXRs and their roles in cholesterol transport. Then, we provide

in-depth descriptions of the downstream genes of LXRs in the regulation of cholesterol transport and LXR agonists that are involved in anti-atherogenic activities. Next, we summarize the cardioprotective roles of LXRs against myocardial ischemia/reperfusion injury, diabetic cardiomyopathy, and myocardial hypertrophy. Finally, we discuss several novel potential directions for future research on LXRs. The presented information is potentially useful for the design of future studies and the advancement of LXRs as therapeutic targets for cardiovascular diseases.

General background on LXRs and their roles in cholesterol transport

LXRs

LXRs are members of the nuclear receptor superfamily of DNA-binding transcription factors. In humans, LXRα and LXRβ are located on the short arm of chromosome 11 (11p11.2) and the long arm of chromosome 19 (19q13.3), respectively. Human LXRα (447 amino acids) and LXRβ (460 amino acids) share 77% sequence homology in both their full-length DNA and their ligand-binding domains (Pannu et al., 2013; Zhao and Dahlman-Wright, 2010). LXRs contain four principal domains, including an N-terminal ligand-independent activation function domain, a DNA-binding domain, a ligand-binding domain that is required for ligand binding and receptor dimerization, and a C-terminal ligand-dependent activation function-2 domain, which stimulates transcription in response to ligand binding and is requisite for binding co-activators or co-repressors (Faulds et al., 2010).

The transcriptional activity of LXRs is dependent on heterodimerization with retinoid X receptors (RXRs) (Peet et al., 1998b). LXR-RXR heterodimers bind to the LXR response element (LXRE), a specific DNA sequence represented by repeated AGGTCA sequences that are separated by four nucleotides (DR4 response elements) (Apfel et al., 1994; Peet et al., 1998b). A genome-wide analysis has confirmed that DR4 response elements are present in the regulatory regions of many targets and that they play a role in determining LXR binding to DNA (Quack et al., 2002). When not bound to a ligand, the LXR-RXR heterodimer remains bound to the promoter region of target genes in a complex with co-repressors, such as silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) or nuclear receptor co-repressor (NCoR) (Hu et al., 2003), to inhibit the activation of target genes (Svensson et al., 2003). Once bound to an agonist, the conformation of the LXR-RXR complex is altered, leading to the release of the NCoRs and the recruitment of nuclear receptor co-activators, such as activating signal co-integrator 2 (ASC2) (Kim et al., 2009) and E1A-associated protein p300 (EP300) (Huuskonen et al., 2004) (Figure 1A). Although both LXRα and LXRβ are activated by the same ligands and are structurally similar, their tissue expression profiles are very different. LXRα is selectively expressed in specific tissues and cell types, such as the liver, intestine, adrenal gland, adipose tissue and macrophages, whereas LXRβ is ubiquitously expressed (Huuskonen et al., 2004; Peet et al., 1998a; Peet et al., 1998b).

LXRs and reverse cholesterol transport

Cholesterol removal from non-hepatic cells and its delivery back to the liver for excretion are processes collectively known as reverse cholesterol transport (RCT) (Francis, 2010). Elevated flux through the RCT pathway is thought to protect against cardiovascular diseases primarily by facilitating the removal of cholesterol from macrophage foam cells in atherosclerotic plaques (Rosenson et al., 2012). Several direct LXR target genes are closely associated with the RCT pathway, including genes encoding membrane lipid transporters, such as ATP-binding cassette subfamily A type 1 (ABCA1), ABCG1, ABCG5 and ABCG8; apolipoproteins (Apos), such as ApoE; and lipid transfer proteins and cholesterol metabolizing enzymes, such as cholesterol 7-α-hydroxylase (CYP7A1) (Pannu et al., 2013).

At the cellular level, ABCA1 and ABCG1 transport cellular cholesterol to ApoA1 and high-density lipoprotein (HDL), respectively. Then, the lipidated lipoprotein particles transport cholesterol back to the liver via low-density lipoprotein receptor (LDLR) and HDL receptor scavenger receptor class B type 1 (SR-B1), which perform selective lipid uptake from HDL into hepatocytes. Finally, the cholesterol is secreted into bile or catabolized into bile acids via a process that is modulated by CYP7A1 (Hong and Tontonoz, 2014). In *Lxr*$^{-/-}$ mice, cholesterol removal from the body is severely impaired (Joseph et al., 2003). In contrast, the systemic activation of LXRs in mice by synthetic LXR agonists reduces plasma cholesterol levels and raises plasma HDL levels (Joseph et al.,

2002; Tangirala et al., 2002; Wang and Tall, 2003) (Figure 1B).

LXRs and trans-intestinal cholesterol excretion

Biliary cholesterol secretion is the primary mechanism for excess cholesterol excretion from the body. However, cholesterol excretion can also be facilitated by a non-biliary pathway known as trans-intestinal cholesterol efflux (TICE) (Khera and Rader, 2010). TICE involves the movement of cholesterol through the plasma to the basolateral surface of enterocytes. Then,

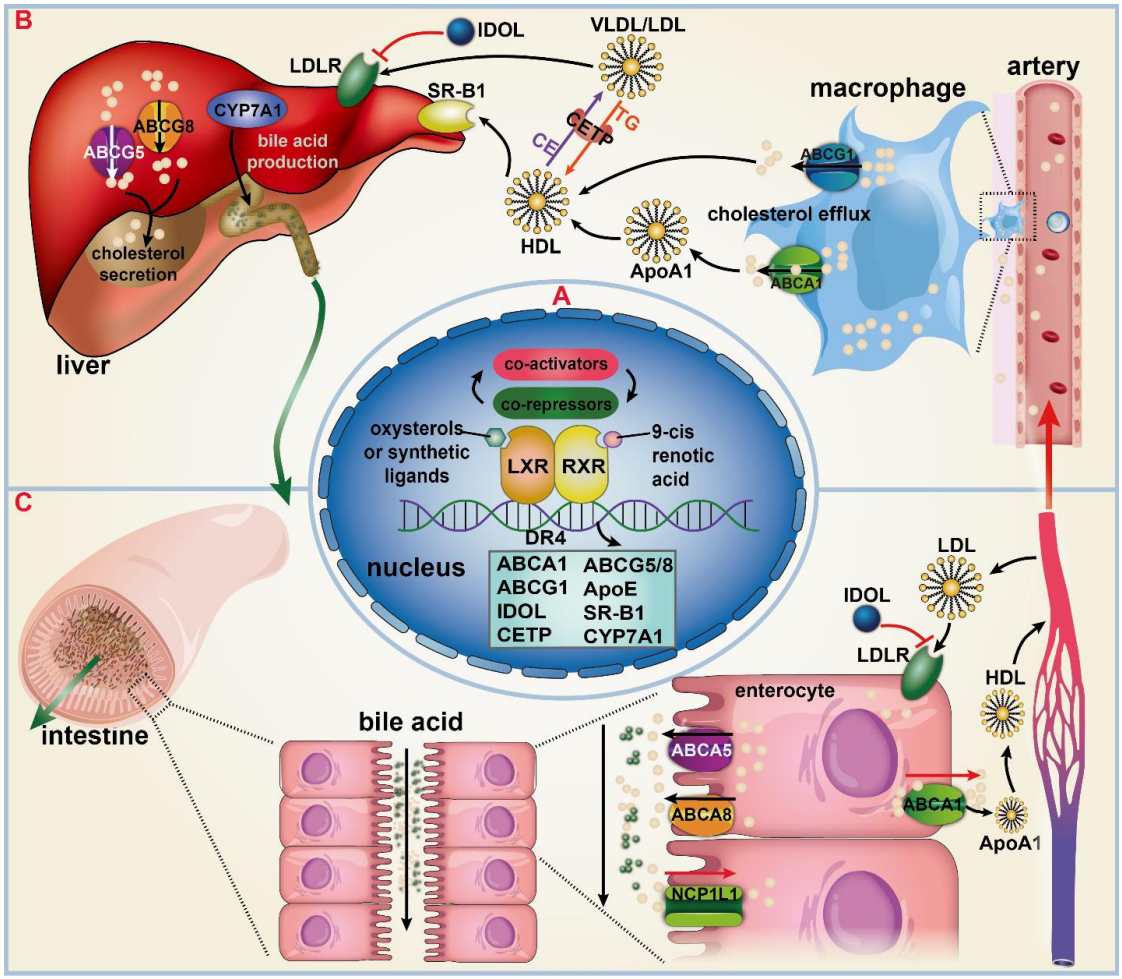

Figure 1. LXR-RXR heterodimers and the actions of LXRs in RCT and TICE. **A.** This diagram summarizes how the LXR-RXR heterodimer regulates the transcription of its target genes. The LXR-RXR heterodimer binds to an LXR response element (LXRE), a specific DNA sequence represented by a repeated AGGTCA sequence that is separated by four nucleotides (a DR4 response element). When unbound, the LXR-RXR heterodimer remains bound to the promoter region of its target genes in a complex with co-repressors, such as SMRT and NCoR. Once bound to an agonist, the conformation of the LXR-RXR complex changes, leading to the release of its nuclear receptor co-repressors and the recruitment of nuclear receptor co-activators, such as ASC2 and EP300. **B.** This diagram summarizes the actions of LXRs in reverse cholesterol transport (RCT), which are described in the **LXRs and reverse cholesterol transport** section. However, the activation of LXRs also promotes the expression of CETP. VLDL and LDL particles bearing ApoB can unload cholesterol from HDL particles through the action of CETP. The blockade of CETP can thus augment HDL levels, but the process is not yet known to produce a clinical benefit. **C.** This diagram summarizes the actions of LXRs in trans-intestinal cholesterol efflux (TICE), which are illustrated in the **LXRs and trans-intestinal cholesterol excretion** section. Abbreviations: RXR, retinoid X receptor; ABCA1, ATP-binding cassette subfamily A type 1; HDL, high-density lipoprotein; LDLR, low-density lipoprotein receptor; SR-B1, scavenger receptor class B type 1; CYP7A1, cholesterol 7-α-hydroxylase; IDOL, inducible degrader of the LDL receptor; CETP, cholesteryl ester transfer protein; CE, cholesteryl ester; TG, transfers triglyceride.

the cholesterol is internalized, trafficked across the cell, and secreted into the lumen of the small intestine (Brufau et al., 2011; Marshall et al., 2014). Several direct LXR target genes are closely associated with the TICE pathway, including ABCA1, ABCG5 and ABCG8. In addition, the activation of LXRs by agonists has been shown to stimulate TICE in mice by 100% (van der Veen et al., 2009).

At the cellular level, lipoprotein receptors that are expressed on the basolateral surface of enterocytes, such as LDLRs, take up circulating cholesterol. Then, transporters on the apical surface, such as ABCG5 and ABCG8, secrete cholesterol into the lumen (Hong and Tontonoz, 2014). Complementing this pathway, Niemann-Pick C1-like protein (NPC1L1), another transporter that is expressed on the apical surface, plays a significant role in the absorption of dietary cholesterol from the lumen (Altmann et al., 2004). Moreover, cholesterol can also be transferred to ApoA1 on the basolateral membrane via ABCA1, thus contributing to HDL formation (Pannu et al., 2013) (Figure 1C).

Downstream genes of LXRs in the regulation of cholesterol transport

ABCA1 and ABCG1

ABCA1, an integral membrane transporter, plays a major role in HDL formation and was originally discovered in patients with Tangier disease. These patients display near-absent HDL levels and a loss of ABCA1 function (Bodzioch et al., 1999). ABCA1 facilitates the movement of cell cholesterol and phospholipids onto exchangeable Apos, in particular ApoA1, to initiate the formation of HDL particles (Oram and Heinecke, 2005). Studies using tissue-specific ABCA1 knockout mice have shown that hepatocyte deletion results in a nearly 80% decrease in plasma HDL cholesterol levels (Brunham et al., 2009), with enterocyte deletion accounting for 30% of the decrease (Brunham et al., 2006), and adipocyte deletion causing 15% of the decrease (Chung et al., 2011). In contrast, the expression of ABCA1 in macrophages and other hematopoietic cells does not contribute to plasma HDL cholesterol levels (Haghpassand et al., 2001). ABCA1 is transcriptionally induced by LXRs, which are activated by the accumulation of oxysterols (Costet et al., 2000). Moreover, the treatment of mice with synthetic LXR agonists upregulated ABCA1 expression and increased plasma HDL cholesterol levels, thus establishing an essential role for LXRs in cholesterol efflux via ABCA1 (Costet et al., 2000).

In contrast with ABCA1, another ABC transporter, ABCG1, has been shown to mediate cholesterol efflux to HDL particles but not lipid-free Apos (Kennedy et al., 2005). ABCA1 and ABCG1 thus have complementary roles in mediating cholesterol efflux to HDL. *Abcg1*$^{-/-}$ mice exhibit a striking lipid phenotype of cholesterol accumulation in macrophages in multiple tissues, particularly in the lungs. However, unlike ABCA1, the role of ABCG1 in atherosclerosis progression is quite complex, and ABCG1 alone does not markedly influence atherosclerosis in mice (Tarling, 2013; Westerterp et al., 2014). When given a high-fat diet, the loss of total body ABCG1 transport does not significantly promote atherosclerosis, and the loss of macrophage ABCG1 in the context of the *Ldlr*$^{-/-}$ or *ApoE*$^{-/-}$ atherosclerosis models has been controversially reported to be either pro- or anti-atherogenic (Baldan et al., 2006; Lammers et al., 2009; Out et al., 2006; Ranalletta et al., 2006; Vaughan and Oram, 2005; Westerterp et al., 2014). Interestingly, the loss of ABCA1 and ABCG1 in mice results in a synergistic increase in tissue lipid accumulation (Out et al., 2008; Yvan-Charvet et al., 2007), and the induction of ABCA1 and ABCG1 expression by cholesterol loading and synthetic LXR agonists suggests a coordinated role for these two transporters in managing cellular cholesterol overload (Aye et al., 2010).

ABCG5 and ABCG8

Two additional members of the ABC transporter family, ABCG5 and ABCG8, form the obligate heterodimer that limits intestinal absorption and facilitates the biliary secretion of cholesterol and phytosterols (Graf et al., 2002; Yu et al., 2014). Consistent with these functions, ABCG5 and ABCG8 are expressed almost exclusively on the brush border membranes of enterocytes and in the canalicular membranes of hepatocytes (Yu et al., 2014). LXRα is regarded as the major regulator of ABCG5 and ABCG8 mRNA expression, and the LXR agonist T0901317 markedly upregulates ABCG5 and ABCG8 expression in the small intestine and liver of wild type but not LXRα knockout mice (Gonzalez-Granillo et al., 2012; van der Veen et al., 2007). Mutations in either of these two genes results in sitosterolemia, which causes the accumulation of plant sterols and cholesterol in the circulation,

leading to premature cardiovascular diseases (Berge et al., 2000). However, the overexpression of ABCG5 and ABCG8 in mice retards diet-induced atherosclerosis by reducing circulating and hepatic cholesterol (Wilund et al., 2004).

ApoE

ApoE is a major component of very low-density lipoproteins (VLDLs), remnant lipoproteins and HDL. ApoE on the surface of lipoproteins serves as a natural ligand for many receptors, including LDLRs, LDLR-related proteins, VLDL receptors and heparin sulfate proteoglycans (Li and Liu, 2014). ApoE-containing HDL participates in cholesterol efflux and contributes to the maintenance of plasma and tissue cholesterol homeostasis (Mahley and Rall, 2000). ApoE is mainly synthesized by the liver but is also produced by the brain, kidney, adipocytes, smooth muscle cells and macrophages (Li and Liu, 2014). Interestingly, LXR activation of ApoE is tissue specific and stimulates ApoE expression in macrophages and adipose tissue but not in the liver (Laffitte et al., 2001). Several other genes that encode Apos, including ApoC1, ApoC2, ApoC4 and ApoD, are also LXR-responsive (Hong and Tontonoz, 2014).

SR-B1

SR-B1 is a physiologically relevant lipoprotein receptor that regulates the selective uptake of lipids in tissues such as the liver, adrenal glands and ovaries. Studies have reported that SR-B1 also localizes to atherosclerotic plaques and the endothelium (Hirano et al., 1999; Yuhanna et al., 2001). SR-B1 is believed to play a major role in the delivery of HDL-derived cholesteryl esters to the liver (Swarnakar et al., 1999). In humans, the *SR-B1* gene (*SCARB1*) is located on chromosome 12 (12q24.31). The *SR-B1* gene contains an LXRE region that responds to oxysterol-stimulated LXR activation (Malerod et al., 2002).

CYP7A1

CYP7A1 belongs to the large family of P450 cytochrome proteins and is the rate-limiting enzyme during the synthesis of bile acid in the liver, which occurs through a classic pathway by producing 7-α-hydroxycholesterol (Iwanicki et al., 2015). In humans, the *CYP7A1* gene is located on chromosome 8 (8q11.12) and consists of 6 exons and 5 introns (Noshiro and Okuda, 1990). However, the regulation of *CYP7A1* by cholesterol varies across species. Although rodents possess an LXRE in *CYP7A1*, humans do not (Goodwin et al., 2003). Moreover, LXRα, but not LXRβ, is regarded as the major regulator of CYP7A1 mRNA expression in the mouse liver (Peet et al., 1998b).

Cholesteryl ester transfer protein (CETP)

CETP is a protein that transfers triglyceride (TG) from VLDL or LDL and in exchange for cholesteryl ester (CE) from HDL, and CETP has also been documented to be regulated by LXRs (Barter et al., 2003; Honzumi et al., 2010; Tall et al., 2008). CETP is synthesized in the liver and secreted into the plasma, and its activity influences the atherogenicity of the lipoprotein profile and cholesterol efflux in macrophages (Tall et al., 2008). In humans, CETP deficiency characteristically exhibits high HDL and low LDL levels (Brown et al., 1989; Inazu et al., 1994), but the correlation of plasma CETP level with atherosclerosis remains controversial and has not yet been clearly demonstrated in studies involving human populations (Dullaart et al., 2007; Quintao, 2016). Some species, such as rats and mice, do not express CETP, and transfer of cholesterol and CE to the liver mainly via HDL (Kingwell et al., 2014). Therefore, CETP-containing species, such as hamsters, monkeys, and ApoB/CETP double-transgenic mice, have been used to evaluate the effects of CETP expression on lipid metabolism and atherosclerosis development (Honzumi et al., 2010; Kingwell et al., 2014). Numerous studies suggest that CETP may have pro- or anti-atherogenic properties depending upon the pathophysiological settings, and whether the CETP inhibition-induced lipoprotein changes exert anti-atherogenic activities remains a matter of debate (Dullaart et al., 2007; Ghosh and Ghosh, 2012; Quintao, 2016). LXRα has an essential role in the induction of CETP, whereas LXRβ activation has only a minor effect (Honzumi et al., 2010). T0901317 enhanced plasma CETP activity and resulted in increased non-HDL and decreased HDL, which suggests that the activation of CETP may potentially be an unwelcome side effect of pan-LXR agonists (Honzumi et al., 2010).

Inducible degrader of the LDLR (*IDOL)*

IDOL is an E3 ubiquitin ligase that mediates the ubiquitination and degradation of LDLR. IDOL expression is controlled at the transcriptional level by LXRs, which are independent of sterol regulatory element-binding protein (SREBP) and

proprotein convertase subtilisin/kexin type 9 (PCSK9) (Zhang et al., 2012). When cellular cholesterol levels rise, oxysterols are formed and serve as ligands for LXRs (Janowski et al., 1999). Activated LXRs induce IDOL production, which further limits the uptake of exogenous cholesterol by the LDLR pathway (Zhang et al., 2012). Synthetic LXR agonists are powerful regulators of RCT that inhibit the development of atherosclerosis in mice (Naik et al., 2006). However, synthetic LXR agonists also induce hepatic IDOL expression, reduce LDLR levels and raise plasma LDL levels in primates, which are undesirable side effects of LXR agonists (Hong et al., 2014). In addition, the effects of the LXR-IDOL pathway on LDLR protein levels are both tissue- and species-specific. In mice, LXR agonists induce IDOL expression in peripheral tissues but not in the liver and do not change plasma LDL levels. By contrast, LXR agonists raise plasma LDL cholesterol levels in primates through an IDOL-dependent mechanism, and IDOL is highly induced by LXR agonists in human hepatocyte cell lines (Hong et al., 2014; Zelcer et al., 2009). Therefore, the LXR-IDOL pathway may be a potential target for the modulation of LDL cholesterol levels, and IDOL inhibition may mitigate the undesirable effects of synthetic LXR agonists on plasma LDL cholesterol levels in humans.

Promoting the cholesterol removal activity of LXR agonists against atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the vasculature that is characterized by lipid accumulation and plaque development within arteries. LXRs function as whole-body cholesterol sensors, and their activation contributes to protection against atherosclerosis. LXRα/β double-knockout mice exhibited increased aortic foam cell accumulation after 18 months of being fed a normal chow diet (Schuster et al., 2002). In contrast, the activation of LXRs resulted in the removal of cholesterol from the body and the amelioration of plasma lipoprotein levels by mobilizing cholesterol from the periphery, reducing cholesterol uptake in the intestine, promoting cholesterol hepatic/intestinal excretion and enhancing its conversion to bile acids (Bonamassa and Moschetta, 2013).

Oxysterols and synthetic LXR agonists

Oxysterols and synthetic LXR agonists are powerful regulators of RCT and TICE that inhibit the development of atherosclerosis in mice. The natural ligands of LXRs are oxygenated forms of cholesterol and certain bile acids, such as 22(R)-hydroxycholesterol (22R-OHC), 20(S)-hydroxycholesterol (20S-OHC), 24(S)-hydroxycholesterol (24S-OHC), 25-hydroxycholesterol (25-OHC), 27-hydroxycholesterol (27-OHC), and 24(S),25-epoxycholesterol (24S,25-EC) (Beyea et al., 2012; Jakobsson et al., 2012; Pannu et al., 2013). The rank order of serum LXR ligand oxysterols levels is 27-OHC > 22R-OHC > 25-OHC (ng/mg) in healthy populations (Ikegami et al., 2012; Koschack et al., 2009), and the order of the ability to activate LXRs is 27-OHC > 24S, 25-EC > 22R-OHC > 20S-OHC > 24S-OHC > 25-OHC (nM or μM) (Beltowski, 2008; Fu et al., 2001; Janowski et al., 1996). The development of LXR agonists can be traced back to the 2000s, as well as the discovery of the first two agonists, T0901317 and GW3965 (Collins et al., 2002) (Figure 2). These compounds are full agonists for both LXRα and LXRβ and show anti-atherosclerotic effects. Unfortunately, their positive effects on atherosclerosis are countered by the induction of hepatic lipogenesis, which leads to hypertriglyceridemia and liver steatosis (Schultz et al., 2000), and other undesirable side effects of LXR agonists, such as the degradation of hepatic LDLRs through the LXR-IDOL pathway (Hong et al., 2014).

In addition to their central role in cholesterol homeostasis, LXRs are also important regulators of hepatic lipogenesis. In response to natural or synthetic ligands, the activation of LXRs induces the expression of two master transcription factors, sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP), which control hepatic lipogenesis and triglyceride secretion and cause hypertriglyceridemia (Faulds et al., 2010). Although both LXRα and LXRβ are capable of inducing hepatic lipogenesis, studies in LXR knockout mice have suggested that LXRα is the dominant isoform in this pathway (Repa et al., 2000). Therefore, the specific targeting of LXRβ has been proposed to achieve anti-atherosclerotic benefits while avoiding hepatic lipogenesis and consequent hepatosteatosis.

Members of the first generation of synthetic LXR agonists, including T0901317 and GW3965, were unsuitable for clinical development due to their pleotropic effects, but these compounds are valuable to the research community. In recent

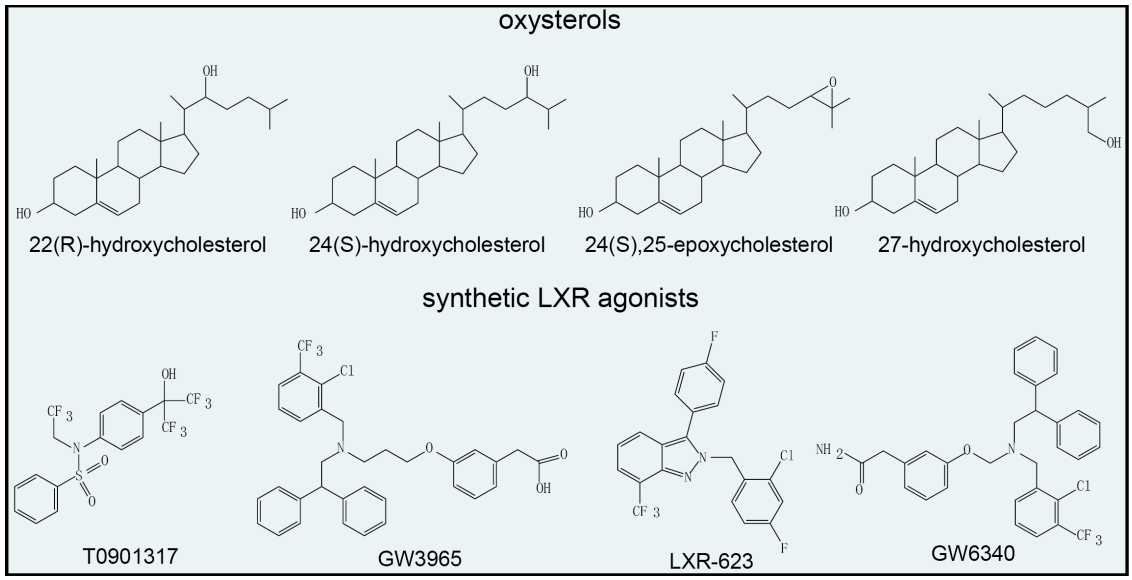

Figure 2. Molecular structures of oxysterols and synthetic LXR agonists.

years, several studies have been devoted to identifying new synthetic LXR agonists. *N,N*-dimethyl-3β-hydroxy-cholenamide (DMHCA) represents a gene-selective LXR modulator that mediates the potent transcription activation of ABCA1 gene expression while exhibiting minimal effects on SREBP-1c both in vitro and in vivo in mice (Quinet et al., 2004). Moreover, AZ876 is a newly developed dual LXRα/β partial agonist that has been shown to reduce atherosclerosis in mice without affecting liver or plasma TG levels when administered at low doses (van der Hoorn et al., 2011). However, these agonists have been evaluated in clinical trials, and whether they are safe for humans requires further investigation. LXR-623 (WAY-252623), an LXRα/β partial agonist, was the first LXR-targeted compound to enter clinical trials, and published data described a dose-dependent increase in ABCA1 and ABCG1 expression in peripheral blood cells of healthy individuals (Katz et al., 2009). LXR agonists could cross the blood-brain barrier and exert advantageous effects on the central nervous system (CNS) (e.g., effects against neuroinflammation and Alzheimer's disease) (Li et al., 2010; Stukas et al., 2012; Wu et al., 2016). Unfortunately, the published Phase I clinical results of LXR-623 suggested that further development of the compound was discontinued due to adverse CNS effects, and it is still unknown whether the adverse CNS observations were off-target effects specific to LXR-623 or were related to LXR modulation in the brain (Hong and Tontonoz, 2014; Katz et al., 2009; Li et al., 2010). To date, similar adverse CNS events in humans have not been reported for other LXR agonists. Moreover, several other compounds, including CS8080, BMS-779788 (XL-652) and BMS-852927 (XL-041), entered Phase 1 clinical trials and exhibited limited success but were terminated due to their unexpected adverse effects (Loren et al., 2013) (Table 1). More detailed reviews of those patented compounds can be found elsewhere (Li et al., 2010; Loren et al., 2013). In addition, the intestine-specific LXRα/β agonist GW6340, an ester form of the systemic LXRα/β agonist GW3965, was discovered. GW6340 significantly upregulates ABCA1, ABCG5 and ABCG8 expression in the small intestine but not in the liver, and it avoids the hypertriglyceridemia effects induced by systemic LXR ligands (Yasuda et al., 2010).

Interestingly, in addition to the genomic effects of LXRs on cholesterol transport, several studies have reported that LXRs also play non-genomic roles in macrophage cholesterol homeostasis. Huwait and colleagues demonstrated that the JNK/c-Jun/AP-1 pathway participates in the LXR

Table 1. The synthetic LXR agonists.

Synthetic agonists	Company	Type	ClinicalTrials.gov Identifier	Comments	References
T0901317	Tularik (now Amgen)	Pan-agonist	None	The first described synthetic LXR agonist Used as a tool compound in research	(Schultz et al., 2000)
GW3965	GlaxoSmithKline	Pan-agonist	None	Used as a tool compound in research	(Collins et al., 2002)
LXR-623 (WAY-252623)	Wyeth (now Pfizer)	High affinity for LXRβ but partial LXRα agonist	NCT00366522 (Phase 1, completed) NCT00385489 (Phase 1, completed) NCT00379860 (Phase 1, terminated)	The first LXR-targeted compound to enter clinical trials Potential treatment of atherosclerosis and dyslipidemia Trial was terminated due to adverse CNS effects (150 and 300 mg/day)	(Hong and Tontonoz, 2014; Katz et al., 2009; Li et al., 2010)
AZ876	None	LXRα/β partial agonist	None	WIPO Patent Application WO200607336 Anti-atherosclerosis effects in mice without affecting triglyceride levels when administered at low doses	(Cannon et al., 2015; van der Hoorn et al., 2011)
GW3640	GlaxoSmithKline	Intestinal-specific LXR agonist	None	Promoting macrophage RCT in vivo in mice	(Yasuda et al., 2010)
CS8080	Daichii Sankyo	Not known	NCT00613431 (Phase 1, completed) NCT00796575 (Phase 1, terminated)	By late 2008, the project was terminated due to undisclosed safety concerns	(Li et al., 2010; Loren et al., 2013)
BMS-779788 (XL-652)	Exelixis and Bristol-Myers Squibb	Not known	NCT00836602 (Phase 1, completed)	Potential treatment of atherosclerosis Proved safety but data have not yet been reported	(Li et al., 2010; Loren et al., 2013)
BMS-852927 (XL-041)	Exelixis and Bristol-Myers Squibb	Not known	NCT01651273 (Phase 1, terminated)	Potential treatment of dyslipidemia Terminated for undisclosed reasons in 2013	(Li et al., 2010; Loren et al., 2013)

agonist-mediated induction of ApoE and ABCA1 expression, which are implicated in the control of macrophage cholesterol efflux (Huwait et al., 2011). Moreover, Buono and colleagues reported that the synthetic and natural LXR agonists, T0901317 and 22-OHC, downregulate macrophage LDL uptake by inhibiting the fluid-phase pinocytosis of native LDL (Buono et al., 2007). Therefore, the activation of LXR transcription factors may be atheroprotective via other novel mechanisms.

Mitochondrial translocator protein (TSPO)

The mitochondrial 18 kDa TSPO [peripheral benzodiazepine receptor (PBR)] is located at the contact sites between the outer and inner mitochondrial membranes and may be therapeutically useful for reducing atherosclerosis (Allen et al., 2013). TSPO has five transmembrane domains and a high affinity for the cholesterol/interaction recognition amino acid consensus (CRAC)-binding C-terminal domain (Allen et al., 2013). TSPO knockdown induced the arrest of mitochondrial cholesterol transport. In contrast, the activation of TSPO by its ligands plays a role in fasting metabolism by reducing

lipogenesis and hepatosteatosis in obese mice (Allen et al., 2013; Gut et al., 2013). Moreover, recent work by Taylor *et al.* showed that TSPO overexpression or ligation induced the expression of LXRα, peroxisome proliferation-activated receptor α (PPARα), ApoE, ABCA1, and ABCG4, but not ABCG1, and contributed to the increased efflux of cholesterol to ApoA1 and HDL into macrophages. In addition, the overexpression or ligation of TSPO also caused a decline in macrophage total neutral lipid mass without inducing lipogenesis and effectively prevented foam cell formation by limiting cholesterol esterification following exposure to acetylated LDL (Taylor et al., 2014). Mechanistically, the coordinated induction of PPARα and LXRα by TSPO may be a key factor in abrogating the side effects associated with the activation of LXRα by agonists, such as T0901317. TSPO ligand treatments have been previously reported to promote mitochondrial 27-OHC production (a natural ligand of LXRs, as mentioned above) (Tsankova et al., 1996); however, whether this effect contributes to LXR activation remains unknown. Altogether, targeting mitochondrial TSPO activity or maintaining its function could be therapeutically useful for reducing atherosclerosis, and determining whether it will be useful in primates or humans warrants further investigation.

Heme and metformin

Mhem is a novel adaptive macrophage phenotype that was discovered in human plaques (Boyle et al., 2009). Mhem exhibits coordinated properties, including increased heme oxygenase-1 (HO-1) expression, reduced oxidative stress, a suppressed inflammatory response, increased lipid export and resistance to foam cell formation (Boyle et al., 2009). Recent work by Wan *et al.* has shown that the concentration of heme or metformin drives macrophages to exhibit the Mhem phenotype (Wan et al., 2013).

Metformin is a relatively safe oral hypoglycemic agent that is effectively used to treat type 2 diabetes mellitus without causing hypoglycemia (El Messaoudi et al., 2011). Metformin also shows cardioprotective effects by improving myocardial remodeling and protecting against ischemia-reperfusion injury (El Messaoudi et al., 2011). In addition, Wan *et al.* demonstrated that a pathophysiological concentration of heme (10 μmol/L) and a pharmacologically relevant concentration of metformin (also 10 μmol/L) increased activating transcription factor 1 (ATF1) expression via the activation of 5'-AMP-activated protein kinase (AMPK). The AMPK-ATF1 pathway drives the transcriptional co-induction of HO-1 and LXRβ, which allows the interlinking of antioxidant activity resistance to foam cell formation and macrophage deactivation (Wan et al., 2013). Accordingly, AMPK-ATF1 pathway modulation by metformin may provide an effective therapeutic strategy for the treatment of atherosclerosis. Whether this pathway protects against vascular disease *in vivo* and what downstream genes of LXRβ are induced by metformin remain to be determined.

Other potential LXR agonists

In addition to the previously mentioned LXR agonists, other mediators of LXRs also confer protection against atherosclerosis (Table 2). Fibroblast growth factor-21 (FGF21), a member of the FGF superfamily, is an important endogenous regulator of systemic glucose and lipid metabolism. Elevated serum FGF21 levels have been reported in subjects with coronary heart disease and carotid artery plaques (Habegger et al., 2013). Recent work by Lin *et al.* has demonstrated that FGF21 promotes cholesterol efflux by upregulating ABCA1 through the extracellular signal-regulated kinase 1 and 2 (ERK1/2)/PPARγ/LXRα pathway in THP1 macrophage-derived foam cells in a dose- and time-dependent manner (Lin et al., 2014). Soluble guanylyl cyclase (sGC) is a key intracellular signaling acceptor of endothelial nitric oxide synthase-derived nitric oxide (NO) in smooth muscle cells (Angelone et al., 2008). Tsou *et al.* reported that the activation of sGC by 3-(5'hydroxymethyl-2'furyl)-1-benzyl indazole (YC-1) leads to the LXRα-dependent upregulation of ABCA1 in macrophages and may confer protection against atherosclerosis (Tsou et al., 2014). In addition, Campia and colleagues observed that cardioactive glycosides, such as digoxin and ouabain, activate LXRs and increase the expression of ABCA1 but not ABCG1 (Campia et al., 2012). Accordingly, glycosides may be novel therapeutic targets for use against atherosclerosis. However, little is known about the anti-atherogenic biological interaction between sGC activation and LXRα. Whether the activation of LXRα by FGF21, YC-1 and glycosides induces hepatic lipogenesis may warrant further investigation.

Table 2. Potential LXR agonists involved in anti-atherogenic effects.

LXR agonists	Experimental models	Mechanisms	References
TSPO	Human THP-1 monocytes	Inducing the expression of LXRα, PPARα, ApoE, ABCA1 and ABCG4	Taylor et al., 2014
Metformin	Human blood-derived macrophages	Promoting HO-1 and LXRβ expression through the AMPK-ATF1 pathway	Wan et al., 2013
FGF21	THP-1 macrophage-derived foam cells	Upregulating ABCA1 through the ERK1/2/ PPARγ/LXRα pathway	Lin et al., 2014
YC-1	TIB-67 cells and ApoE KO mice injected with YC-1	Promoting the expression of ABCA1 and LXRα	Tsou et al., 2014
Digoxin	H9c2 cells and FVB male mice injected with digoxin	Promoting the expression of ABCA1 and LXRs	Campia et al., 2012
Lactobacillus plantarum PCS26	Human small intestinal fetal epithelial cells	Upregulating ABCG5/ABCG8 and downregulating NPC1L1 expression	Gorenjak et al., 2014
Pravastatin	Human Hep3B cells	Promoting CYP7A1 and ABCG5/ABCG8 expression through the PPARγ/LXRα pathway	Byun et al., 2014

TSPO, translocator protein; HO-1, heme oxygenase 1; ATF1, activating transcription factor 1; AMPK, 5'-AMP-activated protein kinase; ERK1/2, extracellular signal-regulated kinases 1 and 2; YC-1, 3-(5'hydroxymethyl-2'furyl)-1-benzyl indazole

Several studies have shown that probiotic bacteria exert positive effects on hypercholesterolemia by lowering serum cholesterol and improving lipid profiles, which in turn leads to a reduced risk of atherosclerosis and coronary heart disease (Sanders, 2000). *Lactobacillus plantarum* PCS26 may act as an LXR agonist and may therefore promote ABCG5/ABCG8 expression and downregulate NPC1L1 expression in small intestine cells (Gorenjak et al., 2014). Accordingly, *Lactobacillus plantarum* PCS26 has the potential to promote biliary cholesterol efflux and inhibit intestinal absorption, but it also warrants study in *in vivo* clinical trials for further assessment. Statins are extremely effective at reducing cardiovascular risk, as demonstrated in clinical studies that have confirmed the inhibition of the onset and progression of atherosclerosis (Zhao et al., 2015). Byun *et al.* reported that pravastatin enhances hepatic bile acid synthesis via the PPARγ-LXRα-CYP7A1 pathway and activates PPARγ-LXRα-ABCG5/ABCG8, which excretes cholesterol into bile (Byun et al., 2014). In addition, Ginnarelli and colleagues reported that LXR-623 significantly reduces the progression of atherosclerosis and induces plaque regression when used in combination with simvastatin, a finding that may drive the future development of novel anti-atherosclerotic therapeutic approaches (Giannarelli et al., 2012).

Generally speaking, the potential LXR agonists mentioned above may exert anti-atherogenic activities via the stimulation of LXRs. However, as with statins, their anti-atherogenic effects may be largely or partially related to LXR activation. Therefore, the use of cells or animals with genetic alterations related to LXRs may reveal whether LXR-dependent pathways play major roles in the anti-atherosclerosis effects exerted by those agents.

Anti-inflammatory activity of LXRs against atherosclerosis

Macrophages

Inflammation plays a significant role in the development of atherosclerosis. In addition to their beneficial effects on macrophage cholesterol efflux, LXRs may also modulate atherosclerosis through their anti-inflammatory roles (Steffensen et al., 2013; Zelcer and Tontonoz, 2006). Mechanistically, these effects of LXRs are attributed to the nuclear inhibition of nuclear factor κB (NF-κB) signaling via transrepression (Calkin and Tontonoz, 2010; Ghisletti et al., 2007). Interestingly, ligand-bound LXRs are not recruited to LXREs in pro-inflammatory gene promoters but are conjugated to a small ubiquitin-like modifier (SUMO) protein. In the promoters of NF-κB target genes, SUMOylated LXRs prevent the removal of the NCoR/SMRT co-repressor from NF-κB, which results in the maintenance of downstream inflammatory genes, such as *matrix metallo-*

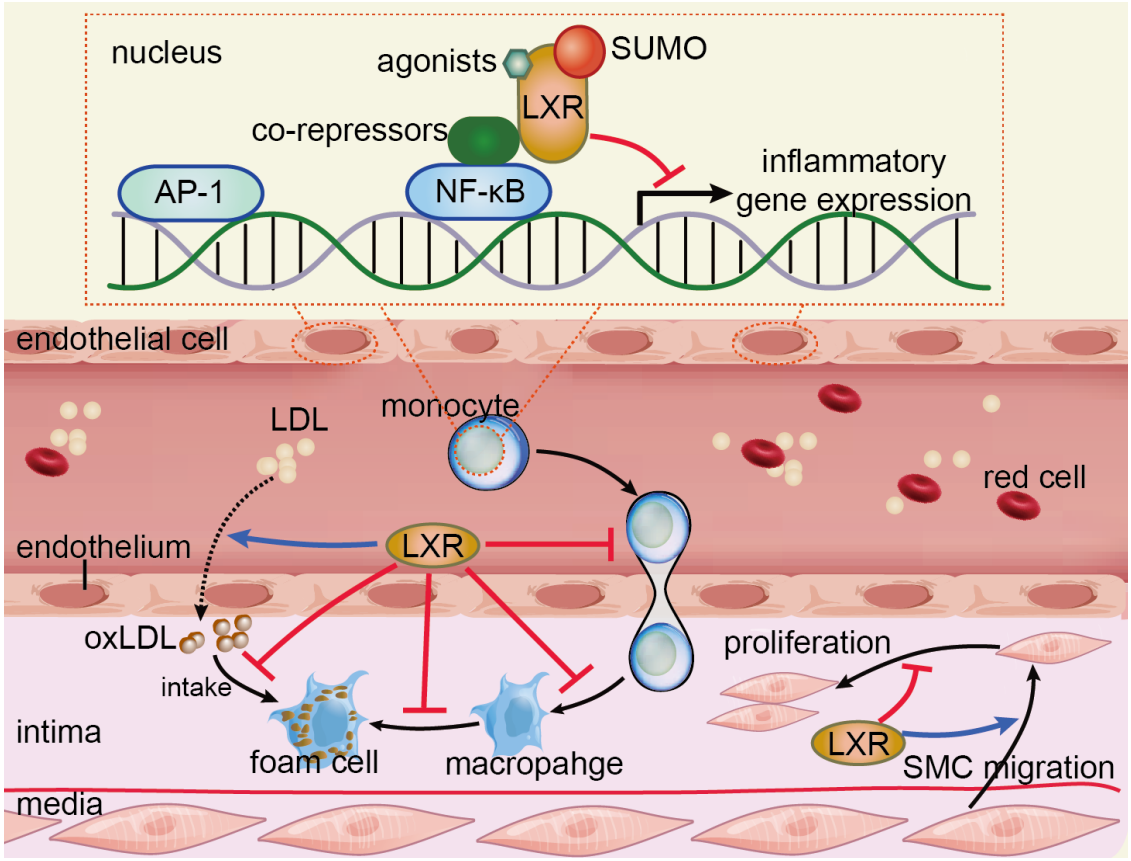

Figure 3. Molecular mechanisms of LXR transrepression and anti-inflammatory actions of LXRs in atherosclerosis. Inflammatory gene promoters are most often associated with the binding sites for NF-κB or AP-1 transcription factors. SUMOylated LXR is recruited to a pro-inflammatory gene promoter, where it binds and stabilizes the transrepression complex, thereby repressing pro-inflammatory signaling in macrophages and endothelial cells. Moreover, LXR activation increases macrophage cholesterol efflux and decreases macrophage infiltration and SMC proliferation. The roles of LXRs in macrophages and SMCs are highlighted with blue arrows. Abbreviations: NF-κB, nuclear factor κB; AP-1, activator protein 1; SMC, smooth muscle cell.

proteinase-9 (*MMP-9*), in a repressed state (Ghisletti et al., 2007; Im and Osborne, 2011; Khan et al., 2016) (Figure 3). Recent work by Kappus *et al.* showed that LXR activation by T0901317 decreased inflammatory gene expression *in vitro* in lipopolysaccharide (LPS)-stimulated macrophages that lacked ABCA1 and ABCG1. These inflammatory genes included *interleukin-1β* (*IL-1β*), *IL-6*, *monocyte chemo-attractant protein 1* (*Mcp-1*) and *macrophage inflammatory protein 1α* (*Mip-1α*). Moreover, LXR activation decreased atherosclerosis and macrophage infiltration in *Ldlr*$^{-/-}$ mice that received transplanted *Abca1*$^{-/-}$ *Abcg1*$^{-/-}$ bone marrow (Kappus et al., 2014). These findings suggest that selective LXR agonists that mediate inflammatory transrepression without directly targeting cholesterol efflux, IDOL or lipogenic genes should be further developed as anti-atherosclerosis therapeutic methods.

Endothelial cells and smooth muscle cells

In contrast with the well-studied role of LXR signaling in macrophages, the impact of LXR activity on other cells that directly affect atherosclerosis, including endothelial cells and smooth muscle cells (SMCs), has been investigated to a lesser extent. Blaschke *et al.* have demonstrated that both LXRα and LXRβ are expressed in human coronary artery SMCs and that the activation of LXRs by T0901317 and GW3965 inhibits SMC proliferation and neointima formation after balloon injury (Blaschke et al., 2004). Moreover, 4-1/2 LIM protein 2 (FHL2), interacting with and regulating several transcription factors, is not only involved in the phenotypic modulation of SMCs but also

inhibits SMC proliferation and migration. A recent study by Kurakula *et al.* showed that FHL2 is a co-activator of LXRs in SMCs and enhances ApoA1- and HDL-mediated cholesterol efflux via regulating ABCA1 expression at the transcriptional level (Kurakula et al., 2015). Thus, those studies suggest that LXR activation may exert atheroprotective roles in SMCs.

Vascular endothelial dysfunction occurs during the early onset of atherogenesis, and inflammation is an important trigger of endothelial dysfunction. Endothelin-1 (ET-1), which is primarily produced by vascular endothelial cells, plays an important role in the pathogenesis of inflammatory diseases. Gao *et al.* reported that the activation of LXRs significantly attenuates LPS-induced ET-1 expression by inhibiting AP-1 or NF-κB signaling via transrepression (Gao et al., 2012). Spillmann and colleagues reported that LXR agonists (T0901317 and GW3965) directly improve TNFα-induced endothelial dysfunction by their anti-apoptotic, anti-inflammatory, and anti-oxidative properties and their capacity to restore NO bioavailability, independent of their cholesterol-modulating effects (Spillmann et al., 2014). Although the roles of LXRs in endothelial cells and SMCs are less understood, these results suggest that LXRs exert an anti-atherosclerotic effect in these cells, and these findings warrant further investigation.

Role of LXRs against myocardial diseases

Myocardial ischemia/reperfusion injury

Previous studies have demonstrated that both LXRα and LXRβ are expressed in the cardiovascular system, where they may play significant roles in protecting against myocardial ischemia/reperfusion (MI/R) injury (Lei et al., 2013). Recent work by Wang *et al.* demonstrated that T0901317 enhances the functional survival of transplanted adipose-derived mesenchymal stem cells (AD-MSCs) in infarcted myocardium via the modulation of the Toll-like receptor 4/myeloid differentiation factor 88/NF-κB and Kelch-like ECH-associated protein 1/Nrf2/heme oxygenase-1 signaling pathways. Moreover, a combined therapy consisting of an LXR agonist and AD-MSCs had a synergetic effect on cardiac repair and functional improvement after infarction (Wang et al., 2014). Using an MI/R model of LXR-deficient (LXRα knockout, LXRβ knockout or LXRα/β double-knockout) and LXR-overexpressing male mice, He *et al.* reported that LXRα, but not LXRβ, is significantly upregulated after MI/R. Moreover, the activation of LXRα instead of LXRβ plays a cardioprotective role in MI/R injury by reducing endoplasmic reticulum stress- and mitochondrial-mediated apoptosis. Mechanistically, LXRα activation significantly decreases myocardial NADPH oxidase expression, attenuates superoxide generation, and reduces iNOS and tissue nitrotyrosine content in myocardium after ischemia/reperfusion (He et al., 2014a). In addition, several members of the nuclear hormone receptor superfamily, such as PPARs, estrogen receptors and androgen receptors, have been proposed as the endogenous protective receptors that act against myocardial apoptosis and MI/R injury, while the activation of the farnesoid X receptor and Nur77 exacerbates myocardial apoptosis and MI/R injury (Lin et al., 2009; Pu et al., 2013; Tsang et al., 2008). Therefore, it is conceivable that potential regulatory cross-talk among LXRα and these nuclear factors may maintain a delicate homeostatic balance between cellular death and survival in the heart.

Diabetic cardiomyopathy

Diabetic cardiomyopathy (DCM) is defined as diabetes-induced pathologic abnormalities and includes myocardial metabolic disturbances, oxidative/nitrative stress, inflammation, cardiomyocyte apoptosis, left ventricular dysfunction and structural remodeling (Huynh et al., 2014). Several studies have suggested that LXRα may be involved in cardioprotection against DCM (Cheng et al., 2011; He et al., 2014b; Huynh et al., 2014). Recent work by He *et al.* demonstrated that LXRα, instead of LXRβ, is selectively upregulated by hyperglycemia in male diabetic mice (He et al., 2014b). Moreover, GW3965 exerts a cardioprotective effect against DCM by attenuating insulin resistance, restoring Akt phosphorylation, inhibiting mitogen-activated protein kinase (MAPK) phosphorylation, reducing oxidative/nitrative stress and suppressing NF-κB activation and inflammation (He et al., 2014b). Therefore, LXR is a potentially attractive molecular target for the treatment of DCM. In addition, further studies are warranted to define whether the activation of LXRα, LXRβ, or both is involved in myocardial protection against DCM and to better define the complex mechanisms involving LXR during the regulation of oxidative stress and inflammatory responses.

Myocardial hypertrophy

Myocardial hypertrophy is a major risk factor for the development of heart failure. It occurs in response to a wide range of pathological stimuli, such as arterial hypertension, valvular heart disease, myocardial infarction, inflammation and sarcomeric dysfunction by compensatory growth (Lorell and Carabello, 2000). LXR activation by T0901317 plays a cardioprotective role in attenuating myocardial hypertrophy *in vivo* (Kuipers et al., 2010a). LXRα activation suppresses the renin-angiotensin-aldosterone system (RAAS) by reducing the renin, angiotensin II type 1 receptor (AT1R) and angiotensin converting enzyme (ACE) expression in the heart and kidneys (Kuipers et al., 2010b). Moreover, Wu *et al.* reported that LXRα is upregulated in hypertrophic hearts induced to undergo left ventricular pressure overload, and the administration of T0901317 suppresses angiotensin II- and LPS-induced cardiomyocyte hypertrophy by inhibiting NF-κB signaling in mice. Both LXRα and LXRβ may inhibit NF-κB signaling, whereas LXRα may be more responsive to hypertrophic stimuli (Wu et al., 2009). Recent work by Cannon *et al.* demonstrated that LXR activation by AZ876 significantly reduces transverse aortic constriction-induced increases in heart weight, myocardial fibrosis, and cardiac dysfunction without affecting blood pressure. Mechanistically, AZ876 suppresses the upregulation of hypertrophy- and fibrosis-related genes and further inhibits pro-hypertrophic and pro-fibrotic transforming growth factor β (TGFβ)-Smad2/3 signaling (Cannon et al., 2015). Accordingly, LXRs represent a potential molecular target for anti-hypertrophic and anti-fibrotic therapies, and more selective LXR agonists should be further explored for their cardioprotective potential.

Future perspectives and conclusions

Thus far, emerging LXR agonists have been shown to be potential therapeutic targets for many diseases; however, the clinical translation of novel, safe and effective synthetic LXR agonists has become the biggest obstacle to overcome in the future. As characterized by the treatment for atherosclerosis or dyslipidemia, LXR-623, CS8080, BMS-779788 and BMS-852927, entered Phase 1 clinical trials and exhibited limited success but were terminated due to their unexpected or undisclosed adverse effects (Katz et al., 2009; Li et al., 2010; Loren et al., 2013) (Table 1). Unfortunately, there are still no clinically available synthetic LXR agonists. The discovery of LXRα/β partial agonists, such as LXR-623 and AZ876, is exciting (Katz et al., 2009; van der Hoorn et al., 2011). A balance between efficacy and several side effects, including hepatic lipogenesis, increases in TG, VLDL, CETP and IDOL levels, could be achieved with compounds that have partial activity for LXRα. However, the discovery of truly selective LXRβ agonists and safe LXR agonists requires further investigation.

Instead of atherosclerosis, synthetic LXR agonists play a protective role against several cardiovascular diseases. Using mice with multiple genetic alterations related to LXRs suggested that activation of LXRα, instead of LXRβ, participates in cardioprotection against MI/R injury and DCM development via its inhibitory roles on apoptosis, oxidative stress, and inflammation (He et al., 2014a; Huynh et al., 2014). However, there are some differences between humans and rodents in the LXR gene expression profile. Using other animal models, such as non-human primates and transgenic mice, which have LXR gene expression profiles more similar to that of humans (Honzumi et al., 2010), could provide more convincing evidence that LXRs are cardioprotective molecules. Moreover, such studies provide a theoretical basis for developing LXRα/β partial agonists to treat cardiovascular diseases.

Notably, several recent findings have indicated potential directions for future studies of treatments for atherosclerosis. MicroRNAs (miRNAs) modulate gene expression post-transcriptionally via base-pairing to target mRNAs, and they regulate a wide range of cellular processes (Ambros, 2004). Emerging evidence has suggested that several miRNAs participate in the regulation of the lipid metabolism. Recent work by Liu *et al.* showed that the translational activation of LXRα by miR-28-5p is involved in miR-28-5p-mediated ABCA1 upregulation in HepG2 cells and THP-1-derived macrophages, which may contribute to cardioprotection against atherosclerosis (Traini et al., 2014). Moreover, Ramírez and colleagues demonstrated that T0901317 increases miR-144 expression in macrophages and mouse livers. The overexpression of miR-144 reduces ABCA1 expression, while the silencing of miR-144 in mice increases the expression of ABCA1 and plasma HDL levels (Ramirez et al., 2013). Thus,

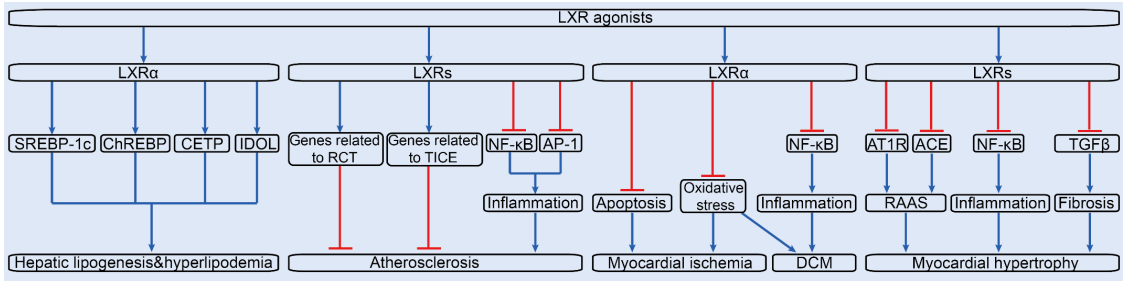

Figure 4. Mechanisms of the cardioprotection mediated by the LXRs signaling network. SREBP-1c, sterol regulatory element-binding protein-1c gene; ChREBP, carbohydrate response element-binding protein; CETP, cholesteryl ester transfer protein; IDOL, inducible degrader of the LDL receptor; NF-κB, nuclear factor κB; AP-1, activator protein 1; TICE, trans-intestinal cholesterol efflux; RCT, reverse cholesterol transport; DCM, diabetic cardiomyopathy; AT1R, angiotensin II type 1 receptor; ACE, angiotensin converting enzyme; RAAS, renin-angiotensin-aldosterone system; TGFβ, transforming growth factor β.

the modulation of miRNAs may represent a potential therapeutic approach for treating dyslipidemia and atherosclerotic vascular disease. In addition, other miRNAs, including miR-33 (Rayner et al., 2011), miR-785 (Ramirez et al., 2011), miR-106b and miR-26 (Kim et al., 2012; Sun et al., 2012), have also been shown to regulate the expression of ABCA1, and whether there are interactions between LXRs and these miRNAs remains to be determined.

In 2015, Zhang and colleagues demonstrated that nanoparticles (NPs) containing GW3965 (NP-LXR) inhibit the development of atherosclerosis without causing hepatic steatosis (Zhang et al., 2015). The engineered NPs are self-assembled in a biodegradable diblock poly(lactide-*co*-glycolide)-*b*-poly(ethylene glycol) (PLGA-*b*-PEG) copolymer (Swami et al., 2014). PLGA-*b*-PEG copolymers are biocompatible, biodegradable, and used in many products that are already approved by the US Food and Drug Administration (FDA). Moreover, NP-LXR is significantly more effective than free GW3965 at inducing LXR-targeted gene expression and suppressing inflammatory factors in macrophages *in vitro* and *in vivo*. In addition, the NPs elicit negligible levels of lipogenic gene stimulation in the liver (Zhang et al., 2015). Therefore, NPs encapsulating LXR agonists may be a promising nano-therapeutic approach for combating atherosclerosis without side effects.

We propose a model of the probable processes involved in LXR-related protection against several cardiovascular diseases by connecting all of the effectors and pathways described above. LXR agonists, such as oxysterols, synthetic LXR agonists, and non-classic LXR agonists, regulate the expression and function of LXRs. Furthermore, the activation of LXRs plays a cardioprotective role in promoting RCT and TICE, suppressing inflammation, and decreasing oxidation and apoptosis, as well as suppressing insulin resistance and hypertrophy. Taken together, these activities may constitute an interactive network that protects the heart against atherosclerosis, MI/R injury, DCM or myocardial hypertrophy. In this network, we may find that distinct pathways intersect, and these intersections may suggest new targets for exploring cardioprotection (Figure 4).

In summary, increasing evidence produced by basic research suggests that the activation of LXRs protects the heart from several cardiovascular diseases. Numerous regulators and signaling targets of LXRs have provided researchers with many opportunities to explore their underlying mechanisms. With the in-depth understanding of LXR biology and the development of innovative drug discovery strategies, several LXR isotype-selective agonists have emerged and even entered clinical trials. Unfortunately, no compounds have yet been approved by the US FDA for clinical treatment due to several unexpected side effects, such as adverse CNS events. However, it is undeniable that LXRs are important therapeutic targets for cardiovascular diseases and maintaining cholesterol homeostasis. Thus, the advancement of drug discovery programs could promote the development of safe and

effective LXR agonists for future clinical applications.

Disclosure
The authors declare no competing interests.

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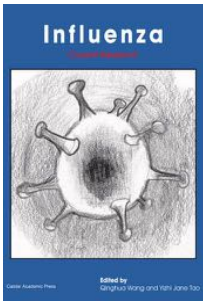

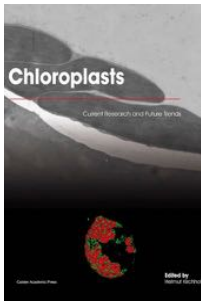

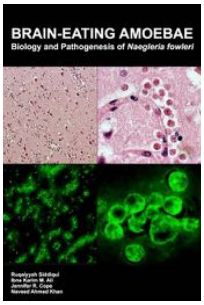

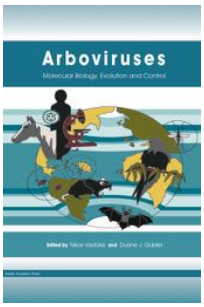

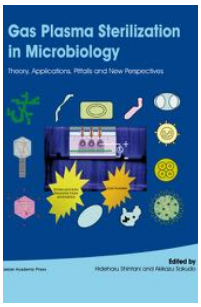

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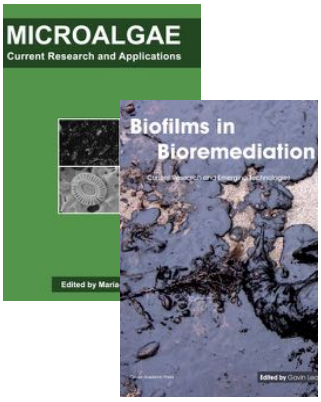

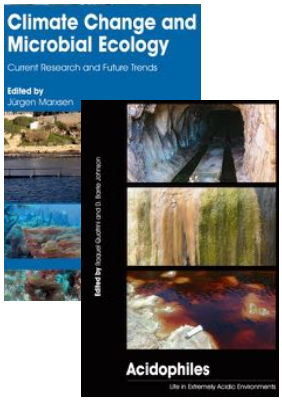

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