

Lipid droplet-associated proteins in atherosclerosis (Review)

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Abstract. Accumulation of atherosclerotic plaques in arterial walls leads to major cardiovascular diseases and stroke. Macrophages/foam cells are central components of atherosclerotic plaques, which populate the arterial wall in order to remove harmful modified low-density lipoprotein (LDL) particles, resulting in the accumulation of lipids, mostly LDL-derived cholesterol ester, in cytosolic lipid droplets (LDs). At present, LDs are recognized as dynamic organelles that govern cellular metabolic processes. LDs consist of an inner core of neutral lipids surrounded by a monolayer of phospholipids and free cholesterol, and contain LD-associated proteins (LDAPs) that regulate LD functions. Foam cells are characterized by an aberrant accumulation of cytosolic LDs, and are considered a hallmark of atherosclerotic lesions through all stages of development. Previous studies have investigated the mechanisms underlying foam cell formation, aiming to discover therapeutic strategies that target foam cells and intervene against atherosclerosis. It is well established that LDAPs have a major role in the pathogenesis of metabolic diseases caused by dysfunction of lipid metabolism, and several studies have linked LDAPs to the development of atherosclerosis. In this review, several foam cell-targeting pathways have been described, with an emphasis on the role of LDAPs in cholesterol mobilization from macrophages. In addition, the potential of LDAPs as therapeutic targets to prevent the progression and/or facilitate the regression of the disease has been discussed.

Contents

1. Introduction
2. Lipid droplets
3. Lipid droplet-associated proteins
4. Cholesterol trafficking in macrophages
5. Approaches to target foam cells
6. Conclusion

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1. Introduction

On average, every 34 sec someone in the United States suffers from a coronary event, and every 40 sec someone has a stroke (1). Cardiovascular disease and stroke are pathologies associated with the development of atherosclerosis, a chronic inflammatory process that affects the walls of large- and medium-sized arteries. The systemic risk factors associated with a higher prevalence of atherosclerosis-related diseases include dyslipidemia, hypertension, chronic kidney disease, metabolic syndrome and diabetes (1). Unstable plaques may rupture and block the bloodstream, ultimately leading to myocardial infarction or stroke. Atherosclerotic plaques consist of fatty materials, predominantly cholesterol; necrotic cores; calcified regions; and various types of cells, including smooth muscle cells, endothelial cells, immune cells, monocytes and foam cells. Among these cells, lipid-laden macrophages, which are commonly known as foam cells, are central components of the plaques, which have an important role in the process of atherosclerotic plaque development from the early to late stages.

According to the 'response to injury' theory by Ross *et al* (2,3), atherosclerosis is initiated by maladaptation of the blood vessel to endothelial cell damage. Injured endothelial cells produce adhesion molecules, which cause monocytes and T lymphocytes to adhere to endothelial cells and migrate to the subendothelial space (2,3). However, later observations have indicated that endothelial injury is not a common feature, and uninjured endothelial cells are actually more common in developing plaques (4). The alternative 'response to retention' hypothesis, postulated by Williams and Tabas (4), proposes that atherogenesis is initiated without endothelial denudation. Low-density lipoprotein (LDL) that enters the intimal space is retained by the subendothelial extracellular matrix molecules, predominantly proteoglycans. Proteoglycan-bound LDL aggregates and is prone to undergo several modifications. In particular, LDL binding to proteoglycans increases susceptibility of LDL to oxidation. Subsequently, oxidized LDL (oxLDL) triggers the synthesis of monocyte chemoattractant protein (MCP-1) by endothelial and smooth muscle cells, and oxLDL is also directly chemoattractive to monocytes (4). Therefore, the final outcome of both hypotheses is that monocytes migrate to the arterial wall and differentiate into arterial macrophages, which take-up oxLDL to become foam cells.

Following endocytosis of oxLDL by macrophages, the cholesterol ester (CE) carried by these particles is hydrolyzed

to free cholesterol (FC) in the lysosomes, which is subsequently released into the cytosol. Elevated FC levels in macrophages due to uncontrolled LDL uptake can cause membrane damage and cytotoxicity (5). However, toxicity can be prevented by increasing FC efflux to high-density lipoprotein (HDL), or by esterifying FC to CE, which is subsequently stored in the core of cytoplasmic lipid droplets (LDs) (6) (Fig. 1). As a result of LD accumulation in the cytosol, macrophages form 'foamy' looking shapes, hence their alternative name, foam cells. While this mechanism may initially be protective, the overwhelming accumulation of foam cells caused by unfettered LDL uptake at the arterial wall results in inflammation and necrosis. Therefore, foam cells have been the object of extensive research efforts aiming to identify novel therapeutic strategies against atherosclerosis. In this review, the general mechanisms of foam cell formation are described, genes associated with LDs and their roles in atherosclerotic development are investigated, and the prospect of targeting foam cells to prevent and/or intervene against atherosclerosis is discussed.

2. Lipid droplets

LDs are fat depots found in all eukaryotic cells (7). In mammalian adipocytes, LDs reserve large amounts of lipid to provide energy in the event of scarcity; therefore, the LD was initially considered to be a mere fat reservoir. However, numerous studies have demonstrated that LDs are not only lipid reservoirs but also dynamic organelles that provide lipids for metabolic processes, membrane synthesis, cell signaling and intracellular vesicle trafficking (8-10). In non-adipocytes, LDs protect cells from cytotoxicity caused by a surplus of free fatty acids or FC by storing neutral lipid esters in the cytosol (11,12). However, the excessive storage of lipids in cells is closely associated with metabolic diseases, including obesity, diabetes, fatty liver disease and atherosclerosis (13).

LDs consist of a phospholipid monolayer, lipid droplet-associated proteins (LDAPs), and an inner core of neutral lipids, including triacylglycerol (TAG), sterol esters, retinyl esters, waxes and ether lipids (7,14). A lipidomic study previously revealed that LDs are complexes that contain >160 species of phospholipid. The most abundant phospholipid is phosphatidylcholine, followed by phosphatidylethanolamine, phosphatidylinositol and ether-linked phosphatidylcholine (14). The composition of neutral lipids in the core of LDs varies in different cell types. For example, yeast cells contain almost an equal proportion of TAG and CE, whereas adipocytes contain mostly TAG; however, macrophages/foam cells contain mostly CE that originates from LDL (14). In eukaryotes, the prevalent theory for LD biogenesis is that LDs bud off the endoplasmic reticulum (ER), where the majority of enzymes for neutral lipid synthesis are located, including acyl-CoA:cholesterol acyltransferase (ACAT) for sterol esters and acyl-CoA:diacylglycerol acyltransferases for TAG (15,16). Following synthesis of neutral lipids within the interspace of the lipid bilayer of the ER membrane, lipids are enclosed by a monolayer of phospholipids, which originates from the cytoplasmic leaflet (17-20). The newly formed LDs increase in size by incorporating lipids that are synthesized *in situ* by enzymes localized at the LD surface, or by the fission of pre-existing LDs (7,21).

3. Lipid droplet-associated proteins

LDAPs are usually located at the surface of LDs and have an important role in the formation and degradation of LDs (22). Proteomic analyses on LD fractions of lipid-loaded cells have identified numerous LDAPs (23,24). Relatively well-characterized LDAPs include members of the perilipin, ADFP and Tip47 (PAT), and cell death-inducing DNA fragmentation factor-like effector (CIDE) families. In mammals, the PAT family comprises five members: Perilipin 1 (PLIN1), Perilipin 2 (PLIN2/adipophilin/adipose differentiation-related protein/ADFP), Perilipin 3 (PLIN3/Tip47), Perilipin 4 (PLIN4/S3-12), and Perilipin 5 (PLIN5/lipid storage droplet protein 5/myocardial lipid droplet protein/XPAT/PAT1) (25). The CIDE family comprises three members: CIDEA, CIDEB and CIDEA (human)/fat-specific protein of 27 kDa (mouse). While all cells have the ability to accumulate LDs, the expression of LDAPs varies depending on cell and tissue type. Therefore, different LDAPs are expected to replace the function of others based on their expression pattern. PLIN1 was the first LDAP to be identified (26), and is highly expressed in white and brown adipose tissues, and steroidogenic cells (26,27). In addition, PLIN1 is expressed in detectable amounts in macrophages (28); however, its expression in mouse macrophages remains controversial (29). Four splicing variants of PLIN1 have been identified, namely perilipin A-D (26,30). Perilipin A and B are expressed in adipocytes, whereas the C and D isoforms are predominantly expressed in steroidogenic cells. Under non-hydrolytic conditions, interaction of PLIN1 with comparative gene identification-58 (CGI-58) blocks the access of hydrolases to LDs, and protects TAG in LDs against hydrolysis. Under β -adrenergic receptor activation-induced hydrolytic conditions, both PLIN1 and cytoplasmic hormone sensitive lipase (HSL) are phosphorylated by protein kinase A. Phosphorylated HSL gains access to LDs, whereas phosphorylated PLIN1 dissociates from LDs and releases CGI-58 from the LD surface. Interaction of CGI-58 with adipose triglyceride lipase (ATGL) in the cytoplasm results in translocation of ATGL to LDs, where it primarily hydrolyzes TAG, whereas HSL sequentially breaks down the diacylglycerol generated by ATGL (31,32).

PLIN2 and PLIN3 are ubiquitously expressed (28,33,34). PLIN2 is highly induced by lipid loading, and is expressed in macrophages/foam cells and atherosclerotic plaques (29,35). PLIN2 was initially isolated and characterized during a study on the differentiation of pre-adipocytes into adipocytes (36). Lipid loading increases the transcriptional and post-translational levels of PLIN2, since PLIN2 bound to LDs is protected from degradation by the proteasome pathway (37,38). PLIN2 reduces the affinity of ATGL to LDs, and consequently reduces the degradation of LDs by ATGL (39). A missense polymorphism in PLIN2 (Ser251Pro) has been shown to increase the number of small-sized LDs. The human Pro251 allele was associated with decreased plasma TAG and VLDL concentrations (40).

PLIN3 has been detected on the surface of LDs in HeLa cells using a PLIN3 antibody to track its subcellular localization (41). PLIN3 is involved in the transport of mannose 6-phosphate receptors from endosomes to the trans-Golgi network (42). Unlike PLIN1 and PLIN2, which

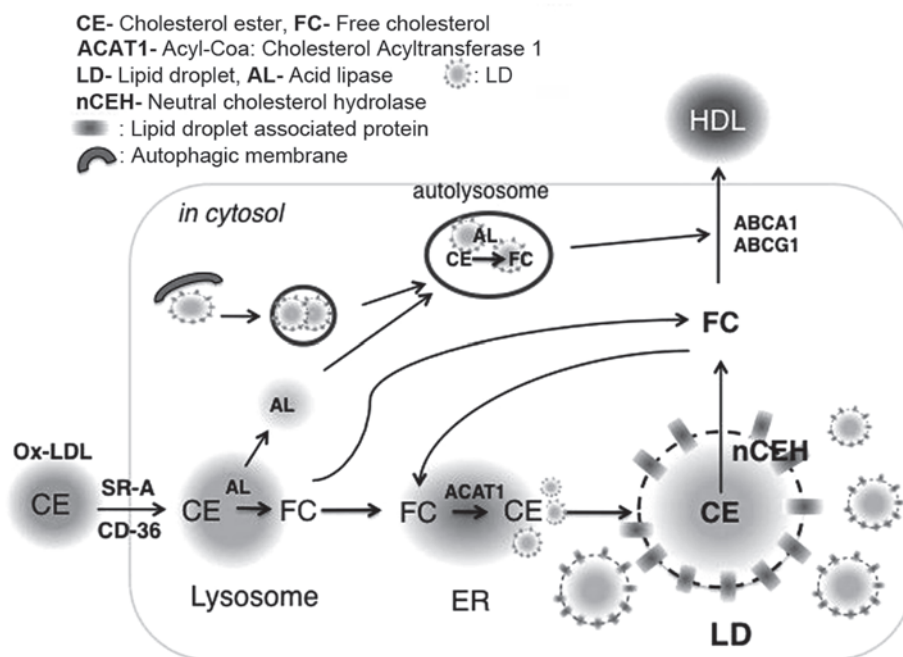


Figure 1. Cholesterol trafficking in macrophages. ABCA1, ATP-binding cassette, sub-family A, member 1; ABCG1, ATP-binding cassette, sub-family G, member 1; ER, endoplasmic reticulum; SR-A, scavenger receptor A; CD-36, cluster of differentiation 36.

are fundamentally associated with LDs, PLIN3 is abundantly found in the cytosol (43,44). Acetylated LDL (acLDL) loading of PLIN2-depleted human THP-1 macrophages was shown to decrease CE levels; however, PLIN3 knockdown reduced TAG levels in acLDL and oleic acid-loaded cells (45). Increased localization of PLIN3 to LDs was observed in PLIN2-depleted THP-1 macrophages, without alterations to PLIN3 expression (29,45). Similarly, Chang *et al* (46) detected increased PLIN3 localization to LDs in PLIN2-deficient hepatocytes. These results suggest a differential and compensatory role of LDAPs in lipid metabolism even within the same cells. PLIN4 is predominantly detected in white adipose tissue, although lower amounts can be detected in heart and skeletal muscle (41,47). PLIN5 is expressed in heart, brown adipose tissue, liver and skeletal muscle (34,47). A previous study, which used microarrays to identify the expression of LDAPs in oxLDL-loaded THP-1 macrophages, demonstrated that PLIN1 expression was not altered, whereas PLIN2 was increased and PLIN3 was decreased. Furthermore, PLIN4 and PLIN5 were upregulated by oxLDL in THP-1 cells (48).

With distinctive tissue distribution, the three members of the CIDE family are involved in TAG metabolism and their functions are highly associated with metabolic disorders. CIDEA and CIDEB are abundantly expressed in brown adipose tissue and in the liver, respectively (49,50). CIDEc is expressed in white and brown adipose tissues, but not in normal liver tissue (49,51,52). Deletion of each CIDE member in mice resulted in leaner mice due to increased energy expenditure, and was associated with resistance to diet-induced obesity and insulin resistance (53). All three members of the CIDE family have recently been detected in THP-1 macrophages loaded with oxLDL (48). However, the role of CIDEs in cholesterol metabolism and atherosclerotic development has yet to be elucidated.

The predominant form of lipid stored in the LDs of macrophages/foam cells is CE. Whereas PAT and CIDE proteins have central roles in LD metabolism, these proteins do not exert TAG or CE hydrolyzing activities. HSL is the best-characterized CE hydrolase in macrophages (54,55). Despite its strong CE hydrolase activity, the absence of HSL in human macrophages and plaques suggests the possible existence of other CE hydrolase(s) to replace the role of HSL in human atheroma (23,55). A recent proteomic analysis on the LD fraction of Raw 264.7 macrophages identified a novel CE hydrolase, lipid droplet-associated hydrolase (LDAH). Overexpressed LDAH in RAW 264.7 macrophages decreased CE levels by increasing FC efflux (23). LDAH was reported to be ubiquitously expressed; however, it is significantly more abundant in white and brown adipose tissues and in the liver. Notably, LDAH is also highly expressed in both human and mouse macrophages and atherosclerotic lesions (23).

4. Cholesterol trafficking in macrophages

In macrophages, oxLDL is notably taken-up via endocytosis mediated by scavenger receptors (SRs), including SR-A and cluster of differentiation 36 (CD36); however, additional mechanisms may mediate oxLDL uptake (56,57). CE derived from oxLDL is hydrolyzed to FC by lysosomal acid lipase (LAL) and is then released into the cytosol. FC in the cytosol is either effluxed by ATP-binding cassette (ABC) transporters, including ABCA1 and ABCG1, or re-esterified by ACAT1 in the ER and stored as CE in cytosolic LDs (Fig. 1). Two LD CE hydrolytic pathways have been reported. In the first pathway, CE in LDs is hydrolyzed to FC by neutral cholesterol ester hydrolases (nCEHs), which associate with LDs. The second pathway involves autophagocytic engulfment of LDs, followed by fusion with lysosomes and CE hydrolysis by LAL (58). FC

generated from both pathways is effluxed via ABC transporters and transferred to extracellular acceptors for reverse transport to the liver and, ultimately, the feces (Fig. 1).

5. Approaches to target foam cells

Due to the central role of foam cells in atherosclerotic development, foam cells have been the target of interventions, in order to identify novel therapeutic strategies. Some of the most significant approaches are described in this review.

Blocking the uptake of oxLDL by knocking down SRs. Native LDL is removed from circulation by the process of endocytosis by LDL receptors (LDLRs); however, modified LDL is recognized and taken-up by SRs (57,59). Among these receptors, SR-A1 and CD36 are responsible for 75-90% of the degradation of oxLDL *in vitro* (60). Therefore, blocking SRs may be a promising strategy to ameliorate the development of atherosclerosis. However, studies in SR-A1^{-/-} or CD36^{-/-} mice with an apolipoprotein E knockout (apoE^{-/-}) or LDLR knockout (LDLR^{-/-}) background have exhibited contradictory results. Suzuki *et al* (61) reported that SR-A1^{-/-}/apoE^{-/-} mice exhibited decreased atherosclerotic lesion development due to reduced cholesterol uptake. However, these mice were more sensitive to infection, since SRs bind a broad range of ligands expressed by bacterial pathogens (61). CD36^{-/-}/apoE^{-/-} mice also exhibited significantly reduced lesion development; however, elevated plasma LDL levels were detected due to loss of LDL uptake (62). In addition, SR-A or CD36 deficiency in macrophages of LDLR^{-/-} mice resulted in reduced lesion development (63). Conversely, Moore *et al* (56) detected increased lesion area in the aortic sinus with abundant macrophages/foam cells in apoE^{-/-} mice lacking either SR-A1 or CD36, presumably due to alternative LDL uptake mechanisms. Manning-Tobin *et al* (64) also reported no changes in lesion size and macrophage/foam cell content, but observed reduced inflammatory gene expression and macrophage apoptosis in SR-A^{-/-}/CD36^{-/-}/apoE^{-/-} mice. Therefore, the benefit of targeting SRs remains controversial.

Inhibiting re-esterification of FC to CE (ACAT^{-/-} mice). Excessive cytoplasmic FC can be re-esterified by ACAT1 in the ER, and the generated CE can subsequently be stored in LDs (Fig. 2). Since only FC, not CE, can enter efflux pathways, blocking re-esterification of FC to CE may be considered a promising strategy to inhibit foam cell formation and facilitate cholesterol efflux. Unexpectedly, ACTA1^{-/-} mice, with either an LDLR^{-/-} or apoE^{-/-} background, exhibited increased lesion size with abundant necrotic cores due to macrophage apoptosis as a result of toxicity from excessive FC accumulation in the ER (65-67). Therefore, the therapeutic inhibition of ACAT1 does not appear to be a desirable strategy for the treatment of atherosclerosis.

Blocking LD formation by knocking down LDAPs. Since LDAPs are structurally or enzymatically involved in LD homeostasis, their roles in pathologies associated with dysfunctional lipid metabolism have been extensively studied. As mentioned previously, abundant expression of certain LDAPs in macrophages is closely associated with foam cell

formation during atherosclerotic development. Among known LDAPs, PLIN2 is highly expressed in macrophages and its expression is increased by lipid loading, whereas the expression levels of other members of the PAT family remain very low or unchanged (29,48). In line with the role of PLIN2 in TAG accumulation in non-monocytic cells, PLIN2 increased CE accumulation in acLDL-loaded THP-1 macrophage by inhibiting cholesterol efflux (68). In addition, PLIN2 mRNA is highly expressed in human and mouse atherosclerotic plaques compared with healthy areas of the same arteries (68,69). A global approach to identify cholesterol responsive genes in the macrophages of LDLR^{-/-} mice loaded with cholesterol *in vivo* detected increased levels of PLIN2 (35). In agreement with these findings, a significant role for PLIN2 in the development of atherosclerosis was verified by Paul *et al* (29) using PLIN2 null mice with an apoE^{-/-} background. PLIN2^{-/-}/apoE^{-/-} mice exhibited decreased lesion development with reduced foam cell formation in lesions due to increased FC efflux (29). In addition, contrary to observations made under ACAT1 deficiency, PLIN2 deficiency was well tolerated by the macrophages, thus indicating that PLIN2 may be a safe target for the amelioration of atherosclerotic development (70).

Macrophages/foam cells in the arterial wall generate proinflammatory cytokines. The secretion of these cytokines is an important predictor of atherosclerotic development. The expression levels of proinflammatory cytokines, including tumor necrosis factor- α , MCP-1 and interleukin-6, were increased by PLIN2 overexpression and decreased following knockdown of PLIN2 in THP-1 macrophages loaded with acLDL (71). Regarding the role of PLIN1 in the development of atherosclerosis, contradictory results have been reported. Langlois *et al* (72) detected increased atherosclerosis in PLIN1^{-/-} mice, whereas Zhao *et al* (28) reported that global and bone marrow-specific PLIN1 deficiency reduced atherosclerosis. With respect to CIDE proteins, CIDEB has been shown to control hepatic cholesterol homeostasis, and CIDEB^{-/-} mice exhibited lower levels of plasma cholesterol and LDL, and increased hepatic cholesterol levels, due to increased LDLR and ACAT expression (73). These observations raise the possibility that, in addition to a potential role in lipid metabolism in macrophages, CIDE family proteins may have a role in atherogenesis by regulating plasma cholesterol levels.

Increasing CE hydrolysis in LDs. Reverse cholesterol transport (RCT) from arteries involves transfer of cholesterol from macrophages/foam cells to HDL (74). In order to be effluxed, CE deposited in LDs must be hydrolyzed to FC; therefore, CE hydrolysis may be considered the first step in RCT (75,76). Since RCT from arteries is considered atheroprotective, enzymes that hydrolyze CE stored in LDs, generally known as nCEHs, may have high therapeutic potential. HSL is an intracellular neutral hydrolase that is able to hydrolyze various esters, including CE in macrophages (77,78). However, HSL knockdown in the bone marrow macrophages of LDLR^{-/-} mice did not induce significant changes in lesion development, indicating the possibility of compensatory mechanisms (79). Unexpectedly, rather than improving atherosclerosis, macrophage-specific expression of transgenic rat HSL in mice with an apoE^{-/-} background accelerated atherosclerosis. This paradoxical effect was not associated with the excessive

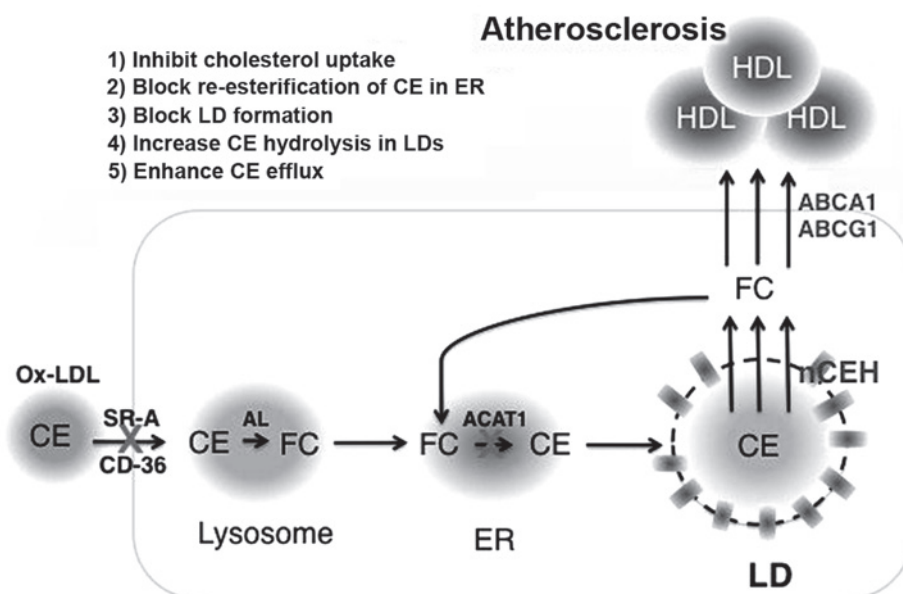


Figure 2. Efforts targeting foam cells to prevent/intervene against atherosclerosis. CE, cholesterol ester; ER, endoplasmic reticulum; LD, lipid droplet; ox-LDL, oxidized low-density lipoprotein; SR-A, scavenger receptor A; CD-36, cluster of differentiation 36; AL, acid lipase; ACAT1, acyl-CoA:cholesterol acyl-transferase 1; nCEH, neutral cholesterol hydrolase; ABCA1, ATP-binding cassette, sub-family A, member 1; ABCG1, ATP-binding cassette, sub-family G, member 1; HDL, high-density lipoprotein.

intracellular accumulation of FC, or with larger necrotic core development within the lesions, but was attributed to coupling of effective re-esterification of surplus FC to CE by ACAT1 and to limited efflux by ABC transporters (77,80). Notably, increasing cholesterol acceptors in HSL transgenic mice reduced aortic lesion development (81). However, regardless of its role in mice, HSL is not expressed in human atherosclerotic lesions (23). Therefore, the identity of the nCEH(s) in human atheroma remains unknown. A possible candidate is LDAH, which is expressed in both human and mouse atherosclerotic lesions, as well as in cultured and primary human and mouse macrophages (23). LDAH overexpression in macrophages has been reported to increase the rate of CE hydrolysis and cholesterol efflux. However, to date, the role of LDAH in genetically engineered mice has yet to be reported (23).

Enhancing cholesterol efflux and acceptors. Although FC can be effluxed by passive diffusion (82), FC from macrophages is mainly effluxed through the ABC transporters, ABCA1 and ABCG1 (83). Therefore, inducing expression or activity of ABC transporters is an attractive strategy to increase RCT from arteries. ABCA1 delivers both FC and phospholipids to lipid-free or lipid-poor apolipoprotein A-I in order to generate nascent or pre-beta HDL, whereas ABCG1 effluxes FC to HDL particles (84). This gives rise to a synergistic relationship in which the nascent HDL generated by ABCA1-mediated FC efflux serves as substrate for FC efflux by ABCG1. ABCA1^{-/-}/LDLR^{-/-} mice exhibit increased atherosclerosis, and human ABCA1 transgenic mice with an apoE^{-/-} background develop markedly smaller and less complex lesions (85). Although plasma HDL levels did not rise, HDL from ABCA1 transgenic mice accepted FC more efficiently than HDL from wild-type mice (86). The role of ABCG1 in atherosclerosis is less clear. The transgenic expression of human ABCG1 in apoE^{-/-} mice did not affect lesion development (87). Conversely,

ABCG1 transgenic expression in LDLR^{-/-} mice increased atherosclerosis (88). In addition, it has been reported that macrophage-specific deletion of ABCG1 in bone marrow-derived cells decreased lesion development in both apoE^{-/-} and LDLR^{-/-} mice (89), whereas another study observed slightly increased lesion development in LDLR^{-/-} mice with ABCG1 deletion in macrophages (90). Notably, compared with the controversial phenotype of single ABCG1 knockout on the development of atherosclerosis, the double knockout with ABCA1 synergistically increased atherosclerosis development (91).

The expression of ABCA1 and ABCG1 is regulated by direct binding of liver X receptor (LXR) (92,93). Administration of synthetic LXR agonists has been reported to successfully attenuate atherosclerotic development (94). However, systemic administration of LXR ligands causes unfavorable effects, including liver steatosis and hypertriglyceridemia due to activation of enzymes associated with fatty acid biosynthesis (95-98). Therefore, several studies have attempted to discriminate the mechanisms of LXR activation between liver and macrophages. Kim *et al* (99) reported that thyroid hormone receptor-associated protein 80 (TRAP80) selectively activates LXR-mediated sterol regulatory element binding protein 1c, which causes liver steatosis, but not LXR-mediated ABCA1 expression. Combinatory treatments to concomitantly reduce TRAP80 activity and increase LXR activity could be of potential therapeutic use against atherosclerosis (99). Furthermore, nanotechnology has recently been employed for local delivery of LXR agonists to macrophages/foam cells without systemic effects (100,101). The delivery of the LXR agonist GW3965 encapsulated in poly(lactide-co-glycolide)-b-poly(ethylene glycol) copolymer nanoparticles to LDLR^{-/-} mice markedly reduced the number of macrophages and decreased the size of atherosclerotic plaques by 50%, without increasing total cholesterol and TAG levels in liver and plasma (100). Alternatively, Lim *et al* (101) developed a

novel site-specific antibody-drug conjugate (ADC) to target and deliver an aminoxy-modified LXR agonist conjugated to anti-CD11-immunoglobulin G through a stable, cathepsin B cleavable oxime linkage. The LXR agonist delivered by ADC was 3-fold more powerful than the conventional LXR agonist T0901317 when tested in THP-1 macrophages, but it did not induce LXR target genes in hepatocytes (101). However, the effect of this delivery system remains untested *in vivo*. Since targeted delivery of LXR agonists effectively prevents atherosclerosis while avoiding the unfavorable side effects of conventional LXR agonists, additional research in this field is strongly supported, and underscores the potential of nanomedicine to treat atherosclerotic cardiovascular disease.

6. Conclusion

Atherosclerosis is a life threatening pathology, which progresses as plaques grow in the arterial wall. Macrophages/foam cells are found in the plaques from the early to late stages of atherosclerotic development. Therefore, numerous efforts to elucidate the mechanisms underlying foam cell formation, and to target foam cells to prevent and/or reverse atherosclerosis have been made. Presumably, given the complexity of advanced plaques, effective interventions against atherosclerosis should involve several pathways. Recent advances concerning the mechanisms underlying foam cell formation have identified several LDAPs in macrophages. Genetic modulation of some of these proteins in mice has supported the hypothesis that LDAPs may represent plausible novel targets for the amelioration of atherogenesis by preventing foam cell formation, and promoting RCT with less side effects than other interventions on non-LD foam cell proteins. The recent identification of novel LDAPs in macrophages leaves much room for research on the role of these proteins in lipid homeostasis and the development of atherosclerosis. Unveiling the function of diverse LDAPs and elucidating their molecular network may lead to novel therapeutic strategies to overcome atherosclerosis.

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