Abstract—HDL functions mainly as a cholesterol scavenger, facilitating transport of cholesterol to the liver for conversion to bile acids and secretion into the bile for elimination or recycling in the enterohepatic bile acid cycle. Because of its major function in cholesterol clearance, HDL is in general considered to be atheroprotective. From cell cholesterol can be removed by efflux especially to apoA-I and HDL as extracellular acceptors which transport the cholesterol to the liver for excretion. This process is called reverse cholesterol transport. In this context the ATP binding cassette transporter protein ABCA1 facilitates cellular cholesterol and phospholipid release to apoA-I–containing HDL precursors. In addition ABCA1 plays a role in vesicular lipid transport mechanisms required for HDL particle formation. In general to maintain intracellular lipid homeostasis, sterols and associated lipids move between cellular compartments by vesicular and nonvesicular pathways. However, cholesterol sorting on vesicle formation is poorly understood. This review summarizes the current knowledge of the molecular mechanisms of HDL and associated vesicular trafficking mechanisms to mediate cellular lipid homeostasis. (Arterioscler Thromb Vasc Biol. 2009;29:1718-1722.)

Key Words: HDL ■ lipid efflux ■ vesicular trafficking

The most prominent role of HDL is its role in the reverse cholesterol transport process by which excess cholesterol is shuttled from peripheral cells to the liver either for elimination via biliary excretion or reuse in the enterohepatic cycle. Especially the uptake of cholesterol from macrophages via ABC-transporters and phospholipid transfer proteins prevents the formation of foam cells in the atherosclerotic lesion, which is one of the first steps in the pathogenesis of atherosclerosis. HDL serves as a depository for the apolipoproteins apoA-I, AII, AIV, E, CI to CIV, LI, M, F, D, and H which are mainly involved in lipid metabolism. ApoA-I–containing HDL particles are antiatherogenic and protect against atherogenesis via reverse cholesterol transport and play an important role in antiinflammatory and antithrombotic response. Therefore, therapeutic HDL elevation may open new avenues for the treatment of atherosclerosis.

Scavenger receptor class B type I (SR-BI) is the receptor for HDL, mediating the transport of cholesterol and cholesteryl esters from HDL particles. It is primarily expressed in the liver and nonplacental steroidogenic tissues and involved in selective uptake of cholesteryl esters from HDL. SR-BI function is also important for cholesterol efflux in the vessel wall.

The free cholesterol taken up by HDL is then esterified by lecithin:cholesterol acyltransferase (LCAT) and the hydrophobic cholesteryl esters are retained into the core of HDL, so that new cholesterol molecules can be translocated on the HDL surface.

Excess free cholesterol is esterified by sterol o-acyltransferase 1 (SOAT1), localized at the ER esterifying cholesterol with oleoyl-CoA for storage in cytosolic lipid droplets. Rehydrolysis of cholesterylesters can take place via neutral cholesterylester hydrolase (carboxylesterase-1, monocyte/macrophage serine esterase-1, CES1). Free cholesterol respectively unesterified cholesterol/phospholipid complexes are effluxed via the ATP-binding cassette transporters (ABCs) ABCA1 and ABCG1. Cholesterol efflux to HDL requires ABCG1, whereas efflux to apoA-I requires ABCA1. The preferred extracellular acceptor of cellular phospholipids and unesterified cholesterol in the process mediated by ABCA1 is a monomolecular, preβ-migrating, lipid-poor, or lipid-free form of apoA-I. This mononuclear form of apoA-I is quite distinct from the preβ-migrating discoidal HDL which contains 2 or 3 molecules of apoA-I per particle and which are present as minor components of the HDL fraction in human plasma. In addition to cholesterol, ABCG1 also mediates the efflux of sphingomyelin and phosphatidylcholine, and it was suggested that ABCG1-mediated cholesterol efflux is sphingomyelin-dependent.
As a consequence ABCG1 expression is upregulated in macrophage cells from patients with Tangier disease, an autosomal recessive disorder which is characterized by mutated ABCA1 and by almost complete absence of plasma HDL, deposition of cholesteryl esters in the reticulo-endothelial system, and aberrant cellular lipid trafficking.

Synthesis and uptake of cholesterol is regulated via feedback mechanisms mediated by sterol-gated transport of the membrane-bound transcription factor sterol regulatory element binding protein 2 (SREBP2) from the ER to the Golgi complex. In contrast, SREBP-1c is mainly involved in fatty acid and phospholipid synthesis. Interestingly, ABCG1 expression is inducible by a SREBP-1c–dependent mechanism.6

PPARα and PPARγ mediate the lipid-lowering actions of fibrates and the antidiabetic effects of thiazolidinediones by forming heterodimers with RXR/LXR.7 LXRs direct transcription of target genes involved in the cholesterol efflux pathway and repress inflammatory genes in macrophages. They regulate lipogenesis and cholesterol homeostasis and stimulate cholesterol efflux from cells to HDL.8 The zinc finger transcription factor ZNF202 represses genes involved in reverse cholesterol transport including ABCA1 and ABCG1 and therefore plays a central role at the interlink of HDL, triglyceride, and glucose metabolism.7 Signaling cascades derived from the interaction of ABCA1 with casein kinases, AMP kinases, and Janus kinases are involved in regulating lipid metabolism (Figure 2).

In addition cholesterol trafficking is regulated by vesicular transport, and this review summarizes the current knowledge of the molecular mechanisms of HDL and associated vesicular trafficking mechanisms to mediate cellular lipid homeostasis.

**Cholesterol Trafficking Regulated by Lipid Transport Proteins**

The lipid transfer/lipopolysaccharide-binding protein gene family includes bactericidal permeability increasing protein (BPI), lipopolysaccharide binding protein (LBP), phospholipid transfer protein (PLTP), and cholesteryl ester transfer protein (CETP) which all protect against systemic inflammation and promote lipid transfer.9 CETP transfers cholesteryl esters from HDL to triglyceride-rich lipoproteins and LDL as well as triglycerides from triglyceride rich lipoproteins to HDL.9 CETP is a facilitator of cholesterol flux in reverse cholesterol transport where cholesteryl esters are transferred from HDL to LDL and taken up by the liver through LDL-receptor–mediated uptake.9 It regulates plasma levels of mature HDL and the size of HDL particles.9 PLTP modulates HDL levels by transferring phospholipids from remnant lipoproteins playing an important role in HDL remodeling and formation of lipid poor apoA-I particles.9 Enhancement of lipid efflux by PLTP involves the ABCA-1 pathway through binding of PLTP and apoA-I to ABCA-1.9 PLTP may also promote cellular cholesterol efflux indirectly by increasing plasma preβ-HDL levels.9 Plasma from insulin resistant subjects revealed a normal capacity for cholesterol efflux despite lower HDL cholesterol levels, because of a higher PLTP activity and increased preβ-HDL formation.9

Oxysterol-binding protein related proteins (ORPs) function also as soluble sterol transporters.10 The human genome contains 12 OSBP (oxysterol-binding protein)/ORP family member genes which are involved in the direct control of lipid synthesis and lipid transport in cells.10 ORP1S is largely cytosolic, whereas ORP1L is involved in endosome trafficking and localizes to late endosomal compartments.

Free cholesterol released by acid lipase from luminal cholesteryl esters is transferred by NPC2 (HE1) to luminal membrane associated NPC1 for cholesterol transport out of late endosomes. The steroidalogenic acute regulatory protein (StAR)-related lipid transfer (START) domain containing protein StarD3 (MLN64) binds cholesterol released from endosomes and might function as a lipid trafficking protein mediating target specific sterol transfer from the endosomal membrane, to acceptor membranes.10 Endolysosomal lipid storage disease Niemann Pick Type C (NPC) is caused by mutations in the NPC1 and NPC2 genes accumulating cholesterol and other lipids in late endocytic organelles.

**Cholesterol Trafficking Regulated by Vesicular Transport**

Cells maintain a cholesterol gradient across the secretary system, with the lowest concentrations in the ER and the highest in the plasma membrane. To maintain a high concentration of cholesterol in the plasma membrane, cholesterol may be sorted into vesicles targeted to the plasma membrane. Little is known how sterols are actively sorted into or excluded from transport vesicles. It has been shown that sphingomyelin and cholesterol are partially excluded from COPI-coated vesicles, which are important for retrograde transport from the Golgi network to the ER and intra-Golgi transport.10,11 Sorting of cholesterol and other lipids could be driven by their affinity to cholesterol- and sphingolipid-enriched microdomains and the influence of membrane curvature.10,11

Treating cells with brefeldin A, which causes Golgi complex disassembly, promotes retrograde transport and blocks movement of proteins from the ER to the plasma membrane but has minor influence on the delivery of newly synthesized cholesterol to the plasma membrane, indicating that cholesterol can bypass the Golgi apparatus to proceed to the plasma membrane.10 However, there is also evidence for a partial contribution of the Golgi complex in the transport of newly synthesized cholesterol to the cell surface involving detergent-resistant membranes.12

Endosomes contain cholesterol in variable amounts, and there is continuous vesicular membrane trafficking between the compartments of endocytic and secretory pathways where cholesterol is sorted during transport vesicle formation.13 Endolysosomal storage disorders like acid lipase deficiency (Cholesterylster storage disease [CESD]/Wolman Disease), NPA/B (acid sphingomyelinase deficiency), NPC, and certain amphiphilic compounds leading to cholesterol accumulation in late endosomes (phospholipidosis), helped to elucidate the pathological effects of cholesterol accumulation on endocytic
ABCA1-Mediated Cholesterol Efflux and Vesicular Trafficking

Lipid-free apolipoproteins including apoA-I, apoE, and preβ-HDL-precursors are extracellular free cholesterol acceptors to which ABCA1 facilitates the transfer of cholesterol and phospholipids, a process that involves also vesicular transport. Another apoA-I high-affinity binding site in the plasma membrane was the β-chain of human ATP-synthase, a major protein complex of the mitochondrial inner membrane also present in the plasma membrane, involved in ATP synthesis.22 It two major domains, F0 and F1, the latter containing 5 different subunits among which the β-chain interacts with apoA-I. It has also been reported that both the β-chain and α-chain of ATP synthase are receptors for apoE-enriched HDL in the plasma membrane.23 The possible involvement of the F0/F1-ATPase in the lipid influx/efflux rheostat together with ABCA1 is shown in Figure 1.

A fraction of ABCA1 is located in internal endocytic compartments where it may facilitate cholesterol efflux from late endosomes.24 Internalization and trafficking of ABCA1 is functionally important in mediating cholesterol efflux from intracellular cholesterol pools.24 Depletion of cell surface lipids induces a signaling cascade to mobilize lipids from late endosomes/lysosomes, via the trans-Golgi network to the plasma membrane. This process is facilitated by NPC1 and defective in NPC1 mutations. As a consequence also ABCA1-mediated efflux is impaired leading to reduced HDL cholesterol levels.24 ABCA1 is rapidly recycled between the plasma membrane and intracellular compartments including late endosomes, possibly containing some apoA-I, and this process also maintains delivery of lipids to the cell surface for HDL

In addition to late endosomes, endosomal recycling routes participate in sterol transport. While recycling endosomes may acquire cholesterol by nonvesicular mechanisms,21 recycling of sterols to the cell surface appears to be regulated by the same molecules that mediate vesicular trafficking of other recycling cargo, such as Rab11 and Rme-1.13

Overexpression of Rab9 or Rab7 has been reported to reduce cholesterol accumulation in late endosomes/lysosomes.18,19 Whereas Rab9 complements the NPC phenotype, Rab7 overexpression acts similarly in 1 study but not in another.18,19 ORP1L appears to influence endosomal trafficking through Rab7 by regulating its GTP-GDP cycling in mammalian cells.20 Rab8 localizes to the Golgi region, vesicular structures, and peripheral membrane ruffles, and functions in polarized membrane traffic to the cell surface involving recycling endosomes as intermediates.17 Rab8 in human fibroblasts regulates the delivery of cholesterol from late endosomal compartments to apoA-I and is stimulated on overexpression of Rab8.17 Rab8 redistributes cholesterol from late endosomes to the cell periphery and stimulates cholesterol efflux to the ABCA1-ligand apoA-I without increasing cholesterol esterification.17

The largest subfamily of Rab GTPases, the Rab proteins, are key regulators of vesicular trafficking that provide compartmental specificity for membrane trafficking. Rab-dependent membrane transport is involved in clearing cholesterol from late endocytic organelles. Perturbation of their function with Rab-guanine nucleotide dissociation inhibitor (GDI) was shown to impair cholesterol removal from late endosomes.13 Membrane cholesterol loading impairs the extractability of several Rabs, including Rab4, Rab5, Rab7, and Rab9, from membranes by GDI.17

The lipid influx/efflux rheostat model maintains lipid uptake and export mechanisms in a balance. ATP synthase is regulated by apoA-I or apoE leading to enhanced conversion of ATP to ADP. The absence of apoA-I would lead to enhanced sinking in phagocytosis because actin can bind ATP, polymerize, and form F-actin, which is essential for type II phagocytosis. Hence apoA-I could lead to increased influx. On the other hand, apoA-I binds to ABCA1 leading to enhanced lipid efflux. Dysfunction of this equilibrium may lead to severe disturbances of cellular lipid traffic. This is obvious in Tangier disease patients where ABCA1 is inoperative and apoA-I-dependent cholesterol is absent. Cholesterol influx, however, is enhanced by apoA-I-dependent stimulation of ATP synthase B leading to cholesteryl ester formation and enhanced foam cell formation.
ADP-ribosylation factors (ARFs) represent Ras-related small regulatory GTPases which control vesicular transport in the secretory and endosomal pathway at the stage of vesicle budding. ARF-like 7 (ARL7) is involved in transport between the trans-Golgi network and the plasma membrane and together with ABCA1 promotes the release of cholesterol- and phospholipid-containing vesicles from the trans-Golgi compartment.\textsuperscript{25}

Retroendocytosis of apoA-I may be involved in ABCA1-related release of cholesterol from endosomes and lysosomes.\textsuperscript{24} In addition, enhanced vesicular transport from the Golgi to the plasma membrane, in response to apoA-I/ABCA1-mediated cholesterol efflux, is absent in ABCA1-deficient fibroblasts leading to trans-Golgi lipid storage.\textsuperscript{24}

Syntaxin 13, a SNARE protein which plays a major role in vesicular transport, associates with ABCA1 and flotillin-1\textsuperscript{27} (Figure 2). Syntaxin 13 deficiency reduces ABCA1-mediated choline-phospholipid efflux.\textsuperscript{26} ABCA1 also plays a role in phagosome regulation because ABCA1 deficiency leads to enhanced phagocytosis.\textsuperscript{26}

The Rho family GTPase Cdc42 being involved in vesicular traffic directly interacts with ABCA1 and controls filopodia formation, actin organization, intracellular lipid transport, and ABCA1 dependent lipid efflux.\textsuperscript{27-29} ABCA1 was found to reside on intracellular vesicles,\textsuperscript{30} and the trafficking of these vesicles may be hindered by an association of ABCA1 to the cytoskeleton via \( \beta 2 \)-syntrophin/utrophin\textsuperscript{31} (Figure 2).

Structural and functional abnormalities in caveolar processing and the trans-Golgi secretory pathway of cells lacking functional ABCA1 indicate that lipid export processes involving vesicular budding between the Golgi and the plasma membrane are severely disturbed.\textsuperscript{32} In summary, cholesterol trafficking to the plasma membrane depends on target-specific vesicular transport systems which mediate cholesterol flux from intracellular pools to ABCA1 located at the plasma membrane, trans-Golgi membranes, and recycling endosomes.

**Conclusion and Perspectives**

ApoA-I/HDL–dependent vesicular lipid trafficking machines involve transporters (eg, ABCA1/ABCG1, NPC1), trafficking ATPases (eg, ATP-synthase), vesicular proteins (HE-1, Rab, etc), and lipid transfer proteins which may be potential targets for the treatment of lipid storage diseases. In addition, signaling cascades involving protein kinase A (PKA), Janus kinase 2 (JAK2), and casein kinases regulate ABCA1 function. Further detailed investigation is necessary to elucidate the functional relation between these HDL pathway constituents and to identify new drug targets promoting cellular lipid homeostasis.

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**Disclosures**

None.
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