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HDL Metabolism and Diseases

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HDL Metabolism and Diseases

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Preface

High-density lipoproteins (HDLs) are first featured by the ultracentrifugation of plasma to separate lipoproteins of different densities. Over the past several decades, major progress on the study of HDLs has been made. HDLs, as a complex mixture, include various lipids, proteins, micro-RNAs, vitamins, and hormones, which endow diverse biological functions beyond mediating reverse cholesterol transport. Many population studies have shown that the level of HDL-c is inversely related to the risk of cardiovascular research. However, it has been demonstrated that increasing HDL-c level by certain drugs is not effective for reducing the risk of disease. Currently, researchers begin to pay more attention to the metabolism of HDLs. The abnormal metabolism of HDLs is known to closely associate with many diseases, including cardiovascular diseases, diabetes, sepsis, kidney diseases, and so on. Moreover, the structural and functional characteristics of HDLs have also made it a natural delivery carrier. In this book, we systematically review HDL metabolism and its related diseases from lab to clinic. Firstly, we review HDLs metabolism from multiple dimensions, including HDLs structure, some key proteins, and RNA relating to its metabolism (CETP, SR-BI, ABCA1, ABCG1, and miRNA), and two metabolic reactions (such as inhibition of oxidation, some lipid metabolism). The endothelial cell, as a dominant role in cardiovascular system, also requires the normal HDLs. Besides, we also not only selectively review some related diseases, but also emphasize some treatments related to HDLs. Although we also try our best to review most important aspects, some parts regarding this theme likely are omitted or not done enough.

We hope that our book can be a handbook for researchers and physicians, and benefit the other audiences, and that this book can contribute to the development of future therapies.

Beijing, China Lemin Zheng

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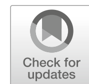

1 HDL Structure

Siying Deng, Yangkai Xu, and Lemin Zheng

Abstract

HDL has various protein components, including enzymes, complement components, apolipoproteins, protease inhibitors, etc. In addition to proteins, lipids are also a significant component of HDL. These components and their structure determine the function of HDL. HDL is heavily involved in the acute response phase, complement regulation phase, hemostasis phase, immune response phase, and protease inhibition phase. Among the apolipoproteins, the predominant component is Apo A-I, which confers various atherogenic activities to HDL. Apo A-II, Apo-C, Apo-D, Apo-F, Apo-H, Apo-J, and Apo-O, which can bind free fatty acids, regulate the activity of many proteins involved in HDL metabolism, inhibit lipid transfer, and control the endogenous coagulation cascade. A major functional component is the enzyme LCAT, which helps catalyze the conversion of cholesterol to plasma-based lipoproteins and then to cholesteryl esters. Another enzyme associated with HDL is human paraoxonase, calcium-, PON1-, PON2-, and PON3-dependent lactone enzyme with catalytic activity, including reversible binding to substrates. PAF-AH is a phospholipase with lipoprotein properties, and HDL and LDL particles are commonly bound to plasma PAF-AH for circulation. As for lipid components, PC is an essential phospholipid subclass and may be a biomarker for constitutive inflammation. Sphingolipids, such as sphingomyelin and ceramide, also play an indispensable role in HDL function. In different physiological and pathological stages and plasma environments, HDL can exhibit different structural features, such as discoid HDL and spherical rHDL.

Keywords

HDL · Structure · Apolipoprotein · Metabolism

1.1 Protein Components

HDL has a variety of proteins as compared to the other lipoproteins. They could be categorized into a number of subgroups which include enzymes, complement components, apolipoproteins, proteins that transfer lipids, proteins with actual

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phase response, and protease inhibitors among many other constituents of proteins [1, 2]. The two subgroups which are primarily considered supplementary provisions involving functional HDL components include enzymes and apolipoproteins. They are also quite significant in preventing infections and acute phase reactions. Among these subgroups, there are several proteins in the components of normal human plasma HDL with a large involvement in the phase of acute response, regulation of complements, hemostasis, immune response, and protease inhibition [3]. This suggests that HDL certainly plays a role in both inflammation and host defense mechanisms.

How the HDL proteomes are composed might be dependent on the method involved in the actual separation of HDL. Ultra-centrifugation in high ionic strength and high-concentration salt solution may play a role in removing some protein components from HDL. While some other HDL methods of separation such as precipitation, immunoaffinity chromatography, and gel filtration make HDL subject to a wide range of plasma proteins pollution or subject HDL to conditions that are non-physiological that can be altered, hence affecting its structure and/or composition [4, 5].

Proteomics of the fractions containing apoA-I that are separated from the human plasma through a method of non-denaturing fast protein liquid chromatography FPLC revealed that each fraction contains up to 115 individual proteins. Of these 115 individual proteins, only 32 can be identified to be related to the HDL isolated by HDL ultracentrifugation. It is worth noting that there are indeed some of the highly dominant plasma proteins within all apoA-I which contain components that are separated using FPLC, including albumin, haptoglobin, and α-2-macroglobulin, indicating that they are partially bound to HDL non-specific, low affinity binding [6]. In fact, plasma proteins that match the size of HDL co-elution are unavoidable in FPLC-based separations. However, in a series of fractions of different sizes that contain HDL and are separated through FPLCE, the presence of specific proteins is based on their association with phospholipids, indicating that this protein is indeed related to HDL [7, 8].

1.1.1 Apolipoproteins

The main functional and structural HDL protein that accounts for roughly 70% of all the HDL proteins is apolipoprotein A-I [9]. There is a strong belief that almost all the particles of HDL have apoA-I. Some of the primary functions of apoA-I include the conferring of various atherosclerotic activities of HDL, intermingling with cell receptors and the activation of cholesterol/ lecithin acytransferase (LCAT) [10]. Circulating apoA-I is a typical representation of amphiphilic protein that does not have disulfide or glycosylation bonds but has eight 22-amino acid α-helix amphiphilic domains and two 11-amino acid repeating sequences [11]. As a result, apoA-I combines with lipid craze and has effective properties similar to those of a detergent. ApoA-I can easily move through lipoprotein and can also be found in lipoprotein with low density (VLDL) and chylomicrons. The principal sites for apoA-I secretion and synthesis are the ileum and the liver [12].

The second major type of HDL apolipoprotein is ApoA-II. It accounts for roughly 20% of the entire HDL protein reserve [13]. It is speculated that nearly half of the particles of HDL might have apoA-II. As compared to apoA-I, ApoA-II is highly hydrophobic and has a circulation that resembles a homodimer that consists of polypeptide chains that are identical and connected through a disulfide bond. ApoA-II forms a heterodimer with other cysteine-containing apolipoproteins and is chiefly synthesized within the liver.

ApoA-II can easily circulate in free form [14, 15]. It is considered as the most hydrophilic apolipoprotein. It can also be easily exchanged between lipoproteins. ApoA-IV has 22-amino acid repeats that are 13 in number. Out of these 13, 9 are structures that are highly alpha helix. A significant portion of these helices can be described as amphiphilic, and they can serve as domains that are lipid binding [16, 17]. In human

beings, apoA-IV is processed in the ileum and expelled out into the circulatory system together with chylomicrons [18].

ApoC constitutes a unit of small apolipoproteins which have an exchangeable nature and are synthesized primarily in the liver. Of all the apolipoproteins that can be associated with VLDL and HDL and can be easily exchanged between them, apoC-I is the smallest. Its charge is positively strong, and this allows it to bind fatty acids that are free and to regulate the activity of many proteins that are involved in the metabolism of HDL [19]. Therefore, apoC-I has an involvement in inhibiting liver lipase and CETP as well as activating LCAT. ApoC-II has an association with VLDL and HDL, and it also helps in activating a lot of triacylglycerol lipases. Apoc-III is primarily found in VLDL with tiny amounts of it being traceable in HDL. The protein also plays a role in the inhibition of lipoprotein lipase (LPL) as well as liver lipase. Additionally, it reduces the uptake of chylomicrons by the cells of the liver. When overexpressed in mice, ApoC-IV tends to lead to hypertriglyceridemia. In plasma that lower lipids, around 80% of the protein exists in the VLDL and a huge portion of the rest remains within the HDL. As compared to other apoC proteins, the content of HDL in apoC-IV is significantly lower.

ApoD is a protein whose expressions can be found in many tissues, such as those in the intestine and the liver. As a glycoprotein, it is highly associated with HDL [20]. One notable thing about apoD is that it lacks the usual apolipoprotein structural expression, and it belongs to the alicyclic family of proteins. It includes uteroglobulin, lactoglobulin, and proteins that bind retinol. Lipoprotein can be defined as a small protein that transfers lipids and has an amino acid sequence identity that is limited but with a uniform tertiary structure. Lipoprotein compounds have many binding hydrophobic ligands, and they also possess a β-barreled fold that is structurally conserved. ApoD involves the transportation of tiny hydrophobic ligands which in essence have a high affinity for arachidonic acid. In plasma, apoD and apoA-II form disulfide-linked homodimers and heterodimers.

Although the content of apoE in HDL particles is much lower than that of apoA-I, it still constitutes an essential glycoprotein component of HDL with a key functional and structural value. The main part of apoE that circulates is usually carried by lipoproteins that contain tri-glyceride where it acts as ligand to apoE or apoB receptors and, at the same time, ensures lipoproteins bind glycosaminoglycans to the cell surface. Like apoA-II and apoA-I, apoE has amphiphilic α-helix repeats and portrays properties similar to those of detergents for phospholipids. ApoE is synthesized in a variety of cell types and tissues which comprise macrophages, endocrine tissue, the liver, and the central nervous system [21].

ApoF can be defined as a sialic acid glycoprotein that has the presence of lipoprotein with low-density (LDL) and in human HDL. Due to its ability to inhibit CETP, it can also be referred to as LTIP, which stands for lipid transfer inhibitor protein. ApoF is synthesized within the liver and is often glycosylated by N-and O-linked glycosyl groups. This process of glycosylation transforms the protein into a highly acidic state and increases its molecular weight by 40%.

ApoH, which is also referred to as β-2-glycoprotein 1, is very versatile. It is an N-O-glycosylated protein. ApoH can interfere with the processes of activating endogenous coagulation cascades. It can do this by binding to phospholipids and various negatively charged molecules (mainly cardiolipin) on the surface of cells that are damaged. These properties of binding are guaranteed by domains, which are positively charged. Apolipoprotein is responsible for the regulation of platelet aggregation, and it usually has its expression in the liver.

ApoJ (which is also known as complement-related protein SP-40, 40 and cluster protein) is a disulfide-bonded heterodimeric glycoprotein that is antiparallel in nature. Human apolipoprotein comprises of two subunits called α (34–36 kDa) and β (36–39 kDa) that have insufficient homology and five disulfide bonds that are connected. The structure of ApoJ is unique, and it binds a variety of cell surface receptors on one hand and hydrophobic molecules on the other.

ApoL-I is an essential element of HDL-related human trypanosomal degrading factors. ApoL-I has a glycosylation site, and it is structurally and functionally similar to the Bcl-2 family of apoptosis regulator proteins. ApoL-I is highly expressed in the spleen, pancreas, prostate, placenta lung, and liver. It also has a high affinity for cardiolipin and phosphatidic acid. ApoM is an apolipoprotein predominantly present in HDL, which has eight antiparallel β-valerilipocalin folds and a small hydrophobic molecule. ApoM shares with apoD, which is also an apolipoprotein member of the lipoprotein family, with a 19% homology. It is synthesized in the liver and kidney. The hydrophobic N-terminal signal peptide of apolipoprotein ensures binding of apoM to lipoprotein [22]. It remains secreted apolipoprotein, an atypical phenomenon of plasma apolipoprotein [23–25].

ApoO is only expressed in some human tissues. Unlike the others, it is a minor component of HDL. It exists not only in HDL, but also in VLDL and LDL. One other thing about it is that it contains chondroitin sulfite chains and belongs to the family of proteoglycans, distinguished from the peculiar features of proteins. ApoO still has an unknown physiological function. Secondary components of apolipoproteins separated in the HDL density range is also a reflection of the existence of lipoprotein (a), taking apoB and apo (a) as examples and are caused by duplication of HDL2 and lipoprotein (a) (Table 1.1).

1.1.2 Enzymes

LCAT helps in catalyzing the conversion of cholesterol into lipoproteins that are plasma-based then into cholesterol esters. Almost 75% of the activities in plasma LCAT are related to HDL. ApoD and plasma LCAT also have close relations, and apoD is usually co-purified. Major expressions of the LCAT gene are found in the liver while lesser expressions can be traced in the testis and brain. The LCAT protein undergoes very heavy N-glycosylation. LCAT has a secondary structure whose maintenance can be attributed to two disulfide bonds, and it contains an active site covered with a lid. LCAT also has free cysteine residues at positions 184 and 31, which are two in number.

Human paraoxonase is a lactone enzyme dependent on calcium, PON1, PON2, and PON3 [27]. When circulating, PON1's association is almost exclusively associated with HDL [28]. This association requires a hydrophobic leader sequence that remains in the producing PON1 and is largely due to the surface phospholipids on HDL. The synthesis of Human PON1 mainly takes place in the colon, liver, and kidney. It has been suggested that the hydrolysis of homocysteine thiolactone represents the main physiological function of PON1. It is worth noting that the name "PON" reflects PON1's capability to hydrolyze the paraoxon of the organophosphate substrate along with other [29]. The enzyme has cataclysm activities which include reversible binding to the substrate.

PAF-AH is a phospholipase with lipoprotein properties that stands for platelet activating factor acetyl hydrolase. It is independent of calcium, and at the same time an N-glycosylate which degrades biological activity through hydrolyzing sn-2 ester bonds Lysozyme PAF to degrade PAF. The enzyme uses short residues to split phospholipid substrates and can therefore hydrolyze proinflammatory oxidized phospholipids with short chains. There is, however, no activity within non-oxidized phospholipids of long chains. The synthesis of PAF-AH takes place in the brain within the placenta and the white adipose tissue. The macrophages are the most significant source of circulating enzymes. HDL and LDL particles usually combine with plasma PAF-AH for purposes of circulation [30].

The crystal-like structure of PAF-AH illustrates the normal lipase α/β-hydrolase folding and catalytic triad. The site of activity is very close to the lipoprotein surface, and it can gain entry in the water phase at the same time. Hydrophobic residues can cluster in twos and constitute a domain that is lipid-binding in nature, hence ensuring a binding to lipoproteins. Plasma glutathione peroxidase 3 (GSPx-3) is different from other members of the GSPx family which are representative of red blood cells and Hepatocyte

Table 1.1 The components and their major functions of HDL [26]

Proteins		Major function
Apolipoproteins	ApoA-I	Functionally and structurally activates LCAT apolipoprotein
	ApoA-II	It is a functional and structural apolipoprotein
	ApoA-IV	It is a functional and structural apolipoprotein
	ApoC-I	LCAT activator CETP activity modulator
	ApoC-II	Activator of LPL
	ApoC-III	Inhibits LPL
	ApoC-IV	Regulator of the metabolism of TG
	ApoD	Helps in playing a binding role to hydrophobic molecules that are small in size
	ApoE	Acts as a ligand to LRP and LDL-R
	ApoF	Inhibits CETP
	ApoH	Helps in the negative binding of hydrophobic molecules
	ApoJ	Interacts with cell receptors Binds molecules that are hydrophobic
	ApoL-I	Serves as factor in the serum of humans
	ApoM	Binds hydrophobic molecules that are small in nature
Enzymes	LCAT	Polymerization of cholesterol to cholesterol esters
	PON1	Serves as a lactonase that is dependent on calcium
	PAF-AH	Acts as an hydrolysis of phospholipids that are oxidized by short chains
	GSPx-3	Helps in reducing hydro peroxides
Lipid transfer proteins	CETP	Helps in PL homoexchange and the heteroexchange of TG and CE
	PLTP	Transportation of LPS and converting of HDL
Acute-phase proteins	SAA1	Reactant with a minor acute phase
	SAA4	Reactant with a minor acute phase
Complement components	C3	Activation of complements

solute enzymes. These constitute GSPx-1 and GSPX-2. In all reactions that involve glutathione, GSPx enzymes usually protect biomolecules from the damage that arises due to oxidation by helping to catalyze the reduction of organic hydro peroxides, lipid peroxides, and hydrogen peroxide. In plasma, GSPx-3 has only one association with HDL [31].

1.1.3 Other Proteins

Within the protein superfamily bactericidal permeability-increasing protein (BPI)/binding protein (LBP)/lipopolysaccharide (LPS), there exist in Phospholipid transfer protein (PLTP). The synthesis of PLTP occurs in the brain, lungs, skeletal muscle, placenta, kidney, liver, pancreas, and heart. During circulation, a strong relation can be derived between HDL and PLTP, which ends up converting HDL into bigger and bigger particles. PLTP also plays another critical role in the transportation of extracellular phospholipids and could also function in the binding of LPS [32]. PLTP potentially plays a role in the innate immune system. Additionally, it is regarded as a reactant with a positive acute-phase.

CETP has several N-glycosylation sites. It also belongs to the protein superfamily of LBP/BPI/Plunc. The primary expressions of CETP can be found in the adipose tissue and the liver. During circulation, CETP promotes the transfer between triglycerides and cholesterol esters in a bidirectional manner. Additionally, it shuttles between apoB-containing lipoproteins and HDL in the process. A look at the structure of CETP reveals a hydrophobic tunnel that is highly filled with

cholesterol ester molecules and on the other hand blocked amphiphilic phosphatidylcholine (PC).

Serum amyloid A (SAA) protein is usually secreted in the acute phase of the inflammatory response. The protein is the main reactant in the acute phase. In human beings, there are three subtypes of SAA whose production takes place in the liver. They include SAA1, SAA2, and SAA4. During the acute phase reaction, the liver expresses SAA1 and SAA2 therefore causing their levels of circulation to increase by almost 1000-fold from a basal concentration of around 1–5 mg/L. In comparison and contrast, SAA4 has a constitutive expression in hepatocytes. Thus, live concert is often known as the constitutive SAA. It is essential to note that SAA1's existence is not in free form. Instead, it is crucial in binding non-HDL lipoproteins without HDL.

Some complement components are associated with HDL. Complement 3(C3) is crucial in activating a complementary system via the classical pathway of activation and the alternative pathway of activation. C3's existence takes two forms (α and β) which disulfide bonds.

1.2 Lipid Composition

1.2.1 Phospholipids

Among phospholipids, phosphatdylcholine is the main plasma phospholipid. It accounts for 32–35 percentage molecules of the total HDL lipids. As a lipid, PC is structural in nature. This is consistent with the distribution of means among the subpopulations of HDL. Some of the primary types of molecules of PC have their representations by 16:0/18:2, 18:0/18, 2 and 16:0/20:4 types. In comparison to various lipoproteins, HDL has a rich content of polyunsaturated fatty acids found in PC.

There exist subclasses of HDL. LysoPC is an essential phospholipid subclass. This is as a result of controlled PC degradation by phospholipases, one of which is LCAT. This is in line with the latter's preference of binding to HDL particles. To be specific, LCAT has previously been reported to have a good association with small high-density particles of HDL with lysoPC enrichment that is approximately twice that of large, light HDL. The production of LysoPC can also be through the hydrolysis of oxidized PC by PLA2 or LpPLA2. It is usually secreted under proatherogenic conditions such as inflammation and oxidative stress, therefore constituting a likely biomarker of inflammation [33]. LysoPC, a predominant species of HDL, contains mainly fatty acid moieties that are saturated with 18 and 16 atoms of carbon. HDL contamination of these two compounds is typical for FPLC separations. As a significant component of serum lysoPC is also associated with albumin. In HDL, however, when separated by ultracentrifugation of isopycnic density, the content of HDL in lysoPC is 2–1/10 [34].

Phosphatidylethanolamine (PE) is reasonably contained within HDL. Its content increases with an increase in the hydration density of HDL. The predominant species of PE are represented by 38:4 and 36:2 residues of fatty acids.

1.2.2 Sphingolipids

Sphingomyelin is a structure that improves the stiffness of surface lipids. It is the main sphingolipid of HDL that circulates (5.6%–6.6% mol of total lipids). It is derived primarily from triacylglycerol-rich lipoproteins and neonatal HDL sphingolipids. Only part of myelin is 16:0 and 24:1. Sphingomyelin usually consumes up to 30% of dense and small cells as compared to light and large HDL. This is unlike a PL that is negatively charged. The results reflect the low content of sphingomyelin in neonatal HDL, which is a precursor of metabolism in HDL3c, hence suggesting a unique pathway for metabolism in the HDL subgroup.

Of the sphingolipids, SIP is of particular interest because of the important role played by the bioactive lipid in vascular biology. Over 90% of circulating sphingosine phosphates were found in the fractions containing HDL and albumin [35]. It is recommended to choose dense particles which are also small in nature. This is in line with the high levels of apoM in the small and dense

particles. HDL is another biologically active glycosphingolipid, represented by lysophospholipids and thioglycolipids.

Ceramide is heavily involved in the signaling of cells, inflammation, and apoptosis. It is a sphingolipid intermediate. It also has insulin sensitivity and mitochondrial function. Lipids are difficult to transport via HDL. HDL usually accounts for only 25 mol % of the entire ceramide in plasma and only 0.022–0.097 mol% of the entire HDL lipid [36]. Just like sphingomyelin, this sphingomyelin hydrolysis produces a product rich in high amounts of light HDL. This suggests that these lipids have a common metabolic pathway. However, the patterns of major ceramide species (24:0 and 24:1) observed in HDL do not support this hypothesis.

For glychosphingolipids, data on gangliodies and sulfite lipids are lacking. Hexosyl- and lactose-based substances make up the major glycosphingolipids of plasma lipoproteins.

1.2.3 Neutral Lipids

On the surface lipid monolayer of HDL particles, non-esterified (free) sterols are located. They regulate their fluid nature, too [37]. These sterols are mainly composed of cholesterol, which reflects the significant function that lipoproteins play in transporting cholesterol throughout the body. In lipoproteins, the content of other sterols is significantly lower, including the amounts of sitosterol, estrogens, oxysterols, ergosterol, and phytosterols. The affinity of sphingomyelin to free cholesterol is now established, and it helps in the preferential binding of both light and large HDL [38].

The tans esterification of PL through the cataclysm of LCAT with cholesterol results in the formation of large amounts of cholesterol ester (CE) in plasma HDL. Such lipids that are highly hydrophobic constitute the lipid crux of HDL and make up around 35% of the entire HDL lipid [39]. Most HDL CE is caused by cholesterol linoleic acid. Pioneering studies on the distributive nature of CE species in high-density lipoprotein subpopulations by gas chromatography have shown that the distribution between lipoprotein 2 and 3 of high densities is very similar. Similar to CE, the TAG type is reserved between HDL2 and HDL3 [40].

1.3 HDL Structure

1.3.1 Discoid HDL

Discotic high-density lipoproteins, which may be present in human plasma, have a very short life because of their nature as good substrates for LCAT. There has been a detection of HDL disks in isolated compartments like interstitial fluid and peripheral lymphatic fluid, and may have activity in LCAT that is low in nature. As stated above, intervertebral discs can be remodeled. Their particles illustrate a lot of properties of native HDL, such as the activation of LCAT, the transfer of lipids, and the binding of receptors.

The most studied rHDL disk is composed of two molecules of apoA-I. These are usually about 96A in diameter and around 47A thick and contain between 150 and 200 phospholipid molecules [41]. This particle can be described or utilized as a benchmark and utilized as the basis for the original dual-band model of molecules. In this model, two circular apoA-I molecules entail the lipid membrane leaflets in an orientation that is not parallel. The helix 5 of these molecules faces one another directly. In this arrangement, there are boundaries between molecules that depend on the manner in which they are stacked. Right to right (RR) and left to left (LL). An analysis by computers shows that the interface of LL of helix 5 of two molecules that are directly opposite each other has the most weighted score of potential salt bridges between molecules of apoA-I [42]. This is known as the LL5/5 (G129j) registry. It is oriented in the same way as the Borhani crystal structure. This orientation has been validated from experiments in three studies that used the cross-linking of chemicals to derive the orientation of molecules in the two apoA-I have a molecule on these particles. The two molecules in our lab are in good agreement with the 5/5 orientation of most molecules, but

some bridges are also in agreement with the second 5/2 registry. Cross-link research also fits the common 5/5 band model for most molecules. However these researchers showed that the C- and N-terminal 40–50 residues are doubles within the molecule. This improvement is known as the "belt and belt buckle." Martin et al. performed an electron paramagnetic resonance study of 96A rHDL particles that is in consistency with a 5/5 antiparallel double band orientation around the particle [43]. They observed that the properties of the residues 134–145 (within helix5) that spin-coupled were in line with a circular region that created an opening locally between the parallel bands. This "loop" is a potential site for LCAT to enter cholesterol and phosphatidyl chains, suggesting that cholesterol that phosphatidyl chains of the cell membrane are embedded in the lipid bilayer.

1.3.2 Spherical rHDL

A good number of HDL particles that exist in human plasma are spherical in nature. From a structural point of view, little work has been done on these particles. The particles possess a neutral lipid core that comprises cholesterol esters and triglycerides, which eliminates the "edges" of particles that bind apolipoproteins like disks. The helical domain can interact with the acyl chains across the phospholipid surface across the phosphate groups.[44] Furthermore, careful NMR studies have shown that the apoA-I structural changes between the recombinant disc and the sphere are mainly confined to the apoA-IN N-terminus.

The cross-linking method was used to study the structure of recombinant apoA-I particles which are spherical in nature and are produced by the inhabiting rHDL discs together with LCAT under conditions that are controlled. These spheres mainly contain a cholesterol ester core, the diameter of which is about the same as the "reference disc" above. In a cross-link experiment, these balls contain three apoA-I molecules, but the disc has only two. Surprisingly, the cross-linking pattern produced by the sphere is almost the same as the disc. The problem we are solving is the "trilobe model." That is, exactly the similar interactions of molecules that occur between the two apoA-Is in the disk and the three apoA-Is in the ball. The scheme adds a twist to the apoA-I helices 5 and 10. Then, according to the double belt model, two antiparallel belts were bent to 120, and then a similarly bent third molecule was inserted. This creates a cage structure that is three-dimensional and in which the helical domains of each molecule undergo a similar salt-bridge interaction. This is considered the original dual band. It is believed that this cage encapsulates the core of neutral lipid esters and provides support to the polar lipids on the surface with open spaces that intervene or thread between molecules.

1.3.3 HDL Particles

One of the important tools for understanding the basic placement of apoA-I on the surfaces of HDL particles currently is reconstructed HDL disks and balls. The use of traditional spectrophotometry is possible because of the homogeneous nature of these preparations. The overall goal, however, has always been to understand the natural plasma form of HDL. Human plasma HDL is separated by density gradients, as described elsewhere.

Ultracentrifugation differs in almost all properties. Some of these properties include the composition of lipids, the composition of proteins, particle charge, particle shape, and particle size. This lack of uniformity does not include the utilization of conventional construction techniques. A good example is the studies related to circular dichroism of systems where apoA-I has presence in multiple conformations of a varied nature or in combination with some other apolipoprotein that averagely yields data that does not fit any particles present within the sample. In an attempt to make these particles simple like the particles containing only apoA-I and no apoA-II (LpA-I) by immunoseparation, the heterogeneity of formulation composition and size was used for information structure studies. Due to these reasons or factors, understanding the structure of HDL in actual plasma HDL particles is

way behind that of forms that have been reconstituted. The utilization of mass spectrometry as a structural tool eventually broke this barrier. Even in the presence of many other proteins, the resolution and accuracy of these modern-day mass spectrometers can be used in the identification of crosslinks unique to a particular protein. This was used to investigate the five density subcomponents of the normal human plasma HDL drawn by the process of ultracentrifugation. To keep the particles as simple as possible, we mainly separated the particles that contained apoA-I. This suggests strongly that the general properties of the apoA-I structure are associated with various lipid-related morphologies of size, shape, and origin. The careful measurement of lipid/protein composition reveals that the size of each particle is in the range of 3–5 apoA-I molecules. In fact, an essential result of this study is the recognition that the surface of HDL particles is predominantly protein. A good example we determined is that apoA-I occupies 87% of the surface of LpA-13c particles. For the LpA-12b particles that are larger, this is reduced to 71%. This is consistent with compositional calculations, which showed that apoA-I accumulates more in native spheres than in typical recombinant particles.

Furthermore, it is recommended that HDL particle size be adjusted by resident apoA-I torsional motion, which is consistent with Segresto's laboratory MD studies. Through this model, apoA-I constitutes an 11 nm cage that stabilizes approximately 170 phospholipid molecules. The cross section shows the correct volume cavity with a CE and a TG measured in the core. These comprise the first molecular-scale models of the proposed native plasma HDL.

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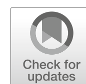

2 HDL and Cholesterol Ester Transfer Protein (CETP)

Siying Deng, Jiewen Liu, and Chenguang Niu

Abstract

Cholesterol ester transfer protein (CETP) is important clinically and is one of the major targets in cardiovascular disease studies. With high conformational flexibility, its tunnel structure allows unforced movement of high-density lipoproteins (HDLs), VLDLs, and LDLs. Research in reverse cholesterol transports (RCT) reveals that the regulation of CETP activity can change the concentration of cholesteryl esters (CE) in HDLs, VLDLs, and LDLs. These molecular insights demonstrate the mechanisms of CETP activities and manifest the correlation between CETP and HDL. However, animal and cell experiments focused on CETP give controversial results. Inhibiting CETP is found to be beneficial to anti-atherosclerosis in terms of increasing plasma HDL-C, while it is also claimed that CETP weakens atherosclerosis formation by promoting RCT. Currently, the CETP-related drugs are still immature. Research on CETP inhibitors is targeted at improving efficacy and minimizing adverse reactions. As for CETP agonists, research has proved that they also can be used to resist atherosclerosis.

Keywords

Cholesterol ester transfer protein · Anti-atherosclerosis · Reverse cholesterol transport · CETP inhibitor · CETP agonists

2.1 Introduction

Cholesterol ester transfer protein (CETP) is a hydrophobic glycoprotein that mediates the bidirectional transfer of cholesterol esters (CEs) and triglycerides (TGs) between plasma lipoproteins in the process of cholesterol reverse transport (RCT). Since CE generally stems from HDLs and TG enters the plasma as a component of VLDLs, CETP activity results in a net mass transfer of CE from HDLs to VLDLs and LDLs, and of TG from VLDLs to LDLs and HDLs [1].

According to observational studies, the risk of cardiovascular disease (CVD) is reduced by about 2–3% for every 1-mg/dL increment in high-density lipoprotein cholesterol (HDL-C). The potential significance of the view is to point out that the HDL-C level in plasma is a reliable

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biomarker for the ability of HDL particles to mediate RCT [2]. In early epidemiological studies and clinical trials, a negative correlation between HDL-C levels and cardiovascular (CV) events has been observed, indicating that low HDL-C levels are recognized as independent risk factors for coronary heart disease [2–4]. Therefore, drug therapies targeting HDL-C levels in plasma, such as CETP inhibitors, emerge with the growing needs of CV health. Regulating CETP activity, as it can directly affect HDL-C levels in plasma and control the occurrence and development of cardiovascular diseases, has become a hot spot in anti-atherosclerotic therapy over the past decades.

However, it has been recently reported that HDL-C levels cannot fully reflect the overall abundance of HDL particles in plasma, the distribution of HDL subspecies, or the ability of HDL-mediated RCT [5, 6]. There is also a lack of correlation between CVD risk and HDL-C levels commonly measured in intervention trials. The biological significance of HDL-C levels might be overamplified. Indeed, some studies have pointed out that although the HDL-C concentration is negatively correlated with CVD and mortality within a specific concentration range, excessively high HDL-C levels may adversely affect the mortality of atherosclerotic cardiovascular disease [7–9]. These findings show that it is unreliable to evaluate the impact of HDL on CVD only from its plasma levels. Data from human genetic researches [10] and the negative results of clinical trials of a large number of CETP-targeted inhibitors have escalated the controversy over the HDL hypothesis. But the altercation should not negate solid experimental evidence manifesting that one of the primary functions of HDL particles is to mediate RCT. When the controversy is approaching, it is also an opportunity for scientists to further investigate the mechanism in order to revise and improve the HDL hypothesis.

This chapter describes the structure of CETP, its mechanism of action, and how CETP regulates HDL-C level under physiological conditions. Progress in research on the relationship between HDL and CETP expression levels in experiments and the results of clinical trials of drugs targeting CETP are summarized.

2.2 Structural and Functional Features of CETP

The human CETP gene is located on chromosome 16, and the gene fragment is about 25 bp, containing 16 exons and 15 introns, similar to the LCAT gene locus [11]. The gene is mainly expressed in the liver, spleen, adipose tissue, heart, kidney, adrenal gland, and small intestine. Human plasma mature CETP is a hydrophobic glycoprotein composed of 476 amino acids with high lipophilicity, of which crystal structure observed under an electron microscope has the shape of a flyer.

In vitro, kinetic data show that HDLs, VLDLs, and LDLs regularly exchange in the hydrophobic channels within CETP through the shuttle mechanism or ping-pong mechanism and complete the transformation from HDLs to VLDLs and LDLs [12–14].

In 2007, Qiu et al. [13] constructed the structural model of CETP and pointed out that CETP has a tunnel structure with four structural components:

- The N-terminal β-bucket region.
- The C-terminal β-bucket region.
- The central β-fold.
- The C-terminal extension (the C-terminal involves the twisted amphiphilic helix of Glu465-Ser476, i.e., the helix X).

The structure model demonstrates that CETP is able to hold two neutral lipids (which can be either two cholesterol esters, one cholesterol ester and one triglyceride, or two triglycerides) and two phospholipids on specific binding sites. The tunnel openings of the N-terminal domain and the C-terminal domain are each covered by a phospholipid. The tunnel allows neutral lipids to run through their entire length rather than stay in two separate pockets [15]. Zhang et al. [16] found that CETP connects high-density lipoprotein and low-density lipoprotein to form a ternary complex. The N-terminal β-Barrel domain is inserted into the surface lipid monolayer of high-density lipoprotein. Ternary complexes' structural characteristics support the "tunneling

mechanism" of CETP transferring neutral lipids between different lipoproteins. Lei et al. [17, 18] used molecular dynamics simulations to study CETP. The distal flexibility of the N-terminal β-Barrel domain in solution is much greater than that in crystals. It can penetrate to the surface of high-density lipoprotein, thus promoting the absorption of cholesterol esters. Under the action of a series of driving forces, the hydrophobic tunnel inside the CETP is sufficient to allow the CE molecule to pass through the whole CETP in the predicted transport time. The simulated transport rate is similar to that of physiological measurements [19]. Parallel studies using microsecond-scale coarse-grained molecular dynamics simulations also show that CETP has a high degree of conformational flexibility and can form a continuous tunnel across its long axis without additional driving force, through which CEs and TGs can be directionally transferred [20].

2.3 CETP and Reverse Cholesterol Transport (RCT)

The anti-atherosclerotic effect of HDL makes it an essential cardiovascular protective factor. Typically, HDL has a variety of anti-atherosclerotic biological activities, such as promoting RCT, reducing the oxidative modification of LDL by oxidative free radicals, inhibiting the pathological remodeling of chronic inflammation on the arterial wall, improving endothelial function, repairing endothelial cell, anti-thrombosis, anti-apoptosis, anti-diabetes, and anti-infection [21–24]. Each of these mechanisms may play an imperative role in HDL-mediated cardiovascular disease protection. Particularly, its ability to promote RCT is considered to be the key to tackling atherosclerosis. HDL can be regarded as both a cellular cholesterol receptor and a cholesterol carrier in the RCT pathway. RCT is an indispensable step in the transformation of plasma HDL. Controlling the biological process has a far-reaching effect on anti-atherosclerosis. In the RCT pathway, as one of the critical proteins in the modification of HDL, it has been clear that the regulation of CETP activity can further change the level of plasma HDL-C.

Cholesterol exists in all mammalian cells, with the highest concentration in the plasma membrane and the lowest in the endoplasmic reticulum(ER) membrane. A steady-state of cholesterol in cells is vital for normal cell functions. RCT refers to the process in which cholesterol moves out from the cells of peripheral tissue (including foam cells in atherosclerotic plaques), enters the circulation, and is excreted out of the body with feces. Cholesterol extracted from human peripheral cells enters the liver through RCT in two pathways: (1) the direct way is the interaction between HDL and scavenger receptor B1 (SR-B1) on the surface of hepatocytes, (2) the indirect way is mediated by CETP. Cholesterol is converted between HDLs, LDLs, VLDLs, and finally enters hepatocytes. CE is hydrolyzed in the liver, and free cholesterol is either converted into bile acid or transported into bile by ABCG5 and ABCG8 and then excreted into feces [25].

The indirect way of RCT mainly includes three steps. Firstly, nascent HDL can obtain free fatty acid (FC) from extrahepatic tissue cells through two methods: (1) passive diffusion of fatty acids; (2) cholesterol outflow through ABCA1 and ABCG1 transporters on the cell membrane. The second step is that the lecithin cholesterol acyltransferase (LACT), activated by Apo-AI on the surface of HDL, transforms FC into cholesterol ester into the core of HDL, and the nascent HDL is transformed into HDL_3 with higher density and smaller particles. Under the mediation of CETP, phospholipid transporter (PTP), and other proteins, about 80% of esterified cholesterol esters are transferred from HDL to VLDL and LDL, 20% the core of HDL. At the same time, TG is transferred from VLDL to HDL core, which causes HDL to change from HDL_3 with small particles to HDL_2 with large particles. CE enters the core of VLDL and further forms LDL to complete TG and CE redistribution between lipoproteins. Finally, HDL is degraded in the liver [26–28]. There are two main ways of degradation of LDL formed in this process: (1) to bind to the LDLR on the surface of hepatocytes to enter intracellular catabolism, (2) to be swallowed

by monocytes-macrophages. If plasma LDL is pathologically oxidized, it can be consumed by macrophages and vascular smooth muscle cells to form foam cells, which gradually accumulate and lead to atherosclerotic lesions.

In sum, it can be seen that regulating CETP activity to control these lipid transfers governs the concentration of CE in HDLs, VLDLs, and LDLs, and ultimately affects the formation of foam cells.

2.4 The Inconclusive Effect of CETP on Atherosclerosis

Species lacking CETP, such as rodents, are considered to be immune to arterial disease, and the plasma contains high HDL [29, 30]. Early epidemiological cohort studies revealed an increase of HDL-C levels and a decrease of LDL-C levels in patients with CETP deficiency, whose lipoprotein profile has potential anti-atherosclerotic effects [31, 32]. These findings boost the development of CETP inhibitors. Nonetheless, a comprehensive analysis of animal experiments and cell experiments conducted among different species indicates that whether reducing CETP can reduce atherosclerosis occurrence is still controversial. The impact of CETP gene regulation on blood lipid levels and cardiovascular diseases remains to be determined in the future.

2.4.1 Inhibition of CETP Activity Is Beneficial to Anti-Atherosclerosis

The difference between rodents and humans is the lack of CETP genes. Accordingly, to use rodents to study the mechanism of CETP function, transgenic models must be constructed. Marotti KR et al. [33] bred transgenic C57BL/6 mice expressing different levels of CETP in crab-eating monkeys and fed a high-fat diet with C57BL/6 mice (no CETP activity), UCTP-45 (low CETP activity), and UCTP-20 (high CETP activity) mice, respectively. Compared with wild-type mice, UCTP-45 and UCTP-20 mice showed lower levels of plasma HDL-C and Apo-AI and aggravated aortic atherosclerosis, suggesting that CETP may have an atherogenic effect. Compared with low-activity UCTP-45 mice, the degree of proximal atherosclerosis in high-activity UCTP-20 mice increased, and the results showed that CETP expression level was positively correlated with atherogenic lesions. Plump et al. [34] crossed the human CETP transgene into the classic atherosclerosis model Apo-E$^{-/-}$ mice and LDLR$^{-/-}$ mice and fed them with a high-fat diet. The results indicated that CETP expression led to a moderate increase of atherosclerosis in Apo-E$^{-/-}$ and LDLR$^{-/-}$ mice. It suggested that CETP activity played an atherogenic role in the metabolic environment of severely damaged (LDLR$^{-/-}$) clearance of residues (Apo-E$^{-/-}$) or LDL.

Apo E*3-Leiden transgenic mice are also a classic hyperlipidemia animal model. The target gene Apo E*3-Leiden (a variant of Apo E3) was microinjected into the male pronuclei of fertilized eggs with the same genetic background (C57BL/6JxCBA/J). The transgenic mice were mated with C57BL/6 J mice to produce transgenic mice. This mouse model of hyperlipidemia and AS can be employed to investigate the influence of environmental factors and genetic factors on hyperlipidemia (HLP). It can be used to screen blood lipid regulating drugs and anti-AS drugs as well. This transgenic mouse is highly prone to hyperlipidemia and AS induced by a high-fat diet (or even a low-fat diet). This model has a significant advantage in studying the relationship between serum TC exposure and AS area. Westerterp et al. [35] evaluated the effect of expressing human CETP on atherosclerosis in Apo E*3-Leiden (E3L) mice using a humanized lipoprotein map. They hybridized E3L mice with human CETP transgenic mice and fed E3L mice (control group) as well as CETP.E3L mice (experimental group) with the western diet containing 0.25% cholesterol. The results displayed that the expression of CETP in E3L mice strengthened cholesterol transfer from high-density lipoprotein (HDL) to very-low-density lipoprotein/low-density lipoprotein (VLDL/LDL) and decreased plasma-mediated SR-BI-dependent cholesterol efflux.

This implied CETP was an apparent atherogenic factor in E3L mice. And they expected that CETP.E3L mice would become a valuable model for preclinical evaluation of elevated high-density lipoprotein intervention in atherosclerotic progression.

Because it is necessary to model complex diseases in different species, there are also preliminary studies in other rodents such as rats. To explore the direct effect of CETP on HDL structure and composition in vivo, Zak et al. [36] expressed CETP in Fisher rats to produce CETP transgenic rats, a new CETP-Tg rat strain. In contrast to other previously reported animal models for CETP transgene (including C57BL/6 mice and Dahl rats [37]), Fisher rats have higher plasma levels of large-sized HDL_1. They fed the rats with typical food. The results showed that compared with the control group, the plasma HDL-C level of the experimental group was significantly reduced by 48%. Among different plasma high-density lipoprotein subgroups, HDL_1 decreased the most. In this study, continuous expression of varying levels of CETP in Fisher rats with high HDL_1 can comprehensively and physiologically reflect the effect of CETP on HDL. Moreover, Zak et al. [38] constructed a CETP transgenic rat model, and rats were fed with a standard diet, high-fat diet, and a high-sugar diet, respectively. Consequently, CETP had different effects on lipoprotein profiles under different diets. Compared with wild-type rats, the HDL-C concentration of transgenic rats fed with a standard diet was lower, but the concentration of non-HDL-C was similar. The levels of total cholesterol and non-HDL-C in wild and transgenic rats fed with a high-fat diet increased substantially but did not affect CETP expression. Compared with wild-type rats fed with sugar, the sugar diet only increased the non-HDL-C of transgenic rats by 82% and reduced HDL cholesterol by 80%. It is suggested that the effect of CETP on lipid metabolism may be different due to different basic blood lipids.

Unlike rodents, rabbits have CETP gene. Their plasma CETP activity is higher than that of humans, and the characteristics and metabolism of lipoproteins are similar to those of humans. It is an ideal model to study the inhibitory effect of CETP, and the results are consistent. Zhang et al. [39] obtained $CETP^{-/-}$ rabbits by zinc finger nuclease gene-editing technique and fed them a high cholesterol diet. Compared with wild-type rabbits, plasma HDL-C levels increased, and aortic and coronary atherosclerosis were significantly alleviated in $CETP^{-/-}$ rabbits. In addition to CETP gene knockout, previous studies also inhibited the content and activity of CETP in rabbits by injecting CETP antisense oligodeoxynucleotide [40] or CETP vaccine [41] aimed at the liver or by applying small molecule inhibitor [42, 43]. The results proved that the level of plasma HDL-C in the experimental group was higher than that in the control group, and the atherosclerotic lesions were alleviated.

Other animal models of atherosclerosis include zebrafish, miniature pigs, dogs, etc. In recent years, zebrafish have gradually become a model animal suitable for studying atherosclerosis because of its unique physiological advantages. Zebrafish possess a set of genes related to lipid metabolism that are conservative and highly similar to humans. Its lipoprotein distribution is parallel to that of humans and is sensitive to high-fat foods. It is crucial that zebrafish also have CETP genes.

For this reason, zebrafish can be an ideal model for lipid metabolism and atherogenesis research [44–46]. Jin et al. [47] used high-cholesterol zebrafish to analyze and test the anti-atherosclerotic activity in vitro and in vivo on water extracts of ground pepper, cinnamon, rosemary, ginger, and cloves. As a result, cinnamon or clove extracts affect CETP. The inhibitory effect is concentration-dependent, showing blood lipid-lowering activity. The study also demonstrated that hydrophilic components of cinnamon and cloves show effective anti-atherosclerosis and anti-diabetes abilities by preventing Apo-AI glycosylation and LDL phagocytosis, inhibiting CETP, and lowering blood lipid activity. Kim et al. [48] studied the molecular mechanism of human cellular atherosclerosis and senescence induced by HDL in zebrafish model through the modification and dysfunction of $CdCl_2$ and the changes in embryo

survival rate, plasma lipoprotein profile, CETP activity, and fatty liver. Cadmium can be ingested and inhaled through smoking, so it is easy to interact with lipoprotein, making this modification directly related to the production of dysfunctional high-density lipoprotein. CETP activity in each group fed with $CdCl_2$ was higher than that in the control group and damaged the beneficial function of HDL, resulting in a loss of antioxidant, anti-atherosclerotic, and anti-aging activities.

Over the past few years, cellular studies in vitro have found that CETP promotes atherosclerosis by enhancing cellular inflammation and lipid metabolism. Zhang et al. [49] performed immunohistochemical analysis on the expression of CETP in human atherosclerotic lesions. In their study, CETP was expressed massively in foam cells of human aorta and coronary atherosclerotic lesions but not in normal arterial walls. Foam cells in lesions are mainly derived from macrophages. Studies have suggested that CETP may enhance cellular inflammatory response, thereby promoting the formation of atherosclerotic lesions.

Furthermore, Gao et al. [50] bred transgenic rabbits overexpressing the human CETP gene to detect the effect of CETP on atherosclerosis development and fed them with a high cholesterol diet. The number of macrophages in atherosclerotic lesions of CETP transgenic rabbits significantly increased, representing that CETP overexpression can increase inflammatory response and increase the accumulation of macrophage-derived foam cells in atherosclerotic lesions, thus promoting atherosclerosis. It is still unclear whether CETP encourages the accumulation of macrophage-derived foam cells in atherosclerotic lesions by inhibiting cholesterol efflux from macrophages yet, and other molecular mechanisms need to be studied. To further clarify the role of CETP in cell lipid metabolism, Izem et al. [51] used plasmid transfection to overexpress full-length CETP in liposarcoma cells SW872 stably. Compared with the control group, lipid dynamics was consequently disrupted by CETP overexpression: balanced, intracellular triglycerides were reduced, and smaller, more metabolically active lipid droplets were formed in the cell. This also suggests that CETP may cause abnormal blood lipid metabolism in the body, thereby promoting atherosclerosis.

2.4.2 The "Self-Contradictory" Anti-Atherosclerotic Effect of CETP

However, some studies have found that CETP may have an anti-atherosclerotic effect. Foger et al. [52] hybridized LCAT transgenic mice with CETP transgenic mice and gave them a high-fat diet. The results showed that the plasma HDL-C level of the hybrid group was lower. The average aortic lesion area was 41% less than that of simple LCAT transgenic mice, suggesting that CETP has an anti-atherosclerotic effect. Besides, Hildebrand et al. [53] introduced the human CETP gene into SR-B1$^{-/-}$ mice and given a high-fat diet to explore whether CETP can normalize HDL-C transport and reduce atherosclerosis in SR-B1$^{-/-}$ mice. The results revealed that CETP could restore HDL-C levels in SR-B1$^{-/-}$ mice and partially normalize abnormally large HDL particles but did not change the susceptibility and other typical atherosclerosis characteristics associated with SR-B1 deletion.

2.4.3 Further Analysis of the Relationship Between CETP and Atherosclerosis by Human Gene Research

In the past years, observational studies have shown that HDL-C level is negatively correlated with the risk of myocardial infarction, while LDL-C is positively correlated. However, observational studies cannot determine the causality and underlying pathophysiological signs in the pathological process. These two aspects can be distinguished in humans by changes in cholesterol components or genetic DNA variation in large-scale randomized trials [10].

Nomura et al. [54] tested whether the Protein-Truncating Variants (PTV) at the *CETP* gene was associated with blood lipid levels and coronary heart disease. PTV carriers of *CETP* manifested higher HDL-C, lower LDL-C, lower triglycerides, and lower risk of coronary heart disease than non-carriers. Although SNP that reduces CETP expression or impairs its function may play a role through changes in LDL-C or non-HDL-C, the *CETP* mutation itself does not affect coronary heart disease risk. Still, it is closely related to the degree of reduction of LDL-C. Iwanicka et al. [55] examined whether *CETP* gene polymorphisms (rs1532624, rs247616, and rs708272) were associated with coronary heart disease (CAD) in the Polish population. Blood lipid levels and single nucleotide polymorphism of the *CETP* gene were detected in 494 premature infants with coronary heart disease (CHD) and 246 blood donors (control group). In this cross-sectional study, the *CETP* gene rs247616 and rs1532624 polymorphisms might regulate coronary heart disease risk in the Polish population. Vargas et al. [56] assessed whether *CETP* and *LCAT* gene polymorphisms were statistically associated with an HDL size distribution, HDL subclass cholesterol levels, or acute coronary syndrome (ACS) susceptibility. They analyzed two polymorphisms in the *CETP* gene (rs4783961 and rs708272) and one polymorphism in the *LCAT* gene (Rs2292318) in 619 ACS patients and 607 normal controls. Studies have confirmed that the *CETP Taq1 BA5454G* (Rs708272) polymorphism of the *CETP* gene is associated with an increased risk of ACS. It is also possible to distinguish a haplotype (*AGA*) associated with an increased risk of developing ACS. On the other hand, *LCAT A21892G* (Rs2292318) and *CETP Taq1 BA5454G* (Rs708272) gene polymorphisms are statistically significant with lower cholesterol levels in specific HDL subclasses.

2.5 Application of CETP-Related Drugs Therapy

Lipid-lowering therapy is the preferred strategy for the treatment of cardiovascular disease in clinical application. Statins are hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors, which can considerably reduce LDL-C. But many patients are still experiencing cardiovascular events. This residual risk highlights the need to develop additional treatment strategies for high-risk patients. According to the results of animal and cell experiments and the randomized Mendelian analysis of CETP gene polymorphism, the effect of CETP level on atherosclerosis has not been determined. Although clinical trial results of four CETP inhibitors are not ideal, they are still hot new drugs for anti-atherosclerotic therapy. What's more, the researches on CETP agonists are also in full swing.

2.5.1 Animal Experiments and Clinical Trials of CETP Inhibitors

Since the advent of CETP inhibitors, after continuous improvement of the structural formula, there are four major kinds of targeted inhibitors of CETP: neutralizing antibodies, antisense oligodeoxynucleotides, vaccines, and small molecular inhibitors. And four small molecular inhibitors have completed phase 3 clinical trials. They have been systematically evaluated respectively, but the performance of all compounds in reducing the risk of cardiovascular events is not satisfactory. The correlation between plasma HDL-C levels and the incidence of CVD needs to be further verified.

Zhang et al. [15] explored the mechanism of CETP inhibitors and observed a significant increase in CETP binding to HDL induced by inhibitors (from ~9.9% to ~50.5%). Like HDL, inhibitors significantly increased the binding rate of CETP to LDL (from ~12.9% to ~21.8%). Unexpectedly, inhibitors increased CETP binding

to each type of lipoprotein (HDL or LDL, to form binary complexes), but did not increase CETP binding to these two types of lipoproteins (forming ternary complexes), which reduced the formation of ternary complexes. The efficiency of Torcetrapib in lowering the proportion of CETP ternary complex is similar to that of Anacetrapib, and they are higher than that of Dalcetrapib. These effects seem to be consistent with the corresponding degree of lipid regulation effectiveness observed in large clinical trials.

ILLUMINATE trial [7] assessed the benefits of CETP inhibitor Torcetrapib in patients with ACS and moderate cardiovascular risk of stroke. The evaluation results give that HDL-C increased by 72.1% compared to the baseline, and LDL-C decreased by 24.9%. However, the trial was terminated prematurely due to the increased risk of death and cardiac events in patients treated with Torcetrapib. Now it is generally believed that the reason may be related to the off-target effect of Torcetrapib, which can lead to an increase of systolic blood pressure, a significant growth of corticosteroids, and a decrease of serum potassium. The subsequent Dal-OUTCOMES trial [57] evaluated whether Dalcetrapib could benefit ACS patients. As a result, Dalcetrapib had no noticeable off-target effect. The plasma HDL-C level in the experimental group increased. Yet it could not reduce the risk of cardiovascular events and was terminated precipitately, which might be related to the relatively weak effect of Dalcetrapib. ACCELERATE trial [58] evaluated the efficacy and safety of Evacetrapib in patients at high risk of cardiovascular diseases such as ACS and cerebrovascular atherosclerotic disease. Although Evacetrapib significantly increased HDL-C levels, decreased LDL-C levels, and enhanced cellular cholesterol outflow capacity, Evacetrapib treatment did not reduce cardiovascular events and had an early termination of a small number of subjects and short follow-up time [59]. REVEAL trial [60], the only test with positive results, included 30,449 adult patients with atherosclerotic vascular disease receiving intensive Atorvastatin. During the median follow-up period of 4.1 years, the average HDL-C of the Anacetrapib group was 104% higher than that of the placebo. Still, the intermediate level of non-HDL-C was 18% lower. The average level of apolipoprotein B was 18% lower than that of the placebo, and the average level of lipoprotein (A) was 25% lower than that of the placebo. Major endpoint events, including coronary artery death, myocardial infarction, or coronary revascularization, were 10.8% in the Anacertrapib group and 11.8% in the placebo group. Despite these results, Merck decided not to continue to ask regulators to approve Anacetrapib. That is probably because of the long persistence of the drug in blood and adipose tissue [61]. The newly reported CETP inhibitor is TA-8995. During the 12-week treatment alone, LDL-C decreased by 45%, and HDL-Mel C levels increased by 180%. But in order to seek a more accurate judgment on its effectiveness, a cardiovascular clinical outcome trial is needed to determine whether these effects translate into reduced ASCVD events [62].

It can be deduced that in the future, the development of CETP inhibitors is to reduce the molecular weight and fat solubility while maintaining the activity. Moderate lipophilicity and appropriate molecular weight of inhibitors are conducive to further structural optimization and drug efficacy, and this method can minimize missed targets and adverse reactions [63]. Some studies have pointed out that the cardiovascular effect of CETP inhibitors in the REVEAL trial may reduce non-HDL-C levels [64].

In comparison with the clinical trials, the positive effects of CETP inhibitors in animal experiments are more apparent. In recent years, the synthesized small molecule CETP inhibitor K-312 increases HDL-C and inhibits the proprotein convertase subtilisin 9. Compared with CETP inhibitors such as Anacetrapib and Evacetrapib, its efficacy is more robust. Miyosawa et al. [65] fed the rabbits with a high-fat diet. The research showed that K-312 could increase HDL-C, lower LDL-C, and reduce aortic atherosclerosis. Brodeur et al. [66] studied the effects of two CETP inhibitors Dalcetrapib and Anacetrapib, on the distribution of serum HDL particles and cholesterol efflux in rabbits and

monkeys. In rabbits, CETP increased HDL-C and increased ABCA1-induced cholesterol efflux. While Anacetrapib generated similar results in horsetail monkeys, Dalcetrapib had an opposite effect, as LDL-C levels rose by 42% and HDL-C levels fell by 48% (<0.01). Adverse effects of Dalcetrapib in different species indicate that its impact on HDL metabolism may vary greatly depending on the metabolic environment. To determine whether CETP inhibition has different effects due to obesity, Lin et al. [67] applied short-term Anacetrapib treatment on CETP transgenic mice fed with a high-fat diet. Anacetrapib increased HDL-C levels, improved HDL function, including reverse cholesterol transport, and had better antioxidant capacity in high-fat-fed mice than in chow diet-fed mice. Anacetrapib decreased the anti-inflammatory ability of HDL in fed mice. The HDL proteome of HFD mice treated with Anacetrapib was distinct from that of chow-fed mice. Although it is beneficial to HDL, anacetrapib leads to triglyceride accumulation and insulin resistance in the liver of high fat-fed mice. Overall, their findings support the physiological importance of CETP in protecting the fatty liver and suggest that background selectivity for CETP inhibition may be necessary for obese subjects.

2.5.2 Research Progress of CETP Agonists

The CETP pathway can also be activated by reagents such as probucol which, however, was initially labeled as an antioxidant. Probucol is very hydrophobic. In plasma, probucol is generally carried by low-density lipoprotein, and its single oral absorption is limited and changeable. After chronic administration, the steady-state plasma level was reached a few months later. Probucol is retained in fatty tissue, and the concentration in adipose tissue is 100 times higher than that in plasma [68].

Early studies have shown that probucol in low-density lipoprotein can delay the beginning of lipid oxidation, which can contribute to anti-atherosclerosis [69]. The preventive effect of probucol on the progression of atherosclerosis was preliminarily determined by the study of WHHL rabbits. In the experiment, the drug had an impressive delaying impact on aortic atherosclerosis development in cholesterol-fed rabbits [70]. It has been proved that the anti-atherosclerotic properties of probucol can be attributed to many molecular mechanisms, i.e., promoting the formation of pre-low-fat HDL 1-β involved in cellular cholesterol efflux, improving PON1 activity, and increasing cholesterol efflux by activating CETP and liver SR-B1 to promote RCT, thereby reducing HDL-C levels [71–75]. Probucol-increased CETP could accelerate CE from HDL to form small cholesterol ester deficient HDL, with strong cholesterol removal ability.

Additionally, probucol may increase the expression of SR-B1 protein through species-specific protein stabilization [74, 76]. Probucol significantly antagonized the decrease of liver SR-B1 after a high cholesterol diet, increased liver HDL-CE uptake by two times, and reduced atherosclerosis. Yamamoto et al. [77] researched on wild-type and SR-B1 KO mice fed with a 0.5% probucol diet and regular diet. In wild-type mice, probucol reduced HDL-C by more than 80% but maintained macrophage RCT. In gene knockout mice, probucol not only massively decreased HDL-C but also considerably increased macrophage RCT. In addition, probucol significantly increased the excretion of HDL-C in feces of wild type and SR-B1 knockout mice. It can be concluded that the positive effect of probucol on CETP is only one of its anti-atherosclerotic mechanisms. At present, there are still a few agonists that simply target CETP to resist atherosclerosis.

2.5.3 Reasonable Regulation of HDL

In addition to HDL-C levels, in recent years, many studies have shown that other characteristics of HDL, such as its structure, subtype distribution, and levels, can influence the HDL-mediated RCT pathway. It is reported by Nanab et al. [78] that Apo-A1, the main

constituent protein of HDL, can be destroyed by mechanisms such as oxidation. The oxidation process could reduce the proteins mediating cholesterol efflux in HDL, affecting its cholesterol efflux ability. Simultaneously, other types of HDL proteins can also be affected by oxidative environments such as acute stress. Changes in the composition of these proteins can significantly vary the anti-atherosclerotic properties of HDL. Khera et al. [79] found that cholesterol efflux from macrophages was slightly better at predicting coronary heart disease than HDL-C (odds ratio, 0.75 vs. 0.85). They believed that the heterogeneity of high-density lipoprotein in particle size, charge, and protein composition makes HDL-C levels a deficient substitute for cholesterol outflow, reducing the predictive value of HDL-C in carotid intima-media thickness and coronary heart disease. Williams et al. [80] investigated the relationship between lipoprotein subfractions and CHD and pointed out that HDL_2 plasma levels had the most significant reduction in coronary heart disease risk among all HDL particles. Besides, macrophage cholesterol efflux is positively correlated with HDL_2 concentration but has nothing to do with plasma total HDL concentration [81]. Hence, it is instructive to observe the effect of plasma HDL_2 level on coronary heart disease and determine the relationship between cholesterol efflux capacity and carotid intima-media thickness.

HDL-C ‘s Mendelian randomized (MR) trial also showed a neutral event between plasma HDL-C levels and coronary heart disease risk [82]. A more comprehensive assessment of the potential relationship between a given biomarker and cardiovascular risk is to be conducted in order to further develop expensive drugs. Before starting clinical development, genetic research should be more integrated to provide further assurance [83].

2.6 Conclusion

CETP is a hydrophobic glycoprotein with high lipophilicity. The “tunneling mechanism” realizes CE and TG conversion between HDLs, LDLs, and VLDLs and completes the redistribution. The vital relationship between CETP and lipoproteins such as HDL and LDL is an essential target for anti-atherosclerotic therapy in academia.

Over the years, scientists have conducted animal experiments, cell experiments in different species, and several population studies. Recently, they have explored CETP and have also analyzed the relationship between CETP gene polymorphism, but the results are controversial. On the one hand, some studies have shown that inhibiting CETP to increase plasma HDL-C levels can have an anti-atherosclerotic effect. On the other hand, some studies support that CETP weakens atherosclerosis formation by promoting RCT, so increasing CETP can also be beneficial. This may be caused by the heterogeneity of lipoprotein, the complex mechanism of reverse cholesterol transport, different animal genetic backgrounds, different blood lipid baselines, etc.

As a result, the state of CETP inhibitors and agonists is still a mystery. Although CETP antagonism led to a significant reduction in CV events in some major long-term studies, the tests of many other inhibitors were aborted accidentally. Besides, the only drug whose CV results are positive, Anacetrapib is accompanied by excessive persistence in the blood, especially in tissues. Hence it is considered unsuitable for further clinical development. Given that current inhibitors only inhibit the formation of ternary complexes, it is worth noting whether the development of a new generation of CETP inhibitors can reduce the rate of binary and ternary complexes among HDL, LDL, etc. and make CETP more effective in atherosclerotic protection [15]. While Probucol encounters barriers in some countries, it is prevalent in eastern countries, leading to an increase in CETP activity and the decrease of HDL-C. However, probucol treatment is also associated with its impressive ability to clear cholesterol ester deposits and reduce interleukin-1 β in tissues. Probucol remains an effective way to prevent CV, and the potential development of its analogs is promising.

To sum up, the field of CETP pharmacology remains open, and additional data, such as data

from the ongoing Dalcetrapib gene study [84], will further clarify the potential benefits of CV.

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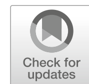

3 HDL and Endothelial Function

Yufei Wu, Yangkai Xu, Jie Chen, Mingming Zhao, and Kerry-Anne Rye

Abstract

Plasmid high-density lipoprotein (HDL) is a critical biomarker in predicting cardiovascular diseases. Endothelial cells are physically located in the intima of blood vessels, which directly contact with circulating substances. Numerous previous studies have demonstrated that HDL exert protective effects on maintaining endothelial integrity and enhance anti-inflammatory functions, etc. In this chapter, we introduced how HDL benefit endothelial functions. We summarized the function of HDL on endothelial cell, such as endothelial permeability, proliferation, migration, apoptosis, etc. In addition, we discussed the effects of HDL on classical endothelial functions, such as coagulation and vasodilation. Although HDL have huge effects on endothelial functions, lots of cardiovascular diseases such as atherosclerosis could not be fully prevented and treated. Thus, a further understanding of the relationship between HDL and endothelial cell is needed, which would create a potential therapeutic approach to cardiovascular diseases.

Keywords

HDL · Endothelial cells · Endothelial barrier integrity · Endothelial function

3.1 Introduction

Endothelial cells (ECs), structurally, line a barrier between blood and tissue and exert pivotal functions in sustaining vascular homeostasis [1]. Endothelial dysfunction or endothelium impaired would initiate and deteriorate the pathogenesis of various disorders, especially atherosclerosis [2, 3].

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Plasma levels of HDL cholesterol have been used as a biomarker in cardiovascular health. It has been reported that a low level of HDL in plasma is an independent risk factor of endothelial dysfunction not only in healthy subjects but also in patients suffered from coronary artery disease (CAD) [4]. As we all know, the best vessel-protective characterization of HDL is its capacity to transport cholesterol from peripheral tissues to liver for bile acid synthesis, which is called reverse cholesterol transport (RCT) [5, 6]. Additionally, many experimental and translational studies have further revealed that HDL from healthy subjects also has a wide variety of protective effects on ECs implicating in the maintenance and repairment of endothelial barrier integrity, the improvement of ECs-dependent vasodilation, anti-inflammation, and anti-thrombosis, thereby exerting anti-atherogenic function.

However, pharmacologically raising the level of HDL cholesterol in plasma fails to reduce cardiovascular events [7, 8], which may be ascribed to HDL dysfunction or heterozygous characteristics in disease states [9]. Here, the focus of this review will in considerable detail summarize those endothelial functions affected by normal HDL and dysfunctional HDL, and molecular mechanisms involved.

3.2 HDL and Endothelial Barrier Integrity

ECs form a semipermeable barrier between the blood and the tissue cells. The vascular endothelial barrier is of the great importance to the cardiovascular and cerebrovascular diseases, in particular to the development of atherosclerosis and thrombotic disorder [10, 11]. In disease conditions, endothelial barrier integrity has been investigated to mainly involve two aspects: the endothelial permeability on a molecular level and the repairment and maintenance of endothelial monolayer after injury in a cellular level [12]. The former, the endothelial permeability, is mediated by paracellular and transcellular transport system [13, 14]. In addition to neighboring-cells junctions, paracellular pathway is also intimately linked to cytoskeletal rearrangement rapidly refilling cell-to-cell gap [15, 16]. Additionally, the later has been thought to associate with endothelial apoptosis, proliferation, and migration. Furthermore, the endothelial permeability has a close relationship with the maintenance of endothelial monolayer not only by affecting cell to cell adhesion but also transferring intracellular signaling. It has been reported that HDL from healthy subjects can protect endothelial barrier integrity from disease factor-induced disruption, by directly or indirectly maintaining cellular junctions, reducing ECs apoptosis, and promoting resident ECs proliferation and migration [11, 12, 17].

3.2.1 HDL and Maintenance of Endothelial Permeability

The integrity of endothelial permeability is associated with a variety of vascular pathologies, such as edema, inflammation, and some vascular diseases [12].

Studies have been shown that sphingosine-1-phosphate (S1P), particularly HDL-containing S1P, is able to protect the vascular barrier integrity. S1P, as one of bioactive lysophospholipids (LPs), produces mainly from three kinds of sphingolipids: sphingomyelin (SM), ceramide, sphingosine metabolism [18]. In detail, SM is catalyzed into ceramide by sphingomyelinase enzyme; then, ceramide is deacylated to form sphingosine by ceramidase; finally, sphingosine is phosphorylated to produce S1P by sphingosine kinase enzyme [18]. The concentration of S1P is present at quite high levels in the blood and lymph but low in tissues [10, 19]. The plasma level of S1P is critical to regulate physiological and pathological progress. In addition to synthesis, there is no doubt that S1P levels are closely related to its degradation which is regulated by S1P lyase, S1P phosphatases, and LPs phosphatase 3 [19]. Interestingly, S1P sources are from various cell types and tissues, such as platelets [20], red cells [21], endothelial cells [21], brain [22], liver [23], and so on. However, rather than

platelets, erythrocytes and endothelial cells may be capable of being major contributors to plasma S1P [24–27]. Furthermore, it has been thought that S1P delivery to the circulatory system occurs through two kinds of specific transporter: ATP-binding cassette (ABC)-type transporters and non ATP dependent transporter Spns2 [28–30]. The former transporters includes ABCA1, ABCC1, ABCG1 [30], and the latter transporter acts in vascular ECs instead of erythrocytes and platelets [28, 29]. Serum albumin and HDLs/apolipoprotein M (ApoM) are as S1P carriers in plasma, accounting for about 35% and 65% respectively, but only S1P of HDL/ApoM has a crucial role for maintenance of ECs integrity [31, 32]. Compared to carrier serum albumin, S1P is greater stability when binding to HDL [10, 19].

It has been reported that S1P is able to maintain the endothelial permeability by the stabilization of intercellular junctions and cytoskeletal rearrangement [10, 19]. Likewise, the lack of some directly associating-S1P genes (S1P kinases, receptors or ApoM) all lead to vascular leakage [10]. S1P functions via binding to some high affinity receptor instead of itself as second messenger [33, 34]. S1P receptors, including five subtypes (S1P1–5, originally designated Edg1–5), all belong to a class of G protein-coupled protein receptors and differentially expressed in different cell types [19]. In ECs, the expression of S1P1–3 receptors is more abundant compared to other S1P receptors [19, 35]. Functionally, S1P3 is able to promote endothelial integrity similar to S1P1 by activating RAC-1 [36]. On the contrary, S1P2 can increase ECs permeability by activating RHO GTPase [37]. For this three ECs-expressing receptors, however, S1P preferentially binds S1P1 to protect vascular barrier integrity, and S1P1-activting RAC1 can antagonize RHO activity [38]. Mechanistically, S1P significantly upregulates the abundance of VE-cadherin, promotes its translocation to enhance adherens junctions, and reassigns the zona occludens protein 1 to junctions to assist the formation of tight junctions by S1P1/Gi/Akt/Rac pathway [19, 39, 40]. Additionally, S1P promotes endothelial spreading and cytoskeletal rearrangement to fill the inter-endothelial gap by S1P1/phosphatidylinositol-3-kinase (PI3K)/AKT pathway [10].

Moreover, the integrity of endothelial glycocalyx (EG) integrity performs a critical function in vascular permeability. The related clinical studies show that the high level of HDL is inversely related with the EG index in older hypertensive patients, which illustrates that HDLs can play a protective role in the hypertension disorder [41].

Additionally, apolipoprotein E (ApoE), as a main lipoprotein originating from brain forms lipoprotein particles which resemble HDL found in blood plasma [42, 43]. It has been observed that the ablation of mice ApoE and the expression of human ApoE4 directly lead to degradation of endothelial tight junctions and blood-brain barrier (BBB) leakage via a cyclophilin-A-Nuclear factor-κB-matrix-metalloproteinase-9 pathway, which finally results in neurol injury and degeneration [44, 45].

3.2.2 HDL and Maintenance and Repairment of Endothelial Monolayer Integrity

Endothelial monolayer integrity is disrupted generally by gap formation between resident ECs related to internal shear stress or gross denudation linked to external vascular intervention, which makes vessel more susceptible to suffer from vascular disease, especially atherosclerosis [46, 47]. The endothelium monolayer integrity is sustained and repaired at least not only by reducing RECs apoptosis or increasing RECs proliferation and migration, but also by improving endothelial progenitor cells (EPCs) function.

3.2.2.1 HDL and ECs Apoptosis

ECs apoptosis disrupts vascular endothelial monolayer integrity and contributes to the pathogenesis of vascular disorders. Several in vitro studies show that HDLs can reduce ECs apoptosis. Mechanistically, two signaling pathways of apoptosis have been demonstrated in mammals: extrinsic and intrinsic pathway (also called death

receptor pathway and the mitochondrial pathway, respectively) [48].

Both oxidized low-density lipoprotein (oxLDL) and tumor necrosis factor-α (TNF-α) present in atherosclerotic lesion, induce ECs apoptosis by extrinsic pathway, thereby disrupting endothelium integrity and promoting the formation of atherosclerotic plaque [48–51]. In 1997, Suc and colleagues firstly characterized that HDL could inhibit ECs apoptosis [52]. And, two group verified that HDL acted directly on ECs against the proapoptotic effects of oxLDL, which protective role could be mimicked by a major protein component of HDL, apoprotein A-I (apoA-I) [52, 53]. Excessive enoplasmic reticulum (ER) stress and autophagy would trigger cellular apoptosis [54, 55]. Further study showed that disruption of cytosolic Ca^{2+} homeostasis induced by oxLDL in ECs would result in ER stress and autophagy to response to oxLDL, these events could be blocked by HDL [56]. Another in vitro study showed that HDL from healthy subjects prevents human umbilical vein endothelial cells (HUVECs) apoptosis induced by TNF-α, via significantly inhibiting caspase 3 activity [51]. Further mechanistic study showed that the apoA-I-mediated anti-apoptotic effect on ECs was totally blocked by specific inhibitor of its receptor F1-ATPase, independently of scavenger receptor-B type I (SR-BI) and ABCA1, but which was performed on a serum-deprived apoptotic model [57]. The serum deprivation-induced apoptosis model has been suggested to be associated with intrinsic apoptosis pathway [58, 59]. An in vitro study showed that S1P, as a kind of HDL cargos, inhibits caspase 3 activity, cytochrome c release, and DNA fragmentation via S1P/S1P1/endothelial nitric oxide synthase (eNOS) signaling axis, thereby preventing ECs apoptosis induced by serum derivation [59]. Furthermore, another study showed that the HUVECs apoptosis induced by serum deprivation could be reversed also by HDL, and the cytoprotective effect mediated by HDL was roughly paralleling to the S1P/Erk signaling pathway [60]. In addition to extracellular signal-regulated kinase (Erk) and eNOS, the activation of Akt also plays a critical role in HDL-mediated antiapoptotic effects on ECs, and the cytoprotective role of HDL is mimicked by HDL-associated lysosphingolipids [61]. Moreover, further mechanistic study showed that S1P-mediated ECs survival could be markedly inhibited by S1P1 receptor, rather than S1P3 [62]. Subsequently, it has been reported that S1P and apoA-I both are implicated in these two apoptotic pathways [57, 60, 63]. It is controversial that whether eNOS and SR-BI are implicated in HDL-mediated antiapoptotic effect on ECs. A detail study characterized that HDL from health subjects in vitro prevents ECs from apoptosis stimulated by both serum deprivation and TNF-α by PI3K/Akt signaling-mediated Bcl-xL expression upregulation and in part SR-BI, independent of eNOS activation [63].

Consistent with the results of in vitro investigation, in vivo studies also showed that HDL exerts an anti-apoptotic functions on endothelium [63, 64]. In a rat model of type I diabetes, evidences in vivo indicated that apoA-I mimetic peptide D-4F administration could decrease debris in circulating and prevent endothelial cell detachment from vessel [64]. Moreover, injection of HDL to apoE-ablation mice also reduces artery ECs apoptosis evidenced by TUNEL assay, and attenuats caspase 3 activity indicated by western blot assay [63].

3.2.2.2 HDL and ECs Proliferation and Migration

Previous studies have shown that it is migration and proliferation of RECs that play a very necessary and crucial role during the process of vascular repair [65]. Most studies have been reported that HDL can be able to promote ECs proliferation and migration not only in cell culture, but also in animal models, contributing to its cardioprotective potential.

In vitro, Tauber et al. firstly demonstrated that HDL could induce the proliferation of vascular ECs via culturing bovine ECs on dish coated with extracellular matrix in serum-free medium with HDL and are independent on fibroblast growth factor (FGF) [66, 67]. In addition to the regulation of vascular ECs proliferation, Murugesan et al. in 1994 firstly reported that HDL in vitro performs a

novel function, stimulating vascular ECs migration, also by a mechanism distinct from FGF [68]. HDL have been supposed to be soluble complexes of apolipoproteins and lipids. Mechanistically, it is controversial that which components of HDLs exert HDLs-mediated ECs proliferation and migratory activity. As far, there mainly are two paradigms involving this. Firstly, some studies in vitro have been verified that HDL-containing bioactive lipid S1P, rather than apolipoprotein components, regulates ECs (human umbilical vein endothelial cells) proliferation and migration by binding to its receptors S1P1 and S1P3 [62, 69]. In detail, inducing ECs proliferation of S1P is associated with GiGo/RAS/Erk activation [69, 70], and stimulating ECs migration is related to PI3K-mediated Akt [71, 72]/p38 mitogen-activated protein kinase (p38 MAPK) [62, 70, 73], Rho family (Rho [72, 73], Rac [71]) activation, Akt-mediated S1P1 Transactivation [71], and eNOS activation [74]. In addition to the regulation of ECs migration, PI3K can be indispensable for HDL-mediated ECs proliferation as well [74]. Recently, Tatematsu et al. reported in vitro and ex vivo that endothelial lipase (EL) can hydrolyze HDL to modulate S1P/S1P receptor signaling contributing to HDL-stimulated ECs proliferation and migration [75]. Specifically, EL knockdown attenuates HDL/S1P-mediated Akt and eNOS phosphorylation and strikingly inhibits HDLs-induced ECs migration and proliferation [75]. Secondly, Seetharam have shown that bovine aortic ECs migratory activity in vitro induced by HDL is roughly parallel to main apolipoprotein component of HDL, apoA-I, and its receptor SR-BI, rather than S1P [17]. Furthermore, this process is mediated by the Src kinase/PI3K/Akt (Erk)/Rac signaling pathway and independent on the eNOS activation [17]. Moreover, further mechanistic investigation showed that though Src kinase can directly interact with SR-BI, PDZK1, as an adaptor of SR-BI, is required for HDL/SR-BI-mediated Src phosphorylation [76]. Additionally, some studies have been suggested that apolipoproteins of HDL, similar to HDL, also promote ECs proliferation through increasing protein phosphorylation [77, 78]. Importantly, Radojkovic et al. characterized that apoA-I has an effect of proliferative bioactivity on HUVECs by its another receptor F1-ATPase, independently of SR-BI and ABCA1, and this effect can be blocked by its antibody or its specific inhibitor IF1-H49K [57]. Recently, a study in vitro further investigated downstream of apoA-I/F1-ATPase signaling involved in HDL-induced ECs proliferation. This study has been shown that apoA-I can be able to activate F1-ATPase to generate ADP; following activation, extracellular ADP binds to its receptor P2Y1 resulting in activation of PI3K/Akt signaling, which thereby stimulates ECs proliferation [79].

In vivo, preclinical studies found that HDL repairs vascular monolayer injury, which is closely linked to apoA-I and SR-B1-mediated ECs migration [17, 76]. In detail, the ablation of apoA-I and its receptor SR-BI both blunt artery reendothelialization in a model of perivascular electric injury [17]. And, PDZK1, as a specific adaptor of SR-BI, is indispensable for carotid artery reendothelialization in the same model [76]. Additionally, some animal experiments have verified that HDL-containing lipid S1P promotes vessel angiogenesis by inducing ECs migration and proliferation [71, 74, 75].

3.2.2.3 HDL and EPCs Function

Besides resident ECs, EPCs are also implicated in the repairment of vascular monolayer integrity by differentiation and paracrine stimulation [80, 81]. The true EPCs have been redefined in 2005 [82] and are mainly referred to as Endothelial colony forming cells (ECFCs) or late blood outgrowth endothelial cells (BOECs)(also called as “late” EPCs) [81, 83, 84]. However, the majority of early putative EPCs are rather pro-angiogenic hematopoietic cells, “early” EPCs or CFU-Hill [81, 83, 84]. The protective role of the putative EPCs in early studies at least attributes to two aspects [83, 85]. Firstly, due to issue that the EPCs have an ambiguous identification, the putative EPCs possess characteristics of hematopoietic cells, are unstably incorporated into injury vessel, and play an anti-inflammatory and immune role in the repairment of vascular

integrity. Secondly, the putative EPCs have a paracrine effect on RECs and stimulate RECs proliferation and migration in vascular repair, rather than directly replace dysfunctional or damaged ECs.

So far, most preclinical studies have been shown that HDL promote mobilization, differentiation, proliferation, and migration of this putative EPCs, which indirectly or directly promotes HDL-mediated ECs repair by the above mechanisms. In a mouse model of endothelial damage in response to LPS in apoE-deficient background, Tso et al. report that intravenous infusion of reconstituted HDL (rHDL) in mice increases in vivo numbers of EPCs identified by positive Sca1$^+$ cells in aortic endothelium, thereby contributes to aortic endothelial repair [86]. In a mouse model of hindlimb ischemia, intravenous injection of rHDL increases the number of bone marrow-derived EPCs incorporated into new vessel, and rHDL in vitro promotes the differentiation of peripheral mononuclear cells to EPCs via PI3K/AKT signaling pathway, rather than eNOS [87]. EPCs is Identified by a method of cell surface antigen expressing described by Asahara et al. [88], then called "early" EPC [85]. In a mouse model of transplant atherosclerosis, adenoviral human apoA-I (AdA-I) transfer promotes endothelial repair and attenuates neointima formation due to the increasing of the number of EPCs in circulation and bone marrow, and the enhancing of EPCs incorporation into allografts, which protective function is ascribed to apoA-I-mediated EPCs migration and proliferation by PI3K signaling pathway (herein referring to "early" EPC) [89]. Furthermore, the same group report that the attenuation of allograft vasculopathy mediated by apoA-I is dependent on SR-BI expression [90]. In a mouse model of artery denudation, HDL from healthy people promote repairment of endothelium monolayer ascribing to stimulating EPCs differentiation, proliferation, and migration (herein referring to EFU-Hill) [91]. In hypercholesterolemic rats subjected to femoral artery injury, intravenous injection of human plasma HDL contributes to endothelial repair, which attributes to HDL-mediated EPCs proliferation and migration (herein referring to "early" EPC) [92]. The current paradigm is that ECFCs or BOECs represent true EPCs, rather than the above EPCs, early EPCs or CFU-Hill. Feng and colleagues reported that HDL significantly promotes ECFCs migration and adhesion by SR-BI/NO signaling and increasing β1 integrin expression in vitro, thereby contributing to reduce the intimal area in apoE-deficient mice subjected to vein graft to carotid arteries in vivo [93]. Another study investigates the potential hazard effect of HDL on EPCs function and angiogenesis in vitro and in vivo and shows that moderate to high concentration of HDL decreases formation of vessel-like structure, ascribing to impair BOECs tube formation and promote senescence by activating Rho-associated kinase (ROCK) and inhibiting PI3K/Akt and p38 pathways [94].

Most importantly, clinical study has been shown that HDL level from male with no inflammation, coronary artery disease, and myocardial infarction, inversely relates to EPCs number obtained by the CFU-Hill EPCs procedure [95]. Furthermore, another preclinical study has been reported that rHDL infusion could be able to increase circulating EPCs number directly quantified by cell surface antigen CD34$^+$, in patients with type 2 diabetes [96].

3.3 HDL and ECs-Dependent Vasodilation

In addition to common roles in exchange of metabolites, anti-coagulant homeostasis ECs regulate vascular tone by secreting various vasodilators, such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor, and vasoconstrictors, such as endothelin-1, angiotensin II, and thromboxane A2 [97].

Many clinical experiments have been reported that HDL or apoA-I level correlates with ECs-dependent vasodilation. Zeiher *et al.* showed that attenuating vasoconstriction is associated with HDL-cholesterol serum level in atherosclerotic patients. Further clinical study verified that intravenous infusion of rHDL restores ECs-dependent vasorelaxation by increasing NO

bioavailability in hypercholesterolemic patients. Similarly, in the CAD patients with low HDL level, the administration of niacin by raising HDL level in plasma improves endothelial dysfunction evidenced by flow-mediated dilation. In addition, in ABCA1-heterozygous patients with low-HDL level, intravenous infusion of apoA-I also is able to completely restore ECs-dependent vasodilated dysfunction.

An animal study showed that the endothelial-dependent vasodilation impaired in atherosclerosis is correlated with oxLDL [98]. However, it has been reported that ex vivo the preincubation of isolated strips of rabbit thoracic aorta with HDL from healthy human plasma almost completely prevents the oxLDL-induced inhibition of vasodilation to Ach [99].

In addition, on the basal condition, HDL also stimulate ECs-dependent vasodilation [100]. Here, we will review the regulatory paradigms involving two important vasodilators, NO and PGI2, in considerable detail.

3.3.1 NO and eNOS

NO, originally called endothelium-derived relaxing factor, is produced by eNOS from the amino acid L-arginine [101]. Except as a signaling transducer mentioned on the part of "*HDL and Endothelial Barrier Integrity,*" eNOS affects vascular relaxation by NO synthesis and release in ECs. It has been reported that HDL modulates NO production via three manners, including eNOS activation, localization, and abundance.

Firstly, HDL, as a potential agonist of eNOS, regulates its activation at least involving in S1P receptors, SR-BI, and ABCG1. HDL-containing LPs, especially S1P, play an important role in HDL-mediated eNOS activation. As far, there are at least two paradigms for S1P-mediated eNOS activation. On the one hand, caveolin 1 inhibited eNOS activity by the directly binding to eNOS forming heteromeric complex, which could be disturbed by the targeting of calmodulin to eNOS when intracellular Ca^{2+} concentration was elevated, thereby leading to its activation [102]. S1P could activate a non-selective cation (NSC) channel to promote the elevation of intracellular Ca^{2+} concentration in HUVECs [103], and S1P-induced eNOS activation was inhibition by intracellular Ca^{2+} chelator BAPTA [104]. Moreover, the structure inhibition between eNOS and caveolin 1 may be regulated by S1P receptor, as S1P receptor S1P1 directly targeted caveolin 1 verified by immunoprecipitated assay in a heterozygous expression model, thereby potently and robustly activating eNOS enzyme [105]. On the other hand, in vitro mechanistic studies in BAECs culture evidenced that S1P stimulate eNOS phosphorylation at Ser1179 by the S1P1/PI3K/Akt signaling pathway, thereby leading to eNOS activation [104, 106, 107]. Most importantly, HDL-induced vasorelaxation ex vivo could be mimicked by all three HDL-containing LPs, sphingosylphosphorylcholine (SPC), S1P, and lysosulfatide (LSF) [100]. The response to LPs is abolished in S1P3-deficent aorta [100]. In vitro study also showed that Ca^{2+} mobilization and Akt phosphorylation mentioned above are implicated in HDL-mediated NO release of HUVECs [100]. And, in vivo animal study has been shown that arterial blood pressure in rats lowers by vascular injection of LPs [100]. In contrast to its vasodilatory effect on artery, intravenous injection of S1P decreases myocardial infusion and is completely abolished in S1P3-dificient mice [108]. The controversial role remains to be elucidated.

In addition to S1P receptors, Yuhanna *et al.* delineated that HDL could activate eNOS via apoA-I/SR-BI-mediated manner in vitro, and thereby enhancing endothelial-dependent vasorelaxation ex vivo in thoracic arteries from normal mice [109]. Moreover, the response of aorta to HDL is blocked by apoA-I and SR-BI antibodies and does not display in SR-BI-null mice [109]. Further in vitro mechanistic study has been shown that HDL phosphorylated eNOS at Ser-1179 entails the sequential activation of SR-BI-Src-PI3k-Akt/Erk [110, 111]. Subsequent study found that PDZK1 interacting with c-term intracellular PDZ domain of SR-BI is required for Src phosphorylation and eNOS activation [76]. Moreover, HDL or apoA-I incubation with

HUVECs alone activates eNOS by multisite phosphorylation change in HUVECs culture [112]. However, Yuhanna *et al.* found purified apoA-I does not elicit eNOS activation in ovine fetal pulmonary artery ECs [109]. In addition to the different EC lines used in experiments, this controversial results could be likely due to the difference of duration of apoA-I action: the response of NO release to apoA-I is slower than HDL [112]. Most importantly, ex vivo and in vivo studies shown that two apoA-I mimetics, L-4F and D-4F, could restore endothelial dysfunction in hypercholesterolemic LDLR-deficient mice by improving eNOS-dependent vasodilation [113, 114].

This difference of responses to HDL and apoA-I mentioned above gives researchers' hint that HDL can mediate other signaling pathways by apoA-I/SR-BI to activate eNOS. Gong *et al.* found in vitro and ex vivo that HDL from premenopausal women delivers its cargo estradiol by binding to SR-BI, thereby stimulating eNOS activation and arterial relaxation [115]. Another important study delineated that it is necessary for HDL-mediated eNOS activation that the delivery of cholesterol to or from ECs by the targeting of HDL to SR-BI extracellular domain and the binding of cholesterol to SR-BI C-term transmembrane domain [116, 117].

Another HDL receptor ABCG1 also plays a vital role in the eNOS of activation in part by inducing efflux of cholesterol and 7-ketocholesterol (7-KC) to HDL [118, 119]. An animal study reported that ABCG1 is expressed specifically in vascular endothelium, and ABCG1-ablated mice fed with a western diet displays the strikingly reduced eNOS activity in aortic lysates and the markedly decreased EC-dependent vasodilation [118]. This endothelial dysfunction in ABCG1-deleted mice may be correlated with ABCG1-mediated oxysterol 7-KC to HDL [118]. In vitro investigation indicated that HDL treatment to HAECs inhibits accumulation of intracellular 7-KC which stimulates reactive oxygen species (ROS) production to lead to the inhibition of eNOS, thereby promoting eNOS activation [118]. Moreover, ABCG1-mediated cholesterol efflux is also required for eNOS activation similar to SR-BI [119]. Further mechanistic investigation found that the inhibition of efflux to HDL in ABCG1-dependent manner attenuates the inhibitory interaction of caveolin 1 with eNOS [119].

Secondly, HDL prevents oxLDL-mediated translocation of eNOS to the internal membrane, thereby promoting eNOS activation. It is reported that the acylation of eNOS enhances its targeting to plasma specialized domain caveolae [120]. Moreover, Feron el at. demonstrated that eNOS could directly bind to caveolins 1 that is as a critical structure component of caveolae in ECs, by immunoprecipitated assay, which indicated that this subcellular localization is likely required for eNOS activation [121]. Subsequently, oxLDL incubation elicits the translocation of mostly all membrane eNOS from caveolae to intracellular membrane, thereby impairing eNOS activity, but not affecting eNOS phosphorylation [122]. Further study suggested that HDL normalizes eNOS localization to caveolae, which is associated with HDLs/SR-BI-mediated cholesterol efflux against oxLDL/CD36-induced cholesterol deletion in caveolae [123].

Thirdly, HDL also potently regulats eNOS abundance. A basic experiment in 2002 reported that when exposed to HDL, HAECs have an increased eNOS abundance measured by a western blotting method [124]. Consistent with this result, another in vitro detail study showed that incubation of HDL or apoA-I alone with HAECs affects eNOS posttranslational regulation and upregulates eNOS protein abundance by increasing eNOS protein life-half rather than eNOS mRNA level [111]. Subsequently, Terasaka and colleagues also found that the protein level of aortic eNOS is significantly downregulated in ABCG1-ablated mice fed with a western diet [118].

3.3.2 Prostacyclin

In addition to NO, HDL also regulates Prostacyclin (PGI2) production from ECs, thereby affecting vascular tone. PGI2 is generated from metabolism of arachidonic acid via cyclooxygenase (COX)/prostacyclin synthase (PGIS) pathway in the membrane of ER and nuclear envelope, and ECs is a primary source of its production [125]. About three decades ago, an in vitro study reported that human HDL and delipidated apoproteins of HDL incubated with sub-confluent porcine endothelial cells could significantly promotes the biosynthesis of PGI2, and rat HDL containing a higher level of arachidonate elicits a greater release of PGI2 than human HDL [126]. Further data indicated that arachidonate derived from HDL could be delivered into ECs and metabolized to PGI2 [127]. Therefore, HDL promotes PGI2 release, in part by replenishing arachidonate into endothelium. However, the evidence found by Spector and colleagues indicated that albumin-bound arachidonic acid could play a more important role in providing arachidonate for ECs than HDLs [128]. Subsequent study found that HDL component of apoA-I, as a PGI2 stabilizing factor, could prolong its half-life, thereby enhancing its function of vasodilation and inhibiting platelet aggregation [129]. In addition, another study showed that HDL promotes the PGI2 production might be by the upregulated expression of Cox-2 synergizing with cytokine [130].

Importantly, clinical studies showed that 6-keto-PGF1α, a stable metabolite of PGI2, correlated with concentration of HDL cholesterol or HDL subfraction [131].

3.4 HDL and ECs-Related Antithrombotic Function

3.4.1 HDL and Platelets

The rupture of an atherosclerotic plaque in a coronary artery with its subsequent coronary thrombus formation are always thought to be the primary cause of acute coronary syndrome. HDL cholesterol level is proved to be a significant independent predictor of ex vivo platelet thrombus formation through the fact that in an ex vivo flow chamber model for coronary thrombosis the thrombus area is much lower in human subjects with higher HDL cholesterol level [132].

HDL has several favorable effects on platelets that might explain its interference with thrombus formation in the coronary vasculature. First, it has been reported that HDL can block the activation and aggregation of platelets. In a small clinical trail, 13 patients with type 2 diabetes mellitus were randomized to a single infusion of rHDL or placebo. Study results established that a 40% increase in HDL cholesterol level in patients with type 2 diabetes mellitus is associated with a 50–75% reduction in platelet aggregation [133]. If isolated human platelets are incubated with rHDL or natural HDL in vitro, platelet aggregation is similarly reduced. Studies have also found that HDL can regulate platelet aggregation and also prevent platelet degranulation and spread, as well as platelet and fibrinogen adhesion [133, 134]. Related studies have demonstrated that rHDL can limit platelet binding in growing thrombus under in vitro flow conditions [133]. HDL inhibition of platelet reactivity is caused by cholesterol depletion in lipid rafts [133]. HDL-related phospholipids are key to the antithrombotic function of HDL. HDL may also regulate platelet activity by reducing the synthesis of platelet activating factors in ECs [135, 136].

3.4.2 HDL and Coagulation

HDL also affects the exogenous coagulation pathway, which may lead to a reduced tendency for thrombosis. In addition to the fact that HDL can directly inactivate tissue factors and factor Va, HDL can down-regulate tissue factor expression in thrombin-stimulated endothelial cells by inhibiting the expression of RhoA and PI3K, and ultimately affecting the coagulation cascade [137].

High lipoproteins have also been shown to promote the cleavage of anticoagulant-activating protein C by Arg306 to cause factor Va inactivation [138]. However, some studies have shown that the ability of HDL components to enhance the activated protein C system is caused by the co-separation of anionic phospholipid membrane and liposomes [139].

3.4.3 HDL and Antithrombotic Factors

HDL in ECs induces the expression of prostacyclin (PGI2), and prostacyclin is a vasoactive prostaglandin that works in synergy with prostaglandins. It would function to inhibit NO in the vasomotor control and inhibit platelet activaty and slow down the proliferation of smooth muscle cells. In addition, the PI3K–Akt–eNOS signaling avtivated by endothelial SR-BI is involved in HDL-induced COX-2 expression and PGI2 releases [140, 141].

HDL mediates induction of prostacyclin by providing arachnoid acid or in part through induced Cox-2 expression. HDL regulates COX-2/PGI-S activity in ECs through p38 MAPK-dependent COX-2 mRNA stability and transcription and caveolin 1-dependent PGI synthase shuttle and COX-2 coupling [137, 141–143].

HDL can also reduce the expression of ECs adhesion molecules and monocyte adhesion. In terms of cell adhesion-related effects, the downregulation of VCAM-1, ICAM-1, and E-selectin by HDL is mediated by the previously described SR-B1 and S1P receptor transduction signal pathways. HDL signaling pathway inhibits endothelial adhesion molecules, and by binding to SR-BI, it induces a signal cascade to activate PI3-K/Akt phosphorylation and eNOS, therby producing biologically active NO. NO further inhibits TNF-α signaling through the nuclear factor (NF)-κB pathway, thereby inhibiting the transcription of endothelial adhesion molecule (VCAM) -1, such as vascular endothelial cell adhesion molecules. By this way, HDL can eventually mediate antithrombotic effects by affecting cell adhesion [144–146].

Apoptosis of ECs promotes thrombosis [147], and the anti-apoptotic effect of HDL may help reduce thrombosis [47].

3.5 HDL and Endothelial Anti-Inflammatory Effects

There are several mechanisms to explain the inhibitory effect of HDL on endothelial inflammatory activity [148]. HDL can inhibit the activation of endothelial proinflammatory transcription factor NF-κB [149, 150]. Endothelial anti-inflammatory effects of HDL are mediated by SR-BI, PDZK1, PI3K, eNOS, and S1P receptors [151]. HDL can inhibit VCAM-1 expression and endothelial monocyte adhesion, and the production of NO promotes these endothelium anti-inflammatory effects of HDL [152].

HDL main protein component apoA-1 exerts the anti-inflammatory ability. One of the potential mechanisms of apoA-1's anti-inflammatory effect is the release of cellular cholesterol via the ATP-binding transporter ABCA1 [151, 153]. Moreover, apoA-1 can also reduce palmitate-induced NF-κB activation by reducing Toll-like receptor-4 recruitment in lipid rafts [154]. HDL and apoA-1 may be achieved by depleting cholesterol in most cells, including endothelial cells. Cholesterol transporters such as ABCA1 and ABCG-1 on ECs also have anti-inflammatory effects. Decreased expression of CD11b in ECs on the arterial wall is also closely related to HDL and is likely to be mediated by apoE, which is also responsible for reducing the lipid load in monocytes [153].

The lipid component of HDL also has anti-inflammatory effects. The inhibitory effect of HDL on endothelial adhesion molecule expression depends on the type of phospholipids associated with HDL [155]. The lipid composition of HDL affects its anti-inflammatory ability and may be an important determinant of HDL function [148].

HDL protects LDL from oxidation, thereby reducing its effect on inflammatory activation of ECs. The antioxidant effect of HDL is mediated in part by the paraoxonase and arylesterase activities of the HDL-related enzyme

paraoxonase 1 (PON1). Proteomics has shown that HDL can also exert its anti-inflammatory effects by regulating effects in the complement system and inhibiting proteases [156, 157].

Studies have also shown that apoA-1 reduces inflammatory activation of vascular endothelium by upregulating apoA-1. The HDL-dependent SR-B1 activation pathway may be mediated by the 24-dehydrocholesterol reductase (DHCR24) and anti-inflammatory heme oxygenase I (HO-1) mediated by the PI3K/Akt pathway. It also works by inhibiting the NF-kB pathway, which is also independent of the down-regulation of ICAM-1 and VCAM-1 mediated by TNF-α [158, 159].

3.6 Heterogeneity of HDL

HDL, a vasoprotective molecule, has been proven to have a variety of vascular endothelial protective properties in previous studies, such as anti-inflammatory, anti-apoptosis, promoting endothelial cell migration and proliferation, anti-thrombosis, mediating vasodilation, and anti-atherosclerotic properties [47, 109].

Considering the strong vasoprotective properties of HDL, HDL-targeting therapies seem to be promising. Therefore, attempts have been made to increase the HDL content with various interventions. In a clinical trial using torcetrapib, a cholesterol ester transfer protein (CETP) inhibitor that can increase HDL cholesterol levels in plasma [160], to treat patients with high cardiovascular risk, despite patients receiving torcetrapib had increased HDL cholesterol levels by 72.1% from baseline, the risk of cardiovascular events and death also increased, leading to the termination before completion of the trial due to lack of clinical benefit [161]. In another study, investigators used dalcetrapib (another CETP inhibitor) to treat patients with an acute coronary syndrome (ACS), and the results showed that dalcetrapib increased HDL cholesterol levels without reducing the risk of cardiovascular events [162].

All attempts to increase HDL to treat cardiovascular disease have failed, which casts a shadow on the future of HDL-oriented therapies. It is realized that HDL levels in patients with high-risk cardiovascular disease have no clear association with the risk of cardiovascular events, because the vascular effects of HDL can be highly heterogeneous. The alteration of the endothelial protective function of HDL has been observed in patients with various diseases, such as coronary heart disease [63, 152, 163], systemic lupus erythematosus [164], antiphospholipid syndrome [165], diabetes [166–168], obesity [169], and chronic nephritis [170].

The heterogeneity of HDL is reflected in various aspects. First, the capacity of HDL to inhibit LDL oxidation is impaired under certain circumstances. Studies by Navab et al. [171] have shown that HDL can protect LDL from being oxidized by human arterial wall cells and play an anti-inflammatory role, and this protective effect is impaired in patients with hyperlipidic coronary heart disease. Research by Morgantini et al. [172] showed that the ability of HDL to inhibit LDL oxidation and LDL-induced chemotactic and adhesive activity of monocytes to endothelial cells is impaired in type 2 diabetes. In another study, Ansell et al. [163] found that the ability of HDL to inhibit LDL-induced monocyte chemotactic activity in patients with coronary heart disease was improved after simvastatin treatment. However, compared to age- and sex-matched healthy subjects, HDL in patients was still pro-inflammatory after treatment. Persegol et al. [166, 167] also observed that the ability of HDL to reverse endothelial-dependent vasodilation inhibition caused by oxidized LDL was impaired in patients with diabetes.

HDL can regulate the expression of eNOS, stimulate the production of endothelial NO, and then exert endothelial protective effects. However, in patients with diabetes and coronary heart disease, HDL promotes the production of peroxides, inhibits rather than promotes the synthesis of NO [152], greatly reduces the bioavailability of NO [168, 173], and finally loses the endothelial protective ability to promote endothelial repair as well as limit endothelial inflammatory activation.

A study by Riwanto et al. [63] found that, compared with HDL from healthy subjects,

which exerts anti-apoptotic effects on endothelial cells, HDL isolated from patients with coronary heart disease is pro-apoptotic. Failing to induce the expression of anti-apoptotic protein Bcl-xL in endothelial cells, HDL from patients with coronary heart disease stimulates the pro-apoptotic pathway of endothelial cells and promotes the activation of pro-apoptotic protein tBid.

HDL in patients with diabetes has been found to have an impaired ability to stimulate human umbilical vein endothelial cells (HUVECs) proliferation, migration, and adhesion to extracellular matrix (ECM), which are essential for endothelial repair process and maintenance of vascular integrity [174]. Another study by Sajoscha et al. found that HDL from healthy subjects stimulated endothelial nitric oxide production, improved endothelium-dependent vasodilation and early endothelial progenitor cell-mediated endothelial repair, while in HDL from diabetic patients this endothelial repair effect has not been observed [168].

Several reasons lead to the heterogeneity of HDL. The first is the complexity of HDL particles. HDL particles contain more than 50 proteins (i.e. apoA-I, apoE, PON1, PAF-AH, and PAF-AH) and various lipids, the recombination of various components leads to heterogeneity of HDL [175]. In fact, HDL under physiological conditions is also a heterogeneous particle, with a density ranging from 1.063 to 1.21 g/ml and a diameter between 7–12 nm [175]. Such heterogeneity is caused by the difference in the relative content of apolipoproteins and lipids in HDL. ApoA-1, the principal protein in HDL, can switch its conformation according to the amount of bound lipid, and HDL particles are also being reshaped in this process. In humans, there are both small, dense, protein-rich HDL3 particles and large, light, lipid-rich HDL2 particles [175]. Compared with HDL2, HDL3 has stronger anti-oxidation, anti-inflammatory, and anti-endothelial cell apoptosis capabilities, while HDL2 shows stronger ability to inhibit platelet aggregation than HDL3 [5]. It can be seen that different HDL subtypes are also heterogeneous in function.

Another reason is the modification of HDL-related components. Cavigiolio et al. [176] found that apoA-I can spontaneously and rapidly exchange between lipid-associated and lipid-free states. When apoA-I on HDL is oxidized by myeloperoxidase, its exchange kinetics is significantly reduced. Another study showed that paraoxidase 1 (PON1) in HDL inhibits malondialdehyde(MDA) formation. In patients with coronary heart disease, PON1 decreases and MDA content increases, while the ability of apoA-I to promote cellular cholesterol efflux and endothelial NO production is impaired as apoA-I in HDL is oxidized by MDA [152]. ApoA-I can also be replaced by serum amyloid A (SAA), an acute phase protein enriched in HDL in the acute phase of inflammation, which turning HDL into a pro-inflammatory factor, and stimulating the expression of monocyte chemotactic protein 1 (MCP1) [177, 178]. In fact, analysis of human atherosclerotic plaques found that apoA-I contains a large number of modifications associated with impaired atherosclerotic protective function of HDL particles [179, 180]. In diabetic patients, HDL is saccharified and triglyceride content is increased. Researchers have found that HDL triglyceride content is inversely proportional to the ability of HDL to reverse oxidized LDL-induced inhibition of vasodilation [167]. Similar phenomenon was also observed in patients with abdominal obesity [169], in which the content of apoA-I in HDL is reduced, and the proportion of triglycerides in HDL is increased. The study by Riwanto et al. [63] showed that the reduction of clusterin and the increase of apolipoprotein C-III content in HDL in patients with coronary heart disease are the mechanisms of the changes of anti-apoptotic and pro-apoptotic signaling pathways in endothelial cells.

3.7 Conclusion

In general, HDL exerts a variety of endothelial protective functions, including promoting reverse cholesterol transport, improving the availability

of endothelial nitric oxide, anti-inflammatory, inhibiting LDL oxidation, and anti-thrombosis. We know that the formation of atherosclerosis is associated with the change of endothelial cell function, such as reduced availability of endothelial NO, increased apoptosis of endothelial cell, expression of adhesion molecules and chemokines, and pro-thrombotic activation. Studies have shown that low levels of HDL cholesterol are important predictor of atherosclerotic cardiovascular events, thus HDL also exerts direct anti-atherosclerotic properties on endothelial cells, helping to prevent the emergence or progression of atherosclerosis, and even promote repair and regression of the lesion.

Despite such powerful vascular endothelial protection, HDL has not been successfully used in the prevention or treatment of cardiovascular disease, one of the reasons is the complexity of the structure and function of HDL particles. HDL particles are highly heterogeneous, and their vasoprotective effect is impaired in patients with coronary heart disease, diabetes, and chronic renal insufficiency. HDL in patients has lost its ability to promote cholesterol efflux, and changed from anti-inflammatory particles to pro-inflammatory particles, such HDL is called "dysfunctional HDL."Changes in HDL functions are due to changes in its structure and composition, such as oxidative modification of HDL-related lipids and proteins. HDL cholesterol levels provide only information about the size of the HDL pool, but cannot help to predict the structure or function of HDL particles. At present, our understanding of the complex metabolism involved in HDL particles and the dynamic heterogeneity of HDL is still incomplete. It has been determined that plasma HDL cholesterol level is not an appropriate marker of HDL's influence on blood vessels, and that only HDL-cholesterol level is not enough as a therapeutic target for patients with cardiovascular disease. A better understanding of the relationship among HDL metabolism, structure, and biological functions will be the target for future clinical and basic research, and the development of new HDL-targeted therapies may need to consider the change in the vascular effects of HDL in cardiovascular disease. The mechanism underlying protective effects of HDL on ECs and the loss of it, rather than plasma HDL cholesterol levels, may be a promising target.

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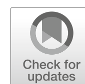

4 HDL and Lipid Metabolism

Qi Zhang, Yilang Ke, and Huashan Hong

Abstract

Mediating reverse cholesterol transport (RCT) is the most classic function of HDL. HDL and HDL-C participate in the entire process of RCT, including cholesterol removal from cells, cholesterol transport in circulation, and cholesterol excretion. As cholesterol is a component of lipid rafts and lipid droplets in cells, HDL and RCT can influence cell activity. HDL has also been shown to be related to the metabolism of some other biological lipids, such as S1P and ox-PL. Here we will introduce in detail the molecular mechanism of HDL participation in RCT and its significance.

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Keywords

Reverse cholesterol transport · ABCA1 · Bile acid · Transintestinal cholesterol excretion · Lipid droplets · S1P

4.1 HDL Mediates Reverse Cholesterol Transport

Mediating RCT is the most classic function of HDL. Early in the 1960s, Dobbins et al. [1] proved the presence of cholesterol acyltransferase in HDL, and then the pathway of RCT was identified. Now RCT is defined as the process by which cholesterol moves out of cells in peripheral tissues, enters the circulation, and is excreted in the feces [2]. HDL is both the acceptor of cholesterol from cells and the carrier of cholesterol in circulation, and, therefore, plays an important role in RCT (Fig. 4.1).

4.1.1 HDL Participates in Cholesterol Removal from Cells

Cellular cholesterol homeostasis is essential for normal cell function. As it was said, HDL acts as the specific cholesterol acceptor that transports excess cholesterol stores within peripheral tissues to the plasma [2]. ABCA1 and ABCG1 are critical receptors for the initial step of RCT, which mediate cholesterol efflux out of cells. Combined

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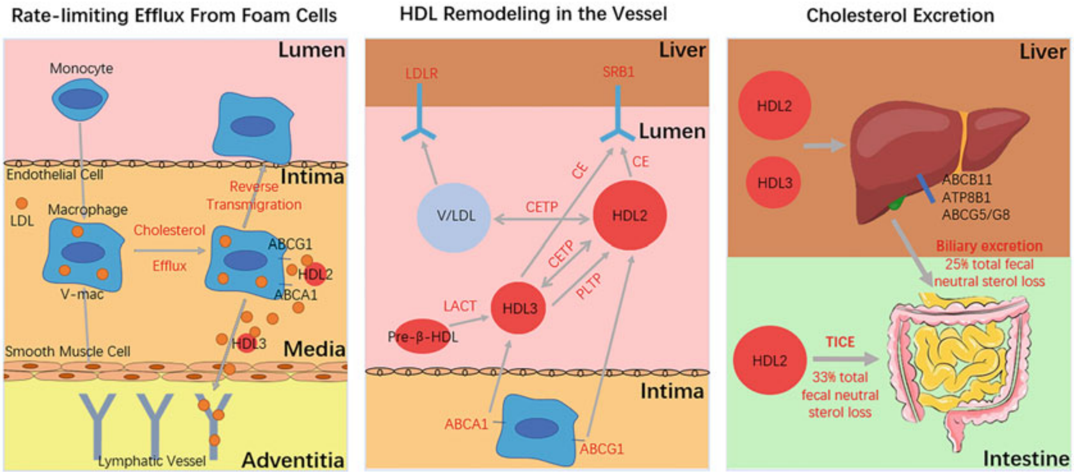

Fig. 4.1 Key steps of reverse cholesterol transport (RCT). The first step of RCT is the removal of cholesterol from macrophages or vascular smooth muscle cells. In this step, free cholesterol efflux to acceptors such as HDL. This is the rate-limiting step of RCT. In macrophages, ABCA1/ABCG1 (ATP-binding cassette proteins A1/G1) is required to pump out cholesterol, but the efflux mechanisms in vascular smooth muscle cells are not well understood. In the next step, the cholesterol on HDL is transported to the liver. SR-B1 (scavenger receptor class B type 1) is responsible for binding HDL and selective cholesterol intake. Another part of cholesteryl ester is transferred to VLDL or LDL by CETP (cholesterol ester transfer protein) and PLTP (phospholipid transfer protein) and cleared by LDLR. Finally, cholesterol is excreted into the feces. Biliary cholesterol excretion mediates about 25% of total fecal neutral sterol loss, while transintestinal cholesterol efflux (TICE) mediates about 33% under normal circumstances. *LCAT* lecithin-cholesterol acyltransferase, *OSBP* oxysterol-binding protein. Adapted from Ouimet, M. et al. [2]

ABCA1 and ABCG1 deficiency in macrophages resulted in impaired cholesterol efflux to HDL and decreased apoE secretion in vitro. This deficiency also increased secretion of inflammatory cytokines and chemokines. Mouse models showed the same trend [3, 4]. ABCA1 preferentially synthesizes small HDL particles, specifically apoA-I to form nascent HDL [5], at the same time ABCG1 stimulates net cholesterol efflux to larger HDL but not to lipid-poor apoA-I [2, 6]. The classical hypothesis is that free cholesterol generated by lipid lipolysis reaches the ABCA1 and ABCG1 by vesicular or non-vehicular trafficking pathways and then becomes effluxed [7]. In fact, ATP-binding cassette transporters themselves can be motile. ABCA1-mediated cholesterol efflux to apo A-I was found to take place in endosomes [8]. ABCA1 can also shuttle between the plasma membrane and late endosomes, and between the endolysosomal compartments and plasma membrane [9]. ABCG1 relocalizes from the Golgi and ER to the plasma membrane following LXR activation to stimulate efflux to HDL [2, 10].

Another cholesterol trafficking pathway is mediated by a conserved family of lipid-binding/transfer proteins, which is constituted by oxysterol-binding proteins (OSBPs) and its related protein homologs, OSBP-related proteins (ORPs). ORPs are ubiquitously expressed in eukaryotes, and their ligand-binding domain accommodates cholesterol and oxysterols [11]. Thus, ORPs facilitate non-vesicular transfer of cholesterol between lipid bilayers and coordinate lipid signals with a variety of cellular regimes. Recent study found ORP6 may contribute to HDL homeostasis and regulate cholesterol efflux, although the mechanism is not clear [12]. Other proteins related to lipid trafficking could also influence cholesterol efflux to HDL,

such as steroidogenic acute regulatory protein (StAR) and Niemann-Pick type C (NPC) [13].

4.1.2 HDL-C Mediates Cholesterol Transport in Circulation

After cholesterol transferred to HDL particles, the next step is the esterification to form cholesteryl ester (CE), which depends on lecithin-cholesterol acyltransferase (LCAT). Overexpression of LCAT in mice raises HDL-C levels, though results in unaffected atherosclerosis [14]. Similarly, LCAT knockout mice showed extremely low levels of HDL-C, but the influence on atherosclerosis remained unclear [15, 16]. Studies have found that the capacity of cholesterol transport is also related to the functional status of HDL itself [17, 18]. Although LCAT has long been believed to be critical for promoting RCT by maintaining a free cholesterol gradient between cells in the periphery and plasma HDL, some current data are inconsistent with it [19]. The clearance of HDL-C is much faster than its esterification. Within the halftimes of HDL-C, only about 2% of free cholesterol is esterified, which means that over 95% of HDL-FC is cleared without esterification, and LCAT may play only a minor role in RCT [20, 21]. Consistently, LCAT knockout mice showed little damage in macrophage RCT [22]. Interestingly, overexpression of LCAT reduced macrophage RCT by reducing the level of lipid poor apoA-1 and the cholesterol efflux via ABCA1, although the HDL-C level was increased in this model [22].

It is generally believed that high plasma HDL-C concentration represents a better rate of cholesterol metabolism and is therefore associated with a reduced incidence of cardiovascular events [23]. Studies such as the Helsinki Heart Study and the Veterans Administration HDL Intervention Trial (VA-HIT) also support this view, which showed that increased plasma HDL-C levels in patients receiving gemfibrozil versus placebo are associated with fewer coronary heart disease events [24, 25]. However, these studies cannot completely rule out the effects of other risk factors, like insulin resistance and plasma triglyceride levels, and other studies showed different results. Especially, high plasma HDL-C level and no CVD incidence reduction were found in patients with cholesteryl ester transfer protein (CETP) deficiency. CETP inhibitors, which profoundly increase plasma HDL-C levels, can't reduce CVD events either [26]. In genetic studies, the Copenhagen City Heart Study showed patients with genetically elevated plasma levels of HDL-C did not have a reduced atherosclerotic cardiovascular disease (ASCVD) risk [27]. According to a Mendelian randomization study, an HDL-C-raising endothelial lipase variant was not associated with reduced myocardial infarction [28]. In fact, new views hold that HDL's functional changes are more important than its quantitative changes, and the abnormal high HDL-C level is caused by HDL dysfunction. Moreover, some epidemiological studies in humans showed the correlation between plasma HDL-C levels and ASCVD hazard ratio is U-shaped, with the extremes of high and low HDL-C concentrations being associated with more all-cause and ASCVD mortality [29–31], suggests that HDL may derive its dysfunctionality from both high free cholesterol content and high plasma HDL concentration.

Recently, a lot of evidences support that the most important site from which HDLs act to promote cholesterol efflux from cells is in the extracellular matrix of tissues, rather than in plasma [32]. Since one of the major roles of the lymphatic system is to drain macromolecules from the interstitial space back to the circulation, it is generally believed that the return of lipoproteins from the interstitium to plasma may occur mainly via the lymphatic system and less via the venous capillaries [33]. Analysis of animal and human lymph fluid composition showed that lymph fluid was rich in cholesterol and HDL [34]. The major apolipoprotein of HDL is apoA1, and approximately half of all apoA1 in the body is extravascular and found within interstitial fluid of peripheral organs [35]. Total cholesterol level in lymph HDL was about 30% higher than plasma, indicating that periphery cholesterol clearance has relevance to lymph and extravascular compartment [34]. Whereas plasma

mainly contains α-HDL particles that are the predominant carriers of CE to hepatocytes, interstitial fluid provides a metabolic environment that drives the conversion of α-HDL to pre-β-HDL, the main acceptor of free cholesterol from peripheral tissues [2, 36]. And this conversion may due to the high specific activity of phospholipid transfer protein (PLTP) in lymph, along with the absence of cholesterol esterification [15]. Furthermore, studies have found mice with impaired lymphatic drainage showed impaired RCT, and lymphatic endothelial cells could express functional HDL transporters such as SR-BI, which directly demonstrated that lymphatic drainage is required for RCT and HDL function [37].

4.1.3 CE in HDL Can Be Transferred into Cells

In humans, CE in HDL can be transferred to triglyceride-rich lipoproteins by cholesteryl ester transfer protein (CETP) for elimination via hepatic clearance in the liver through the LDLR, or selectively taken up via scavenger receptor class B type 1 (SR-B1) acting as a hepatic receptor for CE on HDL [2]. Notably, this process in mice differs from that in humans.

CETP is a 74-kDa hydrophobic plasma glycoprotein that has an established role in mediation of neutral lipid transport among lipoproteins [38]. The main effect of CETP activity is a transfer of CEs from HDLs to apo B containing lipoproteins in exchange for triglycerides. People with loss-of-function mutations in both CETP alleles have extremely high levels of HDL-C, indicating the importance of this pathway in humans [20]. Indeed, studies in humans have indicated that the majority of CEs are transferred to the liver by apo B containing lipoproteins and not HDL, which was shown by injection of HDL labeled with a CE tracer [20]. As said before, CETP is a therapeutic target for inhibition to raise plasma levels of HDL-C. Mice overexpressing CETP experience a substantial reduction in HDL-C levels, as well as increased macrophage RCT rate, consistent with the concept that CETP promotes the delivery of HDL-C to the liver [39]. Besides, the function of CETP is also influenced by LDL receptors and the clearance of apoB-lipoproteins [19].

SR-BI is a high affinity receptor for HDL, and it also binds LDL, VLDL, and phospholipids [40]. Studies by Pagler et al. have shown that SR-BI can transfer cholesterol from lipoproteins to cells through whole particle uptake/endocytosis [41]. However, SR-BI can also uptake cholesteryl esters without parallelly uptaking the entire lipoprotein particles, which is the mainly known function of SR-BI [42]. In this selective uptake process, HDL-C firstly binds to the loop domain of SR-BI, and then cholesterol esters transfer to the plasma membrane, finally HDL releases back to the circulation [43, 44]. Large population-based studies revealed that heterozygous carriers of the P376L variant of SR-BI have significantly high levels of plasma HDL-C [45]. SR-BI expressed in both hepatocytes and liver endothelial cells may mediate the uptake of HDL-C into the liver [46–48]. In fact, the highest relative expression level of SR-BI was found in steroid hormones producing cells despite the major focus on SR-BI in the liver, as cholesterol is a substrate for steroid hormone synthesis [49]. Interestingly, along with its classical function of cholesterol transcellular transport, SR-BI initiates signaling in some cell types [50]. For example, the binding of HDL to SR-BI will activate endothelial NO synthase (eNOS) in endothelial cells [51].

Although immune cells like most cells can utilize intricate feedback mechanisms of cholesterol uptake and synthesis, they also express numerous unregulated scavenger receptors [52, 53]. Macrophage foam cells have been a sign of early atherosclerosis in humans and animal models. These cells contain numerous droplets of CE, which could be marked by lipid stain such as Oil red O [54]. The imbalance in the rate of cholesterol uptake and efflux results in CE accumulation in immune cells [52].

How cholesterol internalized from HDL is made available to the cell for storage or modification is poorly understood. Aster proteins are ER-resident proteins that bind cholesterol and facilitate its removal from the plasma membrane,

providing a new mechanism to mobilize HDL-derived cholesterol. Mice lacking aster-B are deficient in adrenal cholesterol ester storage and steroidogenesis because of an inability to transport cholesterol from SR-BI to the ER [55].

4.1.4 Cholesterol Is Excreted Through the Liver and Intestines

Although cholesterol itself can be secreted into the bile for excretion from the body, synthesis and excretion of bile acids comprise the major cholesterol catabolism pathway in mammals [2, 56]. Studies in healthy normolipidemic humans in vivo show that unesterified cholesterol in HDL can be rapidly and directly taken up by the liver and targeted to bile [20]. Biliary cholesterol excretion is driven by the two half ABC transporters, Abcg5 and Abcg8, which form an active heterodimer [57]. Mice lacking either Abcg8 or Abcg5 have an 80% reduced biliary cholesterol output, indicating that Abcg5/8 is essential for the largest part of biliary cholesterol excretion [58, 59]. And many other factors have been found taking part in regulating the classic biliary pathway of RCT. For example, ATP8B1, a phosphatidylserine flippase in the canalicular membrane, is demonstrated to inhibit biliary cholesterol excretion, which is independent of Abcg5/8 activity [60].

However, there is now evidence that HDL-driven movement of cholesterol back to the liver can't fully correlate with how much cholesterol is lost in bile or the feces [61, 62]. Similarly, mice genetically lacking apoA-I or ABCA1 have normal biliary and fecal cholesterol loss [63, 64], indicating that there exist cholesterol excretion pathways not through bile. RCT can also proceed through a non-biliary pathway known as transintestinal cholesterol excretion (TICE), which involves the direct secretion of plasma lipoprotein-derived cholesterol by the small intestine [65]. Current evidence suggests that the non-biliary branch of RCT can be initiated by either re-uptake of biliary cholesterol via the canalicular sterol transporter Niemann-Pick C1-like 1 (NPC1L1) [66, 67], or by blocking cholesterol acyl-CoA:cholesterol acyltransferase 2 (ACAT2)-driven cholesterol esterification [68]. Both conditions are expected to cause the accumulation of free cholesterol in the liver, but these excess free cholesterol will be repackaged into new apoB-containing lipoproteins. These lipoproteins are then secreted from the liver into the circulation and are recognized by the proximal small intestine through lipoprotein receptors such as LDLR [69]. Since TICE can still occur in LDLR −/− mice, there may be other mechanisms contribute to it, but SR-BI is not included in them considering the current results [68, 69]. The transport route of cholesterol within the intestinal enterocyte is not well understood in TICE. However, as apoB-containing lipoproteins are the main particles participating in TICE, endosomal or lysosomal compartments are probably to be involved [65]. After efflux from TICE associated lipoproteins, this cholesterol can be transported by ATP binding cassette transporters ABCG5/ABCG8 and ABCB1a/b [69–71].

When evaluating the relative contribution of non-biliary routes to RCT, it is important to clear whether in normal physiology conditions or not. Under normal physiological conditions, the biliary pathway mainly contributes to the RCT efflux, while the non-biliary route typically makes up less than 30% of the total cholesterol found in the feces [72, 73]. However, the cholesterol efflux ability of non-biliary route is highly dynamic and can be stimulated by some pathophysiologic conditions and drugs. In transgenic mouse models that lacked the ability to normally secrete cholesterol into bile, or in the case of surgical removal of the common bile duct, fecal cholesterol loss remained the same or increased in some cases [58, 65, 74]. These studies suggest that the non-biliary TICE could be a reserve to response to the decrease of fecal cholesterol loss level under the pathophysiologic condition of biliary cholesterol insufficiency (Fig. 4.2).

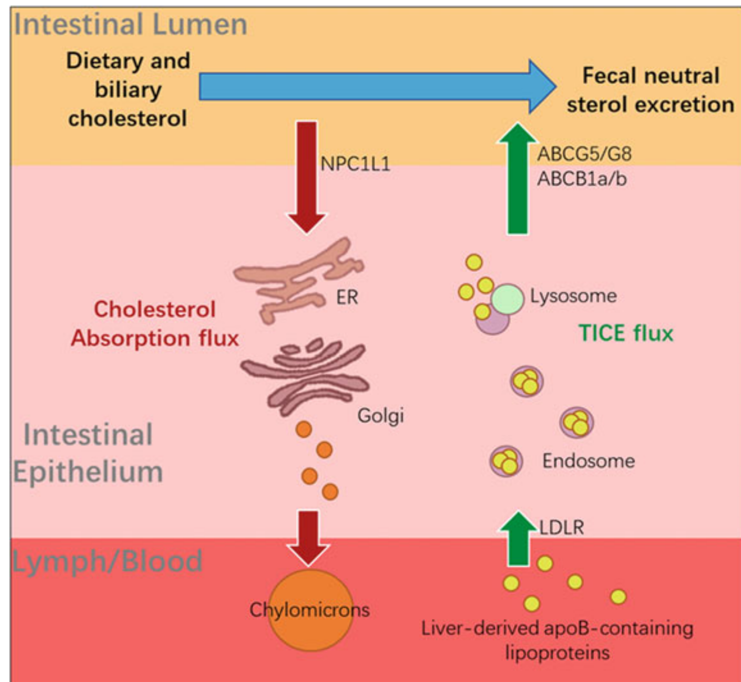

Fig. 4.2 Model for integrated biliary and non-biliary reverse cholesterol transport. Adapted from Temel, R. E. & Brown et al. [65]

4.2 Metabolism of Cholesterol Is Essential to Cell and Life

Cholesterol plays a role in many essential biochemical processes. Cholesterol is a special kind of plasma lipids for it is a precursor of steroidogenic steroids, which could regulate metabolism. And cholesterol in the form of LDL-C and HDL-C has been proven to be associated with ASCVD [23].

4.2.1 Lipid Droplets in Cells

Lipid droplets are intracellular organelles specialized for the storage of neutral lipids. Extensive evidences show that FC accumulation within cells is toxic, pro-inflammatory, and pro-atherogenic [75, 76]. It is shown that cholesterol load in macrophages leads to cholesterol crystal and the formation of NLRP3 inflammasome, which then produce pro-inflammatory mediators such as IL-1β [77]. The conversion of excess FC to CE by ACAT is thought to represent a protective mechanism against the toxic effects of excess FC in cells [78]. CE droplets are usually found in fat store tissues, but in fact the formation of droplets can be induced in any cell type when cholesterol is overloaded [79]. However, CE accumulation could also cause cell damage. For instance, the accumulation of CE in the central nervous system is related to neurodegeneration and the accumulation of β-amyloid peptide in Alzheimer's disease [80]. Although ACAT inhibition indirectly enhances the movement of the nascent amyloid precursor protein molecules into the early secretory pathway [52, 81], the amyloid plaque load in mouse model is decreased and the cognitive function is improved [82].

Moreover, some evidences suggest that CE rich lipid droplets are dynamic rather than passive inert structures and can be pro-inflammatory. In leukocytes these lipid bodies consist of a neutral lipid core, which is surrounded by a phospholipid monolayer [83]. These lipid droplets are found to be metabolically active since the CE in droplets undergoes a continual cycle of hydrolysis and re-esterification to release FC for membrane lipid raft maintenance and efflux [84]. There are

also proteins found in lipid droplets of not only adipocytes but also all cells, such as the ancient ubiquitous proteins which play roles in the degradation of HMGCoA reductase [85]. Recent studies suggest that lipid bodies can be formed in response to inflammation and inflammatory cell activation. Leukocyte lipid droplet formation has been observed following infectious by Hepatitis C, *Trypanasoma cruzi*, and exposure to various bacterial products, though whether blocking the formation of lipid body in leukocytes can change the progression of disease remains to be shown [52, 86, 87].

4.2.2 Membrane Cholesterol and Lipid Raft Microdomains

FC, together with glycerophospholipid (GPL) and sphingomyelin form a highly ordered 5–500 nm diameter structure, called lipid rafts in the plasma membrane [88]. These rafts are detergent-resistant membrane complexes that can organize and separate many different protein components. The FC content of these structures is usually 3–5 times that of the surrounding membrane. These FC can help stabilize the raft through their hydrophobic combination with other components [89]. A number of critical enzymes and signaling systems are active when concentrated within these microstructures, modulating cell activation and function, such as eNOS, SR-B1, Ras, CD36, Rho, MAP kinase, G-protein coupled receptors, and Ca2+ regulatory proteins [52]. In addition, although lipid rafts are typically studied at the cell membrane, similar structure can also be found in organelles containing membrane components, such as the Golgi, mitochondria, lysosomes, and lipid droplets [52, 90, 91].

The cholesterol required for the formation and maintenance of lipid rafts can come from exogenous sources, such as lipoproteins or from cell synthesis, through the mevalonate pathway in the ER and then transport to the plasma membrane [76, 92]. Another source of cholesterol are intracellular lipid droplets [93]. Extrinsic signals which promote CE hydrolysis can lead to the cholesterol efflux from lipid droplet. FC can also be moved to the substrate pool to be exported through ABCA1. ABCA1 is the uniquely sensitive master controller of membrane cholesterol that regulates lipid raft composition [52], which is under the control of the liver X receptor (LXR) [94]. Further studies showed that cholesterol efflux and lipid raft cholesterol maintenance were the same process, and the lipid composition of nascent HDL was similar to that typically found in microdomains from cell membranes [95, 96].

The addition of β-cyclodextrin can consume membrane cholesterol, while squalene replenishes it. By this way, the importance of cholesterol and lipid raft to cell activation and polarization has been extensively studied in many different conditions [97, 98]. Especially in the bone marrow, lipid rafts have been proven to regulate the retention and quiescence of hematopoietic stem cell in bone marrow niches and further participate in regulating their mobilization and homing [52, 99, 100]. Therefore, the regulation of the formation, composition, and decomposition of lipid rafts in cells is of great significance and can be applied in the field of cardiovascular disease.

4.3 HDL and Other Bioactive Lipids

For many years, phosphatidylcholine and sphingomyelin, along with small amounts of phosphoglycerol, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol, have been considered the main components of the HDL surface. Later, several other classes of glycosphingolipids were found, such as galactosylceramide, glucosylceramide, lactoseceramide, sulfatides, globulins, and gangliosides. Subsequently, phospholipid lysoderivatives were identified and soluble phospholipase A2 was found in HDL [101]. Sphingosine 1 phosphate (S1P), as a kind of lysosphingolipid, was found to be mostly carried by HDL and also positively correlated with HDL-C, apoA-I and apoA-II levels [102].

4.3.1 HDL and S1P

Moreover, HDL-related S1P is now considered to be the main determinant of S1P concentration in plasma. The source of S1P in circulation remained uncertain. Sphingosine kinase is the main enzyme that phosphorylates sphingosine to produce S1P. It is expressed in platelets and various peripheral blood cells, including red blood cells, neutrophils, and monocytes [103]. Platelets could store a large amount of S1P and release them under the stimulation of thrombin or calcium, but other studies have shown that S1P derived from red blood cells accounts for the largest proportion in the blood [104, 105]. Although erythrocytes cannot produce sphingosine and display relatively low levels of sphingosine kinase activity, they efficiently convert exogenous sphingosine to S1P [106]. Lack of S1P-degrading enzymes leads to the accumulation of S1P in erythrocytes. S1P may transport from erythrocytes to HDL through ATP-binding cassette transporters. Although the association with HDL seems to explain the atherosclerotic protective effect of S1P, it is not clear whether this effect also depends on the morphology of HDL and the ratio of apoA-I and apoA-II. A recent report indicated that S1P was enriched in small and dense HDL3 particles which contained apoA-I [107]. The main plasma apolipoprotein that S1P physically binds is apolipoprotein M (apoM), most of which are contained in HDL [108]. Only the apoM-containing fraction of human HDL carries S1P, and apoM-deficient mouse HDL contains almost no S1P, indicating that the presence of apoM determines HDL-S1P [109]. However, there is no correlation between apoM and S1P in human plasma or HDL [110], which suggests that there may be other molecules besides apoM involved in the relationship between S1P and HDL [111]. Interestingly, there is recent evidence that S1P may perform different biological functions, depending on whether it functions in an HDL-related form or an albumin-related form. In cultured endothelial cells, it was found that HDL-S1P helped more in reducing S1P internalization though had the similar half-life, which means HDL-S1P was more effective than albumin-S1P in maintaining endothelial function [112]. The function of HDL-S1P was shown to have changed in human cardiovascular, kidney, and metabolic diseases. A number of studies have linked plasma and HDL-S1P to the incidence of coronary artery disease [113–115].

4.3.2 HDL and Ox-PL

Phospholipids contain polyunsaturated fatty acids that are easily peroxidized. Ox-PL is involved in cell signaling and is associated with a variety of inflammatory processes, as well as atherosclerosis. Plasma levels of ox-PC have been found to be associated with increased risk of cardiovascular disease. Oxidized-PL in plasma is mainly associated with apoB100-containing lipoproteins, including the low-density lipoprotein variant Lp (a). The role played by HDL in oxidative-PC metabolism is unknown, but given the anti-inflammatory potential of HDL, it is expected to be beneficial [116]. Based on this, Gharavi et al. [117] have reported that HDL inhibited the proinflammatory pathway induced by ox-PL signaling in human endothelial cells, possibly by reducing superoxide production. This suggests an additional mechanism for the anti-atherosclerotic function of HDL.

4.4 Conclusions

HDL is of great significance to lipid metabolism, especially cholesterol metabolism. Mediating RCT is the most classic function of HDL. RCT includes the efflux, transport, and excrete of cholesterol. ABCA1 and ABCG1 mediate the efflux of cholesterol from cells to HDL, especially in vascular smooth muscle cells and macrophages. After cholesterol transfer to HDL particles, the next step is the esterification of cholesterol to form cholesterol ester, which depends on LCAT. The combined HDL-C is transported through lymph and circulation. CE in the HDL core can be transferred to triglyceride-rich lipoproteins by

CETP or be selectively taken up via SR-B1 to enter the cells again. Synthesis and excretion of bile acids comprise the major cholesterol catabolism pathway in mammals. This process is mainly carried out through the liver, but transintestinal cholesterol excretion is equally important, especially under certain pathological conditions. As cholesterol is a component of lipid rafts and lipid droplets in cells, HDL and RCT can influence cell activity. Problems in RCT are closely related to immune cells and atherosclerosis. HDL has also been shown to be related to the metabolism of some other biological lipids, such as S1P and ox-PL. Although some clinical experiments of using HDL-C concentration as a direct target failed, the importance of HDL in lipid metabolism and its potential clinical value are significant.

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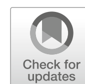

5 HDL and Oxidation

Qi Zhang, Zongzhe Jiang, and Yong Xu

Abstract

In this chapter, we will focus on HDLs' activity of inhibiting LDL oxidation and neutralizing some other oxidants. ApoA-I was known as the main antioxidant component in HDLs. The regulation of antioxidant capacity of HDL is mainly exhibited in regulation of apoA-I and alterations at the level of the HDL lipidome and the modifications of the proteome, especially MPO and PON1. HDL oxidation will influence the processes of inflammation and cholesterol transport, which are important processes in atherosclerosis, metabolic diseases, and many other diseases. In a word, HDL oxidation might be an effective antioxidant target in treatment of many diseases.

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Keywords

Oxidation · ApoA-I · Myeloperoxidase · Paraoxonase 1 · HDL oxidation

5.1 Antioxidant Activity of HDL

5.1.1 HDL Inhibits LDL Oxidation

While participating in cholesterol reverse transport, some types of HDL also show antioxidant activity. This activity is mainly reflected in the inhibition of LDL oxidation [1]. LDL's chemical modifications including aggregation, enzymatic digestion, and oxidation are important to induce this process. As a vascular protection mechanism, HDL-induced reduction of ox-LDL can be observed both in vitro and in vivo [2]. Exogenously supplementation of apoA-I, the main antioxidant component in HDLs, could reduce ox-LDL deposition in mouse atherosclerotic plaques by approximately 75% [3]. When both lipoproteins are co-incubated under oxidizing conditions, HDL prevents the accumulation of lipid hydroperoxides in LDL, but does not in parallel increase the HDL lipid hydroperoxides content [4, 5]. Due to the protein-based activity of HDL, this kind of inhibition of oxidation can last for several hours, without chain-breaking of antioxidants or chelation of transition metals [6]. HDL can inhibit the formation of oxidized LDL by inhibition of 12-lipoxygenase or other

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enzymes which function in different steps of LDL oxidation [7]. Besides, HDL particles with free radicals protect LDL from oxidative damage and generating pro-inflammatory oxidized lipids. HDL-mediated inactivation of lipid hydroperoxides (LOOH), such as phosphatidylcholine hydroperoxides (PCOOH), involves their initial transfer from LDL to HDL and subsequent reduction to inactive hydroxides by redox-active Met residues of apoA-I. Addition of a hydroperoxyl group to a phospholipid or cholesteryl ester molecule greatly increases hydrophilicity. As a result, LOOH molecules have a higher surface activity than their non-peroxidised counterparts [8]. Their exposure to the aqueous phase at the HDL surface facilitates their removal from the lipoprotein [9]. HDL cholesteryl ester hydroperoxides can be removed by liver cells rapidly and selectively, which indicates that antioxidant function of HDL is mediated by a transport mechanism of oxidant molecules [10]. Furthermore, the removal effect of HDL on LOOH is also found in membranes of erythrocytes and astrocytes, which is associated with cellular membrane phospholipid rigidity [11, 12].

5.1.2 HDL Antioxidant Ability-Associated Oxidants

Traditionally, research has focused on the ability of HDL to protect LDL from free radical-mediated oxidation, whereas research on non-radical oxidants in this area remains scarce. There are two types of naturally occurring oxidant compounds, those with unpaired electrons and those with all electrons paired [5]. The former is called single-electron oxidants or radicals which include hydroxyl radicals, lipoxyl radicals, superoxide, etc. The latter, two-electron oxidants or radicals, include HOCl, ONOO-, etc. Ahmed et al. found that apoA-I reduced oxidative damage of HDL particles by ONOO- in both reconstituted and isolated HDL [13]. Introduction of His and Cys residues at the positions 2/3 and 12, respectively, of apoA-I significantly enhanced its ability to prevent LDL oxidation and remove HOCl [14]. However, evidence suggests that natural HDL is barely resistant to HOCl-mediated oxidation. HOCl could target and oxidize Tyr, Met, and Trp residues in apoA-I, resulting in its loss of function [15]. Therefore, the ability of HDL to prevent non-radical-induced oxidation seems to depend on the natures of the oxidant molecules [5].

5.1.3 Antioxidant Activity of HDL Subfractions

ApoA-I, the main protein in HDL, was found to play a major role in this process [7]. As mentioned before, apoA-I Met residues 112 and 148 can reduce LOOH to redox-inactive lipid hydroxides, thereby terminating chain reactions of lipid peroxidation [16]. Molecular dynamics simulation shows that Met112 and Met148 interact with Tyr115 to create a microenvironment unique to human apoA-I that may be optimal for such redox reactions [17]. Recombinant HDL which contained only purified apolipoprotein A-I and phospholipids (POPC) was observed to have an antioxidant capacity close to that of natural HDL. This suggests that apoA-I plays a key role in HDL-mediated protection of LDL against oxidation by one-electron oxidants [16]. Another main protein associated with HDL especially large particles, apoA-II, shows less antioxidant ability, however [18]. LOOH can also be slightly inactivated by apoA-II. Thus, apolipoprotein A-II enrichment will impair HDL's antioxidant properties, and HDL derived from human apoA-II transgenic mice fails to protect LDL from oxidative modification [19]. Besides, apoA-IV, apoE, apoM, apoD, apoF, apoJ, and apoL-1 can also display partially antioxidative properties [20–23]. Several HDL-associated enzymes are present at elevated concentrations in HDL3 and light HDL2, which can be involved in the inactivation of short-chain oxidized phospholipids. Structural defects in surface lipid packaging which allows insertion of exogenous molecules and become more pronounced as HDL particle size decreases, may account for this property [5, 24]. Besides, reduced levels of sphingolipids

and free cholesterol in small, dense HDL may lead to increased mobility of the surface lipid monolayer, thereby promoting the admixture of exogenous oxidized lipids [25]. Therefore, HDL represents a multimolecular complex capable of acquiring and inactivating oxidant.

5.1.4 HDL Antioxidant Capacity in Endothelial Cells

In endothelial cells, HDL also inhibits oxidation by activating NO production [26]. When HDL binds to SR-BI, interaction of the C-terminal PDZ-interacting domain of SR-BI with the adaptor PDZ domain-containing protein PDZK1 will start a signaling in the endothelium, which finally leads to endothelial nitric oxide synthase (eNOS)-Ser1177-phosphorylation and eNOS-Thr495-dephosphorylation [27–29]. ABCG1 mediated cholesterol efflux can prevent the deposition of cholesterol and 7-oxysterols in the endothelium, which is another pathway to preserve active eNOS dimer levels [30]. Therefore, HDL improves the bioavailability of NO and reduces the production of reactive oxygen species in endothelium overall.

5.1.5 HDL Antioxidant Dysfunction

HDL's antioxidant function is one of its important functions and will affect HDL's role in anti-inflammatory, plasma transport, and other aspects. For example, it was reported that HDLs isolated from mice infected with influenza A virus lose their ability to protect LDLs against oxidation in human artery wall cells, which suggests a relationship between HDL antioxidant and anti-inflammatory [31]. Lipids in cells and plasma, including those incorporated in HDL particles, may undergo oxidative modification [32]. Peroxidized HDL particles lose their ability to take part in reverse cholesterol transport [33]. Therefore, the determination of HDL antioxidant activity can be one of the ways to identify HDL dysfunction [34].

5.2 Regulation of HDL Function

HDL's antioxidant function is regulated by multiple pathways. Recent studies have indicated that extensively oxidized HDLs, especially apoA1, are dysfunctional. Directly lipid peroxides may interfere with HDL's antioxidant, anti-inflammatory, and cholesterol acceptor activities [35].

Alterations at the level of the HDL lipidome and the proteome modification which could impact antioxidative properties of HDL. These lipidome alterations include enrichment in sphingomyelin and depletion of polyunsaturated fatty acids, which would result in elevated rigidity of the phospholipid surface monolayer of HDL [5]. This structural abnormality may result in HDL3's inability to obtain LOOH from LDL and prevent the production of ox-LDL.

5.2.1 MPO Participates in the Oxidative Modification of HDL

A most studied mechanism of HDL oxidation is the myeloperoxidase (MPO) modification of apoA-I [34]. MPO is an enzyme that processes hydrogen peroxide, which mainly expresses in neutrophils and monocytes to exercise anti-infective immune function. Besides, MPO was also found to be enriched in arterial plaque and could also modify host proteins and lipids [36]. For example, LDL exposed to the complete myeloperoxidase-H2O2-Cl system underwent chlorination of its protein tyrosyl residues [37]. Bergt et al. demonstrated that co-incubation of HDL and MPO in a HOCl-containing environment (at a 100:1 ration of HOCl and apoA-I) resulted in the loss of unsaturated fatty acids in phospholipids and cholesteryl esters, as well as the loss of cholesterol receptor activity [38]. Although some evidences suggest that selective apoA-I methionine oxidation does not disrupt its structure and leads to an increase in lipid binding and cellular lipid acceptor activities [39], apoA-I Met-148 modification by MPO was

showed to be associated with loss of LCAT activation [40]. In addition, Panzenboeck et al. also showed that MPO- or HOCl-modified HDL was more readily taken up and degraded by macrophages, thereby converting HDL from lipid-receiving lipoproteins to lipid-loaded lipoproteins [41]. Combination with studies in Smith, Hazen, and Kinter labs at the Cleveland Clinic demonstrated that apoA-I is a selective target for MPO-catalyzed nitration and chlorination in vivo [42]. They identified apoA-I as a nitrated protein in serum and found probable contact site between the apoA-I moiety of HDL and MPO by cross-immunoprecipitation studies in plasma. Studies demonstrated that MPO can bind to the region of residues 190–203 in apoA-I helix 8 [42, 43]. And Wu et al. demonstrated MPO binding to apoA-I induced loss of LCAT activation, correlating with loss of the Tyr166 containing parent peptide [43]. The Oram labs also examined the MPO induced molecular alterations in apoA-I and agreed that MPO modification of apoA-I is physiologically relevant [44, 45]. Although plasma levels of MPO are usually very low in healthy individuals, significant elevations may occur in patients with coronary syndromes and those at risk of major adverse cardiac events [46]. As MPO is the only human enzyme known to generate hypochlorous acid, protein-bound chloropyrazine content can be a specific molecular marker of MPO-catalyzed oxidation in isolated HDL [37]. Interestingly, 4WF isoform, a restructured apoA-I created by replacing four Trp residues with Phe, is markedly resistant to the increasing oxidant stress from MPO or HOCl [47, 48]. If this strategy is successful, apolipoproteins that are more beneficial can be built.

5.2.2 PON1 Increase Antioxidant Function of HDL

Another important molecule involved in the regulation of HDL oxidation is paraoxonase 1 (PON1). PON1 is a calcium-dependent esterase known to catalyze the hydrolysis of organophosphate esters and is widely distributed in tissues such as liver, kidney, and serum. PON1 is capable of hydrolyzing a variety of substrates such as lactones, thio-lactones, glucuronide drugs, aryl esters, organophosphorus pesticides, cyclic carbonates, and nerve gases. It gets its name from the ability to hydrolyze the pesticide paraoxon [49]. In humans, serum PON1 activity and HDL susceptibility to oxidation are inversely correlated. And adding PON1 to HDL can reduce HDL peroxide and aldehyde formation by up to 95%. Mice overexpressing human PON1 exhibit reduced production of reactive oxygen species and reduced foam cell formation [50]. A single cohort study (Cleveland Clinic GeneBank study) involving 3668 patients who underwent coronary angiography showed that the lowest quartile of serum PON1 activity was associated with a more than two-fold higher risk of new cardiovascular events compared to the highest quartile [51]. Regarding the genetic evidence, it was shown that PON1 192QQ homozygotes showed a high antioxidant activity of HDL [52]. Further research found that PON1 can promote the hydrolysis of hydrogen peroxide (H2O2). Since H2O2 is a major reactive oxygen species produced under oxidative stress during atherogenesis [5], this hydrolysis induced by PON1 is a possible mechanism for its inhibition of HDL oxidation [53]. However, some studies don't support PON1 to be an antioxidant enzyme, which showed that PON1 failed to hydrolyze oxidized phospholipids (oxPLs) and is weakly reactive towards LOOH in vitro [54–56]. Thus, Khersonsky et al. suggested that lactones but not oxPLs constitute physiological substrates of PON1 [57], which suggests that PON1 may protect HDL from oxidative modification through other mechanisms. Rosenblat et al. proposed that PON1 increased the ability of HDL to remove cholesterol from macrophages, which is thought to be an important step in reverse cholesterol transport, and therefore increased the free radical scavenging capacity of monocyte/macrophages and lowered oxidative stress [58].

In addition, the effects of PON1 and MPO on HDL oxidation are considered interrelated. It is demonstrated that MPO, PON1, and HDL bind to one another, forming a ternary complex. Hazen

labs proved the complex forming by immunoprecipitation studies, in which antibodies against apoA1 pulled down both MPO and PON1 from plasma. They then revealed functional interaction sites on apoA1 of HDL and PON1 by hydrogen deuterium exchange mass spectrometry and mutagenesis studies. Above this, MPO oxidizes PON1 on tyrosine 71 (Tyr71) to inactivate PON1, and MPO activity is partially inhibited due to the binding of PON1 at the same time [59] (Fig. 5.1).

5.2.3 LCAT Increase Antioxidant Function of HDL

Lecithin-cholesterol acyltransferase (LCAT) is an enzyme that catalyzes the conversion of free cholesterol into cholesteryl ester, thereby increasing its hydrophobicity [5, 60]. LCAT also hydrolyzes the oxidized acyl chains of oxPLs, producing lysophosphatidylcholine and oxidized free fatty acids. In addition, LCAT can function as a chain-breaking antioxidant through its Cys residues at positions 31 and 184 [61]. Evidence suggests that LCAT mutations may reduce the antioxidant capacity of HDL but do not lead to increased plasma concentrations of lipid peroxidation products [62]. Furthermore, LCAT inhibition did not reduce the ability of HDL to scavenge lipid peroxides from LDL [16]. Taken together, the effect of LCAT on the antioxidant function of HDL may be based on its independent decomposition of oxPLs.

5.2.4 EL Increase Antioxidant Function of HDL

Endothelial lipase (EL) belongs to the triacylglycerol lipase family and is mainly synthesized and secreted by vascular endothelial cells [5, 63]. It is known that EL can effectively

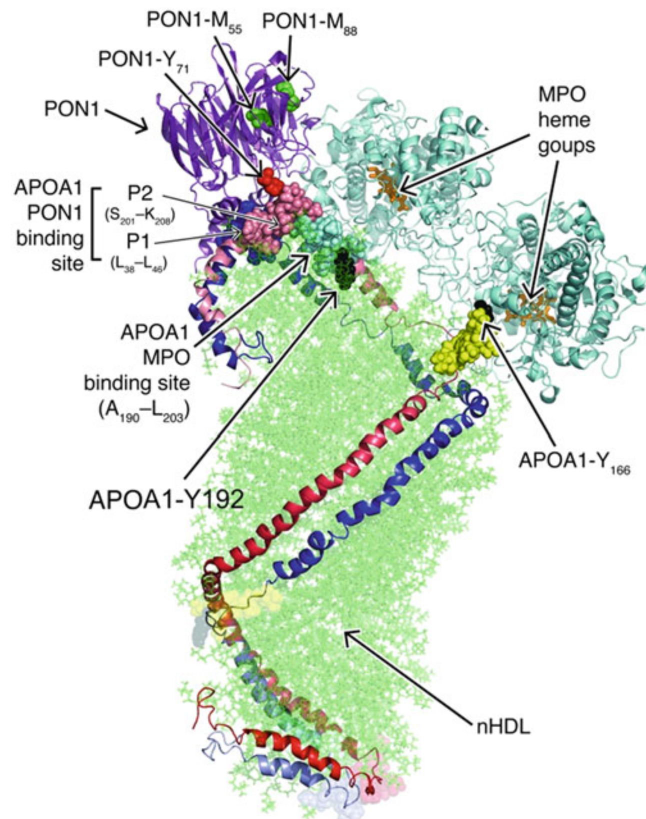

Fig. 5.1 Structural model of a hypothetical ternary complex of MPO and PON1 bound to nHDL [59]

reduce HDL plasma levels and regulate HDL lipid composition at the same time [64, 65]. By its pronounced phospholipase activity EL can consume phospholipids (PL) in HDL and produce lysophospholipids and fatty acids [66]. HDL isolated from EL-deficient mice showed enhanced antioxidant capacity, which may be due to the depletion of HDL-PL mediated by EL [67]. EL modification of HDL was shown to produce particles with altered composition and enhanced antioxidant capacity in vitro [68]. Chloramine T, which could modify Met residues of apoA-I and free SH groups in other HDL associated proteins, profoundly attenuated the capacity of both EV-HDL and EL-HDL [5, 9, 63]. Thus, the enhanced antioxidant capacity of EL-modified HDL may be due primarily to the enrichment of HDL in EL-generated lipolytic products and, to a lesser extent, to the reduced HDL particle size and increased activity of chloramine-T-sensitive mechanisms.

5.2.5 PAF-AH Increase Antioxidant Function of HDL

PAF-AH is another hydrolase present in HDL which can hydrolyze oxidized short-chain phospholipids but not long-chain non-oxidized phospholipids [69]. PAF-AH is mainly from macrophages, and plasma PAF-AH circulates in the association with LDL and HDL particles, with the majority of the enzyme bound to small, dense LDL and to lipoprotein (a) [5, 70, 71]. Studies have shown that PAF-AH is the main oxPL hydrolase in HDL, thus free fatty acid hydroperoxides are transferred to HDL and subsequently reduced to the corresponding hydroxides [56, 72]. It is worth mentioning that PAF-AH has overlapping substrates with PON1, so its activity is also linked to PON1 [73]. However, the majority of PAF-AH is carried by LDL, where it is associated with the release of lysophospholipids and becomes harmful to cells and membranes in the subendothelial space [74].

5.2.6 Other Molecules Participating in the Oxidation Regulation of HDL

Besides, there are other molecules thought to be involved in the oxidation regulation of HDL. For example, HDLs modified by 15 lipoxygenases were reported unable to contrast the formation of reactive oxygen species in cells incubated with TNF-alpha. This modification therefore impairs HDL-mediated cholesterol efflux and anti-inflammatory effects [75].

Advanced oxidation protein products (AOPPs) are carried by oxidized plasma proteins, especially albumin, and might be able to serve as a new marker of oxidative stress in cardiovascular disease. It is showed that AOPP-albumin produced in vitro could bind to SR-BI and block the binding of HDL to SR-BI, thereby inhibiting HDL function [76].

Some other molecular components, such as cholesteryl ester transfer protein (CETP), serum amyloid A (SAA), phospholipid transfer protein (PLTP), and PON3, are showed to enhance the antioxidative activity of HDL, but the specific mechanism has not been revealed [77–80].

5.3 HDL Oxidation and Disease

5.3.1 HDL Oxidation in Cardiovascular Diseases

As atherosclerosis is an inflammation-related and lipid metabolism-related disease, HDL and HDL oxidation play important roles in it. LDL-derived LOOH display multiple pro-oxidative and pro-inflammatory properties [5]. Short-chain oxPL and aldehydes produced by the breakdown of LOOH also have proatherosclerotic effects. Pro-oxidants increase in local oxidative stress compared to antioxidants and cause oxidation in the intima of arteries. These highlight the relevance of HDL-mediated neutralization of lipid peroxidation products and atherosclerosis [81]. Data from both experimental and human studies support the involvement of HDL in

reducing oxidative damage [35, 82]. The lack of other antioxidant pathways in the arterial endothelium makes HDL antioxidant activity particularly important in this class of microenvironment. Increased oxidative stress is characteristic of coronary atherosclerosis and frequently leads to damaging oxidative modifications [1, 45]. Cellular oxidative systems involved in atherosclerosis include MPO, NADPH oxidase, nitric oxide synthase, and lipoxygenase. They produce a variety of reactive chlorine, nitrogen, and oxygen species in the form of one-electron (free radical) and two-electron oxidants [83]. Based on studies using an in vitro artery wall model, it was concluded that LDLs deposited in arterial plaque receive additional lipid hydroperoxides produced by the lipoxygenase, as well as MPO pathways operating in local cells, which in turn promoted ox-LDL formation and inflammation [7, 18]. Oxidation of apoA-I by MPO occurs mainly within the subendothelial compartment and results in the increased oxidation of multiple residues, which have been defined by mass spectrometric analysis [84–86]. The levels of chlorinated Tyr192 and oxidized Met148 are higher in patients with stable coronary artery disease (CAD) or ACS than in control individuals [82]. MPO-catalyzed oxidation of HDL and apoA-I results in selective inhibition in ABCA1-dependent cholesterol efflux from macrophages, which could also be a potential mechanism for MPO-dependent generation of a proatherogenic dysfunctional form of HDL in vivo [42]. Systemic PON1 activity was also reduced within the acute coronary syndrome cohort compared with the healthy controls [59] (Fig. 5.2).

5.3.2 HDL Oxidation in Metabolic Diseases

In metabolic diseases like diabetes, HDL function may also be mediated by oxidation. Studies show that HDL3 from patients with metabolic syndrome(MetS) have impaired ability to reduce LOOH accumulation in LDL particles in vitro, up to 23% compared with nonobese normolipidemic controls [87]. Similar result was obtained in patients with type 2 diabetes (T2D), whose HDL3 also had reduced PON1 and PAF-AH activity [88]. Altered phospholipid composition of HDL in patients with T2D leads to an increased ratio of sphingolipids to phosphatidylcholine and increases the rigidity of the HDL surface, which is one of the major determinants of the antioxidative activity of HDL [16, 89]. Another study showed that HDL2 exhibited a reduced ability to protect LDL from macrophage-mediated oxidation when the hyperglycemia was poorly controlled [90]. Interestingly, recent studies have found that MPO-mediated oxidative stress impaired HDL function even before appreciable changes in HDL levels in diabetes [91]. As for hyperlipidemia, HDL subfractions from patients with isolated low HDL-C levels showed reduced antioxidative activity compared with normolipidemic controls, after excluding the interference of blood glucose [5, 92]. In patients with hyper-alpha lipoproteinemia associated with high HDL levels of triglycerides and low hepatic lipase activity, the antioxidative activity reduced more significantly [93].

5.3.3 HDL Oxidation in Neurodegenerative Disorders

Besides, due to the diversity of HDL functions, HDL antioxidant capacity changes occur in many diseases. Emerging evidences suggest that HDL and apoA-I may play a pivotal role in preserving cognitive function under normal and pathological conditions [94], suggesting that antioxidant properties of apoA-I/HDL may play a significant role in neuroprotection just like that in cardiovascular disease. Functional changes of apoA-I have been reported in various diseases such as Alzheimer's disease and Parkinson's disease [95, 96]. In Parkinson's disease (PD), the activity of PON-1 has been shown to be decreased, which indicated that HDL oxidation could play a direct role in its pathogenesis [97]. Several studies have also reported that individuals with the PON1 gene polymorphism in reducing enzyme activity have an increased risk of developing PD. [98, 99]

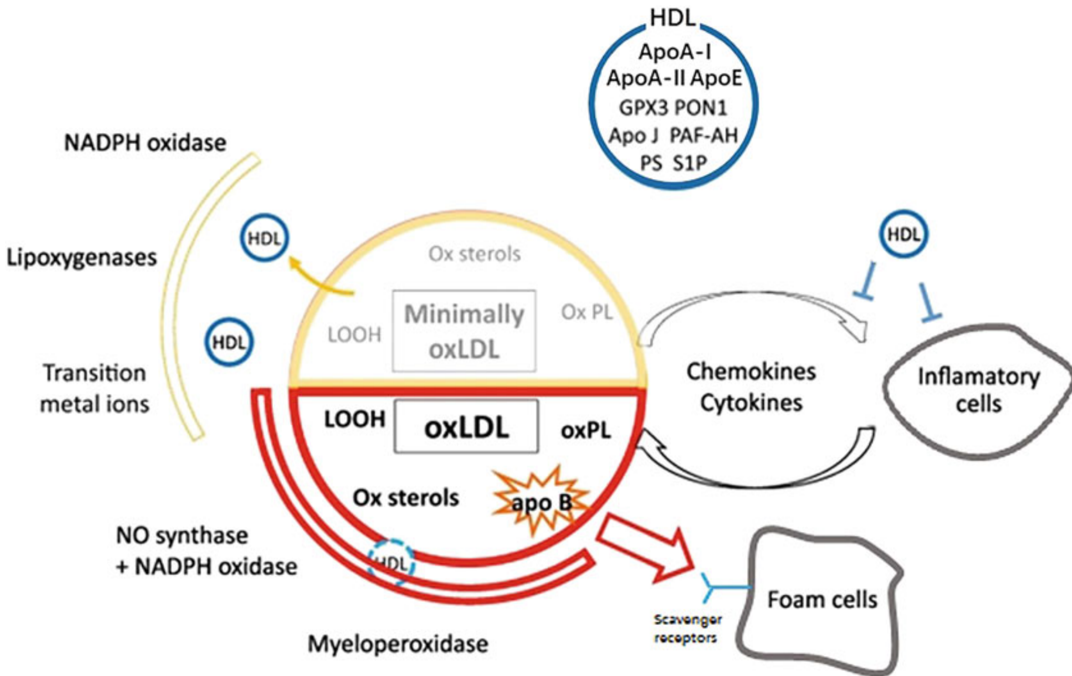

Fig. 5.2 Mechanisms of HDL-mediated protection against LDL oxidation in the arterial wall (Adapted from Brites, F. et al. [5])

Consistently, some results showed that mutation and haplotype of SNPs of PON cluster were significantly associated with sporadic amyotrophic lateral sclerosis (ALS) in populations [100, 101]. Since oxidative stress plays a central role in the pathogenic process of sporadic ALS, these findings implicated that enhancing HDL function may help to mitigate ALS [102].

5.3.4 HDL Oxidation in Immune Diseases

Some evidences support that HDL oxidation also involves in some immune diseases. A recent study found that patients with rheumatoid arthritis (RA) with high levels of inflammation (hsCRP > 10 mg/L) had reduced antioxidant activity of HDL3 [103]. Consistently, McMahon et al. observed reduced antioxidant activity of total HDL in patients with systemic lupus erythematosus (SLE) [104]. These may result in the binding of HDL and lectin-like oxidised LDL receptor 1 and in turn induce proinflammatory responses in macrophages [105]. In addition, the increasing of MPO-mediated HDL oxidation and the decreasing of PON1 activity were also reported in these diseases [106, 107].

5.3.5 HDL Oxidation in Other Diseases

Patients with polycystic ovary syndrome, a disease characterized by insulin resistance, showed a reduced ability to protect LDL from oxidation in serum [5], as well as decreased PON1 activity was found in these patients in another study [108, 109]. Patients with iron deficiency anemia (IDA) also showed reduced antioxidant activity of total HDL associated with impaired PON1 activity [110]. Similar results were observed in postmenopausal women with PON1 heterozygous activity phenotype [111].

5.3.6 Therapeutic Perspectives in HDL Oxidation

Based on the association between HDL oxidation and disease, improving antioxidative activity of HDL may become a new therapeutic approach, although the evidence is still limited.

Lifestyle is a known factor which will affect HDL composition and functionality. Studies have investigated the effects of diet and exercise habits on HDL antioxidant activity. Significant improvement in HDL3c antioxidant activity in patients with metabolic syndrome and normal LDL cholesterol plasma levels was observed after 12 weeks of individualized diet and exercise intervention [112]. Moreover, dyslipidemic patients showed increased antioxidant potential of HDL2a and 3b, while PON1 activity increased after 3 months of moderate physical activity [113]. Besides, increased antioxidative activity of total HDL was showed in trained triathletes with the PON1 heterozygous activity phenotype, reflecting an adaptive mechanism of oxidative stress which results from increased oxygen consumption [114]. As for unhealthy habits like smoking, Park et al. found impaired HDL antioxidant activity and elevated LDL oxidation in smokers compared to nonsmoking controls [115]. Smoking induced phospholipid depletion in HDL3 fractions and causes the production of dysfunctional HDL3 particles, which was measured by ferric-reducing capacity [116].

Traditional HDL therapy targeted to raise HDL-C levels. Nevertheless, several pharmacological alternatives were found to improve antioxidative activity of HDL potentially at the same time. Atorvastatin increased PON1 activity while enhancing plasma antioxidant capacity in hypercholesterolemic patients [117]. Pitavastatin could increase the transcription of PON1 [118]. Other statins have also been shown to enhance PON1 activity in some other studies [119, 120]. Fenofibrate could cause an increase in activity of antioxidant enzyme, glutathione peroxidase, together with a decrease in systemic oxidative stress in dyslipidemia patients, as well as improvements in PON1 activity [121, 122].

Niacin intake is negatively associated with plasma oxLDL levels in healthy adults and results in a decrease in systemic oxidative stress [123, 124]. Extended-release niacin (ERN) is the most effective agent for increasing HDL-C, but studies have shown that the increase in HDL-C achieved with ERN does not match the increase in antioxidant capacity. These results may explain why Niacin and some lipid lowering agents fail to reduce cardiovascular risk [125].

The using of apoA-I mimetics and reconstituted HDL (rHDL) could modify the antioxidative capacity of HDL directly. Navab et al. tested the ability of a series of apoA-I peptides to inhibit LDL oxidation and found significant effects of peptides 4F and 5F [7]. Another apoA-I mimetic, L-6F, also showed antioxidant properties in several mouse models [126]. Compared with normal HDL, rHDL containing apoA-I mutant peptide V156 K and R173C showed higher antioxidant activity in vivo and in vitro [127]. In addition, rHDL containing apoA-I Milano showed higher antioxidant activity than wild-type rHDL in rats, thereby protecting against endotoxin-induced multi-organ damage [128].

5.4 Conclusion

Overall, we have known that HDLs have the activity of inhibiting LDL oxidation and neutralizing some other oxidants. ApoA-I is the main antioxidant components in HDLs. The antioxidant capacity of HDL and apoA-I is regulated by alterations at the level of the HDL lipidome and the modifications of the proteome, especially MPO and PON1. HDL oxidation is an important process in atherosclerosis, metabolic diseases, and other diseases. Although the influence of some molecules on HDL oxidation and its mechanisms mediated in HDL oxidation remain inconclusive, HDL oxidation might become a disease marker and therapeutic target in many diseases as research continues.

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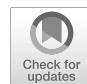

6 HDL and Scavenger Receptor Class B Type I (SRBI)

Hong Yu

Abstract

The scavenger receptor class B type I (SR-BI) is a versatile HDL receptor protein. It is highly expressed in liver and steroidogenic tissues. SR-BI regulates selective uptake of cholesterol ester (CE) from HDL, revealing its role in mediating reverse cholesterol transport (RCT) and steroid hormone synthesis. In addition, SR-BI is involved in cholesterol transport, cellular inflammatory response, platelet reactivity, and HDL-initiated signaling in the vascular system in several mouse models. Mutations in the human SR-BI gene (*SCARB1*) have been found to be associated with abnormally high plasma HDL-C levels and an increased risk of atherosclerotic cardiovascular disease. At present, the key regions of SR-BI transmembrane structure and the regulatory mechanisms of SR-BI expression still need to be further studied. In this chapter, the structural, functional, and regulatory characteristics of SR-BI are reviewed, and the importance of SR-BI in related metabolic diseases was expounded.

Keywords

Scavenger receptor class B type I (SR-BI) · HDL metabolism · Reverse cholesterol transport · Cholesterol transport · Cellular inflammatory response · Platelet reactivity · HDL-initiated signaling · Atherosclerotic cardiovascular disease

The scavenger receptor class B type I (SR-BI), a member of the CD36 superfamily, is a membrane glycoprotein that functions as a HDL receptor [1]. SR-BI is well conserved between species and abundantly expressed in the liver and steroidogenic tissues where it mediates selective uptake of cholesteryl ester (CE) from HDL. Hepatic SR-BI plays an important role in reverse cholesterol transport (RCT) and is closely related to HDL metabolism [2, 3]. Meanwhile, several studies from mouse models support that SR-BI regulates cholesterol trafficking, cell inflammatory responses, platelet reactivity, and HDL-initiated signaling in the vasculature, which is related to its role in anti-atherogenesis [4, 5]. Recently, some large population based studies have demonstrated that the human SR-BI gene (*SCARB1*) has single-nucleotide polymorphism (SNP) and subjects heterozygous for the P376L mutant form of SR-BI have abnormally high levels of HDL-C and increased risk of cardiovascular disease [6, 7]. Here, we summarize the structural, functional, and regulatory characteristics of SR-BI for a better

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understanding that SR-BI-mediated pathway may be a potential gateway for therapy and diagnosis in related diseases.

6.1 Structural Features of SR-BI

6.1.1 SR-BI Gene Structure

The SR-BI gene sequence was originally obtained from a Chinese hamster ovary (CHO) cell variant [8]. Mouse and rat SR-BI genes were found to be localized on chromosomes 5 and 12q15–16, respectively. The human *SCARB1* gene is located on chromosome 12, with a full-length of 86 kb and consisting of 13 exons and 12 introns. The exon structure of SR-BI is very similar to that of CD36. Exon 1 comprises the 5’-UTR of cDNA and a short coding sequence, and exon 13 contains all the 3’-UTR. The length of human SR-BI mRNA is about 2.8 kb. Due to exon alternative splicing, SR-BI gene can be transcribed to produce multiple mRNA species. For example, Exons 2 and 3 of human SR-BI can be selectively spliced, resulting in deletion of 300 bp mRNA. The jump of exon 12 (129 bp) also generates a C-terminal variant mRNAs encoding SR-BII, in which three residues at C-terminus are shorter than SR-BI and the last 39 amino acids (467–506) are different.

At present, studies found a number of essential regulation elements in the *SCARB1* promoter. The human *SCARB1* promoter has two E-box consensus sequences (CANNTG) at the proximal (−160 bp) and the distal (−1145 bp) regions of the transcription start site, respectively, which bind to transcription factors with the basic helix-loop-helix (bHLH) motif and sterol regulatory element (SRE)–binding protein (SREBP). There are also two conserved CAAT elements that bind enhancer-binding protein (C/EBP) at −476 bp and −1046 bp regions upstream of the transcription starting site, and a proximal (−217 bp) binding site for steroidogenic factor-1 (SF-1). Meanwhile, a liver receptor homolog-1 (LRH-1) response element and a liver X receptor (LXR) response element are located in the promoter at −77 bp and − 1139 bp, respectively. Additionally, two SREs, four estrogen response element (ERE) half sites, seven simian virus 40 protein-1 (Sp1)–binding sites, and eleven E-boxes in proximal (−238 bp) and distal (−1958 bp) regions are found in the rat SR-BI promoter [9].

6.1.2 SR-BI Protein Structure and Localization

Human SR-BI protein was early named CLA-1 (CD36- and LIMPII-analogous-1) because of its structural similarity. UniProt predictions indicated that selective splicing of human *SCARB1* mRNA might produce five SR-BI isomers. Isoform 1 was the first to be identified and named SR-BI, which contains 509 amino acid residues and has a predicted molecular weight (MW) of 56.9 kDa. In fact, due to glycosylated modification, it is usually detected with a relative MW of 82–83 kDa. SR-BI has a hairpin-like topological structure, the 409 amino acid residues in the middle segment form the extracellular domain, which is anchored to the plasma membrane by two transmembrane domains (aa 12–32 and 441–461), and is adjoined two short cytoplasmic domains at the N-termini (aa 1–11) and C-termini (aa 462–509), respectively [9].

Structure-function analyses identify that the extracellular domain binding to HDL particle contains several Cys residues that form disulfide bridges and four proven N-glycosylation sites (Asn-102, Asn-108, Asn-173, Asn-330). Nearly all Cys (251, 280, 321–323, 274–329, 334, 384) are conserved in a variety of species, such as human, cow, pig, rabbit, hamster, and mouse, among which four Cys in the ectodomain (280, 321, 323, and 334) are important for structural integrity, the intracellular transport, HDL-binding, and lipid-transporter activity of SR-BI [10]. It has been reported that Cys-384 is involved in the binding site for block lipid transport 1 (BLT-1), an inhibitor of SR-BI synthesis [11]. Mutational studies in human SR-BI have shown that mutation of Asn-108 or Asn-173 causes an inability of SR-BI to locate to the

plasma membrane and reduce lipid transfer from HDL to cells. In addition, the glycine dimerization motif (G15_G18_G25) exists in the N-terminal transmembrane domain of SR-BI, which is a necessity for normal homo-oligomerization and lipid-transporter activity [9]. The N-terminus and C-terminus of the extracellular domain of SR-BI, as well as Trp-415 combined with other Trp residues, are also critical for mediating lipoprotein binding and HDL-C selective uptake [12]. Moreover, membrane localization and SR-BI-mediating signaling are highly dependent on the VLQEAKL motif at the tail of the C-terminal cytoplasmic domain, which interacts with PDZK1, a multi-PDZ domain–containing adaptor protein [13]. It is worth noting that human and rodent SR-BI motifs share a number of common features, including three potential phosphorylation sites (Ser481, Ser4, and Ser477), a leucine zipper (aa 427–455), and a possible peroxisomal-targeting sequence (PTS-1) (aa507–509), which may be modulated by phosphorylation and by dimerization. Recently, it has been reported that the C-terminal cytosolic domain contains a Ser-496 that is phosphorylated by the kinase SIK-1, which modulates SR-BI efficiency for selective HDL-CE uptake [14]. Additionally, early studies found that mouse SR-BI could undergo fatty acylated at Cys462 and Cys470, which may be palmitoylation or myristoylation.

The cellular and tissue distribution of SR-BI protein is closely related to the function of lipid transport and metabolism. Early studies have been reported that SR-BI protein in human, mouse, and rat showed a similar tissue distribution. SR-BI is predominantly expressed in the liver and steroidogenic cells of the adrenal gland, ovary, and testis, little expression in testes, mammary gland, heart, and intestine, but no expression in the uterus, muscles, brain, kidney, spleen, and lungs. Later studies have shown that unlike in rodents, SR-BI distribution in humans is characterized by low expression in ovaries and high expression in placenta. Besides parenchymal hepatic cells and steroidogenic cells, SR-BI is also expressed in various cell types, including monocytes, macrophages, smooth muscle cells, endothelial cells, platelets, adipocytes, keratinocytes, and various epithelial cells [9].

At present, it has been recognized that SR-BI is located on the plasma membrane with a unique structure for mediating the flux of CE, free cholesterol, and related lipids into and out of cells [15]. Studies using electron microscopy indicated that SR-BI could preferentially locate in the microvillar domains to form hydrophobic channels for capturing various lipoproteins, including HDL. Of interest, about 60% of SR-BI is present in caveolae-rich membrane invaginations, which is usually associated with cellular signaling, potocytosis, and transcytosis. Some SR-BI also existed within the caveolin-poor membrane component [16].

6.2 Functional Features of SR-BI

Early experiments evidenced that scavenger receptors, as cell surface membrane proteins, could bind native lipoproteins and chemically modified lipoproteins, including oxidized LDL (ox-LDL), acetylated LDL (ac-LDL), phospholipids, phosphatidylserine, and many other types of ligands [17]. However, after the discovery of the *SCARBI* gene encoding the SR-BI in 1994, SR-BI was first identified by Krieger's laboratory as a physiological HDL receptor, which could selectively remove cholesterol esters from HDL through high-affinity binding to HDL [1, 5]. Subsequently, a series of studies demonstrated that mice with SR-BI deficiency had abnormally high plasma levels of HDL-C, while the adrenal cholesterol content in mice decreased by 72%, and SR-BI knockout (SR-BI$^{-/-}$) mice showed the increased diet-induced atherosclerosis susceptibility, which was paralleled by pathological phenomena such as reticulocytosis, anemia, thrombocytopenia, splenomegaly, and female infertility [3, 18]. Recently, the growing evidence reveals that SR-BI not only has a crucial role in HDL metabolism, steroid metabolism, and cholesterol homeostasis in the body, but also has an important impact on cell homeostasis, immune inflammation, and reproductive development [5].

6.2.1 SR-BI and HDL Metabolism

Numerous studies have identified that SR-BI plays an important role in cholesterol and steroid metabolism, especially in RCT and HDL metabolism. SR-BI regulates the first step and the last step of RCT by promoting cholesterol efflux from peripheral tissues and the selective delivery of HDL-CE to the liver. Through the selective uptake of HDL-CE, hepatic SR-BI mediates plasma cholesterol clearance for routing to the bile, thereby modulating HDL composition and function [5]. Because the lipoproteins as donors of CE for SR-BI-regulated selective CE uptake differ in characteristics, the processes for SR-BI selective uptake of CEs from lipid-rich lipoproteins have not been completely understood. Based on several lines of evidence obtained using CD36/SR-BI chimeras, SR-BI mutant (N173Q) and apoAI-deleted HDL particles, it seems that the initial binding of HDL to SR-BI and subsequent selective CE transport into cells can be divided into two independent steps: the first step involves the binding of cholesterol-rich lipoprotein to the extracellular domain of SR-BI, and the second step refers to SR-BI-mediated transfer of CEs from HDL to the plasma membrane and the release of the cholesterol-poor HDL particles back into the circulation. Among this process, SR-BI mediates selective uptake of HDL-CE without degradation of HDL protein via a holoparticle internalization and HDL recycling way, which differs from that of endocytosis through the caveolae and vesicle structure [5, 19]. Although the exact mechanism is not fully clear, a proposed model suggests that SR-BI forms a hydrophobic channel between HDL and the cell membrane, and then CEs move through the channels in a concentration-gradient manner.

Studies from mouse model have revealed that hepatocytes in SR-BI$^{-/-}$ mice completely lose the ability to selectively uptake CE from plasma HDL, and the secretion of biliary cholesterol is impaired due to RCT pathway blocking, resulting in a 2.5-fold increase in plasma levels of HDL-C and total cholesterol (TC), and accumulation of abnormally large HDL particles in mice. Especially, SR-BI$^{-/-}$ mice have a high ratio of plasma free cholesterol (FC) to TC increased by 2–3 times versus wild-type mice, which may be closely related to the decreased activity of lecithin-cholesterol acyltransferase (LCAT) [5, 20]. A series of studies demonstrated that compared with the relative amount of protein in HDL from wild-type mice, that in SR-BI$^{-/-}$, HDL was reduced by about one quarter, and the capacity of functional component apoAI and paraoxonase 1 (PON1) in SR-BI$^{-/-}$ HDL was significantly decreased although the relative level of plasma apoAI in mice was not changed, suggesting that abnormally high levels of HDL-C and unesterified properties of HDL are closely related to the accumulation of dysfunctional HDL in SR-BI$^{-/-}$ mice [21, 22]. Other studies also demonstrated that overexpression of SR-BI in mouse livers with adenoviral vectors or transgenes decreased HDL-C levels and promoted secretion of cholesterol into the bile, resulting in reduced atherosclerosis [23]. Besides CEs, SR-BI also mediates the bidirectional flux of other lipids, including FC, phospholipids, and triglycerides (TGs) between HDL and cell plasma membranes.

Human SR-BI is also abundantly expressed in the liver for regulating RCT and HDL-C levels, but the plasma lipoprotein profile in humans is different from those in mice. In contrast with rodents, humans express cholesteryl ester transfer protein (CETP) that can transfer CE from HDL to VLDL for conversion to LDL, and LDL is then internalized via hepatic LDL receptors mediating route, providing an alternative pathway for HDL-CE delivery from the circulation to the liver. It had been considered that functional defects of human SR-BI do not cause serious consequences of abnormal HDL metabolism, evidenced by the study that about one-third of HDL-CE may be directly taken up by hepatic SR-BI in human subjects. However, a number of clinical studies have reported the association between SR-BI polymorphisms and alterations in plasma HDL-C levels, HDL functional changes, and cardiovascular diseases. Vergeer et al. identified that an *SCARB1* missense

mutation (889C > T) led to a functional mutation SR-BI (P297S), resulting in elevated HDL-C levels in carriers and a 56% decrease in HDL-CE uptake in hepatocytes [6]. The earliest identified genetic variation of SR-BI gene (*SCARB1*) is a single-nucleotide polymorphism (SNP) of exon 1 (rs4238001), which results in an SR-BI variant (G2S), the subjects who carried this variant show a significant negative correlation between SR-BI protein levels and HDL-C levels, and have an association with incident coronary heart disease (CHD) in multiethnic populations [24]. Subsequently, carriers of SR-BI missense mutations (S112F and T175A) also showed increased HDL-C levels and impaired function of SR-BI binding to HDL [25]. In 2016, large population-based studies reported by Zanoni et al. revealed that individuals who are heterozygous carriers harboring a P376L missense mutation in SR-BI are associated with an increased risk of CHD albeit increased plasma levels of HDL-C. The P376L variant disrupts posttranslational processing of SR-BI and abrogates selective uptake of HDL-C in hepatocyte-like cells [7]. Therefore, SR-BI is atheroprotective through promoting RCT and affecting HDL metabolism.

In addition to CE uptake from HDL, liver SR-BI may also have roles in the clearance of VLDL and atherogenic lipoprotein (a) [Lp(a)]. Studies identified that VLDL clearance was decreased by two-thirds in SR-BI$^{-/-}$ mice compared to wild-type mice, and that hepatic SR-BI binds VLDL. SR-BI internalizes VLDL through mechanisms involving heparin sulfate proteoglycans and lipoprotein lipase. Recently, the genetic role of *SCARB1* gene in impacting plasma levels of apoB was found between rs4765615 and apoB, whereas rs11057844 demonstrated the strongest association with HDL-C level in a population-based investigation [26].

6.2.2 SR-BI Action in Vascular Cells

SR-BI, as a HDL receptor in the cell surface, plays different roles in a variety of cells. Besides SR-BI regulates selective HDL-CE uptake by high-affinity binding HDL/apoAI in hepatocytes and steroidogenic cells, SR-BI in other cells from the vasculature also mediates pleiotropic effects. For example, SR-BI is expressed in endothelial cells and macrophages where it functions to reduce atherosclerosis.

6.2.2.1 SR-BI in Endothelial Cells

SR-BI expressed by endothelial cells plays a crucial role in HDL-induced activation of endothelial nitric oxide synthase (eNOS) through HDL binding to SR-BI in endothelial cell caveolae, which is evidenced by the experiments wherein antibodies against SR-BI or apoAI completely abrogate HDL effects [27]. Frank et al. found that SR-BI expression markedly increased the stabilization of caveolin-1, an integral protein of caveolae, which contributes to the regulation of cellular cholesterol homeostasis and participates in transcytosis and cell signaling [16]. In endothelial cells, the role of SR-BI in the regulation of cholesterol efflux is involved in the activation of eNOS by HDL. It has been found that the interaction of SR-BI C-terminal PDZ-interacting domain with Src leads to Src phosphorylation, which further activates PI3K, Akt, and eNOS [28]. In vivo data from SR-BI$^{-/-}$ mice are consistent with increased endothelial cell apoptosis, suggesting that SR-BI has a protective effect against apoptosis of endothelial cells. Kimura et al. have demonstrated that SR-BI plays an important role in the inhibition of adhesion molecule expression (VCAM-1, ICAM-1) induced by HDL. A recent study demonstrates that SR-BI binds with apoAI/HDL and negatively regulates sphingosine 1-phosphate (S1P) and its receptor-mediated inflammation in endothelial cells through activating PI3K/Akt signaling pathway [29]. Taken together, these effects contribute to decreasing monocyte adhesion and recruitment into the intima, thus preventing the progression of atherosclerotic lesions.

In addition, SR-BI has an important role in HDL-induced endothelial cell migration and re-endothelialization. Seetharam et al. have determined that HDL promotes migration of endothelial cells in a nitric oxide (NO)–independent manner through SR-BI-mediated Rac GTPase

activation. Rapid initial stimulation of lamellipodia formation by HDL via SR-BI, Rac, and Src kinases has been demonstrated. Moreover, impaired re-endothelialization was observed in SR-BI$^{-/-}$ mice. Therefore, HDL promotes the migration of endothelial cells through SR-BI-initiated signaling, and these mechanisms enhance endothelial monolayer integrity in vivo [30].

Studies have revealed that endothelial SR-BI is actively participated in transcytosis of HDL from the apical to basolateral side of a cell to stimulate cholesterol efflux from intimal macrophages and lymphatic vessels. However, SR-BI regulates the transmembrane effect of HDL in brain microvascular endothelial cells, which differs from the receptor-mediated lipid uptake and signaling pathways in other cells [31].

Additionally, SR-BI is also expressed in endothelial progenitor cells (EPCs) and plays a critical role in HDL-mediated EPC number and function. It has been shown that EPC migration and mobilization is mediated via NO and that enhanced NO production requires signaling via SR-BI [32].

6.2.2.2 SR-BI in Macrophages

SR-BI is also expressed in macrophages in tissues and atherosclerotic lesions. Macrophage SR-BI protects against atherosclerosis by affecting cellular cholesterol homeostasis, inflammation, apoptosis, and efferocytosis. Early evidence has shown that macrophage SR-BI acts as a multiple ligand receptor and regulates the uptake of native (HDL, LDL, and VLDL) and modified (oxLDL and acetylated LDL) lipoproteins, resulting in foam cell formation [17]. On the other hand, SR-BI interacts with HDL with high affinity to regulate intracellular FC efflux to HDL particles, which is a passive, nonenergy, concentration-dependent transport. The study from bone-marrow-derived macrophages (BMMs) revealed that SR-BI expression could enhance both cell cholesterol influx and efflux from HDL in the early stage of cell differentiation, but did not result in altered cellular cholesterol mass [33]. Therefore, SR-BI plays a unique role in promoting bidirectional cholesterol flux in nonlipid-loaded cells and facilitates net efflux from the cell when a favorable cholesterol gradient exists.

Linton et al. conducted BMT studies in the apoE$^{-/-}$ mouse model to examine the role of macrophage SR-BI in the pathogenesis of atherosclerosis in vivo. The results demonstrated that the loss of macrophage SR-BI expression increased atherosclerotic lesion area without alterations in plasma lipid and lipoprotein profiles. The removal of cholesterol from macrophages is one mechanism through which macrophage SR-BI could alleviate atherosclerosis [5, 34]. Some evidence suggests that macrophages could not restrain the excess uptake of cholesterol, and therefore, macrophages rely on an active cholesterol efflux process to sustain cholesterol homeostasis within the cell. Moreover, macrophage SR-BI acts similarly to ABCA1 and might account for the cholesterol efflux to HDL. SR-BI overexpression in mouse macrophages significantly enhanced the efflux of cholesterol to HDL, whereas inhibition of cholesterol transport by SR-BI decreases cholesterol efflux of macrophages [4, 33]. A recent report found that macrophages enriched in cholesterol from human carriers of the *SCARB1* gene P297S variant displayed significantly decreased efflux of cholesterol to HDL, in comparison with macrophages from control subjects [6]. However, previous researches have demonstrated that ABCG1 and ABCA1 are the main mechanisms of cholesterol efflux from macrophages in mice, whereas SR-BI only makes a very limited contribution [35]. These inconsistent findings suggest that cholesterol flux between HDL and cellular SR-BI is modulated by the cholesterol status as well as phospholipid species of both the macrophages and HDL.

Additionally, SR-BI has been reported to mediate inflammatory responses through modulating the conversion of macrophages to inflammatory M1 versus anti-inflammatory M2 phenotype. SR-BI mediates cholesterol efflux from macrophages to HDL, contributing to the anti-inflammatory effect of HDL. Recent lines of evidence have showed that the anti-inflammatory effects of HDL in macrophages depend on SR-BI [36]. SR-BI knockdown or inhibition of SR-BI

ligand binding abrogated the anti-inflammatory effects of HDL. When binding to its ligand HDL, SR-BI can activate Akt signaling and inhibit the inflammatory responses to LPS by dramatically decreasing NF-κB activation, resulting in elevated anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β) and interleukin (IL)-10. Exposure of SR-BI-deficient macrophages to LPS showed increased expression of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) and reduced expression of TGF-β [37]. Although SR-BI is a multiligand receptor that can bind to modified lipoproteins, oxidized phospholipids, and native HDL, it has been reported that inhibiting SR-BI expression or function potentiated glycated HDL induced generation of TNF-α, indicating an important anti-inflammatory effect for SR-BI. Meanwhile, SR-BI also regulates macrophage inflammation by reducing P38, and JNK signaling.

Recent lines of evidence have demonstrated that SR-BI also affects apoptosis susceptibility and efferocytosis in macrophages [5, 38]. Akt-mediated SR-BI activation plays a role in inhibiting macrophage apoptosis, since Akt promotes survival by activating Bad phosphorylation, which prevents Bad from inhibiting the anti-apoptotic factor BCL-XL. SR-BI interaction with HDL also inhibits cell apoptosis by suppressing ox-LDL-induced endoplasmic reticulum stress (ERS) [39]. In agreement with a role for SR-BI in inhibiting cell death in atherosclerotic lesions, Tao et al. demonstrated that transplantation with SR-BI-deficient bone marrow to either apoE$^{-/-}$ or LDLR$^{-/-}$ mice led to remarkably elevated numbers of lesion TUNEL-positive cells, compared to lesions in mice with control bone marrow transplantation. Macrophage SR-BI can also regulate the removal of apoptotic cells or efferocytosis via the Src/PI3K/Akt/Rac1 signaling pathway, leading to enhanced survival of phagocytes [38].

Furthermore, SR-BI appears to have a role in autophagy in macrophages [5]. Recent lines of evidence showed that in the setting of infection, impaired autophagy was observed in SR-BI$^{-/-}$ mouse macrophages, and the formation of subcellular membrane cholesterol domains participates in the SR-BI-induced autophagy. This raises the possibility that SR-BI mediates cytoplasmic cholesterol mobilization. Interestingly, it was found that SR-BI trafficked to lysosomes with Rab7, a key protein, which promotes autophagosome-lysosome fusion and localizes to lysosomes [40]. Lysosomes enriched in FC and engorged in neutral lipid are contained in SR-BI-deficient macrophages.

Additionally, SR-BI also exists in platelets and lymphocytes. As a functional HDL-binding receptor on platelets, the direct interaction between SR-BI and HDL is a necessity for maintaining normal platelet function. SR-BI deficiency leads to platelets' hyperreactivity and high sensitivity of thrombosis [41]. Meanwhile, SR-BI$^{-/-}$ mice also exhibit an imbalance of lymphocyte homeostasis, such as the proliferation of spleen T and B lymphocytes, and led to an imbalance of interferon γ (INF-γ) and IL-4 in cells [42]. Taken together, further research applying SR-BI$^{-/-}$ mice will discover more about SR-BI's role in the control of vascular cells and other cell lines.

6.2.3 SR-BI-Mediating Signaling Pathway of HDL

Accumulated studies have confirmed that SR-BI is involved in regulating HDL-dependent activation of signaling pathways, which may lead to its atheroprotective function. As previously mentioned, SR-BI on cell membrane interacts with PDZK1 (multi-PDZ-domain-containing adaptor protein) by its C terminus and directly participates in HDL-mediated signaling pathways. SR-BI is located in caveolae, and the alterations of cholesterol content and distribution in plasma membrane can affect the activities of the serine/threonine phosphatase PP2A and the tyrosine phosphatase HePTP, which stimulate Erk1/2 phosphorylation, influencing the resulting intracellular signaling governing processes in cells. Mutation of a highly conserved C-terminal transmembrane domain (SR-BI-Q445A) is incapable of HDL-induced signaling, suggesting that SR-BI serves as a plasma membrane sensor of

cholesterol. In addition, SR-BI is required for HDL-induced angiogenesis in vivo and HDL activation of endothelial NO synthase and migration in cultured endothelial cells [15]. SR-BI also serves as a mediator in the uptake of bioactive lipids (phospholipids, sphingosine, sterol hormones, etc.) from HDL [27]. For example, SR-BI participates in eNOS activation by HDL-bound sphingosine 1-phosphate (S1P). Therefore, there are at least three predominant characteristics of SR-BI, which are necessary for its signal initiation: its ability to invoke cholesterol flux; the tail of its C-terminal intracellular domain that interacts with PDZK1; and its C-terminal transmembrane domain, which uniquely interacts with plasma membrane cholesterol [15].

It has been reported that HDL-induced activation of AMP-activated protein kinase (AMPK) is dependent on both S1P receptors and SR-BI through calcium−/calmodulin-dependent protein kinase kinase (CaMKK). In endothelial cells, serine-threonine kinase LKB1 may participate in SR-BI signaling. HDL-stimulated endothelial cell migration, activation of Akt and endothelial NO synthase, and suppression of monocyte adhesion and expression of adhesion molecules are dependent on AMPK activation [43].

In addition, SR-BI has also been reported to interact with downstream signaling molecules, for example, the nonreceptor tyrosine kinase, Src, which causes the activation of PI3K and further leads to the independent activation of both mitogen-activated protein kinase (MAPK) / ERK1/2 and Akt kinase (also known as protein kinase B, PKB) signaling pathways, resulting in phosphorylation of eNOS and NO production [44]. Several studies suggest that apoAI of HDL binding to SR-BI could also activate Src kinase–PI3K/ERK-Rac pathway, causing migration and subsequently repairing the endothelial injuries. In endothelial cells, SR-BI-mediated PI3K/Akt/eNOS signaling pathway participated in HDL-induced cyclooxygenase-2 (COX-2) expression and PGI2 release. Besides, HDL-C stimulates the proliferation of bone-derived mesenchymal stem cells by binding SR-BI and activation of PI3K/Akt, MAPK/ERK1/2 pathway [45]. Moreover, HDL augments angiogenesis in hypoxia through hypoxia-inducible factor 1α (HIF-1α)/vascular endothelial growth factor (VEGF) pathway via SR-BI-mediated PI3K/Akt signaling and modulation of the posttranslational regulators of HIF-1α [46].

6.3 Regulations of SR-BI

6.3.1 Regulating Factors of SR-BI

Numerous studies have established that SR-BI expression in hepatocytes can be regulated by various diets, drugs, hormones, glucose metabolism, and lipid metabolism [9, 47]. Studies in hamsters have shown that polyunsaturated fatty acids (PUFAs) in diet promote hepatic SR-BI expression and HDL-CE uptake, and combined treatment of obeticholic acid (OCA) and LXR agonist GW3965 increases the mRNA level and protein expression of SR-BI in the liver. Nevertheless, dietary myristic acid reduces SR-BI expression in hamster liver. It has been reported that the atherogenic diet of cholesterol loading may lead to posttranslational downregulation of hepatic SR-BI expression in mice and rats. Accumulating evidence in vivo and in vitro has indicated that hepatic expression of SR-BI is transcriptionally regulated by endogenous compounds such as insulin-like growth factors, glucose, lipopolysaccharide, leptin, vitamin A, and several synthetic compounds such as disodium ascorbyl phytostanol phosphate (FM-VP4), and isoflavones. All of these compounds are neither primary nor direct activators of transcriptional factors. Interestingly, some studies have identified new upregulators of SR-BI from 6000 microbial secondary metabolite crude extracts, including trichostatin A, pratensein, genistein, daidzein, etc.

Additionally, it has been reported that numerous drugs, such as thyromimetics, dexamethasone, fibrates, probucol, ezetimibe, tamoxifen, pioglitazone, and natural medicine rutaecarpine (RUT), quercetin, etc., affect SR-BI expression in different cells through different mechanisms [47, 48]. For example, RUT triggers the

promoters of CLA-1 genes, upregulating SR-BI to inhibit atherosclerosis in apoE$^{-/-}$ mice.

6.3.2 Regulating Mechanism of SR-BI

A series of studies have revealed that many nuclear transcription factors, such as liver receptor homolog-1 (LRH-1), liver X receptors (LXRs), steroidogenic factor-1 (SF-1), sterol-regulatory-element-binding proteins (SREBPs), and peroxisome-proliferator-activated receptors (PPARs), affect SR-BI gene transcription. Several consensus sequences for nuclear receptor binding have been determined in the flanking promoter region of the human *SCARB1* gene [5, 9, 47]. For example, adrenocorticotropic hormone (ACTH) has been found to upregulate SR-BI expression in steroidogenic tissues of both human subjects and rats. The suppression of ACTH by dexamethasone, a synthetic corticosteroid, decreased SR-BI levels. Human SR-BI expression can be stimulated by steroidogenic hormones through the cellular cAMP-dependent regulation, and SF-1 is one of the primary transcription factors involved in this pathway via binding to its consensus sequence in the *SCARB1* promoter. In hepatocytes, LRH-1 binds to a proximal response element on the human SR-BI promoter in an overlapping manner with SF-1 and activates the SR-BI promoter. Overexpression of LRH-1 induces SR-BI gene expression, which may be related to histone H3 acetylation on the promoter. Moreover, LRH-1 also regulates CETP, which participates in the remodeling of HDL particles and RCT [47, 49].

LXRs, members of the nuclear hormone receptor superfamily, participates in controlling hepatic SR-BI expression [9]. The oxysterols induce activation of both LXRα and LXRβ and increase the expression of SR-BI both in human and murine cell lines. Since LXRα is upregulated by TGF-β1, TGF-β1 significantly increases SR-BI expression in a dose- and time-dependent manner at the transcriptional and translational levels. PPARs, a family of ligand-activated transcription factors, such as PPARα and PPARγ, heterodimerize with retinoid X receptor (RXR) to bind to distal PPARE on the SR-BI promoter and regulate human SR-BI gene expression. It has been found that macrophage SR-BI expression can be promoted by activators of PPARs [50]. Some PPAR ligands, including fatty acids, glitazones, and fibrates, regulate SR-BI expression at the liver level. Mitogen-activated protein kinase (MAPK) Erk1/2 is involved in the regulation of nuclear receptor activity, including PPARs and LXR, to alter the capacity of the cell to export cholesterol, while inhibition of Mek1/2 enhances PPARα-dependent degradation of SR-BI in hepatocytes [51]. It has been reported that SREBP-1 modulates human *SCARB1* gene expression in response to altered levels of intracellular sterols. Cholesterol depletion can trigger an SREBP1a-regulated induction of SR-BI gene transcription in HepG2 cells. Nonetheless, SR-BI mRNA and protein expressions are normal in livers obtained from a mouse model that lacks mature nuclear SREBPs due to gene knockout of the SREBP cleavage-activating protein (SCAP) and from SREBP transgenic mice, indicating that the SR-BI gene in the liver is not under transcriptional regulation by SREBPs [47].

Studies have shown that estrogen can bind to three different estrogen response elements (EREs) on the SR-BI promoter through estrogen receptor α/β to regulate SR-BI gene transcription. In endothelial cells, 17β-estradiol (E2) increased the mRNA expression of the human SR-BI gene as well as the activity of the SR-BI promoter in a protein kinase C (PKC)–dependent manner [52]. Interestingly, rats supplemented with estrogens and HepG2 treated with 17β-estradiol showed a noted reduction in SR-BI expression combined with an increase of SR-BII. Meanwhile, treatment with the synthetic estrogen 17-α-ethinylestradiol in rats stimulates a reduction in hepatocyte SR-BI expression combined with an increase in Kupffer cell SR-BI expression.

Another nuclear receptor in association with the regulation of hepatic SR-BI expression is Farnesoid X Receptor (FXR) [53]. The mRNA level of SR-BI gene was reduced in the liver of FXR-deficient (FXR$^{-/-}$) mice. When treating mice with a diet containing 0.4% of the FXR agonist cholic acid, elevated mRNA and protein

levels of hepatic SR-BI were observed in the wild-type but not in the FXR$^{-/-}$ mice, suggesting that bile acids could upregulate SR-BI gene expression via FXRs. A recent study found a novel pathway in which FXR activation upregulated SR-BI expression in association with alterations in hepatic regulatory factors such as hepatocyte nuclear factor 4α (HNF4α) and p-JNK. It is likely that the contradiction of these results may be due to the activation of other regulatory pathways of gene expression besides selective agonists of FXR. In addition, pregnane X receptor (PXR), known as a xenobiotic receptor in human subjects, was shown to reduce SR-BI mRNA level in response to the PXR agonists, lithocholic acid, rifampicin, or pregnenolone 16-α-carbonitrile, although no consensus sequences for the binding of PXR have been identified in the SR-BI promoter [9, 47].

Additionally, Krüppel-like factor 4 (KLF4), a conserved zinc finger transcription factor, can be induced by HDL and upregulate SR-BI expression, leading to the binding to a putative KLF4-binding element (−342/−329 bp) on SR-BI promoter in macrophages [54]. Another study was reported that Sp1 and Sp3 are also essential transcriptional factors for SR-BI gene transcription through binding GC box at the proximal promoter.

Of note, the inhibitors, which act on the promoter of SR-BI, have been found. Yin Yang protein-1 (YY1), a zinc finger transcription factor that can play both positive and negative regulatory roles, decreases SREBP-regulated induction of SR-BI transcription by directly binding to two YY1-binding sites in the promoter as well as to SREBPs [55]. The sex adrenal hypoplasia congenital critical region on the X-chromosome, gene-1 (DAX-1), an adrenal-specific inhibitor and orphan nuclear receptor (NR0B1), can bind to SREBP-1α and inhibit its binding to SREs. A p21-activated kinase-1 (PAK-1) interacted with small GTPases and regulates lipopolysaccharide (LPS)-induced decrease of the activity of human SR-BI promoter in macrophages.

Recent studies revealed that small noncoding RNAs, including microRNAs (miRNAs, miRs), are implicated in the posttranscriptional regulation of SR-BI gene expression [56]. Analysis of the SR-BI 3′ UTR sequence identified potential binding sites for a number of miRs. When either miR-125a or miR-455 was overexpressed, the level of SR-BI was reduced, leading to a decrease of both HDL-C uptake and steroidogenesis. Both miR-125a and miR-455 as expressed in steroidogenic cells were downregulated by ACTH and cAMP treatment. Studies demonstrated that miR-24, miR-96, miR-185, and miR-223 could bind the 3’-UTR of SR-BI and negatively regulate the expression level of SR-BI in the liver and macrophages, thereby reducing HDL uptake and selective lipid uptake [57, 58]. Therefore, these observations suggest that certain miRs may have potential therapeutic value for atherosclerosis via targeting SR-BI and HDL.

Recent studies have found that in the setting of infection, autophagy of macrophages is impaired in SR-BI$^{-/-}$ mice, and the SR-BI-induced autophagy involved the formation of subcellular membrane cholesterol domains. These findings raise the possibility that SR-BI mediates cytoplasmic cholesterol mobilization for availability to ABCA1 for efflux. Interestingly, it was found that SR-BI could traffic to lysosomes with Rab7, a protein, which is crucial in promoting the fusion of autophagosomes with lysosomes, and localize to macrophage lysosomeshepatic SR-BI protein levels. The stability, localization, and function of SR-BI in the hepatocyte plasma membrane are primarily controlled by PDZK1 containing four PDZ (PDZ1 to PDZ4) domains [59]. PDZK1 is expressed in the liver, gut, and kidney, but not in steroidogenic tissues. Small PDZK1-associated protein (SPAP) overexpression downregulates PDZK1 in a liver-specific fashion, resulting in the subsequent downregulation of hepatic SR-BI. In PDZK1-null mice, protein levels of hepatic SR-BI are markedly decreased by 95%, whereas mRNA levels remain unaltered, leading to an increase in plasma total cholesterol carried in abnormally large HDL particles [13]. The PDZK1 homologs can serve as physiological translation/posttranslational regulators of the

functional expression of SR-BI. In addition, it was shown that posttranslationally activated PI3K/Akt promotes hepatic SR-BI function through mediating the subcellular localization of SR-BI. The Ras/MEK/ERK signaling cascade regulates the protein level of hepatocyte SR-BI, which is associated with PPARα-induced degradation pathways [51].

Other studies have shown that the synonymous variant of rs5888 (C > T), which is frequently found in humans, can alter the RNA secondary structure of SR-BI, and the translation of SR-BI protein in macrophages is significantly reduced, which may be related to SR-BI protein expression and function [60].

6.4 SR-BI and Atherosclerosis

The increasing evidence involving the manipulation of SR-BI expression in mouse models, either using SR-BI$^{-/-}$ mice or using adenovirus-mediated or transgenic hepatic overexpression, indicates that SR-BI expression protects against atherosclerosis [5]. SR-BI$^{-/-}$ mice fed with a Western diet for 20 weeks showed a significant increase of dysfunctional HDL, which contributes to an increased susceptibility to atherosclerosis. Deletion of SR-BI in apoE$^{-/-}$ mice leads to accelerated atherosclerosis causing coronary artery occlusion, myocardial infarction, and premature death at the age of 5–8 weeks. When fed with a modified Western diet, SR-BI/LDLR double knockout mice also shown abnormal profiles of lipoproteins resulting in severe coronary atherosclerosis, myocardial infarction, and reduced survival rate. On the contrary, transgenic mice overexpressing hepatic SR-BI have accelerated clearance of HDL-C, enhanced selective uptake of hepatic cholesterol, and increased content of biliary cholesterol, which reduces atherosclerosis. Meanwhile, studies employing the bone marrow transplantation showed that macrophage SR-BI inactivation promotes the development of atherosclerotic lesion in apoE$^{-/-}$ mouse model. Interestingly, SR-BI deficiency in bone marrow-derived macrophages inhibited the initial formation of atherosclerotic lesions in LDLR$^{-/-}$ mice, but stimulated the atherogenic process in later stages in LDLR$^{-/-}$ mice, suggesting that the double effects of macrophage SR-BI depend on multiple roles in the different stages of atherosclerosis.

As previously mentioned, SR-BI protects against atherosclerosis via one or more of the following mechanisms [5, 9, 19, 61]: hepatic SR-BI is the main effective way to inhibit atherosclerosis by selective uptake of HDL-CE and promoting RCT pathway, stimulating cholesterol secretion from bile. Additionally, hepatic SR-BI is also involved in the clearance of apoB-containing lipoprotein; the anti-inflammatory effect of HDL depends on the expression of SR-BI in cells, which can be indirectly regulated by lipid transport activity or directly by SR-BI as a receptor for cellular inflammatory signaling; SR-BI directly affects the function and status of cells in vessel wall by minimizing the formation of foam cells and by modulating signaling pathways implicated in efferocytosis, apoptosis, cell migration, and inflammation. It mediates the bidirectional flux of unesterified cholesterol between cells and HDL, and modulates the inflammatory response in macrophages. In endothelial cells, SR-BI plays a critical role in cell migration and vascular protection by mediating HDL-dependent eNOS activation and PI3K/Akt signaling. Moreover, SR-BI promotes the release and migration of endothelial progenitor cells. SR-BI modulates lymphocyte homeostasis via regulating cell proliferation, production of INF-γ and IL-4 cytokine, and HDL function [42]. The direct interaction between SR-BI and HDL on platelets is necessary for maintaining its normal function; otherwise, it will lead to platelet hyperreactivity and high sensitivity of thrombosis [41]. Taken together, SR-BI serves as a multifunctional receptor against atherosclerosis, making it a potential therapeutic target.

6.5 SR-BI and Cancer

Given that cholesterol regulates essential signaling pathways involved in cell proliferation, migration, and survival, accumulated evidence

has shown that in addition to mediating the transfer of cholesterol between HDL and healthy cells, SR-BI also promotes the selective cholesterol uptake by malignant cells. Interestingly, compared to normal tissue, SR-BI expression is increased in multiple cancers, such as Leydig cell tumors, breast cancer, prostate cancer, metastatic melanoma, nasopharyngeal carcinoma (NPC), and clear cell renal cell carcinoma (ccRCC) [62]. Therefore, recent lines of evidence have focused on the role of SR-BI in the progression of cancers.

Studies have shown that overexpression of SR-BI can stimulate HDL-induced proliferation of the breast cancer cell line MCF-7 through the PI3K/AP-1 pathway, and a mutant of SR-BI could inhibit proliferation of human breast cancer cells. Meanwhile, higher expression of SR-BI in breast cancer tissue is associated with an increased disease aggressiveness and mortality of patients. Knockdown of SR-BI reduces HDL-mediated activation of MAPK and PI3K/Akt pathways in the two human breast cancer cell lines, MCF7 and MDA-MB-231. Twiddy et al. showed that SR-BI knockdown decreases viability and prostate-specific antigen (PSA) secretion of prostate cancer cells [63]. Research by Kinslechner et al. revealed that SR-BI knockdown perturbs the metastasis-associated epithelial-to-mesenchymal transition (EMT) phenotype, in association with reduced invasion and migration of melanoma cells and decreased xenograft tumor growth [64]. In recent studies, it has been discovered that SR-BI is overexpressed in all investigated NPC cell lines and 75% of NPC biopsies, and has a great effect on cell motility but does not affect cell proliferation [65]. Of note, high expression of SR-BI was closely associated with aggressive features of ccRCC and could predict a poor clinical outcome. Using specific siRNA to inhibit SR-BI in ccRCC cell lines in vitro successfully impaired the growth, colony formation, migration, and invasion, and HDL-C uptake. Furthermore, SR-BI-targeting siRNA was also reported to yield potent in vivo effects [66]. Taken together, the above promising preliminary studies suggest that SR-B1 may serve as a potential biomarker for cancer diagnosis and therapeutic target in the pathogenesis of cancer [67].

6.6 Conclusions

It has been recognized that SR-BI is a multifunctional HDL receptor, whose prominent function is to play a key role in RCT by regulating selective uptake of HDL-CE in the liver. SR-BI also performs many other important functions involved in the atheroprotective effect: hepatic SR-BI plays a key role in the elimination of remnant lipoproteins and atherogenic Lp(a). In endothelial cells and macrophages, SR-BI minimizes foam cell formation through regulating cellular cholesterol flux and participates in HDL-initiated signaling involved in inflammation, cell survival, and efferocytosis. Recent studies using genomic analyses suggest that the SNPs of human *SCARBI* gene could lead to loss-of-function variants of SR-BI and may be involved in the occurrence of abnormal circulating HDL-C and the increased risk of atherosclerotic cardiovascular disease. It is becoming increasingly clear that a bright future for SR-BI as a therapeutic target can be foreseen not only in cardiovascular disease, but certainly also in inflammatory diseases as well as in cancer. However, it remains obscure whether the HDL derived from human carriers of loss-of-function *SCARBI* variants is dysfunctional, and whether SR-BI mutation is an independent risk factor for atherosclerosis in general population. Secondly, how the key domain of SR-BI transmembrane structure regulates the selective transport of cholesterol and signal transduction is still unknown. Next, very limited information is currently available about the mechanisms of SR-BI expression and its regulation in vivo. The direct regulatory elements and key transcription factors of *SCARB1* gene still need to be clarified. Finally, considering a supposed negative effect in cancer versus a positive effect in inflammation and cardiovascular disease, future efforts should focus on increasing the functional efficiency of SR-BI using genetic or pharmacological approaches, and consequently assist

the prevention and treatment of cancer/atherosclerotic cardiovascular disease.

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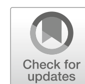

7 ABCA1, ABCG1, and Cholesterol Homeostasis

Xiao-Hua Yu and Chao-Ke Tang

Abstract

Cholesterol is a major component of mammalian cell membranes and plays important structural and functional roles. However, excessive cholesterol accumulation is toxic to cells and constitutes the molecular basis for many diseases, especially atherosclerotic cardiovascular disease. Thus, cellular cholesterol is tightly regulated to maintain a homeostasis. Reverse cholesterol transport (RCT) is thought to be one primary pathway to eliminate excessive cholesterol from the body. The first and rate-limiting step of RCT is ATP-binding cassette (ABC) transports A1 (ABCA1)- and ABCG1-dependent cholesterol efflux. In the process, ABCA1 mediates initial transport of cellular cholesterol to apolipoprotein A-I (apoA-I) for forming nascent high-density lipoprotein (HDL) particles, and ABCG1 facilitates subsequent continued cholesterol efflux to HDL for further maturation. In this chapter, we summarize the roles of ABCA1 and ABCG1 in maintaining cellular cholesterol homoeostasis and discuss the underlying mechanisms by which they mediate cholesterol export.

Keywords

ABCA1 · ABCG1 · apoA-I · HDL · Cholesterol efflux · Atherosclerosis

7.1 Introduction

Cholesterol functions as an important cell membrane component in mammals and is essential for establishing proper membrane permeability and fluidity. Additionally, cholesterol is associated with cell signaling transduction and takes part in the biosynthesis of bile acids, vitamin D, and steroid hormones. Thus, maintaining cholesterol homeostasis is of critical importance for human health. However, cholesterol accumulation can cause cell death and be harmful human body. For instance, hypercholesterolemia has long been regarded as a critical contributor to atherosclerotic cardiovascular disease, the first cause of death globally.

To maintain the dynamic balance of cholesterol, excessive cholesterol must be eliminated from the body. Reverse cholesterol transport (RCT), which is referred to the delivery of cholesterol in arterial wall cells to the liver by high-density lipoprotein (HDL) for further metabolism and excretion, plays a key role in this process [1]. The ATP-binding cassette (ABC)

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superfamily of transporters belongs to transmembrane proteins and contains a variety of members. Among these, ABCA1 mediates initial transport of free cholesterol (FC) and phospholipid molecules to apolipoprotein A-I (apoA-I) for nascent HDL biogenesis, and ABCG1 promotes subsequent continued efflux of FC to these HDL particles [2]. ABCA1- and ABCG1-dependent cholesterol efflux is the first and rate-limiting step of RCT. There is increasing evidence that upregulation of these two transporter expression facilitates RCT and protects against atherosclerosis in animal models [3–5]. This chapter summarizes the role of ABCA1 and ABCG1 in maintaining cellular cholesterol homoeostasis with an emphasis on how they mediate cholesterol efflux from cells.

7.2 ABCA1 and Cholesterol Homeostasis

7.2.1 Structural Features of ABCA1

ABCA1 is a full transporter and an integral membrane protein. Human *ABCA1* gene is mapped to chromosome 9q31.1, spans 149 kb, and contains 50 exons and 49 introns. The mature ABCA1 protein is made up of 2261 amino acids, and its molecular mass is 254 kDa. ABCA1 has two transmembrane domains (TMDs) linked covalently, each containing six transmembrane α-helices (TMs). Also, there are two large extracellular domains (ECDs) and multiple membrane-spanning segments. ECD1 resides between TM1 and TM2, and ECD2 resides between TM7 and TM8. They are connected by two intramolecular disulfide bonds and serve as the binding sites for apoA-I. There are two nucleotide-binding domains (NBDs) in its intracellular region. There are three conserved peptide motifs in each NBD, designated as Walkers A, B, and C. ATP is known to bind to Walker A, and Walker B is associated with subsequent hydrolysis. The hydrolysis of ATP in these sites can alter ECD conformation, thereby leading to the binding of apoA-I. The NBD1 includes a PEST sequence that is composed of proline, glutamic acid, serine, and threonine. Calpain degrades ABCA1 through interaction with this sequence. However, whether Walker C signature is involved in nascent HDL biogenesis remains unclear and needs further characterization.

A recent study revealed the single-particle cryoelectron microscopy structure of human ABCA1 protein at 4.1 Å resolution [6]. The overall structure resembles an elongated torch that is about 200 Å high. Both ECDs are the upward flaring flame, and other domains constitute the handle. There is a shallow pocket enclosed by the intracellular segments of TMs 1/2/5. The amino acid residues within these TMs are mostly polar and charged, which are responsible for binding to the polar heads of phospholipid molecules. Both TMD1 and TMD2 constitute a narrow chamber as an intracellular gate, which drives the transport of lipids from the membrane inner leaflet to the membrane outer leaflet. The helical domains in ECD1 and ECD2 together enclose an elongated hydrophobic tunnel that acts as a temporary storage and delivery passage for lipids. These structural features provide an important framework for better understanding of ABCA1-mediated cholesterol efflux.

7.2.2 Expression and Regulation of ABCA1

Almost all tissues and cells can express ABCA1. However, it is highly expressed in cardiovascular system, digestive system, and central nervous system. Liver X receptor α (LXRα), a member of the nuclear receptor superfamily, plays a critical role in stimulating ABCA1 transcription. LXRα combines with retinoid X receptor (RXR), leading to the formation of an obligate heterodimer. The LXRα/RXR complex then binds to the promoter region of *ABCA1* gene to induce its expression. The increase in intracellular cholesterol levels has been shown to promote the production of oxysterols, which act as a potent LXRα agonist and upregulate the expression of ABCA1 [7]. Exposure of nobiletin to mouse macrophages J774.1 activates the AMP-activated protein kinase/LXRα pathway,

leading to increased ABCA1 expression [8]. In murine bone-marrow-derived macrophages, treatment with DASA-58, an activator of pyruvate kinase M2, downregulates LXRα expression and then decreases ABCA1 levels [9]. Additionally, fargesin facilitates the phosphorylation of CEBPα at Ser21, leading to upregulation of LXRα and ABCA1 expression in macrophages [3].

At the transcriptional level, ABCA1 expression can be regulated in an LXRα-independent manner. Like LXRα, retinoid acid receptor–related orphan receptor α (RORα) is also a nuclear receptor. There is a RORα response element present in the ABCA1promoter. Both CPG52608 and SR1001 have been identified as the specific ligands of RORα. Upon binding to CPG52608 and SR1001 in THP-1 macrophages, RORα exerts a transactivating effect on ABCA1 [10]. Overexpression of prolactin regulatory element-binding (PREB) protein in CRL-2018 cells directly enhances the transcriptional activity of ABCA1 [11]. Heat shock protein 70 impairs ABCA1 expression by preventing Elk-1 from binding to the ABCA1 promoter in THP-1-macrophage-derived foam cells [12]. In contrast, zinc finger protein 202 was shown to directly inhibit ABCA1 transcription [13]. C1q tumor necrosis factor–related protein (CTRP) 1 decreases miR-424-5p levels, which in turn increases Forkhead box O1 expression in the nucleus and inhibits ABCA1 transcription [14]. Overexpression of Kcnq1 overlapping transcript 1, a long noncoding RNA, sponges miR-452-3p to enhance histone deacetylase 3 expression, leading to a significant decrease in the transcriptional activity of ABCA1. Akt is also identified as a transcriptional inhibitor of ABCA1 [15].

At the posttranscriptional level, a variety of agents have been reported to modulate ABCA1 expression. For instance, apoA-I interacts with ABCA1 to suppress the cleavage of ABCA1 protein by calpain. After stimulation with LXRα agonists, syntrophin forms a complex with ABCA1 in macrophages. The resulting complex combines with apoA-I, leading to a significant increase in ABCA1 protein stability [16]. Thus, LXRα can induce ABCA1 expression at the transcriptional and posttranscriptional levels. Tetramethylpyrazine is a biologically active component isolated from the Chinese medicinal plant *Ligusticum wallichii* Franchat. Administration of tetramethylpyrazine markedly enhances ABCA1 protein stability by inhibiting lysosomal degradation in RAW264.7 macrophages. When apoA-I binding protein (AIBP) combines with apoA-I, the degradation of ABCA1 protein mediated by COP9 signalosome subunit 2 is markedly inhibited [17]. In addition, calmodulin and paeonol were shown to protect ABCA1 from calpain-mediated cleavage [18]. Nucleolin significantly increases the stability of ABCA1 mRNA in ox-LDL-treated RAW264.7 macrophages [19]. A similar effect is also observed when extracellular-signal-regulated kinases 1/2 (ERK1/2) are inactivated. As an RNA-binding protein, human antigen R promotes ABCA1 translation by binding to its 3′-untranslated region (UTR) [20]. MicroRNAs (miRNAs) mediate the degradation of target mRNAs and/or inhibit their translational process by binding to the 3’-UTR, leading to silencing of target gene expression. Dysregulation of miRNAs has been linked to abnormal lipid metabolism and the development of atherosclerosis. Interestingly, ABCA1 has been identified as a putative target of a variety of miRNAs, such as miR-33a/b, miR-20a-5p, miR-27a/b, miR-758, miR-19b, miR-17-5p, miR-93, miR-325, miR-25-5p, and miR-361-5p [21].

7.2.3 Biological Functions of ABCA1

The major biological function of ABCA1 is to mediate the translocation of FC from cells, especially macrophages and vascular smooth muscle cells (VSMCs), to lipid-poor/free apoA-I for nascent HDL particle generation. When mouse peritoneal macrophages laden with cholesterol were treated with diluted human serum, ABCA1 mediates approximately half of cholesterol export and ABCG1 mediated 20% of cholesterol export [22]. This finding suggests that ABCA1 is the most important transporter responsible for cholesterol release from cells. Consistently, when the

ABCA1 gene is mutated in humans, Tangier disease occurs. This disease displays extremely low plasma HDL cholesterol (HDL-C) concentration and premature atherosclerosis [23]. Deletion of ABCA1 in mice impairs macrophage-to-feces RCT and promotes atherosclerotic plaque formation [24]; however, overexpression of ABCA1 in mice was shown to increase circulating HDL-C concentration and mitigate atherosclerosis [25]. Thus, targeting ABCA1 is an attractive and promising strategy to prevent and treat atherosclerotic cardiovascular disease.

In addition to taking part in lipid metabolism, ABCA1 has other biological effects. It has been reported that knockout of ABCA1 promotes the expression of proinflammatory cytokines and chemokines in mouse bone marrow–derived macrophages challenged with lipopolysaccharide (LPS) [26]. In contrast, overexpression of ABCA1 significantly attenuates the levels of interleukin-6 and tumor necrosis factor-α in bovine aortic endothelial cells stimulated with LPS [27]. Incubation with AIBP or apoA-I upregulates the expression of ABCA1, thereby leading to decreased secretion of proinflammatory cytokines from THP-derived macrophages challenged with LPS [28]. These observations indicate that ABCA1 exerts an anti-inflammatory action and acts as a critical link between inflammation and lipid metabolism. Additionally, ABCA1 can inhibit retinal ganglion cell apoptosis, promote colorectal cancer cell proliferation, enhance platelet reactivity, and stimulate insulin secretion from pancreatic β-cells [29].

7.2.4 Potential Mechanisms Underlying ABCA1-Dependent Cholesterol Efflux

Currently, several models have been presented to clarify the molecular mechanisms underlying ABCA1-dependent cholesterol efflux. The first model involves channel trafficking [6]. It is suggested that a channel is present in between TMD1 and TMD2 of ABCA1, which plays a central role in controlling lipid access. The chamber is initially open at the bottom and close at the top. In the presence of lipid loading, accumulated phospholipids in the inner leaflet of plasma membrane are laterally transferred to the chamber by binding to amino acid residues within TMs 1/2/5. In this process, cholesterol is concurrently accessible to the chamber with the aid of phospholipids. ATP is then recruited to both NBDs, which leads to the dimerization of NBDs and consequently closes the chamber. ABCA1 then flops the trapped lipids to the outer leaflet of plasma membrane. ATP hydrolysis at both NBDs leads to the formation of an ADP-bound intermediate, which alters the conformation of TMD1 and TMD1 to open the chamber at the top. Cholesterol and phospholipids are egressed from the chamber to the elongated hydrophobic tunnel formed by both ECDs. The ECD conformation can be also changed by the hydrolysis of ATP. This allows the interaction of ECDs with apoA-I. Upon binding to ABCA1, apoA-I takes up lipids from the elongated hydrophobic tunnel to assemble nascent HDL particles. These particles are then released from the cell surface to further accept lipids. Following dissociation of ADP from NBDs, the chamber is restored to the initial open status for lipid uptake. Given that this is a structure-based model, more direct evidence needs to confirm its rationality.

The second model involves ABCA1 dimerization [30, 31]. In this model, ABCA1 monomers constantly transport FC and phospholipids from plasma membrane to their ECDs due to ATP hydrolysis. Following sequestration of sufficient lipids, these monomers undergo conformational changes to dimerize. Subsequently, the membrane-skeletal actin filaments and other stable structure components in plasma membrane are recruited to the lipidated ABCA1 dimers. This interaction is beneficial for apoA-I access. Lipid-poor/free apoA-I combines with the ECDs within the ABCA1 dimers. The lipids reserved by ABCA1 are then delivered to apoA-I. Lipid loading induces a conformational change of apoA-I, leading to its dissociation from ABCA1 and nHDL production. Upon release of the reserved lipids, the ABCA1 dimers transform into monomers, entering next cycle to receive lipids. This model suggests that ABCA1 dimerization is

of critical importance to cholesterol export from cells. Promoting the conversion of ABCA1 monomers to dimers may be another effective strategy for inhibiting intracellular lipid accumulation.

The third model demonstrates that ABCA1 promotes the efflux of intracellular cholesterol to apoA-I through a two-step process [32, 33]. In this model, ABCA1 first facilitates the delivery of phospholipids from the inner membrane leaflet to the outer membrane leaflet. TP is recruited to both NBDs of ABCA1. Then, the hydrolysis of ATP induces a conformational change of ABCA1. This allows an interaction of apoA-I with ABCA1. Phospholipids are loaded onto apoA-I to form a complex, which is a much better acceptor for cholesterol than apoA-I itself. The phospholipid/apoA-I complexes then enter the caveolae, 50–100 nm cellular membrane invaginations enriched in lipids. FC in the caveolae is transported to these complexes in an ABCA1-independent autocrine or paracrine fashion for nHDL production. It is noteworthy that when cells are pretreated with cyclodextrin to deplete intracellular cholesterol, phospholipid efflux and apoA-I binding are not affected, while cholesterol removal disappears [34]. This suggests that both steps are independent of each other. However, a later study showed that phospholipid efflux is tightly coupled with cholesterol efflux [18]. Thus, additional work is required to determine what causes the conflicting findings.

The forth model involves apoA-I-free vesicle [35]. Two apoA-I monomers form a half-circle dimer as revealed by the crystal structure, which is necessary for lipid binding. Interestingly, dimerized apoA-I can acquire lipids from not only cellular membrane but also membrane-derived vesicles shed by ABCA1 during nascent HDL biogenesis. In this process, ABCA1 first translocates FC and phospholipids to the outer leaflet independent of apoA-I. Higher phospholipid levels in this region create mushroom-like protrusions in close proximity to ABCA1 for alleviating surface tension. These protrusions are released from plasma membrane as apoA-I-free vesicles, representing apoA-I-independent lipid efflux. Subsequently, apoA-I docks to plasma membrane through its hydrophobic C-terminus and binds to the ECDs of ABCA1 via its N-terminal domain. The interaction between both molecules leads to the unfolding of apoA-I N-terminus, allowing it to form a dimer. A large amount of FC and phospholipid molecules transported by ABCA1 are loaded onto dimerized apoA-I, leading to the production and release of nascent HDL particles. It is worth noting that these nascent HDL particles can continue to gain lipids from apoA-I-free vesicles to form larger particles.

The fifth model involves retroendocytosis [36, 37]. In addition to plasma membrane, the endosomes are regarded as an important reservoir of cellular cholesterol. ABCA1 resides not only on the cell surface, but also in the endosomal compartments. Consistently, the apoA-I lipidation can occur at these two sites. Several lines of evidence have demonstrated that apoA-I can be internalized to the endosomes for nHDL assembly in a pathway called retroendocytosis. In this model, apoA-I binds to ABCA1 on the cell surface to form a complex, which then enters clathrin-coated pits. The complexes are endocytosed to early endosomes in a Rab5-dependent manner. ABCA1 located in the early endosomal membrane translocates lipids to the endosomal lumen for apoA-I lipidation. ApoA-I is further lipidated when early endosomes become late endosomes. This results in the biogenesis of nascent HDL particles. These particles are then transferred to cycling endosomes that return to the cell surface with the help of Rab4. After fusion with plasma membrane, nHDL is secreted into the extracellular space, and ABCA1 recycles to plasma membrane. This model suggests that stimulating apoA-I internalization provides an alternative approach to enhance ABCA1-dependent cholesterol efflux.

7.3 ABCG1 and Cholesterol Homeostasis

7.3.1 Structural Features of ABCG1

Human *ABCG1* gene is located in chromosome 21q22.3. There are 23 exons in human *ABCG1* gene. This gene comprises alternative start codons, which leads to the generation of multiple transcripts in a tissue-specific manner. The alternative splicing can generate eight different ABCG1 isoforms with 644–785 amino acids in length. The ABCG1 protein is composed of one NBD at the N-terminus and one transmembrane domain containing six α helices. Thus, ABCG1 belongs to a half-transporter. ABCG1 must be dimerized to exert its biological functions.

7.3.2 Expression and Regulation of ABCG1

Like ABCA1, highly expressed ABCG1 is found in arterial wall cells, including macrophages, VSMCs, and endothelial cells. It is worth noting that in addition to cardiovascular system, other organs such as the spleen, lung, kidney, and brain can express ABCG1. At the subcellular levels, ABCG1 predominantly locates in plasma membrane and endosomes.

Similar to ABCA1, LXRα plays a central role in stimulating ABCG1 transcription. Biochanin A is a dietary isoflavone that is extracted from red clover and cabbage and has a cardiovascular protective property. Administration of biochanin A was shown to upregulate ABCG1 expression and inhibit foam cell formation in THP-1-derived macrophages, at least in part, by activating the peroxisome-proliferator-activated receptor γ (PPARγ)/LXRα signaling pathway [38]. CTRP12 is a highly conserved paralog of adiponectin. Overexpression of CTRP12 through lentiviral vector in THP-1-derived macrophages dramatically diminishes miR-155-5p levels, which in turn increases LXRα expression and elevates the transcriptional activity of ABCG1. TP53-induced glycolysis and apoptosis regulator (TIGAR) is a new p53-inducible protein and plays an important role in protecting against oxidative stress. Increased TIGAR expression is observed in macrophage foam cells and atherosclerotic lesions. Importantly, silencing of TIGAR by short hairpin RNA restrains ABCG1 expression and promotes lipid accumulation through the reactive oxygen species/27-hydroxylase/LXRα signaling cascade in THP-1-derived macrophages. In addition, ox-LDL inhibits ABCG1 expression through the mitogen-activated protein kinase/ERK1/2/LXRα pathway in INS-1 cells.

Many agents have been reported to modulate ABCG1 expression at the posttranscriptional level. For example, treatment of Raw264.7 cells with 12-O-tetradecanoylphorbol-13-acetate activates protein kinase C and then phosphorylates ABCG1, leading to a significant increase in its stability and subsequent cholesterol efflux. Activation of adenosine monophosphate–activated protein kinase robustly enhances the stability of ABCG1 mRNA by binding to its 3’-UTR in human and bovine aortic endothelial cells. Treatment with coenzyme Q10 elevates the mRNA and protein levels of ABCG1 through the activator protein-1/miR-378 pathway in peritoneal macrophages of apolipoprotein-E-deficient (apoE$^{-/-}$) mice [39]. In plasma and macrophages of apoE$^{-/-}$ mice with atherosclerosis, miR-23a-5p expression is upregulated. Knockdown of miR-23a-5p promotes cholesterol efflux in macrophages and reduces atherosclerotic burden in apoE$^{-/-}$ mice by directly upregulating ABCG1 expression. Also, miR-33a/b, miR-320b, miR-581, and miR-128-2 have been proven to directly target ABCG1.

7.3.3 Biological Functions of ABCG1

The major biological effect of ABCG1 is to promote the efflux of intracellular FC to HDL for further maturation. There is growing evidence that promoting ABCG1 expression inhibits lipid

accumulation and foam cell formation in THP-1 macrophages as well as increases RCT efficiency and protects against atherosclerosis in apoE$^{-/-}$ mice. Conversely, prevention of ABCG1 accelerates macrophage foam cell formation and the development of atherosclerosis in animal models [40, 41]. Although the majority of studies indicated that ABCG1 plays a beneficial effect on atherogenesis, some reports showed conflicting results. It has been reported that ABCG1 overexpression in apoE$^{-/-}$ mice does not alter atherosclerotic plaque burden. Macrophage-specific ablation of ABCG1 reduces atherosclerotic lesion area in hyperlipidemic low-density lipoprotein receptor-deficient (*Ldlr*$^{-/-}$) mice. In addition. Deletion of ABCG1 in *Ldlr*$^{-/-}$ mice promotes early atherosclerotic lesion formation and slows down the progression of advanced atherosclerotic lesions [42]. Therefore, further research will be required to clarify the precise role of ABCG1 in the development of atherosclerosis.

Given the critical role of ABCA1 and ABCG1 in mediating intracellular cholesterol export, the combined upregulation of these two transporter expression may be more effective in suppressing foam cell formation and atherosclerosis development. As expected, the simultaneous overexpression of ABCA1 and ABCG1 promotes FC efflux from macrophage in a synergistic manner. In contrast, silencing of ABCA1 and ABCG1 leads to more FC release from macrophage and RCT when compared with ABCG1 silencing alone [43]. In line with this study, transplantation of bone marrow lacking ABCA1 and ABCG1 into *Ldlr*$^{-/-}$ mice results in increased foam cell accumulation and atherosclerotic plaque burden as compared to single knockout of ABCA1 or ABCG1 [44]. Loss of ABCA1 and ABCG1 in endothelial cells of mice displays a similar effect [45]. Thus, targeting these two transporters simultaneously may be a more promising approach to improve plasma lipid profiles and treat atherosclerosis-associated diseases.

7.3.4 Potential Mechanisms Underlying ABCG1-Dependent Cholesterol Efflux

Currently, three models have been available to reveal how ABCG1 drives cholesterol translocation. The first model proposed that ABCG1 promotes the delivery of FC from the inner membrane leaflet to the outer membrane leaflet, which makes FC easier to be removed by HDL [46]. The second model demonstrated that ABCG1 functions as an intracellular cholesterol transporter localized in the endocytic vesicles [47]. In this model, ABCG1 first transports FC to the inner leaflet of these vesicles. Subsequently, these vesicles fuse with plasma membrane. Finally, HDL accepts FC from plasma membrane. The third model suggests that ABCG1 takes up HDL for entry into late endosomes, where HDL binds to FC [48]. Thereafter, FC-bound HDL is secreted into extracellular space. Of note, these models can not fully explain the mechanisms by which ABCG1 promotes intracellular cholesterol efflux. Additional work is still needed to precisely clarify the underlying mechanisms.

7.4 ApoA-I and Cholesterol Homeostasis

It is well known that apoA-I accepts cholesterol exported by ABCA1, which is essential for nascent HDL particle formation. Thus, it is not surprising that apoA-I acts as the core and major component of HDL. It is estimated that apoA-I accounts for about 70% of total HDL proteins. ApoA-I is predominantly produced by hepatocytes and enterocytes. Human apoA-I protein is made up of 243 amino acids with a molecular mass of 28 kDa. The last 199 amino acids comprise a series of ten repeating amphipathic α-helices. Among these, eight α-helices contain 22 amino acids, and two α-helices contain 11 amino acids [49]. The ninth and tenth

α-helices are localized to the C-terminal region and have the highest affinity for lipid binding [50, 51]. Thus, these two α-helices are of critical importance for FC transport. Following synthesis, apoA-I is secreted into the circulation and exists in lipid-free, poor, and bound states. Lipid-free/poor apoA-I has a stronger ability to accept FC than lipid-bound apoA-I. Therefore, targeting lipid-free/poor apoA-I is more effective for the therapeutic intervention of hypercholesterolemia and atherosclerosis-associated diseases.

There are two natural mutations of apoA-I in humans, designated as L141RPisa and L159RFIN. When L141RPisa or L159RFIN is expressed in mice, circulating HDL levels are significantly decreased and atherosclerosis occurs at the early stage [52]. In hyperlipidemic mice, dysfunctional HDL with L159RFIN leads to a significant increase in atherosclerotic lesion area. ApoA-I Mytilene is a truncation form of apoA-I and results from a heterozygous nonsense mutation. It has been reported that apoA-I Mytilene leads to a marked decrease in plasma apoA-I concentration and the occurrence of premature coronary artery disease. OSBPL1A p.C39X is a loss-of-function variant of human *apoA-I* gene. It has been shown that this variant dramatically diminishes circulating HDL-C concentration, inhibits cholesterol efflux from macrophages, and impairs RCT. Cathepsin B is known to mediate the cleavage of apoA-I at Ser228 in the C-terminal domain. Overexpression of cathepsin B was shown to impair the atheroprotective effect of apoA-I. On the other hand, injection of recombinant HDL reconstituted with the gain-of-function mutant apoA-I N74C inhibits the development of atherosclerosis in apoE$^{-/-}$ mice fed with a high-fat diet [53]. Local vascular gene therapy with apoA-I not only decreases atherosclerotic lesion area at the early stage but also contributes to the regression of atherosclerotic plaques at the advanced stage in a rabbit model of atherosclerosis [54, 55]. Additionally, AIBP suppresses promotes cholesterol efflux and inhibits lipid accumulation in macrophages as well as is protective against atherosclerosis in mice by interacting with apoA-I. Collectively, the above findings suggest apoA-I as a valuable target for the prevention and treatment of atherosclerosis.

7.5 HDL and Cholesterol Homeostasis

7.5.1 Components and Functions of HDL

As a type of highly heterogeneous lipoprotein particles, HDL has various densities, shapes, sizes, and components. There are about 90 proteins in the HDL particles [56]. In addition to apoA-I, apoA-II, apoE, enzymes, and lipid transfer proteins are identified as the major protein components of HDL. HDL also contents many lipids, including phospholipids, sphingolipids, triglyceride, cholesterol esters, and FC. The major function of HDL is to deliver lipids for the metabolism in the liver. In this process, both cholesterol and phospholipids are transported to apoA-I, resulting in the formation of nascent discoid HDL, namely, pre-β HDL. Subsequently, pre-β HDL is further lipidated to form larger HDL particles, named HDL3. Lecithin:cholesterol acyltransferase promotes the transformation of FC to cholesterol esters, which enter the hydrophobic core of HDL [57]. This results in the generation of mature, spherical, α-migrating HDL particles, termed HDL2. Of note, pre-β HDL is easy to be eliminated through the kidney, whereas the turnover of HDL3 and HDL2 is slower than pre-β HDL [58].

HDL-bound cholesterol is able to be removed from the body through three pathways. Firstly, scavenger receptor class B type I (SR-BI) located in hepatocytes mediates the uptake of cholesterol esters within HDL for hepatobiliary secretion. Secondly, cholesterol esters within HDL are transported to apoB-containing lipoproteins by cholesteryl ester transfer protein in exchange for triglyceride. Cholesterol esters within the apoB particles are taken up into hepatocytes by hepatic low-density lipoprotein receptor. Finally, cholesterol esters within HDL and is directly secreted into the intestinal lumen. This process is termed transintestinal cholesterol excretion. Of note,

HDL also has anti-inflammatory, antioxidative, and antithrombotic effects [59–61].

7.5.2 Role of HDL in Cardiovascular Disease

The basic studies and clinical trials have shown that HDL has an atheroprotective role. For instance, injection of CRE-001, a novel HDL-mimetic, into *Ldlr*$^{-/-}$ mice was shown to increase macrophage-to-feces RCT and decrease atherosclerotic lesion area [62]. Conversely, subcutaneous immunization with heat shock protein 65 in apoE$^{-/-}$ mice causes HDL dysfunction and consequently accelerates the development of atherosclerosis [63]. Administration of miR-19b precursor markedly reduces circulating HDL-C concentration, impairs the whole-body RCT, and aggravates atherosclerosis in apoE$^{-/-}$ mice. When plasma HDL-C levels elevate by 1 mg/dL, the risk of coronary heart disease decreases by 2% in men and 3% in women, respectively. In comparison with the subjects with plasma HDL-C concentration less than 35 mg/dL, the subjects with plasma HDL-C concentration more than 35 mg/dL diminish the risk of coronary artery disease by 70% during a follow-up of 6 years [64]. In addition, the efficacy of statin therapy is better in coronary heart disease patients with high HDL-C levels than those with low HDL-C levels [65].

Even though the majority of clinical studies have demonstrated an inverse correlation between circulating HDL-C levels and cardiovascular disease risk, the elevation of circulating HDL-C levels does not certainly translate into clinical benefits. It has been reported that single-nucleotide polymorphisms can lead to a significant increase in circulating HDL-C concentration, but this increase has no effect on the incidence of myocardial infarction. During a median follow-up of 3.1 years, coronary heart disease patients with low HDL-C (<40 mg/dL) and high HDL-C (≥ 70 mg/dL) have significantly higher risk of all-cause and cardiovascular disease mortality than those with HDL-C between 40 and 49 mg/dL, thereby revealing a U-shape relationship between HDL-C and adverse cardiovascular events [66]. Combined treatment with simvastatin and niacin to treat dramatically increases circulating HDL-C concentration but fails to show an additive clinical efficacy as compared to simvastatin monotherapy in coronary heart disease patients with low LDL-C concentration. Dalcetrapib is an inhibitor of cholesteryl ester transfer protein. Treatment of acute coronary syndrome patients with dalcetrapib does not decrease the risk of recurrent cardiovascular events despite elevated HDL-C levels [67]. A similar effect is seen when fenofibrate is used to treat type 2 diabetes mellitus [68]. In postinfarction patients with high HDL-C and C-reactive protein levels, the risk of recurrent events is still high [69].

Although HDL possesses complex functions, cholesterol efflux capacity (CEC) from peripheral cells is thought to be a key function of HDL. Moreover, CEC has a stronger protective effect on cardiovascular disease than traditional HDL-C concentration. It has been demonstrated that circulating apoA-I levels in individual HDL subpopulations are better to predict the risk of cardiovascular events than simple HDL-C levels [70, 71]. The macrophage-derived CEC is negatively correlated with carotid intima-media thickness and the likelihood of angiographic coronary artery disease, and this correlation is independent of HDL-C [72–74]. The small lipid-poor preβ-1-HDL particles are known to accept cholesterol delivered by ABCA1; however, the large lipid-rich α-1 and α-2 HDL particles predominantly accept cholesterol transported by SR-BI [75]. In healthy subjects, there are strong positive correlations between ABCA1-mediated cholesterol export and preβ-1-HDL levels, and between SR-BI-mediated cholesterol export and α-1/2 HDL levels [76]. Further analysis demonstrated that coronary heart disease patients has increased preβ-1-HDL levels as compared with controls, but their functionality (preβ-1-HDL concentration normalized ABCA1-dependent cholesterol efflux) is significantly decreased [77]. On the

other hand, although large HDL levels in these patients are lower than those in controls, their functionality (α-1/2 HDL concentration normalized SR-BI-dependent cholesterol efflux) is significantly increased. These observations indicate that CEC is dependent on individual HDL particle concentration and their functional properties. Therefore, measurement of the concentration in combination with functionality of HDL particles may be more valuable than traditional HDL-C concentration in predicting the risk of cardiovascular events in the general population. It is worth noting that net cholesterol flux in the body is dependent not only on HDL, but also is associated with ABCA1, ABCG1, and SR-BI. It is not surprisingly that these transporters are also the important determinants of HDL concentration and functionality, while their relative contributions to the functional changes of individual HDL particles are still required to be defined in the future studies.

7.6 Conclusion

ABCA1, ABCG1, apoA-I, and HDL are the critical players of RCT and play important roles in promoting cholesterol homeostasis. At present, some drugs to enhance the functions of these proteins, such as LXRα activators, RVX-208, apoA-I mimetic peptides and reconstituted HDL, are available. The monotherapy of these drugs or combination with statins has shown considerable promise for reducing the incidence of cardiovascular events. Nevertheless, our current understanding of how ABCA1 and ABCG1 mediate cholesterol efflux is still limited. This leads to a delay for therapeutic intervention targeting these agents. As CEC is a better predictor of cardiovascular risk than traditional HDL-C levels, it is also important to establish a standard method to measure CEC in clinic. Collectively, a better understanding of the role of ABCA1, ABCG1, apoA-I, and HDL in regulating cholesterol homeostasis will certainly help us to develop lipid-lowering therapies and improve the prognosis of atherosclerosis-associated disease patients in the future.

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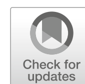

8 HDL and ASCVD

Hongtu Cui and Qian Du

Abstract

In this chapter, we summarize the relationship between circulating high-density lipoprotein (HDL) and atherosclerotic cardiovascular disease (ASCVD). HDL acts in many types of cells, such as endothelial cell, macrophage, T lymphocyte, etc. Recently, novel HDL-related therapies have been developed to treat ASCVD.

Keywords

Atherosclerotic cardiovascular disease (ASCVD) · High-density lipoprotein (HDL) · Molecular mechanism

A strong negative correlation between plasma high-density lipoprotein (HDL) levels and atherosclerotic cardiovascular disease (ASCVD) was found in 1970s, and its relationship is still investigated. Recent studies have shown that HDL inhibits atherosclerosis in many ways, such as promoting cholesterol efflux through the ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1), reducing inflammation, and alleviating lipid oxidation. Apolipoprotein A-I (apoA-I), the main protein component of HDL, can carry and deliver cholesterol to the liver, which seems to be the main function of HDL. Additionally, HDL carries microRNA and regulates intracellular gene expression. HDL also protects endothelial cells by activating the nitric oxide (NO) synthesis. This chapter summarizes the important roles of HDL in atherosclerosis and the latest research results.

8.1 Clinical Research of ASCVD

The content of circulating HDL is closely related to atherosclerotic cardiovascular disease (ASCVD). A recent study shows that composite HDL apolipoproteomic score (pCAD), basing on 5 apolipoproteins in HDL (i.e., apoA-I, apoC-I, apoC-II, apoC-III, and apoC-IV), is closely related to the occurrence of obstructive ASCVD ($\geq$70% lesion in $\geq$1 vessel). Nine hundred and forty three participants were enrolled in this study, all of whom were over 18 years old referred for coronary or peripheral angiography with or without intervention between 2008 and 2011 in the Massachusetts General Hospital. For these participants, the plasma apoA-I, apoC-I,

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apoC-II, apoC-III, and apoC-IV levels were measured during a 4-year follow-up. Finally, in these 943 participants, 587 patients developed coronary stenosis, accounting for 62%. There was a significant correlation between pCAD score and the incidence of coronary stenosis (odds ratio: 1.39; 95% confidence interval CI: 1.14–1.69; $p < 0.001$) independently of traditional cardiovascular risk factors, such as circulating plasma apoA-I and apoB. As for mortality, pCAD is related to cardiovascular death (HR: 1.33; 95% CI: 1.09–1.63; $p = 0.005$) in an unadjusted model. However, this correlation only exists in patients with obstructive ASCVD (HR: 1.36; 95% CI: 1.07–1.73; $p = 0.01$). In another multivariate model, pCAD was not significantly correlated with cardiovascular death after adjusting for the relevant clinical risk factors, such as apoA-I, apoB, and coronary artery stenosis (HR: 1.24; 95% CI: 0.93–1.66; $p = 0.15$). However, under this condition, researchers found a direct relationship between pCAD and cardiovascular death in patients with obstructive ASCVD (HR: 1.48; 95% CI: 1.07–2.05; $p = 0.019$) [1].

ASCVD risk significantly increases in diabetic patients and mice. In animal experiments, it was found that diabetes can lead to the accumulation of macrophages in the plaque, increase the proportion of M1 macrophage, and inhibit the regression of inflammation. All of these are important risk factors that lead to the formation of unstable plaque, thus increasing the possibility of ASCVD. And HDL-C was negatively associated with blood glucose in diabetic mice ($p = -0.427$, $p < 0.05$). What is more, the level of hemoglobin A1c was also negatively associated with HDL-C in diabetic patients ($p = -0.192$, $p < 0.05$). There was positive correlation between the level of hemoglobin A1c or blood glucose and white blood cells ($p = 0.310$, $p < 0.001$ and $p = 0.382$, $p = 0.08$). These findings suggest a link among blood glucose, HDL-C, and leukocytosis in diabetic patients.

Researchers increased HDL levels by overexpressing apoA-I in mice (apo A-I Tg), and found that the increase of apoA1 significantly reduced white blood cells in diabetic mice. And the expression of CD11b and reactive oxygen species (ROS) decreased in leukocytes isolated from apoA-I Tg mice compared to WT mice.

There is an increase in the number of common myeloid progenitors (CMPs) ($0.32 \pm 0.03\%$ vs. $0.22 \pm 0.01\%$; $p < 0.01$) and granulocyte macrophage progenitors (GMPs) ($1.7 \pm 0.04\%$ vs. $1.34 \pm 0.05\%$; $p < 0.01$) in diabetic mice. Overexpression of apo A-I significantly reduced the number of CMPs and GMPs (by 31% and 24%). Furthermore, adding HDL to CMPs and GMPs can restore the damaged cholesterol efflux ability and plasma membrane cholesterol homeostasis of CMPs and GMPs caused by high glucose, which is crucial for the proliferation of CMPs and GMPs. In addition to impairing cholesterol efflux, high glucose also promotes neutrophil activation and S100A8/A9 release. In apoA-I Tg mice, the activation of neutrophil was inhibited and S100A8/A9 levels decreased, which was caused by inhibiting NF-κB pathway. More importantly, the atherosclerotic lesions significantly regressed in diabetic apoA-I Tg mice. However, the regression of plaque in diabetic WT mice was impaired, suggesting that HDL can promote plaque regression. In apoA-I Tg mice, the infiltration of macrophage decreased, whereas the proportion of anti-inflammatory M2 macrophage and the collagen content increased. In addition, neutrophil extracellular traps (NETs) is also an important factor affecting the plaque stability and thrombosis. In apoA-I Tg mice, NETs significantly decreased, indicating that HDL inhibits neutrophil-associated inflammation. In conclusion, HDL inhibits the development of plaque and inflammatory cell infiltration, and maintains plaque stability in diabetic mice. The findings provide a new strategy to reduce the incidence of ASCVD in diabetic patients [2].

8.2 The Critical Molecular Pathway in the Development of Atherosclerosis

In the process of atherosclerosis, phagocytosis of ox-LDL leads to the accumulation of cholesterol

in macrophages and the formation of foam cells. HDL reduces intracellular cholesterol in foam cells by transporting cholesterol efflux. Cholesterol ester (CE) could be transformed to free cholesterol (FC) by HDL, which can be mobilized by membrane-bound transporters, ATP-binding cassette A1 and G1 (ABCA1 and ABCG1) and transported out of cells. Further, HDL-C is absorbed by liver cells through scavenger receptor class B type I (SR-BI). This is HDL-mediated reverse cholesterol transport (RCT), which plays an important role in reducing plasma cholesterol levels. This process is crucial in inhibiting the development of atherosclerosis [3]. If the metabolic pathway of HDL is damaged, it will aggravate the process of atherosclerosis.

The synthesis of HDL is a process of apolipoprotein-mediated solubilization of specific plasma membrane (PM) microdomains in foam cells. Therefore, researchers tried to find the key protein that mediates the binding of apoA-I to PM. The apoA-I-enriched PM was separated by discontinuous sucrose gradient centrifugation. The protein binding to apoA-I was identified by proteomics and desmocollin 1 (DSC1) was screened out. DSC1 is mainly responsible for the assembly process of desmosomes. Through immune-coprecipitation and Time-lapse images, it was found that there was a significant interaction between DSC1 and apoA-I. The combination of apoA-I and DSC1 may lead to sequestering PM cholesterol by DSC1 microdomain, thus resulting in the inhibition of cholesterol efflux mediated by ABCA1. Knockout of DSC1 leads to a significant increase in cholesterol efflux even if overexpression of ABCA1. Further, the researchers also found a significant apoA-I-DSC1 interaction in human atherosclerotic samples. In the late stage of ASCVD, there are a lot of apoA-I and DSC1 in calcification and necrotic nuclei area of advanced-stage lesions. These results suggested that DSC1 takes part in HDL biogenesis, cholesterol deposition, and cell death, thus promoting the progression of ASCVD [4].

Brown adipose tissue (BAT) could be activated by cold stimulation, which changes their metabolism and produces heat. Fatty acids are the main energy source of BAT. There are two main sources of fatty acids. One is the uptake of triglyceride (TG)-rich lipoproteins (TRLs)–associated fatty acids, which are generated from TG by lipoprotein lipase (LPL). The other is the uptake of whole TRLs. Further, the activation of thermogenic adipocytes also reduces blood cholesterol and inhibits the development of atherosclerosis in transgenic mice expressing both a loss-of-function variant of human apolipoprotein E (apoE*3-Leiden; E3L) and human cholesteryl ester transfer protein (E3L. CETP mice) [5].

It is found that the antiatherosclerotic effect of thermogenic adipocytes is related to the content of plasma HDL. Under the stimulation of cold or selective β3-adrenergic receptor agonists, such as CL316,243 (CL), cholesterol is transferred to HDL, and consequently, the level of HDL-C increases in apoA-V−/− mice. In addition to changing the HDL-C content, the activation of thermogenic adipocytes also alters the composition of HDL, including phosphatidylcholine (PC), lyso-PC as well as CE species. When exposed to cold, HDL-related PC34:1 and Lyso-PC18:1 significantly decreased in human, similarly to that of mice. However, changes in HDL components do not directly affect its ability to induce cholesterol efflux. Both clearances from plasma and hepatic uptake of HDL were identical. Interestingly, there was a significant increase in fecal cholesterol and a decrease in plasma and liver cholesterol after cold or CL treatment in WT and hyperlipidemic E3L.CETP mice. These results suggest that LPL-mediated lipolysis may be responsible for the increased HDL turnover and selective cholesterol uptake by the liver. Further, the absence of LPL leads to a decrease in plasma cholesterol clearance. Beyond that, hepatic SR-BI is also responsible for the accelerated HDL-C. Therefore, BAT activation plays an important role in ASCVD by increasing systemic cholesterol excretion. What is more, LPL in adipose tissue can be a potential target for atherosclerosis treatment [6].

T cells play an important role in the pathogenesis of atherosclerosis. The phenotype of T cells may change in the dynamic microenvironment. Hyperlipidemia can lead to T cell dysfunction and

promote inflammation. With the development of atherosclerosis, the number of Treg cells decreases [7]. In addition, the absence of Treg cells will lead to the aggravation of atherosclerosis in mice [8]. In addition, a large number of Treg cells are transformed into Tfh cells in vascular well or spleen in the development of atherosclerosis. Inhibiting the differentiation of Tfh cells will reduce the number of Treg cells and restrain the development of atherosclerosis in mice. The number and percentage of Treg cells increased after the administration of apoA-I in mice. And the number of Tfh cells transformed from exTreg cells significantly decreased. Therefore, apoA-I can inhibit the transformation of Treg cells to Tfh cells. Further, apoA-I increases the expression of IL-2Rα in Treg cells and decreases the level of cholesterol in Treg cells [9]. These findings contribute to further understanding of the pathogenesis of atherosclerosis and provide a new direction for the treatment of atherosclerosis.

8.3 Novel HDL-Based Treatment on ASCVD

HDL is a complex composed of many kinds of molecules with different sizes. The functions of different subcomponents are different. This may be an important reason for the unsatisfactory clinical application of HDL. It is of great significance to understand the function of different components for the recombinant HDL (rHDL).

Recently, researchers designed a novel rHDL, CER-001, which consists of wild apoA-I and sphingomyelin. It has been improved that CER-001 can protect patients with hereditary hyperlipidemia from atherosclerosis [10, 11]. Furthermore, the effect of CER-001 in ASCVD patients treated with statin was also investigated. A total of 301 patients were recruited and randomly divided into two groups. Patients were given CER-001 (3 mg/kg) or placebo by intravenous infusion. Intravascular ultrasound (IVUS) examination within the same coronary artery was completed in 7–21 days after the final injection. However, the results show that the percent atheroma volume (PAV) decreased by 0.41% in the placebo group ($p = 0.005$ compared with baseline), but did not change in the CER-001 group (−0.09%; $p = 0.67$ compared with baseline; between-groups difference, 0.32%; $p = 0.15$). The total atheroma volume (TAV) reduced by 6.6 mm^3 in the control group ($p < 0.001$) and by 5.6 mm^3 in the CER-001 group ($p < 0.001$; between-groups difference $p = 0.64$). All of these showed that administration of CER-001 has no obvious effect on the regression of coronary atherosclerosis in statin-treated patients [12]. This may indicate that the simple increase of HDL content has little effect, and the function of HDL may be related to some of its structures.

The main function of HDL is to promote RCT from macrophages, which requires the participation of ABCA1 and ABCG1. The researchers designed different sizes of rHDL using fixed ratios of phospholipid and apoA-I. Among the different sizes of rHDL, the researchers found that ABCA1-mediated cholesterol efflux to the smallest (7.5 nm) rHDL was the most efficient, compared with other sizes (11.9, 10.9, and 8.7 nm). Furthermore, it was found that ABCA1 is the key protein that mediates cholesterol efflux to HDL3. HDL3b, HDL3c, and lipid-free apoA-I are all fine therapeutic targets for increasing cholesterol efflux [13].

SR-BI is another important protein that mediates RCT. It promotes the removal of cholesterol from HDL by many cells, such as hepatocytes, macrophages, etc. The dysfunction of SR-BI will lead to the accumulation of HDL-C in plasma and increase the risk of ASCVD [14, 15]. In addition, SR-BI deficiency also reduces the antioxidative capacity of HDL.

Phospholipid transfer protein (PLTP) is a widely expressed protein, belonging to the family of lipid transfer/lipopolysaccharide-binding proteins. In plasma, PLTP binds to HDL and mediates the transfer of phospholipids during lipolysis of triglycerides, which is the key step of HDL particle maturation. It was found that knockout of PLTP could reverse the accumulation of cholesterol esters in plasma caused by SR-BI deficiency. In addition, the level of

HDL-C also decreases. PLTP knockout significantly reduces oxidative stress in aorta, but could not completely reverse the development of atherosclerosis caused by SR-BI knockout. This may be due to the presence of severe hypertriglyceridemia, obesity, and impaired glucose tolerance in double knockout mice. Moreover, knockout of PLTP impairs the clearance of very-low-density lipoprotein (VLDL)/chylomicron by the liver [16].

Previous studies have shown that apoA-I can be oxidized by myeloperoxidase (MPO), resulting in the impairment of apoA-I and HDL functions. The oxidation of apoA-I leads to the decreased ability of cholesterol efflux [17]. In clinical studies, it was found that oxidized apoA-I (ox-apoA-I) significantly increased in human atherosclerotic plaque and plasma of patients with ASCVD [18–21]. In the plasma of mice treated with ox-apoA-I, a large number of oxidative cross-linked dimer or trimer forms of apoA-I were observed. However, only 35% of ox-apoA-I was related to HDL, and almost all of native apoA-I was still related to HDL. Compared to native apoA-I, the plasma HDL-C level significantly decreased at 24 and 72 h (by ~40% and ~41.5%, respectively; $p < 0.01$) after the administration of ox-apoA-I. Besides, RCT also obviously reduced after injecting ox-apoA-I. In the short-term treatment period, the administration of apoA-I did not change the size of the plaque. However, compared with ox-apoA-I group and the control group, native apoA-I changes the composition of plaque, reduces the content of macrophage and lipid, and increases the content of collagen, improving the stability of plaque. CCR7-mediated macrophage migration plays an important role in plaque regression. Administration of ox-apoA-I can promote the expression of CCR7. However, native apoA-I significantly decreased the expression of CCR7, leading to the decrease of macrophage migration. Moreover, apoA-I can change the type of macrophages in plaque, decreasing the number of pro-inflammatory M1 macrophages and increasing the number of anti-inflammatory M2 macrophages compared with ox-apoA-I group and the control group. In short, after apoA-I oxidation, many beneficial effects of apoA-I are decreased, such as promoting cholesterol efflux and inhibiting inflammation. Therefore, it might be a new target to inhibit the oxidation of apoA-I [22].

In addition to atherosclerosis, HDL plays key roles in chronic inflammatory diseases. HDL has been shown to reduce the expression of intercellular cell adhesion molecule-1 (ICAM), increase the level of endothelial nitric oxide synthase (eNOS) in endothelial cells, and decrease the content of CD11b in macrophages. In addition, administration of HDL also reduces the content of proinflammatory cytokines in plasma, such as IL-6 and IL-12. HDL significantly increases the expression of ATF3 at the transcription level in macrophages, which binds to the promoter regions of anti-inflammatory factors. Knockdown of ATF3 blocks the anti-inflammatory effect of HDL [23]. ATF3 is a new target molecule of HDL.

There is a small class of HDL-containing apolipoprotein M (apoM), which can carry the bioactive lipid mediator sphingosine-1-phosphate (S1P). Activation of S1P receptor 1 (S1P1) exerts an anti-inflammatory effect. ApoM combining with S1P can inhibit the expression of S1P-related genes to counteract TNF-α. Treatment of endothelial cells with apoM-containing S1P can reduce the expression of E-selectin, ICAM-1, and VCAM-1, inhibit the inflammation of endothelial cells, and maintain the barrier function of endothelial cells [24].

Through transcriptome analysis, HDL extensively inhibits the expression of pro-inflammatory genes and promotes the expression of anti-inflammatory genes. However, HDL also shows a certain pro-inflammatory effect, which is reflected by the activation of chemokine signaling pathway. When the cholesterol content in macrophages changes, the anti-inflammatory function of HDL will also change, indicating that the anti-inflammatory function of HDL depends on cholesterol efflux. The early anti-inflammatory function of HDL is reflected in the downregulation of Toll-like receptor (TLR) 4, while the late anti-inflammatory effect mainly depends on the inhibition of interferon (IFN)

receptor signaling. The pro-inflammatory effect of HDL through the activation of endoplasmic reticulum stress is due to cholesterol depletion. However, in vivo experiments showed that HDL had obvious anti-inflammatory effect and decreased inflammatory factors, and there was no evidence of the pro-inflammatory effect of HDL [25].

Clinical studies have shown that KLF14 is associated with HDL-C levels and ASCVD. A group of highly correlated SNPs have been identified, including rs4731702 and rs972283 located ~14 kb upstream of KLF14 [26]. Previous studies on KLF14 focused on embryogenesis, cell proliferation, differentiation, and development. Researchers found the expression of KLF14 significantly decreased in hyperlipidemia mice. To further verify the role and mechanism of KLF14 in the pathogenesis of atherosclerosis, KLF14 overexpression mice were constructed. It was found that overexpression of KLF14 increased the levels of plasma HDL-C and apoA-I and the capacity of cholesterol efflux in mice. As a transcription factor, KLF14 can bind to CACCC box (−491/−486) in the promoter region of apoA-I through CHIP assay, regulating the transcription of apoA-I and increasing the levels of HDL. Liver-specific knockout of KLF14 reduced the level of apoA-I, indicating that liver KLF14 was involved in the metabolism of HDL. Researchers screened a specific KLF14 agonist, perhexiline, in a chemical library of the NIH/JDRF Custom Collection. Perhexiline can promote the expression of apoA-I in the liver and intestine. After the administration of perhexiline, the levels of HDL-C significantly increased in mice, and the progression of atherosclerosis was inhibited. The discovery of perhexiline provides a new way to intervene KLF14 and explore its clinical potential for the treatment of atherosclerosis [27].

FOXA3 is also an important gene that affects the expression of apoA-I. The expression of FOXA3 is significantly decreased in high-fat-diet (HFD)-fed or diabetic mice and patients with nonalcoholic steatohepatitis. FOXA3 binds to the site of TGTTTAC in apoA-I promoter region, regulating the transcription of apoA-I. Overexpression of FOXA3 can increase plasma HDL levels and inhibit the progression of atherosclerosis in mice [28].

Liver-enriched cAMP-responsive element–binding protein H (CREBH) is a bZiP transcription factor, which takes part in glucose and lipid metabolism. CREBH knockout mice have severe hyperlipidemia, and the ratio of apoB/apoA-I in plasma increases, which aggravates atherosclerosis. CREBH can directly bind to the promoter region of apoA-I, and directly regulate the transcription of apoA-I. Further, overexpression of CREBH can improve the level of various lipoproteins in vivo [29].

Endothelial dysfunction (ED) occurs in the early stage of atherosclerosis, and ED is related to the rupture of plaque, including the increase of the area of necrotic nuclei [30]. In addition, patients with early atherosclerosis showed ED and HDL impairment, and increased cholesterol content in the plaque area. These indicate that there is a certain relationship between ED and HDL function [31, 32].

Haptoglobin (HP) is a binding protein of plasma free hemoglobin, which is a component of HDL and combines with apoA-I to regulate HDL function [33]. Researchers analyzed the function of microvasculature and epicardial coronary endothelium in 338 volunteers with ASCVD. Microvascular ED was defined as $<50\%$ change in coronary blood flow and epicardial ED as $\geq 20\%$ decrease in coronary artery diameter after intracoronary acetylcholine infusion. There are three main subtypes of HP: 1–1, 2–1, 2–2. The proportion of HP 2–2 in diabetic patients with microvascular ($p = 0.01$) and epicardial ED ($p = 0.04$) increased significantly. The HP content of HDL also increased in patients with diabetes [34]. Therefore, different types of HP can be used to predict ED of some diabetic patients with coronary artery disease, and further as an important basis for judging the probability of serious complications such as thrombosis.

Recently, a new mimic peptide of HDL (MDCO-216) has been studied in patients with coronary syndrome. The mimic peptide can be mass-produced due to the improvement of the production process, and can ensure its ability to

promote cholesterol efflux without causing immune response [35].

The researchers selected 126 patients with acute coronary syndrome treated with statins at 22 hospitals in Canada and Europe. Treatment of MDCO-216 can reduce the level of plasma HDL-C (-3.3 vs. 3.0 mg/dL; difference, -6.3 mg/dL; 95% CI, -8.5 to -4.1; $p < 0.001$), but it cannot affect the level of plasma LDL-C (68.6 vs. 70.5 mg/dL; difference, -2.5 mg/dL; 95% CI, -10.1 to 5.0; $p = 0.51$). Most importantly, treatment of MDCA-216 did not significantly reduce plaque area [11]. These results suggest that MDCA-216 has no significant effect on the improvement of acute coronary syndrome during statins administration and cannot significantly inhibit the progression of plaque.

Albuminuria is an important risk factor of cardiovascular disease in diabetic patients, and there is an atherogenic lipid profile characterized by elevated triglycerides, and decrease of the level of HDL-C [36].

The researchers analyzed 46 components of HDL and found that 8 of them were related to albumin excretion rate (AER). This shows that albuminuria can change the composition of HDL. Some of these proteins are also related to estimated glomerular filtration rate (eGFR). The content of paraoxonase (PON) is the only protein that has a direct relationship with coronary artery calcification and AER. However, PON does not regulate the ability of HDL to promote cholesterol efflux from macrophages [37].

Smoking is an important factor increasing the incidence of cardiovascular disease. A study in Japan found that smokers are 2.51 times more likely to have cardiovascular disease in man and 3.35 times more in women than nonsmokers [38]. Preβ1-HDL is an efficient receptor of intracellular free cholesterol, which can be transformed into cholesterol-rich HDL by lecithin–cholesterol acyltransferase (LCAT). In nonsmoking population, the content of HDL in hyperlipidemia patients increased significantly (25.5 ± 6.7 vs. 20.3 ± 4.6 mg/L apoA-I, $p < 0.01$), compared with that in normal person. However, among smokers, the difference disappeared. Smoking can reduce the content of preβ1-HDL in patients with hyperlipidemia, suggesting that smoking may cause more damage to patients with hyperlipidemia [39].

There are different subtypes of HDL in human. By regulating the content and existence of different subtypes of HDL, the downstream metabolism and signal path are regulated through the interaction with receptors and enzymes [40]. Apolipoprotein C-III(apoC-III) is a small lipoprotein found in some HDLs, not all. In addition, it has been found that when apoC-III gene mutation occurs, the level of plasma apoC-III and triglyceride decreases, and the risk of cardiovascular disease decreases [41, 42].

The researchers conducted the study using two different cohorts. It was found that an average of 6–8% of apoA-I contained apoC-III. The total amount of apoC-III was correlated with plasma triglyceride level, but HDL subtypes with or without apoC-III were weakly or not correlated with triglyceride level.

Interestingly, the two subtypes of HDL have opposite associations with the incidence rate of ASCVD. HDL that contains apoC-III was associated with a higher risk of ASCVD (pooled relative risk per standard deviation, 1.09; 95% confidence interval, 1.01–1.18), whereas HDL that lacks apoC-III was associated with lower risk (relative risk, 0.76; 95% confidence interval, 0.70–0.83). The relative risk for HDL lacking apoC-III was even more negative than the relative risk for total HDL (relative risk, 0.80; 95% confidence interval, 0.74–0.87) [43]. These studies indicate that the functions of different subtypes of HDL are very different, suggesting that researchers should consider the important role of different subtypes of HDL in the prediction of ASCVD and the application of HDL analog peptide in the treatment of related diseases.

Recently, it has been pointed out that the concentration of HDL particles (HDL-P), rather than HDL-C, may be a more appropriate index to evaluate the function of HDL. Moreover, HDL-P has a more significant correlation with the risk of ASCVD than HDL-C [44, 45].

Experiments show that cholesterol-overloaded HDL-P is harmful. It can not only inhibit the

ability of cholesterol efflux [46] but also decrease the uptake of cholesterol by hepatocytes [47].

The researchers measured the baseline HDL-C/P levels of 930 volunteers and divided them into four groups (<41.0 (lowest), 41.0–46.9, 47.0–52.9, and > 53.0 (highest).

Finally, it was found that plaque progression in the population with the highest baseline level (>53.0) is 1.56 times (95% confidence interval: 1.14–2.13; $p < 0.006$) higher than that in the population with the lowest baseline level (<41.0) after 5 years [48]. These results explain the possible reasons for the poor effect of HDL-C on ASCVD. In the future, HDL-C and HDL-P should be combined to play the therapeutic role of HDL.

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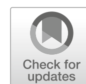

9 HDL and Diabetes

Blake J. Cochran, Bikash Manandhar, and Kerry-Anne Rye

Abstract

Diabetes is a worldwide public health issue, with the number of cases expected to reach 642 million by 2040. Patients with diabetes are at a two- to four-fold increased risk of developing cardiovascular disease. This chapter focuses on the anti-diabetic and cardioprotective functions of plasma high-density lipoproteins (HDLs). HDLs and the main HDL apolipoprotein, apoA-I, improves pancreatic beta cell function. ApoA-I also improves insulin sensitivity. The development of novel, bifunctional HDL-based interventions are a promising therapeutic option for the treatment of cardiometabolic diseases.

Keywords

Diabetes · Antidiabetic · β cell · Insulin sensitivity

9.1 Introduction

In 2015, 415 million people worldwide had diabetes. By 2040, the number of patients with diabetes is expected to increase to 642 million [1]. Treatment of diabetes, along with its complications including cardiovascular disease, retinopathy, neuropathy, and nephropathy, is a major public health priority that is expected to consume more than 15% of the total global health expenditure by 2040 (International Diabetes Federation).

The anticipated rapid increase in the incidence of diabetes, particularly in low- and middle-income countries, highlights a major unmet need to develop new lifestyle modification strategies and therapeutic interventions that will reduce incident disease and reverse established disease. It is well established that a diagnosis of either type 1 diabetes (T1D), which accounts for about 10% of all cases, or type 2 diabetes (T2D), which is the most prevalent form of the disease and accounts for ~90% of all cases, is associated with a two- to four-fold increase in the risk of developing cardiovascular disease [2, 3]. There is thus a compelling case for developing novel, bifunctional therapies that can reduce the incidence of T1D, T2D as well as treat and prevent associated cardiovascular complications.

T1D is an autoimmune disease in which insulin-producing β-cells in the pancreas are selectively destroyed by self-activating autoimmune T cells. β-cell loss can be as high as 70% at the time of T1D diagnosis. Insulin resistance, where uptake of glucose from blood into peripheral tissues is impaired is, by contrast, the hallmark feature of T2D. β-cells compensate for insulin resistance and maintain blood glucose

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levels in the normal range in patients with T2D by secreting extra insulin. However, prolonged compensation eventually leads to β-cell loss, reduced insulin secretion in response to high blood glucose levels, and progression from the prediabetic state (impaired fasting glucose and/or impaired glucose tolerance) to full-blown T2D [4]. Despite this difference in pathogenesis, it is well established that progression of T1D and T2D is driven by the loss of β-cells. This is fortuitous in that development of drugs with the capacity to conserve the function of existing β-cells, or regenerate new β-cells, is likely to be relevant for both forms of the disease.

This chapter is concerned with the recent discovery that, in addition to their potentially cardioprotective functions, plasma high-density lipoproteins (HDLs), and their main apolipoprotein constituent, apoA-I, have antidiabetic properties [5–10]. This raises the possibility that agents that increase plasma apoA-I and HDL levels may have the potential to prevent and reverse T1D and T2D. More importantly, mechanistic insights into such therapies will facilitate the identification of new treatment targets that may potentially lead to new classes of drugs for treating all forms of the disorder. The importance of this approach is further augmented by the knowledge that HDLs and apoA-I have multiple cardioprotective functions that may be enhanced by HDL-increasing agents, leading to a reduction in the risk of developing cardiovascular complications that are responsible for a significant proportion of the morbidity and mortality in patients with diabetes [11, 12].

9.2 Current Treatment Options for T1D

The standard treatment for T1D is insulin replacement. However, patients with T1D often find it difficult to maintain glycemic control using this approach, and many of them remain at increased risk of developing cardiovascular disease, even when glycemic control is achieved [13]. Insulin replacement is also associated with an increased risk of hypoglycemia that can be potentially life-threatening. Pancreas or stem cell transplantation, a further treatment option for T1D, is not used widely due to limited availability of donors and the requirement for permanent immunosuppression of the recipients [4, 14]. Stem cell transplantation is also not used routinely, because it may lead to malignant cell formation [15].

9.3 Current Treatment Options for T2D

Lifestyle modifications, including weight loss and dietary changes that have the capacity to reverse T2D, are the first approach for treating patients with T2D. However, compliance is frequently problematic, and the efficacy of this approach has recently been called into question [16]. The biguanide, metformin, is the first-line therapeutic option in T2D [17]. Metformin inhibits hepatic glucose production and increases insulin sensitivity in skeletal muscle [18]. Other treatment options for patients with T2D include thiazolidinediones, which improve insulin sensitivity and delay T2D onset, but may cause weight gain [19]. Sulfonylureas increase insulin release from β-cells, but may also cause weight gain and increase the risk of hypoglycemia [20]. Long-term sulfonylurea therapy is additionally associated with a significant failure rate [21]. Newer agents, such as glucagon-like peptide 1 (GLP-1) receptor antagonists and dipeptidyl peptidase 4 (DPP4) inhibitors, that increase incretin release from the intestine and improve β-cell function, have fewer side effects than many of the older, more established therapies. GLP-1 receptor antagonists have the added advantage of promoting weight loss and reducing cardiovascular events [22]. Sodium glucose transporter 2 (SGLT2) inhibitors, which reduce glucose absorption in the kidney, also promote weight loss and reduce cardiovascular mortality, but their use can be associated with an increased risk of developing genitourinary infections [23, 24].

Thus, while patients with T2D have a number of treatment options, many of these therapies can have unwanted side effects. Treatment options for

T1D are, by contrast, extremely limited and are all focused on insulin replacement. New multi-functional therapies with minimal side effects that also have the capacity to reduce the rate of β-cell loss, and improve the function and identity of the remaining β-cells in patients with T1D and T2D, would therefore be highly desirable additions to the currently available diabetes treatment armamentarium, as would be agents with the capacity to regenerate new, functional β-cells and reduce insulin resistance. Bifunctional agents that additionally reduce cardiovascular risk while conserving or improving β-cell function represent an even more attractive option for these patients.

9.4 High-Density Lipoproteins

Multiple human epidemiological studies have established that an elevated plasma high-density lipoprotein cholesterol (HDL-C) level is inversely associated with the risk of having a cardiovascular event [25]. HDLs have a number of potentially cardioprotective functions, including an ability to reduce inflammation in endothelial cells, adipocytes, and monocytes/macrophages [26–31]. HDLs also inhibit the oxidation of low-density lipoproteins (LDLs) [32] and play a critical role in reverse cholesterol transport, the pathway whereby excess cholesterol is transported from peripheral cells to the liver for excretion as a constituent of bile [33, 34]. To this end, interventions that increase circulating HDL-C and apoA-I levels have been shown to reduce atherosclerosis in animal models [35, 36], while apoA-I knockout mice have accelerated atherosclerosis [37, 38].

These cell-based and preclinical findings have led to the hypothesis that interventions that increase plasma HDL-C levels represent a potential strategy for reducing the risk of having a major cardiovascular event. This hypothesis has recently been tested in several large-scale clinical outcome trials with interventions ranging from small molecules that inhibit activity of cholesteryl ester transfer protein (CETP) [39–42], through to older drugs such as niacin [43, 44], and infusions of reconstituted HDL (rHDL) preparations consisting of apoA-I complexed with phospholipid [45, 46]. With the exception of the REVEAL trial, in which cardiovascular events were significantly reduced by treatment with the CETP inhibitor anacetrapib in statin-treated patients with atherosclerotic cardiovascular disease [42], these large clinical trials did not reduce cardiovascular events and, in one case, caused harm [47].

Although the underlying reasons for the failure of these clinical trials are not well understood, it is noteworthy that glycemic control was improved in patients with T2D that were treated with the CETP inhibitor torcetrapib in the ILLUMINATE trial [47]. Small randomized, double-blind trials of patients with T2D have additionally revealed that increasing plasma HDL-C and apoA-I levels acutely with a single rHDL infusion, or chronically by inhibiting activity of CETP, improves glycemic control by enhancing pancreatic β-cell function and increasing insulin sensitivity [48, 49]. These studies have thus identified an opportunity to develop new therapies with the potential to improve glycemic control and reduce the risk of developing cardiovascular complications in patients with T1D and T2D. This is of particular importance for patients with T1D in whom insulin replacement is the only currently available treatment option.

9.5 HDLs and β-Cell Function

The first direct evidence that HDLs and the main HDL apolipoproteins, apoA-I and apoA-II (the first and second most abundant HDL apolipoproteins, respectively), improve β-cell function was reported by Fryirs et al. This report showed that incubation of the mouse MIN6 insulinoma cell line and isolated islets from Sprague-Dawley rats under high glucose conditions with isolated human HDLs, rHDLs that contain apoA-I or apoA-II, lipid-free apoA-I, or lipid-free apoA-II, increased transcription of the *Ins1* and *Ins2* genes. This study also showed that insulin secretion by Ins-1E cells was increased in a time- and concentration-dependent manner by incubation with HDLs, apoA-I and apoA-II under basal conditions, and to a greater

extent under high glucose conditions [5]. These effects were additionally found to be dependent on Ca^{2+} mobilization, K_{ATP} channel activation, and glucose metabolism [5]. These outcomes were further extended in an in vivo study of high fat-fed C57BL6 mice that displayed increased insulin secretion and improved glycemic control when treated with lipid-free apoA-I [9].

ApoA-IV, the third most abundant HDL apolipoprotein, also increases the secretion of insulin from isolated mouse islets under high, but not basal glucose conditions in a cyclic adenosine monophosphate (cAMP)–dependent manner [50]. Moreover, glucose tolerance and β-cell insulin secretion are both impaired in apoA-IV knockout mice [50].

Mechanistic insights into the outcomes of these studies have been obtained by showing that the ability of apoA-I and apoA-II to increase insulin secretion in MIN6 cells is dependent on expression of the ATP binding cassette transporter, ABCA1, that regulates cellular cholesterol homeostasis by effluxing cholesterol from cell membranes to lipid-free apoA-I, other HDL apolipoproteins, and small HDLs in the first step of the reverse cholesterol transport pathway [51, 52]. The ability of apoA-I and apoA-II to increase insulin secretion in MIN6 cells was additionally dependent on scavenger receptor class B type 1 (SR-B1), which promotes the bidirectional flux of cholesterol between cell membranes and HDLs [5, 53]. Given that insulin secretion is impaired in cholesterol loaded β-cells, and restored by incubation in the presence of cyclodextrin, which acts as a cholesterol sink and depletes cells of cholesterol [54], these observations raise the possibility that the antidiabetic effects of HDLs and apoA-I and apoA-II may simply reflect their ability to export cholesterol from MIN6 cells via ABCA1 and ABCG1.

However, additional experimentation has confirmed that incubation with apoA-I or apoA-II does not change unesterified or total cholesterol levels in MIN6 cells, leading to the conclusion that the mechanism by which apoA-I and apoA-II increase glucose-stimulated insulin secretion in MIN6 cells is not related to cellular cholesterol homeostasis, or to their ability to accept the cholesterol that is exported from MIN6 cells via ABCA1.

An insight into these apparently contradictory observations was obtained in a subsequent in vitro study in which the rat Ins-1E insulinoma cell line was used to elucidate the role of ABCA1 in the apoA-I-mediated increase in insulin synthesis and secretion [6]. Those results established that the interaction of apoA-I with the β-cell surface facilitates the colocalization of ABCA1 with the heterotrimeric G-protein $G\boldsymbol{\alpha}_s$ subunit, which results in activation of a transmembrane adenylyl cyclase and increases intracellular cAMP levels. This leads to downstream activation of protein kinase A (PKA), which increases intracellular Ca^{2+} levels and the acceleration of insulin secretion [6]. The activated PKA can also translocate to the β-cell nucleus, where it phosphorylates and excludes the transcription factor forkhead box protein O1 (FoxO1) from the nucleus, leading to derepression of insulin gene transcription and increased expression of the *Ins1* and *Ins2* genes [6]. An equally important outcome from this study was that incubation with apoA-I increased expression of the transcription factor, pancreas duodenum homeobox protein 1 (*Pdx1*), a key determinant for pancreas development and β-cell survival [6, 55–57]. This result provided evidence that apoA-I may have the capacity to conserve β-cell identity and prevent β-cell loss, an observation that has significant implications for slowing disease progression in patients with T1D and T2D.

Further evidence that the outcomes of the aforementioned in vitro studies are physiologically relevant has emerged from mice in which ABCA1 and the related intracellular cholesterol transporter ABCG1, that effluxes cell cholesterol to HDLs, were both conditionally deleted in β-cells (β-DKO mice) [58]. Loss of β-cell ABCA1 and ABCG1 in β-DKO mice leads to a doubling of islet cholesterol levels. As expected, the insulin secretory capacity of the β-cells in β-DKO mice was severely impaired due to the increased cholesterol levels. Furthermore, as these animals have normal insulin sensitivity,

they are an ideal model in which to study the specific contribution of β-cells to the regulation of glycemic control by apoA-I in vivo [58]. To this end, it has recently been reported that treatment of β-DKO mice with apoA-I improves GSIS but does not alter islet cholesterol levels [8]. While this result recapitulates the overarching outcomes of the in vitro studies in MIN6 and Ins-1E cells [5, 6], the mechanism by which apoA-I increased insulin secretion in the β-DKO mice, while not yet understood, clearly differs from the ABCA1-dependent pathway that was described in the earlier in vitro study [6].

9.6 HDLs, apoA-I, and Insulin Sensitivity

Evidence that apoA-I and HDLs also improve insulin sensitivity is beginning to emerge. Increasing plasma HDL-C and apoA-I levels in patients with T2D by inhibiting CETP activity improves insulin sensitivity according to the homeostasis model assessment of insulin resistance [47]. A single rHDL infusion also reduces plasma glucose levels in patients with T2D, which is indicative of an improvement in insulin sensitivity [48].

The outcomes from these clinical studies have been recapitulated in vitro in incubations of human primary skeletal muscle cells with lipid-free apoA-I [7]. These studies established that apoA-I increases glucose uptake by phosphorylating the insulin receptor and insulin receptor substrate-1, downstream activation of the PI3K/Akt/AS160 pathway, and translocation of the glucose transporter, GLUT4, to the skeletal muscle cell surface, which results in increased uptake of glucose into the cells [7]. It is noteworthy that the apoA-I-mediated uptake of glucose into skeletal muscle cells occurs in an insulin-dependent as well as an insulin-independent manner, with evidence that both of these mechanisms are regulated by ABCA1 and SR-B1 [7].

An insight into the underlying mechanism by which apoA-I increases glucose uptake into skeletal muscle independent of insulin was obtained by Han et al., who established in a series of in vitro studies that apoA-I phosphorylates AMP-activated protein kinase (AMPK) and acetyl coenzyme A carboxylase in association with clathrin-dependent apoA-I internalization and localization in endosomes [59]. These findings were subsequently confirmed in a small double-blind, placebo-controlled cross-over study of subjects with T2D in whom acetyl coenzyme A carboxylase phosphorylation and AMPK activation were increased following a single infusion of apoA-I-containing rHDLs [48].

The outcomes of these studies have been further recapitulated in vivo with [(^{18}F)]FDG imaging, which has established that treatment of *db/db* mice, a model of T2D, with lipid-free apoA-I improves glycemic control and insulin sensitivity [60]. Subsequent ex vivo analyses in these mice provided direct evidence that treatment with apoA-I increases glucose uptake by skeletal muscle in an insulin-dependent and insulin-independent manner [60].

Indirect evidence for a reduction in hepatic gluconeogenesis has also been reported in apoA-I knockout mice. These animals have increased mRNA levels of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, two enzymes that are critically important for glucose production in the liver [59]. ApoA-I knockout mice are also hyperglycemic and hyperinsulinemic, which is consistent with reduced AMPK activation and a major role for apoA-I in hepatic glucose metabolism in these animals [59]. This conclusion was further confirmed with the evidence that hepatic gluconeogenesis is similarly reduced in apoA-IV knockout mice [61].

A recent report has also indicated apoA-I treatment reduces the insulin resistance that develops in rats during pregnancy by increasing glucose uptake by skeletal muscle and adipose tissue [62]. Increased insulin resistance is a hallmark feature of pregnancy, leading to gestational diabetes in about 14% of all cases. As is the case for T2D, gestational diabetes manifests when β-cells are no longer able to keep up with the demand for additional insulin as insulin resistance increases [63]. Given that the incidence of gestational diabetes is increasing rapidly, and many affected

women subsequently go on to develop T2D and cardiovascular disease, it follows that interventions that increase HDL and apoA-I levels and improve insulin sensitivity in this cohort have the potential to reduce the burden of disease and alleviate future vascular complications.

9.7 Concluding Comments

The clinical implications of the antidiabetic functions of HDLs and HDL-associated apolipoproteins are far-reaching for all forms of the disease, including people with T2D that are refractory to current therapies as well as those with T1D and gestational diabetes, for whom treatment options are limited. Although there are few existing drug development pipelines for agents that raise HDL and apoA-I levels, there is strong proof-of-principal evidence from human and preclinical studies that such approaches may be highly efficacious in a diabetes setting.

Future strategies for exploiting these properties of HDLs and their constituent apolipoproteins with new antidiabetic pharmacotherapies include the synthesis of peptides that mimic the antidiabetic functions of HDL-associated apolipoproteins, the identification of molecular targets with the potential to increase apolipoprotein gene transcription, and infusions of rHDL preparations. The latter approach is currently under investigation in the Phase 3 clinical trial, AEGIS-II, for reducing subsequent events in acute coronary syndrome patients. Although rHDL infusions are not appropriate for long-term treatment, assessment of how such an intervention impacts on glycemic control in the short term in study subjects with diabetes would undoubtedly be highly informative and may provide the impetus that is needed for further development of these and related HDL-increasing agents.

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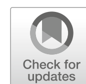

HDL and Sepsis

10

Huanhuan Cao and Wei Huang

Abstract

Sepsis has been recognized as a global health burden in the year 2017 for its high morbidity and mortality. HDL, a cholesterol transporter, also plays vital role in inflammation besides its typical role in reverse cholesterol transportation. In septic patients, HDL levels dropped significantly. HDL exerts a variety of protective effects in the pathophysiology of sepsis including acting on pathogens, inhibiting macrophage inflammatory reaction, and modulating endothelial function in sepsis. Studies have shown that rHDL or apoA-I mimetic peptides could be therapeutic potentials in sepsis. HDL has caused increasing concern as a potential therapeutic agent.

Keywords

HDL · Sepsis · rHDL · apoA-I mimetic peptides

10.1 Introduction

Sepsis is a leading cause of death worldwide. It has been recognized as a global health burden in 2017 for its high morbidity and mortality [1]. In the USA, the reported incidence of sepsis was about 750,000 cases each year [2]. In the UK, it was reported that the prevalence of sepsis was 27% in ICU [3]. According to the reported data, it has been estimated that 30 million episodes happen per year worldwide [4]. However, the true incidence of sepsis worldwide is underestimated. Because the available data were almost from developed countries, no data were found from the developing countries where 87% of the world's population lives. The mortality of sepsis is also high. It is estimated that there are six million deaths per year [4].

Sepsis is defined as a life-threatening organ dysfunction caused by a deregulated host response to infection [5]. Compared to simple infection, sepsis is characterized by a much more complex, variable, and prolonged host response triggered by infection. The host response includes inflammatory response and anti-inflammatory response. The initial inflammatory response leads to subsequent

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anti-inflammatory response syndrome. On the one hand, both pro-inflammatory and anti-inflammatory mechanisms contribute to the clearance of infection and tissue recovery. On the other hand, pro-inflammatory and anti-inflammatory homeostasis imbalance leads to multiorgan dysfunction and sepsis [6].

Sepsis can be caused by virtually any infecting organism, including bacteria, virus, and parasites. Among these, the most common pathogen is bacteria. Bacteria can be divided into Gram-positive and Gram-negative bacteria. Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Pseudomonas* species and *Escherichia coli* are the most frequently identified organisms. The Sepsis Occurrence in Acutely Ill Patients study shows an equal prevalence of Gram-positive and Gram-negative bacterial infections [7]. While another study reported a higher prevalence of Gram-negative bacterial infections than Gram-positive bacterial infections in the USA [8]. Therefore, both Gram-positive and Gram-negative bacterial infections play important roles in sepsis. Except for infecting organisms, the site of infection also varies considerably in sepsis. However, the most common site is the lung and the abdomen, which takes up to 64% and 20% respectively [9, 10].

The pathophysiology of sepsis is characterized by a deregulated host response to infection. The pathogen can be recognized by immune cells through pattern-recognition receptors, like Toll-like receptors [11]. The interaction between pathogen and immune cells triggers the release of proinflammatory mediators, including cytokines (TNF-α, IL-6, IL-1β) and others. These mediators cause neutrophil–endothelial cell adhesion, activation of coagulation cascades, and the generation of microthrombi. Simultaneously, anti-inflammatory responses are involved in the process. Endothelial cell dysfunction then leads to organ dysfunction: endothelial cells are closely involved in vasodilation, disturbances in microcirculation, and distribution of blood flow to organ systems [12].

Unlike the diagnosis of diabetes or hypertension, which has gold criterion, the diagnosis of sepsis is complicated, for no clinical or biochemical marker can be found to provide the gold standard criterion for diagnosis. Presently, the diagnosis focuses on clinical presentation, especially organ dysfunction. Organ dysfunction can be evaluated by the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score. The Sepsis-3 Criteria emphasized that the change of 2 or more points in the SOFA score, called quick SOFA (qSOFA), facilitated earlier recognition of sepsis. Moreover, the Sepsis-3 Criteria removed the systemic inflammatory response syndrome (SIRS) criteria, with inadequate specificity and sensitivity [5]. In the future, more researches are needed to explore biomarkers that enable to indicate early sepsis, or predict the risk, severity, outcomes, or response to therapy.

The combined management measures, including removal of infected tissue, antibiotic therapy, hemodynamic stabilization, the application of vasoactive drugs, glycemic control, and nutritional support, are cornerstone for the success in the treatment of patients with sepsis [13]. Timely antibiotics administration can improve outcomes of patients. A study showed an association between delay in first antibiotic administration and increased in-hospital mortality [14]. Besides, the appropriateness of antibiotics administration based one antibiotic selection is driven by many factors, such as site of infection, patient-specific factors, and pathogen factors. However, a 48-h delay as well as a false-negative cultivation result may affect the determination of pathogens as well as the antibiotic choice. Therefore, the selection of antibiotics in clinical practice is mostly based on experience, which may increases the risk of death due to inappropriate initial antibiotic therapy [15, 16]. This is just an example of antibiotic therapy, and there are also many problems with other measures. Although with general patient management, outcomes of patients with sepsis have certainly improved, the improvement does not attribute to any specific measure in the process. Therefore, it is still a challenge to find a much more effective therapeutic target.

In recent years, interest has arisen in the ability of high-density lipoproteins (HDLs) to modulate the inflammatory response to sepsis, and, hence,

in their potential as novel therapeutic agents in sepsis.

10.2 The Clinical Evidence of HDL in Sepsis

HDL, a cholesterol transporter, also plays vital role in inflammation besides its typical role in reverse cholesterol transport. Therefore, great interest has arisen in the role of HDL in inflammatory diseases, especially in sepsis. Multiple clinical investigations have suggested that HDL is associated with sepsis, and low HDL is a risk factor for sepsis.

HDL levels drop significantly in septic patients. A clinical observational study by van Leeuwen et al. has shown that lipoprotein concentrations can rapidly be reduced to 50% of initial concentrations in patients with severe sepsis, especially LDL and HDL. In the study, 17 patients with severe sepsis were included, and serial blood samples for total cholesterol, lipoprotein cholesterol, apolipoprotein A-1, and so on were collected over 28 days. Researchers found that HDL concentrations decreased rapidly from 0.84–0.92 mmol/L to 0.42–0.35 mmol/L on day 3, while during the following 4 weeks, it increased slowly to 0.84–0.42 mmol/L [17]. Another study compared lipid profiles in septic patients versus trauma patients, which is also associated with a systemic inflammatory response syndrome. Interestingly, lipid profile of septic patients was completely different from the trauma group. HDL levels were low in septic patients, which is consistent with the previous study. Interestingly, the concentration of HDL was not altered in trauma patients. This difference emphasizes the therapeutic potential of HDL in sepsis [18].

Decreased serum HDL level is a poor prognostic factor for sepsis. In a prospective study including sixty-three consecutive patients with severe sepsis, Chien et al. found that overall and sepsis-attributable 30-day mortality rates were increased among patients with day 1 levels of HDL cholesterol of <20 mg/dL. To further assess the ability of HDL levels to predict the prognosis of severe sepsis, patients were divided into two groups using cutoff value: 20 mg/dl. Compared to the “high” (>20 mg/dl) HDL group, patients with “low” (<20 mg/dL) HDL have lower survival rate (a sensitivity of 92%, a specificity of 80%). Therefore, an HDL cholesterol level of <20 mg/dL on day 1 is an independent predictor of the overall 30-day mortality rate [19]. Another study including 151 septic patients showed similar results. A significant negative correlation was found for apoA-I/HDL and 30-day-mortality [20]. In addition, some authors explored several genes involved in HDL metabolism. Both Derivation Cohort and Validation Cohort identified CETP genes (rs1800777, allele A), which was remarkably associated with lower HDL levels, also increase the risk of sepsis-associated AKI [21]. So far, no satisfactory biomarkers have been available yet, the diagnosis of sepsis or the prediction of severity becomes difficult. Cirstea M et al. compared HDL levels with other routine clinical markers, such as white blood cell, lactate, or platelets, and found that HDL levels had superior predictive ability for both development of MODS and 28-day mortality [22]. These studies indicated that HDL levels could be an early biomarker for prediction of the sepsis severity.

In addition to decreased serum levels of HDL, dysfunctional HDL is also associated with outcomes of patients with sepsis. As we all know that researchers have been paying intense attention to study HDL in the therapeutic potential in cardiovascular diseases. The assumption is that high HDL level is an independent protective factor for cardiovascular diseases. However, HDL-raising therapies did not result in improved cardiovascular outcomes in most clinical trials. Therefore, researchers began to shift the focus from HDL levels toward HDL function [23]. Similarly, the correlation between dysfunctional HDL and sepsis is worth being explored. Dysfunctional HDL is referred to as a proinflammatory, dysfunctional state caused by alterations of HDL surface proteins and lipid cargo. Apo A-I, an essential surface protein of HDL structure, could be catalyzed by myeloperoxidase (MPO) during sepsis, which results in dysfunctional HDL. In addition, Apo A-I could be replaced by serum

amyloid A (an acute phase protein) during sepsis. This phenomenon was observed in up to 45% of patients at 1 day after admission for sepsis. In addition, Faheem W et al. also observed that mean percent cholesterol efflux, which showed functional properties of HDL, was significantly reduced in older patients with sepsis [24]. These researches demonstrated that dysfunctional HDL was present in inflammatory state of sepsis. To further prove the ability of dysfunctional HDL to predict adverse outcomes of sepsis, Faheem W et al. used HDL inflammatory index (HII) to express dysfunctional HDL, and found a remarkable difference in change in HII over the first 48 h between those with adverse outcomes and those without. The result suggested an inverse correction between increasing HII and outcomes of sepsis. (OR 5.2) [25].

However, some authors showed that baseline HDL levels were not associated with long-term sepsis rates. The researchers included 29,690 subjects with available baseline HDL-C and LDL-C, and used Cox models to assess the association between quartiles of HDL-C or LDL-C and first sepsis event. Surprisingly, the Low LDL-C level, but not the HDL level, was associated with higher long-term rates of community-acquired sepsis [26].

Given the previous clinical studies, HDL plays an essential role in the pathophysiology. We propose that low HDL level or dysfunctional HDL is associated with outcomes of sepsis. However, the association between HDL and morbidity of sepsis needs further study in the future. Thus, targeting HDL may be an effective and potential therapy for sepsis.

10.3 HDL with Pathogens

HDL could interact with pathogens, including Gram-negative bacteria and Gram-positive bacteria, which plays an essential role in sepsis.

Gram-negative bacteria infection is the most common cause of sepsis. The major pathogenic factor of Gram-negative bacteria is lipopolysaccharide (LPS). LPS could induce an inflammatory response when it is released into blood. HDL can bind and neutralize Gram-negative bacterial LPS, which plays an important role in protection against sepsis. Rall et al. found that an unknown component of serum could reduce the febrile response to bacterial LPS in rabbits [27]. Later studies found that the unknown serum component is HDL, and HDL was shown to interact with LPS to form HDL-LPS complexes [28]. Following these, a multitude of studies of HDL neutralizing LPS appeared. Ulevitch et al. isolated and analyzed the lipopolysaccharide-HDL complex formed in rabbit plasma, and found that most LPS in circulation exists in LPS-HDL complex form [29]. It is well known that LPS triggers a downstream signaling cascade by binding to TLR4, producing pro-inflammatory cytokines like TNF-α and IL-6. Flegel et al. showed that LPS-HDL complex reduced LPS-TLR4 inflammatory signaling in macrophages [30]. There are also some direct evidences; for example, Dai et al. showed that in vivo administration of fluorescently labeled LPS was found in the HDL fraction [31].

Multiple proteins associated with HDL facilitate the binding of HDL and LPS, such as apoA-I, LBP, PLTP, and so on. ApoA-I, as a major component of HDL, is directly responsible for LPS neutralization by protein-protein interaction. Henning et al. reported the specific domain of apoA-I responsible for neutralizing, and the specific domain is the C-terminal of apoA-I. LBP is an LPS-binding protein. Lamping et al. suggested that LBP protected mice from LPS-induced septic shock. In this study, recombinant murine LBP significantly reduced mortality in LPS-induced septic mice, and decreased the release of proinflammatory cytokines [32]. Additionally, another study showed that removal 99% of LBP from human plasma led to 50% reduction in transfer of LPS to HDL. These findings suggest that LBP facilitates binding of LPS with HDL [33]. PLTP is cholesteryl ester transfer protein and phospholipid transfer protein, and it was also shown to affect the transfer of free LPS to HDL [34].

Interestingly, the clinical trial showed that simply neutralization of LPS by using anti-LPS monoclonal antibodies failed to protect against

sepsis. Anti-LPS monoclonal antibody cannot inhibit LPS-induced proinflammatory cytokine production in human monocytes. Delayed clearance of LPS was observed [35]. The finding implies that in addition to its role as an LPS neutralizer, HDL could effect through other mechanisms. Recent studies found that HDL could also promote LPS clearance. The scavenger receptor BI(SRBI), an HDL receptor, plays an essential role in LPS clearance together with HDL. SRBI is well known for its role in reverse cholesterol transport, selectively taking up cholesterol esters from HDL. Besides, SRBI also affects the regulation of inflammation [36]. In SRBI-null mice, inflammatory cytokine response increased and survival rate markedly increased in response to LPS, although plasma HDL levels elevated. Control and SR-BI-null mice exhibited a similar ability to neutralize LPS, while the LPS clearance of SR-BI-null mice decreased compared with normal mice. While in SR-BI-transfected HEK cells, LPS clearance increased by fourfold. These findings suggest that LPS clearance could be mediated in an SRBI-dependent manner [37]. More specific mechanism is demonstrated in other studies. Vishnyakova et al. used the human SRBI orthologue Cla-1 to study SRBI. It was observed that Cla-1-trasfected Hela cells had a three- to four-fold increase in Cla-1-LPS-binding capacity and LPS uptake. Moreover, in Cla-1-overexpressing HeLa cells, LPS uptake was increased by five- to ten-fold when associated with HDL. The study indicated that SRBI could bind and internalize monomerized and HDL-associated LPS, and HDL promotes SRBI-mediated LPS uptake and clearance [38].

The effect of HDL on pathogens also involves Gram-positive bacteria. Lipoteichoic acid (LTA) is an essential component of the cell membrane and wall of Gram-positive bacteria. It has similar structure and similar ability to induce an inflammatory response with LPS. Study has showed that LTA could induce systemic inflammatory response syndrome, organ dysfunction, and septic shock [39]. In inflammatory state, LTA can bind all lipoproteins, of which HDL has the highest affinity capacity [40]. HDL protects against sepsis by neutralizing LTA, similar to the mechanism on LPS. However, HDL alone does not inhibit the inflammatory response induced by LTA, which is different from LPS. Other cofactors are also required to inactivate LTA beside HDL, like LBP. LBP could also bind LTA, and it facilitates the binding of HDL with LTA. The inhibition of LBP markedly decreased (53%) activation of LTA, which also indicated that there are some other cofactors to promote the binding of HDL with LTA [41]. Considering the similarity between LPS and LTA, it is possible that HDL could also inactivate LTA via promoting LTA clearance in an SRBI-mediated manner.

Studies have also shown that LTA could be inhibited by apoA-I, the major component of HDL. Jiao et al. found that apoA-I could bind LTA in vitro directly and LTA-induced proinflammatory cytokine production decreased, resulting in reduced LTA-induced acute lung injury in mice [42]. These suggested that HDL inhibited LTA activation by a variety of mechanisms.

Collectively, there is a wide range of interactions between HDL and pathogens (including gram-positive and gram-negative bacteria) through interactions with LPS or LTA. However, binding of pathogens is supposed not the only mechanism underlying the beneficial effects of HDL. In fact, HDL is multitargeted. Interaction with pathogens is only one step in the action of HDL in sepsis. In addition to neutralizing LPS and LTA, HDL can also target inflammatory response, which makes HDL have a greater opportunity of being an intervention for patients with sepsis.

10.4 HDL in the Pathophysiology of Sepsis

Both immune response and endothelial dysfunction play important roles in the pathophysiological process of sepsis. HDL, as a multiprotective factor in sepsis, could regulate both inflammatory response and endothelial dysfunction.

In immune response, macrophage is major immune effector cell in sepsis besides multiple

other inflammatory cells. Macrophages are widely distributed, varying from peripheral blood to multiple tissues including vascular endothelium. Activation of TLR signaling of macrophages is beneficial for clearance of pathogen, by the production of inflammatory cytokines, including TNF-α, IL-6, IL-1β, etc. However, dysregulation of macrophages plays an essential role in both excessive inflammatory response and immunosuppression, leading to endothelial dysfunction, clotting disorders, and organ injury in sepsis [43]. A multitude of evidence suggests that HDL could be an important modulator of macrophages in sepsis. Nardo et al. found that HDL could inhibit TLR-induced production of proinflammatory cytokines from macrophages. Mechanistically, HDL inhibits the transcription of proinflammatory genes in macrophages, and it is in an activating Transcription Factor 3 (ATF3)–dependent manner. ATF3 is a transcriptional repressor induced by HDL, resulting in suppression of the inflammatory response caused by macrophages in sepsis [44]. Moreover, HDL could regulate cholesterol efflux and TLR signaling of macrophages in sepsis. HDL promotes cholesterol efflux through ABCA1. ABCA1 deficiency contributes to increased surface expression of TLR4 in macrophages and an increased response to LPS.

Immunometabolism of macrophages is altered during sepsis. Macrophage immunometabolism has been widely studied in recent years. The phenotypes of macrophages include proinflammatory phenotypes of macrophages (M1 macrophages) and anti-inflammatory phenotypes of macrophages (M2 macrophages). It refers to the change in the immune function of macrophages, including anti-inflammatory and proinflammatory function, via affecting its metabolic stages. LPS-stimulated macrophages undergo metabolic reprogramming, switching from oxidative phosphorylation to glycolysis. The shift promotes the production of proinflammatory M1 macrophages [43]. Macrophage immunometabolism exerts its regulatory role in sepsis. For example, AMP-activated protein kinase (AMPK) is an essential modulator of regulation of OXPHOS in macrophages. AMPK activator exerted its protective effect on LPS-induced sepsis model in mice. Additionally, AMPK deficiency in macrophages promoted the development of sepsis in mice, due to increase in aerobic glycolysis of macrophages and release of HMGB1. The study showed that AMPK played its protective role in sepsis through regulating metabolic reprogramming in macrophages [45]. Targeting of macrophage immunometabolism is a therapeutic measure for sepsis. Sirtuin 1 inhibitor called EX-527 improved the pathogen clearance during sepsis via changing the glycolysis pathway in macrophages, providing a way to treat sepsis in its hypoinflammatory state [46]. It was worth noting that a previous study demonstrated that HDL could affect macrophage immunometabolism, resulting in M2 polarization. The previous study showed that HDL could shift monocyte-derived macrophages to M2 phenotype in mouse atherosclerotic plaques, and promoted regression of atherosclerosis [47]. These findings indicate that macrophage immunometabolism may be one of mechanisms of HDL in the treatment of sepsis. However, there is little research about the mechanism. In the future, more research is needed on the effect of HDL on macrophage immune metabolism in sepsis.

In the pathophysiological process of sepsis, HDL exerts a variety of protective effects. In addition to acting on pathogens, inhibition of macrophage inflammatory reaction, HDL also modulates endothelial function in sepsis. First, HDL was showed to inhibit the expression of adhesion molecules in endothelial cells. Cockerill et al. found that physiological concentrations of HDL inhibited the expression of leukocyte adhesion molecules on endothelial cells induced by TNF-α. The adhesion molecules included V-CAM-1, ICAM-1, and E-selectin. It was observed that HDL was effective to inhibit VCAM expression on endothelial cells in vitro only 5 min after TNF-α stimulation. Moreover, the inhibitory effect is cellular specific. HDL could not inhibit TNF-α-induced expression of ICAM-1 on human foreskin fibroblasts [48]. Michelle et al. found a similar phenomenon in a rat endotoxic shock model. Compared to control, LPS-induced rat pretreated with

reconstituted HDL expressed lower levels of P-selectin and intercellular adhesion molecule-1 in the renal glomerulus. The attenuation of adhesion molecule expression caused by rHDL was associated with reduced organ dysfunction [49]. The transcriptional mechanism by which HDL decreases the expression of endothelial adhesion molecules has been uncovered. Park et al. showed that HDL inhibited TNF–αinduced VCAM-1 expression on human umbilical vein endothelial cells (HUVECs) via transcription factor NF-kappaB. Reduced nuclear translocation and transactivation of NF-kappaB and AP-1 transcription factors were observed. The study revealed the regulatory function of HDL at transcriptional levels [50].

Moreover, HDL could activate the endothelial nitric oxide synthase (eNOS), beneficial to microvascular vasodilation and endothelium repair. eNOS is responsible for promoting the production of nitric oxide (NO). NO is a key modulator of inhibiting the adhesion of monocytes to the endothelium, inhibiting thrombosis and promoting vasodilation. Studies showed that HDL regulated NO-dependent endothelial diastolic by activating eNOS, and this process is in a SR-BI-dependent manner. Yuhanna et al. found that HDL stimulated eNOS in cultured endothelial cells. In wild-type mice, HDL enhanced endothelium- and NO-dependent relaxation in aortae. Whereas in SR-BI-null mice, the effect of HDL disappeared [51]. Furthermore, HDL-induced eNOS activation is mediated by src- and PI3 kinase-mediated signaling, resulting in parallel activation of Akt and MAP kinases [52]. The human study showed that NOS3 (E298D) SNP was associated with the development of human sepsis by impairing NO production by NOS3(eNOS) in the microcirculation [53]. We all know that there are three types of nitric oxide synthases: neuronal (nNOS), endothelial (eNOS), and inducible nitric oxide synthase (iNOS). However, iNOS-derived NO contributes to multiple organ dysfunction in sepsis [54].

Last, HDL prevented endothelial thrombotic activation. HDL effects on prostacyclin and cyclooxygenase-2 (Cox2). Norata et al. found that HDL promoted prostacyclin and Cox2 production, which are inhibitors of platelet activation [55]. Mechanistically, Liu showed that the effect is through the p38 MAPK, ERK1/2, and JAK2 pathways in an ABCA1-dependent manner in endothelial cells [56]. Furthermore, it was reported that HDL-induced Cox-2 expression and prostacyclin release in endothelial cells was associated with SR-BI-mediated PI3K-Akt-eNOS signaling [57]. On the other hand, HDL effects on tissue factor, and increased tissue factor expression on platelets and endothelial cells have been identified as an essential factor in the initiation of thrombus formation. Hema et al. found that rHDL inhibits thrombin-induced human endothelial tissue factor expression, and this process is mediated through inhibition of RhoA and activation of PI3K [58].

Totally, HDL plays essential roles in suppressing inflammatory response in macrophages and regulating endothelial dysfunction, which implies that HDL is a multiprotective factor against sepsis.

10.5 HDL in the Therapeutic Applications of Sepsis

Sepsis is gradually becoming a leading cause of death worldwide due to its high mortality. The treatment of sepsis is still a challenge. Although with general patient management, outcomes of patients with sepsis have certainly improved, the improvement does not attribute to any specific measure. Due to the phenomenon observed in clinical and experimental studies, like decreased HDL levels and dysfunctional HDL in inflammatory state of sepsis, or antipathogen and anti-inflammatory properties of HDL, HDL has been paid much attention to be potential therapeutic agents.

Since apolipoprotein A-I (apoA-I) is the major protein component of HDL, giving apoA-I is a way to increase HDL levels. ApoA-I-KO mice had lower circulating HDL levels, and were more susceptible to CLP-induced septic death. The survival of apoA-I-KO mice is 47.1%, whereas the survival of control mice is 76.7% ($p = 0.038$). On the contrary, using apoA-I

transgenic mice as a model for increasing plasma HDL levels, it was shown that the mortality of LPS-induced mice decreased. The study implies that apoA-I is important for HDL function, and infusion of apoA-I could be a potential therapy for sepsis [59].

Naked ApoA1 purified from human plasma is effective to both rat- and mouse-LPS-induced septic models. In a study, rats were given 5 mg/kg LPS to induce endotoxemia model, and then 10 mg/kg human apoA-I was injected i.p. The 5-day survival rate was observed. Not surprisingly, the administration of human apoA-I suppressed the TNF-α release and increased the 5-day survival rate from 0% to 90%. In another study, BALB/c mice were challenged with LPS, followed by human apoA-I intravenous (IV) infusion at 100 mg/kg. In result, 3-day survival rate and overall survival time increased in apoA-I administration group, and apoA-I inhibited LPS-induced increases in the IL-1β and TNF-α levels in serum [60]. These studies suggested that human ApoA-I could effectively protect against LPS-induced endotoxemia.

ApoA1 Milano is a natural variant of human ApoA1. Individuals with ApoA1 Milano have lower risk of cardiovascular diseases than with wide-type ApoA1, although their plasma HDL levels are lower. Recently, there have been increasing interest in the therapeutic efficacy of ApoA1 Milano in many inflammatory-based diseases, such as atherosclerosis, inflammatory bowel diseases, and obesity [61]. Since sepsis is also an inflammatory-based disease, it seems that ApoA1 Milano could be an effective agent to treat sepsis. Animal experiments suggest that recombinant high-density lipoprotein (rHDL) with apoA-I (Milano) could be useful for the treatment of sepsis. In order to investigate the role of rHDL reconstituted with apoAI Milano ($rHDL_M$) on sepsis, Zhang et al. used endotoxin-challenged rat models and found that pretreatment with $rHDL_M$ at 40 mg/kg was effective against inflammation, significantly decreasing inflammatory cytokines TNF-α, IL-1β, and IL-6. Moreover, administration of $rHDL_M$ protected LPS-induced rats from multiorgan dysfunction [62].

Additionally, synthetic apoA-I mimetic peptide is also widely studied in sepsis. 18A Peptide (DWLKAFYDKVAEKLKEAF) is the first synthetic apoA-I mimetic peptide. Levine et al. found that prophylactic infusion of 18A sHDL increased the survival rate three- to four-fold, in comparison with saline-treated control LPS-induced mice [63]. Another synthetic apoA-I mimetic peptide 4F, derived from 18A, also showed protective effect on sepsis. Zhang et al. showed that 4F administered by IP injection is efficacious in cecal ligation and puncture (CLP)–induced rat sepsis model. CLP rats were received vehicle or 4F (10 mg/kg) by intraperitoneal injection, 6 h after sepsis induction. Compared to control, 4F treatment prevented the sepsis-induced reduction in plasma HDL, increased plasma IL-6, improved cardiac output, and reduced mortality in CLP rats [64]. Moreira et al. found that 4F could attenuate kidney and heart injury in a rat model of sepsis, inhibiting inflammatory responses and preventing endothelial dysfunction. The protective function was associated with increased plasma HDL [65]. Moreover, Woon et al. suggested that 4F attenuated acute lung injury and improved survival in an LPS-induced rat model. This was associated with the stimulation of S1P1, the activation of Akt, the downregulation of the NF-κB pathway, and the suppression of cell adhesion molecules [66]. Notably, 4F is much closer to clinical application, because it is administered after infection, which is more analogous to the clinical situation.

Multiple animal experimental studies showed that infusion of reconstituted HDLs (rHDLs) could be an effective therapy in septic models. CSL-111is an rHDL, made of purified human ApoA1 and soybean phosphatidylcholine (1:2). Tanaka et al. assessed the effects of CSL-111 intravenous injection in three models of sepsis. In CLP-induced mice, CSL-111 or saline solution was administrated 2 h after the sepsis. Compared to saline treatment, CSL-111 treatment significantly improved survival (81% VS 38%). The other two models are induced by intraperitoneal injection of *Escherichia coli* or *Pseudomonas aeruginosa* pneumonia, and CSL-111 or saline solution was also administrated after that. The

survival rate of saline treatment is 0%, whereas the survival rate of CSL-111 treatment is 40%, which is consistent with the CLP-induced model. Moreover, in all models, inflammation markers in both plasma and organs reduced and bacterial count decreased. This suggested the therapeutic potential of CSL-111 (rHDL) in experimental sepsis [67].

rHDL has been tested in human endotoxemia. In a double-blind, randomized, placebo-controlled, cross-over study, 8 healthy male volunteers were included. rHDL was given as a 4-h infusion at 40 mg/kg before endotoxin challenge (4 ng/kg). As a result, infusion of rHDL elevated HDL levels, reduced endotoxin-induced clinical symptoms, and inhibited endotoxin-induced inflammatory response, such as reduced plasma cytokine levels of TNF-α, IL-6, and reduced monocyte CD14expression [68]. Additionally, rHDL also has differential effects during human endotoxemia. In this study, eight healthy male volunteers were injected with LPS (4 ng/kg) to induce human endotoxemia model. rHDL (40 mg/kg,) or placebo was given as a 4 h infusion starting 3.5 h prior to LPS injection. rHDL treatment significantly reduced activation of coagulation and the fibrinolytic activity, which indicated that rHDL infusion ameliorated human endotoxemia through reducing collagen-induced platelet aggregation [69]. Although there has not been a clinical study using rHDL in septic patients yet, previous studies have shown encouraging results, and the studies of rHDL in human endotoxemia imply that rHDL could be a therapeutic potential in septic patients.

In summary, considering that there is increasing number of researches on rHDL and cardiovascular diseases, our understanding of rHDL or apoA-I mimetic peptides would be improved. Both clinical and experimental studies have shown encouraging result of rHDLs or apoA-I mimetic peptides, which make them promising therapeutic potentials in sepsis. However, further efforts are needed to study the relationship between the properties and the composition of rHDL to use them as better treatment for sepsis in the future.

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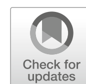

11 HDL Mimetic Peptides

Jie Chen, Jiewen Liu, and Baoqi Yu

Abstract

Since therapeutic strategies designed to raise HDL failed to exert the expected effective cardiovascular disease (CVD) outcomes in clinical trials, how to improve HDL function rather than its plasma level has become a new focus of scientists' attention. Numerous HDL mimetic peptides have been designed and investigated in various animal models in recent years. Although the underlying mechanisms are not fully understood, the peptides' antiatherogenic effects, such as acceleration of RCT and improvement of natural HDL function without necessarily raising its level, showed a promising therapeutic role in the prevention of atherosclerosis and other diseases. This chapter reviews recent studies on the roles and potentials of HDL mimetic peptides in atherosclerosis-related CVD.

Keywords

Cardiovascular disease · HDL · Mimetic peptide · Reverse cholesterol transport

11.1 Introduction

As the leading cause of mortality, 50% of the population in western countries died from cardiovascular disease (CVD) [1], and the number of people with CVD is also going up in developing countries. Atherosclerosis, mainly caused by high plasma lipid levels, is the most common expression of CVD. Accordingly, the application of lipid-lowering drugs is a main strategy to reduce the risk of CVD [2].

High-density lipoprotein (HDL) plays a role in protecting against atherosclerotic development through various mechanisms, including the reverse transport of the excess cholesterol from macrophages and the inhibition of the oxidative modification of LDL [3]. There is considerable evidence demonstrating that the plasma level of HDL is negatively correlated with CVD incidence [4].

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Despite the important role of HDL in maintaining cardiovascular health, a series of clinical trials designed to raise HDL in human failed to show the expected effects on CVD patients' outcomes [5–7]. Similarly, other agents containing niacin, fibrates, or statins showed inapparent cardiovascular benefit in clinical trials [8–11]. These frustrating results suggest that simply elevating the plasma level of HDL is insufficient to protect against CVD. As a result, more attention has been paid to the improvement of HDL functionality rather than their plasma levels [4, 7].

Recently, numerous mimetics of HDL and its components showed promise and were dramatically effective in animal models [12]. Without necessarily raising HDL plasma levels, they can stimulate reverse cholesterol transport (RCT) and improve HDL functionality. Thus, they offer a different approach to protect against atherosclerosis, which also provides an extensive application in other diseases beyond atherosclerosis [13].

11.2 ApoA-I Mimetic Peptides

ApoA-I, the major structural and functional protein with 243 amino acid residues in HDL, exerts atheroprotective function in various aspects, including promoting cholesterol efflux and participating in RCT [14]. In animal models and human clinical trials, apoA-I showed promising roles in amelioration of atherosclerosis [15–17]. However, apoA-I, as a large protein, requires parenteral administration and is expensive in clinical practice, which makes it challengeable for large-scale clinical application [17, 18]. Thus, it seems shorter peptides that can mimic apoA-I would provide more efficacious therapeutic strategies [9].

There are 10 amphipathic α-helices in mature apoA-I and each one has both hydrophobic face and hydrophilic face [14]. This structure is essential for apoA-I to bind lipid and promote cholesterol efflux. Many peptides are designed based on this feature of the amphipathic α-helices to mimic apoA-I functions. And next, we will introduce the main mimetic peptides in chronological order.

11.2.1 Peptide I and II

Based on the opinion that the fragments of a protein may exert some of the properties of the native protein itself, the first mimetic peptide of apoA-I, called peptide I, was designed by Kaiser and Kezdy in 1979 [19]. The peptide I corresponds to the residues 158–168 of apoA-I, which are 11 amino acids repeating units. They used hen egg lecithin single bilayer vesicles to examine the lipid-binding ability of peptide I by measuring free and bound peptide, and found peptide I bound very poorly to the vesicles.

In another experiment, Kaiser and Kezdy designed a new peptide called peptide II corresponding to residues 147–168 of apoA-I, the fragment of apoA-I, which has the highest helix-forming potential [20] and a terminal Pro was included. Peptide II was also tested for its ability of binding to vesicles, and it was found to bind more tightly than peptide I, suggesting that it is possible to improve the peptide's binding ability to phospholipid surfaces [17, 20].

11.2.2 18A

In order to determine the meaning of the number and specific location of charged residues in the amphipathic domains of plasma lipoproteins on protein lipid binding, Segrest et al. [21] designed 18A in 1980, a series of peptides with only 18 amino acid residues forming a class A amphipathic helix. Their sequences have no homology to any of the natural proteins or peptide sequences, and two of them (18Aa,18As) associated tightly with liposomes and the others failed to mimic lipid-binding properties of apoA-I [8, 17, 22, 23]. This result revealed that the appropriate charge distribution of peptides is important for their lipid affinity.

11.2.3 2F

Scientists continue to modify 18A to obtain new peptides, which can better mimic apoA-I in its

antiatherogenic effects. By adding an acetyl group at the amino terminus and an amide at the carboxyl terminus, Venkatachalapathi et al [24] obtained Ac-18A-NH2 in 1993, which also is named 2F for the two phenylalanine residues on the hydrophobic face. It showed improved lipid-binding abilities and efficiency in inducing cholesterol efflux but failed to reduce lesions in a mouse model of atherosclerosis [4, 17, 23].

11.2.4 4F

4F, as the most studied peptide [23], has four phenylalanine residues at the hydrophobic end, which showed optimal hydrophobicity when interacting with phospholipids in the form of a discoidal HDL-like structures [8]. It can activate lecithin:cholesterol acyltransferase (LCAT) at 50% efficiency of apoA-I and inhibited LDL-induced monocyte chemotactic activity [25]. 4F can promote cholesterol efflux to improve pathological process, and also exert multiple functions, including anti-inflammation, anti-oxidation, and anti-thrombosis in many animal models [26].

Many studies demonstrated that 4F is an atheroprotective peptide [27]. The early nascent atherosclerosis in ApoE$^{-/-}$and Ldlr$^{-/-}$mice was reduced after the intraperitoneal administration of 4F peptide [28]. 4Fs synthesized from L or D amino acids are called L-4F or D-4F, respectively. L-4F was rapidly degraded in the circulation and excreted in the urine, and oral administration of D-4F alleviated atherosclerosis development by 79% in Ldlr$^{-/-}$ mice [29] and 50% in diabetic ApoE$^{-/-}$ mice [30]. This marked atheroprotective effect was independent of rise in total HDL level [29].

The anti-inflammatory effects of 4F include reduction of lipid hydroperoxides, paraoxonase levels, and HDL inflammatory index(HII) [23, 31]. One study manifested that compared to apoA-I, L-4F can bind to part of oxidized lipids with higher affinity and remove them from plasma [32]. The binding of 4F to oxidized lipids, which are proinflammatory, might account for its prominent anti-inflammatory functions [23]. Another study showed that the treatment of D-4F in mice promoted pre-β HDL particles formation and improved the efflux of cholesterol to HDL [33].

Furthermore, 4F can increase NO production and diminish oxidative stress. It has been revealed that 4F could improve the migration of endothelial cells and significantly increase endothelial nitric oxide synthase (eNOS) activity via AKT phosphorylation, and SR-B1 expression [26]. It also reduced the number of lipid rafts and the expression of Toll-like receptor 4 on macrophages surface (thus restoring eNOS expression) and upregulated vascular endothelial growth factor expression [34, 35]. A study by Graham et al. [36] found that the products of lipid oxidation suppressed endothelial cells migration in vitro, and preincubation with L-4F could reverse this effect on endothelial cells migration. Treatment with D-4F accelerated re-endothelialization in a mice model with high cholesterol diet. Furtherly, 4F could improve the inhibited endothelial cells migration by lysoPC treatment in vitro [26], which suggested that 4F was able to promote the migration of endothelial cells and protect endothelium from injury in CVD.

ApoA-I is prone to oxidative modification [37], which can change HDL from anti-inflammatory into pro-inflammatory [38]. Anantharamaiah et al. [38] showed that 4F was able to protect the tyrosine residues in apoA-I from oxidation. Furthermore, 4F enhanced the cholesterol efflux ability of apoA-I to a greater extent. Compared with HDL isolated from saline-treated mice, HDL from mice treated with D-4F was more effective in inhibiting LDL oxidation [29]. He et al. [26] found that 4F could improve the impaired function of HDL in re-endothelialization and endothelial cells proliferation, migration, and lamellipodia formation and further improve the repair of endothelium. 4F appeared to work through SR-B1 and induce the phosphorylation of eNOS and Akt. When given together with a statin, 4F significantly alleviated lesion formation in young ApoE$^{-/-}$ mice, and reduction of existing lesions in old ApoE$^{-/-}$ mice was also observed, and apoA-I

synthesis in intestine increased by 60% [18]. In studies using Cynomolgus monkeys [39], oral administration of D-4F in addition to pravastatin also repaired the anti-inflammatory effects of HDL. Above researches suggested that the combination of 4F with statin might be a promising anti-atherosclerosis treatment strategy.

11.2.5 FAMP

Fukuoka University ApoA-I-mimetic peptide (FAMP), developed in 2013, consists of 24 amino acids corresponding to residues 196–219 of the apoA-I. FAMP formed an amphipathic α-helical conformation in solution [40]. This peptide significantly enhanced the function of HDL and reduced aortic plaques formation by 48.2% in ApoE$^{-/-}$ mice fed with a high-fat diet [41]. FAMP can effectively remove cholesterol through the ABCA1 and ABCG1 transporters, generate nascent pre-β HDL particles, and further transform HDL into small HDL particles, which enhances HDL biological functions to inhibit atherosclerotic plaque formation without increasing HDL cholesterol levels [40, 42, 43]. Besides its therapeutic potential, FAMP is also promising in the diagnosis of atherosclerotic plaque development [44]. It can be used to trace the lipid burden in atherosclerosis plaques after specific modifications in clinic by its ability to penetrate atherosclerotic plaques.

11.2.6 Bihelical Peptide

In addition to monohelical peptides, tandem amphipathic α-helical peptides have also been studied [14]. 37pA, a peptide with two 18A peptides and a single proline residue (18A-PRO-18A), promoted macrophage cholesterol efflux through both ABCA1-dependent and ABCA1-independent mechanisms [45]. 37pA exerted protective effects on cardiac function by reducing TNF-α-induced adhesion molecules expression on endothelial cells in an ischemia-reperfusion injury model [46]. Moreover, 37pA can be applied in HDL-based MRI-targeted contrast agents for atherosclerotic plaque composition detection [47].

mFc-2X4, a peptide fusing two tandem repeats of 4F to the C-terminal of a murine IgG Fc fragment [48], could promote cholesterol efflux better than 4F and apoA-I in a dose-dependent manner in vitro. And it was also able to form nascent HDL particles in vitro [23].

ELK-2A2K2E is another kind of bihelical peptide that induces small dense HDL particles formation in ApoE$^{-/-}$ mice plasma. It induced cholesterol efflux efficiently and led to a reduction in aortic lesion area. ELK-2A2K2E can also apparently reduce the levels of lipoprotein and triglyceride in plasma [8, 49].

Besides symmetric amphipathic, there are also studies about asymmetric tandem peptides. Remaley et al. [50] designed a peptide by substituting five hydrophobic residues in one of the 18A helices in 37pA to Ala, and named this bihelical amphipathic peptide as 5A [8]. 5A promoted cholesterol efflux more efficiently than 37pA [14]. In different disease models, 5A was demonstrated to exert the properties of reducing atherosclerotic lesion formation and attenuating inflammation [51–53].

11.2.7 2F *

Owing to nonspecific cellular cholesterol efflux, helical peptides would induce membrane lipid solubilization and hemolysis. To avoid these side effects, Okuhira et al. [54] inserted the photocleavable 4,5-dimethoxy-2-nitrobenzyl group at the glutamic acid in the peptide 2F and developed a photo-activatable peptide with 18 amino acid, named 2F*. After light stimulation, 2F* could form an α-helical structure and trigger cells cholesterol efflux. And the ability of 2F* to form α-helices was limited without light stimulation, so it could not bind to lipids to induce cholesterol efflux [44]. This makes it possible to control cholesterol efflux at the site of atherosclerosis lesions and avoid systemic hemolysis after mimetic peptide administration by a light stimulus.

11.2.8 P12

Recently, Jingman et al. [55] developed a new apoA-I mimetic peptide named P12. They designed and synthesized 17 new apoA-I mimetic peptides by gradually increasing their hydrophobicity based on apoA-I 221–240, which is essential for cholesterol efflux and ABCA1 binding. Among them, P12, a lipid-free 20-amino acid AMP featuring an amphiphilic α-helix structure, is found to possess the lowest cytotoxicity and the greatest effects on cholesterol efflux. It significantly reduced the relative intimal thickening and atherosclerosis plaque size in $ApoE^{-/-}$ mice via its lipid-lowering, anti-inflammatory, and anti-oxidative effects. Moreover, P12 prevented lipid accumulation and peroxidation as well as decreased HDL levels in blood vessels of $ApoE^{-/-}$ mice. It also reconstructed α-HDL into nascent preβ-HDL particles to further promote RCT process.

11.2.9 Reconstituted HDL

Reconstituted HDL (rHDL) particles are made by self-assembly of various lipids and apoA-I [56]. They possess main anti-atherogenic capacities of native HDL, such as cholesterol efflux, anti-inflammation, anti-oxidation, cytoprotection, vasodilation, anti-infection, anti-thrombosis, anti-diabetes, and so on [57]. The ability of loading various substances, like drugs, nucleic acids, signal-emitting molecules, or dyes, makes rHDL an efficient nanocarrier for therapeutic applications or medical diagnosis. As a substitute of full-length apoA-I, the mimetic peptides are used to synthesize rHDL particles, because the cost is lower for purification or recombination of apoA-I mimetic peptides in HDL therapy [57, 58]. On the other hand, some apoA-I mimetic peptides even exert a higher affinity with oxidized phospholipids and fatty acids than apoA-I [56, 59]. Nanodisc HDL particles synthesized from apoA-I mimetic peptides can ameliorate atherosclerosis by promoting cholesterol and phospholipid efflux through ABCA1 transporter-related mechanism similar to native HDL [50, 51, 56, 60]. In clinical diagnosis, rHDL particles followed by different modifications can also be used for imaging of atherosclerotic plaques [56].

11.2.10 Oral Applications

Animal studies suggested that it does not matter which approach, such as injection, infusion, or oral administration, was performed to give an apoA-I mimic in vivo, as long as the enterocytes receive a certain dose of the peptides [61]. Zhao et al. [9] demonstrated that plasma exposure to D-4F was unrelated to its ability to treat atherosclerosis. Rader et al. [61] found that oral D-4F peptide dose could predict HII suppression, whereas plasma D-4F concentration was dissociated with it. These findings indicated that the intestine is the major region where apoA-I mimetic peptides take action. One study demonstrated that D-4F might inhibit inflammation in the villi, thereby suppressing the formation of dysfunctional HDL and inducing transintestinal cholesterol efflux [61]. In another study, D-4F was found to reduce levels of arachidonic and linoleic acids free metabolites in the small intestine, which was associated with enhanced PON1 activity and decreased inflammation in $Ldlr^{-/-}$ mice [18]. Fogelman et al. reported that the L-4F-niclosamide complex, formed by tight binding of niclosamide and L-4F, could protect peptide from trypsin digestion in the small intestine and obviously improve HII and reduce aortic lesion and macrophage lesion area [23, 62].

Another promising preclinical study is on 6F, which exerts powerful anti-inflammatory, antioxidant, and antiatherogenic effects in $Ldlr^{-/-}$ mice. Specifically, 6F prevented Western Diet(WD)–induced systemic inflammation and dyslipidemia by alleviating the increase of unsaturated LPA levels in the small intestine [56, 63–65].

11.3 ApoE Mimetic Peptide

It has been widely believed that apoE is an attractive therapeutic target in cardiovascular disease from both preclinical and clinical evaluations. Unlike the RCT stimulation of apoA-I mimetics, apoE mimetics exert an antiatherogenic function in hepatic clearance of circulating atherogenic lipoproteins via interaction with lipoprotein receptors and enhancement of macrophages cholesterol efflux [2, 66]. Usually, apoE mimetic peptides can mimic the dual-domain structure of the native human apoE(hapoE), and therefore contain both the LDLR-binding domain and the lipid-associating domains of hapoE [66]. The former is indispensable for the hepatic uptake of triglyceride-rich lipoprotein remnants and their clearance from the circulation, whereas the latter mediates lipid efflux [66–68].

11.3.1 Ac-hE18A-NH2

Ac-hE18A-NH_2 is a dual-domain peptide composed of a putative receptor-binding domain of apoE linked to a lipid-associated peptide 18A [66, 67, 69]. It targeted chylomicron remnants, VLDL-C, and LDL-C to hepatocytes for clearance via binding to HSPG [8, 70, 71]. It is reported that Ac-hE18A-NH_2 administered intravenously remarkably reduced plasma VLDL and LDL levels in different models [72, 73]. This peptide could increase phospholipid affinity [73] and form phospholipid-enriched particles to mediate cellular cholesterol efflux efficiently [23]. Furthermore, Ac-hE18A-NH_2 could also induce paraoxonase 1(PON1) and preβ-HDL particles release from hepatocytes [67]. Like apoE, Ac-hE18A-NH_2 can reduce plasma cholesterol but exhibit anti-inflammatory properties independent of its cholesterol-lowering effect [69].

11.3.2 ATI-5261

In 2010, Azhar et al. [74] created a novel single-helix 26-mer apoE mimetic peptide named ATI-5261, which stimulated cellular cholesterol efflux from lipid-loaded macrophages with high efficiency to reduce atherosclerosis in mice [14, 23, 75]. As an ABCA1 agonist, ATI-5261 competed with apoA-I for ABCA1 and promoted efficient ABCA1-mediated cellular lipid efflux [75]. Functional HDL-ATI-5261 lipoprotein particles were generated by the combination of the peptide with ABCA1 and plasma membrane microdomains [76]. However, in C57BL/6 mice, ATI-5261 was found to cause muscle toxicity and increase triglyceride (Tg) levels due to aromatic phenylalanine residues on the hydrophobic face of the peptide, together with positively charged arginine residues at the lipid-water interface [76].

11.3.3 Cs-6253

Due to the side effects of ATI-5261, a variant of this peptide, named CS-6253, was designed by substituting phenylalanine and arginine for leucine and citrulline residues, respectively [14]. CS6253 exerted the equal anti-atherosclerotic properties of its precursor, but without toxicity in vivo [76, 77]. This peptide-mediated ABCA1 cholesterol efflux potently and stabilized ABCA1 concentration on cell surface [78]. Lipid-free CS-6253 interacted with ABCA1 and formed nascent HDL-CS-6253 particles, which matured after recombination in plasma and then delivered cholesterol to hepatocytes via SR-BI in vitro. The addition of CS-6253 in plasma dose-dependently remodeled α-HDL into nascent preβ-HDL particles that stimulated ABCA1-dependent cholesterol efflux efficiently. CS-6253 can also promote the transfer of cholesterol from HDL to LDL by binding with both of them in human plasma [76].

11.4 Other Mimetic Peptides

Although most researches have investigated the effects of apoA-I and apoE-derived peptides, other apolipoprotein mimetic peptides, such as apolipoprotein C-II (apoC-II) or apolipoprotein J

(apoJ) mimetic peptides, have also been studied [2].

D-[113–122]apoJ, a 10-residue apoJ mimetic peptide, can inhibit LDL aggregation in vitro [79] and retard atherosclerosis in ApoE$^{-/-}$ mice [2]. It enhanced the antioxidant and cholesterol efflux effects of HDL, and reduced hepatic inflammatory genes expression [2]. As it can simultaneously take effects on both HDL and LDL, D-[113–122]apoJ would be a promising peptide against atherosclerosis.

C-II-a is an apoC-II mimetic peptide. ApoC-II deficiency in human can lead to hypertriglyceridemia. Injection of C-II-a was demonstrated to reduce plasma TG, indicating that C-II-a could be a potential new therapy for apoC-II deficiency–related diseases [80, 81].

11.5 Effects of HDL Mimetic Peptides on Other Diseases

HDL mimetic peptides can also be used in the treatment of other diseases besides atherosclerotic cardiovascular disease [82].

ApoA-I mimetic peptide 5A altered the protein composition of HDL in the circulation and improved some potentially harmful changes to the protein component in a mouse model of age-related macular degeneration (AMD). HDL mimetic peptide HM-10/10 was designed to reduce oxidative stress, which also played a key role in AMD development [83].

Dual-domain peptides AEM-28, AEM-28-2, and HM-10/10 were found to inhibit cell viability of mouse or human ovarian and colon cancer cell lines, and therefore decrease tumor burden, tumor growth, and tumor dissemination in BALB/C mice [84].

L-4F ameliorated insulin resistance and diabetes by reducing adiposity, improving insulin sensitivity and glucose tolerance, increasing plasma adiponectin, and reducing IL-1β and IL-6 levels in obese mice [85]. Similarly, D-4F dramatically reduced white fat mass, slightly improved insulin sensitivity, and enhanced energy expenditure in mice [56, 86].

In addition to circulating lipid homeostasis, apoE also plays a key role in cerebral lipid homeostasis. COG133, an apoE mimetic peptide that comprises the apoE LDLR-binding domain, could improve clinical dysfunction and inflammatory infiltration of the spinal cord in a murine model of multiple sclerosis. COG112, a modified COG133, showed high therapeutic efficacy in autoimmune encephalomyelitis and traumatic spinal cord injury mouse models [66].

The lack of apoA-I increased airway hyperresponsiveness and the infiltration of inflammatory cells into lungs after stimulation [87]. ApoA-I mimetic peptides have been found to play a protective role in asthma models [88]. ApoA-I mimetic peptide could reduce neutrophils and eosinophils in airway inflammation, and reduce airway hyperresponsiveness and mucocyte metaplasia in allergic asthma mouse model [89]. 5A attenuated the development of airway inflammation and hyperresponsiveness in house dust mite (HDM)–induced asthma mouse model [53].

11.6 Conclusion

HDL mimetic peptides have a simple structure that can be manufactured with a lower cost compared with the complete lipoprotein. They exert anti-atherosclerotic effects in atherosclerotic cardiovascular disease as well as other diseases, which offers incredible opportunities in drug design. However, more researches are needed to better investigate the underlying mechanisms and prove the feasibility of using these peptides clinically.

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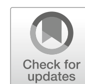

12 HDL and microRNAs

Hongtu Cui, Kaixuan Lv, and Nana Yang

Abstract

In previous chapters, we know that high-density lipoproteins (HDLs) could act at multiple cell lines and then trigger intracellular molecular pathway to prevent several metabolic diseases. Besides the classic genes regulating cholesterol efflux and reverse cholesterol transport (RCT), microRNAs (miRNAs) could also affect HDLs biogenesis, metabolism, and functions. This chapter summarizes the miRNAs, which regulate HDLs functions in table. In addition, HDLs are good vectors for miRNAs. They could carry miRNAs in circulation and take them into several cells such as macrophages and endothelial cells. Complete understanding of the miRNAs associated with HDL regulation would give us broader insights to prevent and treat metabolic diseases.

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Keywords

HDLs · microRNAs · Circulating microRNAs · Metabolic diseases

MiRNAs are small, noncoding RNAs that consist of 21–30 bases. MiRNAs play an important role in pretranscriptional, cotranscriptional, and post-transcriptional gene expression control. Among all kinds of noncoding RNAs, miRNAs are the most widely researched and play a regulatory role in numerous biological processes.

The primary miRNA transcripts are generated from miRNA gene transcription, and then the pri-miRNA is processed to form the miRNA precursors with stem loop structure. Finally, under the action of Dicer digestion, the mature miRNA is generated from the pre-miRNA. miRNAs can bind to the 3′ untranslated region (UTR) of the target mRNA and suppress the expression of gene through mRNA degradation or translation inhibition.

In the past year, the importance of miRNAs in regulating HDLs biogenesis, metabolism, and functions has emerged (Table 12.1). Next, we discuss how these miRNAs can regulate the key genes in these pathways and what the content of these miRNAs are regulated by. With the help of these findings, we can explore the new treatment of metabolic diseases, such as atherosclerosis and diabetes.

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Table 12.1 MiRNAs regulate HDLs biogenesis, cholesterol efflux, and HDL-C uptake

miRNA	Cell type	Target gene	Biological pathway	Related disease	Reference
miR-33	Hepatocyte	ABCA1	HDLs biogenesis	AS	[1, 2]
	Macrophage	ABCA1	Cholesterol efflux	AS	[1, 2]
	Macrophage	ABCA1/ ABCG1	Cholesterol efflux	AS	[17]
miR-758	Hepatocyte	ABCA1	HDLs biogenesis	AS	[5]
	Macrophage	ABCA1	Cholesterol efflux	AS	[5]
miR-302a	Hepatocyte	ABCA1	HDLs biogenesis	NAFLD, AS	[3, 4]
	Macrophage	ABCA1	Cholesterol efflux	AS	[4, 7]
miR-20a/b	Hepatocyte	ABCA1	HDLs biogenesis	AS	[6]
	Macrophage	ABCA1	Cholesterol efflux	AS	[6]
miR-26	Hepatocyte	ABCA1	HDLs biogenesis	AS	[7]
	Macrophage	ABCA1	Cholesterol efflux	AS	[7]
miR-144	Hepatocyte	ABCA1	Cholesterol efflux	AS	[8]
miR-128-2	Hepatocyte	ABCA1/ ABCG1	HDLs biogenesis/ cholesterol efflux	AS	[9]
miR-223	Hepatocyte	ABCA1	HDLs biogenesis/ cholesterol efflux	AS	[13]
	Hepatocyte	SR-BI	HDL-cholesterol uptake	AS	[12, 13]
miR-185	Hepatocyte	SR-BI	HDL-cholesterol uptake	AS	[12]
	Macrophage	SR-BI	HDL-cholesterol uptake	AS	[12]
miR-96	Hepatocyte	SR-BI	HDL-cholesterol uptake	AS	[12]
	Macrophage	SR-BI	HDL-cholesterol uptake	AS	[12]
miR-145	Hepatocyte	ABCA1	HDLs biogenesis, cholesterol efflux	Diabetes	[16]
miR-143&miR-145	Hepatocyte	ABCA1	HDLs biogenesis	AS	[26]
miR-125a	Hepatocyte	SR-BI	HDL-cholesterol uptake	Cholesterol metabolism	[14]
miR-455	Hepatocyte	SR-BI	HDL-cholesterol uptake	Cholesterol metabolism	[14]
miR-24	Hepatocyte	SR-BI	HDL-cholesterol uptake	AS	[11]
	Macrophage	SR-BI	HDL-cholesterol uptake	AS	[11]
miR-21	Macrophage	ABCG1	Cholesterol efflux	AS	[24]
miR-33b-5p	Macrophage	ABCA1	Cholesterol efflux	AS	[25]
miR-23a-5p	Macrophage	ABCA1/ ABCG1	Cholesterol efflux	AS	[20]
miR-10b	Macrophage	ABCA1/ ABCG1	Cholesterol efflux	AS	[22, 23]
miR-19b	Macrophage	ABCA1	Cholesterol efflux	AS	[21]
miR-96-5p	Caco-2/TC7	SR-BI	HDL-cholesterol uptake	Type 2 diabetes	[31]
miR-9-5p	Macrophage	ABCA1	Cholesterol efflux	AS	[15]

NAFLD nonalcoholic fatty liver disease, *AS* atherosclerosis, *SR-BI* hepatic scavenger receptor class B type 1, *ABCA(G)1* ATP-binding cassette transporter A(G)1

12.1 miRNA Regulation of HDLs Biogenesis

HDLs are divided into discoidal HDLs and spherical HDLs during the process of HDLs biogenesis. Apolipoprotein A-I (ApoA-I) synthesized mainly in the liver interacts with ATP-binding cassette transporter A1 (ABCA1) to generate discoidal HDLs with phospholipids and unesterified cholesterol. Phospholipids in discoidal HDLs are hydrolyzed by cholesterol

acyltransferase (LCAT) to generate fatty acyl groups, which are transferred to unesterified cholesterol and generated cholesteryl esters. Cholesteryl esters partition into the center of the discoidal HDLs, which are converted into spherical HDLs. Therefore, ABCA1 is a major regulator of HDLs biogenesis.

MiR-33 is the most widely researched miRNAs and the mechanisms of miRNAs are complicated. miR-33a/b is coded by the introns of the sterol regulatory element–binding proteins (SREBPs) gene. In humans, miR-33a is present in intron 16 of the SREBP-2 gene on chromosome 22 and miR-33b is in intron 17 of the SREBP-1 gene on chromosome 17. miR-33a/b downregulates the expression of ABCA1 through targeting the 3′-UTR of ABCA1. More importantly, the inhibition of miR-33a can maintain the levels of ABCA1, thus increasing HDL cholesterol in vivo [1]. To further investigate the effect of miR-33, several studies have been conducted. Anti-miR33 treatment can increase ABCA1 levels in the liver, downregulate several inflammation genes, and inhibit the formation of atherosclerosis and increase the stability of atherosclerotic plaque in mice models [2]. However long-term inhibition of miR-33 results in high levels of circulating triglyceride (TG) and lipid accumulation in liver. Long-term treated with miR-33 antisense oligonucleotide (ASO) in the mice fed with high-fat diet causes the increased expression levels of genes associated with cholesterol and fatty acid synthesis, thus resulting in hepatic steatosis. MiR-302a levels in hepatocytes are decreased in western-type diet LDLR−/− mice compared with chow diet group, and the relative expression of ABCA1 targeted by miR-302 is increased [3]. Anti-miR302 to high fat diet induced mice increases liver ABCA1 levels as well as circulating plasma HDLs levels [4]. MiR-758 levels are found to be increased in the liver of mice fed with a high fat diet, and it can regulate posttranscriptional ABCA1 levels [5]. MiR-20a/b-treated ApoE−/− mice decreases ABCA1 levels in the liver and HDL cholesterol (HDL-C) levels in plasma, and inhibits RCT in vivo to promote atherosclerotic development [6]. Using LDL to mimic the high fat dietary, the results show that miR-26 expression is diminished in LDL-loaded HepG2 compared with no LDL-loaded HepG2, while its targeting ABCA1 levels are upregulated, which implies that miR-26 might regulate HDLs biogenesis [7].

Activation of Farnesoid-X-Receptor (FXR) by GSK2324, a water-soluble FXR agonist, can upregulate hepatic expression of miR-144. MiR-144 can target 2 binding sites in ABCA1 gene to decrease hepatic ABCA1 expression. Overexpression of miR-144 in mice decreases hepatic ABCA1 levels and the level of plasm HDL-C. Downregulation of miR-144 in mice increases the level of ABCA1 in hepatocytes and the plasma HDL-C in vivo. Further, researchers proved the regulation of miR-144 and FXR-dependent hypolipidemia requires hepatic, but not intestinal, FXR through tissue-specific FXR-deficient mice [8].

Regulation of the ability of cholesterol homeostasis is related to metabolic disease as well as multiple types of cancer. Overexpression of miR-128 induces apoptosis in HepG2 and MCF-7. MiR-128-2 increases the expression of SREBP2 and downregulates the level of SREBP1 in mRNA and protein levels. What is more, miR-128-2 directly targets ABCA1, ABCG1, and RXRα 3′UTR and downregulates the expression of these genes in an SIRT1-dependent and SIRT1-independent manner. The content of miR-128 influences the cholesterol efflux capacity. The expression of miR-128-2 in C57BL6 mice treated with a high-fat diet decreases significantly compared with control mice [9].

12.2 MiRNA Regulation of HDLs Metabolism

Hepatic scavenger receptor class B type 1 (SR-B1) is important to selective HDL-C uptake, which is a critical step in RCT. Therefore, SR-B1 exerts atheroprotective effects through uptaking HDL-C. SR-B1 also promotes the bidirectional flux of free cholesterol (FC) and phospholipids between HDLs and cells. SR-BI is highly expressed in a high percentage of the

liver and the steroidogenic cells of the adrenal gland, ovary, and testis.

MiR-24 plays an important role in cardiovascular disease (CVD) progression. MiR-24 directly inhibits SR-B1 levels through targeting its 3′UTR. MiR-24 decreases HDLs uptake in HepG2 and THP-1 macrophages, and promotes atheromatous plaque formation [10]. Supplement of miR-24 suppresses HDLs uptake. Apply of miR-24 attenuates hepatic SR-B1 levels and promotes the progression of atherosclerosis in ApoE−/− mice [11].

The expression of SR-B1 in HepG2 cells is significantly decreased after slicing miR-185, miR-96, and miR-223. MiR-185, miR-96, and miR-223 can reduce the expression levels of SR-B1 through targeting the sites in SR-B1 3′UTR. Further, the binding sites of miR-185 (DEL1, 84 bp to 89 bp, and/or DEL2, 274 bp to 281 bp), and miR-96 (461 bp to 468 bp) are found through the bioinformatic miRNA target prediction and further verified through luciferase reporter assay. The ability of HDL-C uptake is decreased obviously after treated with mimic of these miRs, while using the antagonists of miRNAs significantly increases the uptake of HDL into HepG2 cells. Further, the expression of miR-96 and miR-185 is regulated by the levels of plasma cholesterol, and these 3 miRNAs can also regulate the expression of SR-B1 in macrophage [12].

What is more, the expression of miR-223 is closely related to intracellular cholesterol levels. After stimulated with LDL (100 ng/mL), the miR-223 gene expression increases significantly. miR-223 can reduce HDL-C intake and SR-B1 in Huh7 cells through the target the SR-B1 3′UTR. What else, methylsterol monooxygenase 1 (SC4MOL) and 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) are also the miR-223 targets in humans. Overexpression of miR-223 can suppress the expression of SC4MOL and HMGCS1; thus, miR-223 can repress cholesterol biosynthesis. Finally, miR-223-null mice have higher plasma (total) cholesterol levels compared with WT mice [13]. Transfection of the liver with pre-miR-125a reduces SR-B1 expression and the ability of HDL-C uptake [14].

12.3 MiRNA Regulation of HDLs Functions

Metabolic syndrome (MS) relates to atherosclerosis, fatty liver, type 2 diabetes, and cancer. MiRNAs, which play an important role in MS, promote the expression of inflammatory cytokines. MiRNAs can be potential targets in treating MS. The remarkable feature of MS is low HDLs and decreases capacity of RCT. The expression of ABCA1 in MS is reduced, and positively correlated with HDL-C ($P < 0.05$, $r = 0.3$), while being negatively correlated with body mass index (BMI) ($P < 0.01$, $r = 0.4$), insulin-resistance (HOMA-IR) ($P < 0.01$, $r = 0.4$), and TG ($P = 0.01$, $r = 0.3$) [15].

ABCA1 plays an important role in liver, macrophage, and pancreas. But the function of ABCA1 in these tissues is different. In liver, ABCA1 regulates the biosynthesis of HDLs. In macrophage, ABCA1 regulates cholesterol efflux, while in islets, ABCA1 improves glucose-stimulated insulin secretion [16]. Recent studies have shown that miRNAs take part in posttranscriptionally regulating ABCA1 expression and multiple cholesterol efflux pathway–related genes.

Older macrophages play an important role in age-associated diseases such as atherosclerosis and macular degeneration. The abnormal polarization in older macrophages is related to the reduction of ABCA1. The expression of ABCA1 is obviously decreased in aging macrophages. Aging-macrophage cholesterol efflux capacity is impaired and the expressions of proinflammatory factors, such as tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), IL1β, prostaglandin-endoperoxide synthase 2 (PTGS2), C-C motif chemokine ligand 2 (CCL2), and matrix metallopeptidase (MMP) 9, are significantly increased in old macrophages compared to young macrophages, whereas expressions of anti-inflammatory agents IL-10 and CD163 are significantly downregulated in old macrophages compared to young macrophages. The reduction of ABCA1 in aging macrophages is associated with the increase of miR-33. Knockout of the ABCA1 or

cholesterol-rich diet can accelerate a senescent macrophage phenotype and suppress the capacity to regulate pathological angiogenesis and vascular proliferation [17].

Macrophage energy metabolism plays an important role in the function of macrophages in atherosclerosis. Mitochondria take part in cholesterol efflux in macrophages and are predicted to be adjusted by miR-33. Anti-miR-33 de-repressed the expression of mitochondrial genes, such as peroxisome proliferator–activated receptor gamma coactivator 1 alpha (PGC-1α), pyruvate dehydrogenase kinase 4 (PDK4), or SLC25A25, in mouse peritoneal and human THP-1 macrophages. Anti-miR-33 treatment increases oxygen consumption rates and ATP production through regulation of multiple mitochondrial genes. As we know, anti-miR-33 promotes cholesterol efflux in macrophages. However, suppression of mitochondria ATP production restrains the phenomena of anti-miR-33 to promote cholesterol efflux. Anti-miR-33 treatment slows progression of the atherosclerosis independent of HDL in vivo. To further confirm their results, researchers detect the expression of related genes in atherosclerotic plaques from patients and control arteries. The expression of miR-33 (both copies, miR-33a and miR-33b) is obviously elevated in human carotid atherosclerotic plaques, and the expression of PGC-1α, SLC25A25, and SLC25A23, as well as in the indirect markers of mitochondrial biogenesis nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) are decreased [18].

Although suppression of miR-33 inhibits the progression of atherosclerosis, knockout of miR-33 results in the development of obesity and metabolic dysfunction. Researchers use CRISPR/CAS9 technology to produce the mice in which the 3 miR-33-binding sites with ABCA1 3′UTR are mutated to stop miR-33 binding. The capacity of cholesterol efflux is significantly increased and foam cells formation is decreased in ABCA1-binding site mutant mice. Moreover, interference of ABCA1-binding site can delay the progression of atherosclerosis without negative effects caused by deficiency of miR-33 [19].

There still are other miRNAs that can regulate the expression of ABCA1 except miR-33. It has been reported that the expression of miR-758 is high in mice, and treatment of high-fat diet can increase its levels in mice. MiR-758 can directly target the human ABCA1 3′UTR to inhibit the expression of ABCA1, thus suppressing expression of ABCA1 and cellular cholesterol efflux. MiR-758 is widely expressed in many tissues, particularly in brain, neurogliomas, and the astrocyte cell line CCF-STTG1. The expression of miR-758 is upregulated in mice fed with high-fat diet. Overexpression of miR-758 in glioblastoma H4 cells reduces the content of ABCA1 and inhibition of miR-758 increases cholesterol efflux. Thus, miR-758 plays an important role in maintaining the neuronal cholesterol homeostasis. What is more, miR-758 can suppress the expression of genes associated with amino acid synthesis, such as solute carrier family 38 member 1 (SLC38A1), insulin-like growth factor 1 (IGF1), nontuberculous mycobacteria (NTM), syntaxin-binding protein 1 (STXBP1), and ephrin type-A receptor 7 (EPHA7) [5]. Other miRNAs are found to suppress the expression of ABCA1 and cholesterol efflux, such as miR-223 [13], miR-26 [7], miR-23a-5p [20], miR-302a [4], miR-9-5p [15], and miR-20a [6].

miR-223 can promote cholesterol efflux. Gene Sp1 (66 actual/21.17 expected, $P = 2.25 \times 10^{-11}$) and Sp3 (24 actual/8.55 expected, $P = 6.5 \times 10^{-6}$) are both the targets of miR-223. But overexpression of miR-223 is found to suppress Sp3 mRNA levels, but not Sp1 mRNA levels. So miR-223 can increase the expression of ABCA1 through inhibition of Sp3, which antagonizes Sp1-mediated ABCA1 activation [13]. MiR-19b represses the expression of ABCA1 through targeting the ABCA1 3′UTR in foam cell–derived THP-1 and mouse peritoneal macrophages, and slicing miR-19b promotes the expression of ABCA1. miR-19b inhibits the capacity of apo-AI to remove cholesterol from cells and increases the accumulation of cholesterol in macrophages. MiR-19b promotes the progression of atherosclerosis in mice [21]. MiR26 also can inhibit the levels of ABCA1 and ADP-ribosylation factor-like 7 (ARL7) to depress

cholesterol efflux [7]. MiR-23a-5p has been proved to repress ABCA1/G1 3′UTR, and MiR-23a-5p inhibitor promotes macrophages cholesterol efflux through upregulation of ABCA1/G1 levels [20].

MiR-9-5p is negatively correlated to ABCA1 ($P < 0.05$, $r = -0.3$); researchers find a negative correlation between miR-9-5p expression and HDLs levels ($P < 0.05$, $r = 0.3$), and positive correlation with BMI ($P = 0.01$, $r = 0.3$), HOMA-IR ($P < 0.01$, $r = 0.3$) and TG ($P < 0.01$; $r = 0.4$). What is more, compared with healthy controls, the cholesterol efflux capacity of monocytes derived from peripheral blood mononuclear cells of patients with MS is decreased. And the cholesterol efflux capacity is positive correlation with the expression of ABCA1 ($P < 0.01$; $r = 0.8$) and negative correlation with the expression of miR-9-5p ($P < 0.01$; $r = 0.7$) [15]. Furthermore, miR-302a and miR20a can target ABCA1 3′UTR to inhibit the expression of ABCA1, and contribute to decrease cholesterol efflux in macrophages [4, 6].

Protocatechuic acid (PCA) is a metabolite derived from human gut microbiota metabolism of cyanidin-3-O-β-glucoside (Cy-3-G). PCA could inhibit the atherogenic process. PCA but not Cy-3-G can increase cholesterol efflux from mouse peritoneal macrophages and human THP-1 macrophages. PCA can increase the expression of ABCA1 and ABCG1 on mRNA and protein levels through downregulation of miR-10b. miR-10b can reduce the 3′UTR activity of ABCA1 and ABCG1. Further, PCA and Cy-3-G can promote cholesterol efflux in vivo [22].

The expression of miR-10b is increased in the arteries with advanced but not early atherosclerotic plaques. The typical feature of the transition from early to advanced atherosclerosis is the accumulation of free cholesterol-induced apoptotic macrophages (FC-AMs). Supplement of FC-AM increases the expression of miR-10b in mouse resident peritoneal macrophages (RPMs). The increase of miR-10b results from the upregulation of Twist1/2 stimulated by FC-AM. Interestingly, anti-miR10b treatment selectively promotes the expression of ABCA1 in the macrophages of advanced rather than early atherosclerotic plaques. Anti-miR10b treatment depresses the expression of ABCA1 in mRNA and protein levels only in resident peritoneal macrophages (RPMs) engulfing FC-AM but not in RPM- or RPM-derived foam cells. Similarly, Anti-miR10b treatment promotes cholesterol efflux only in RPM engulfing FC-AM not in RPM or RPM-derived foam cells. Antagonism of miR-10b suppresses the progression of advanced atherosclerosis but not the formation of early atherosclerosis [23].

MiR-21 is highly expressed in macrophages/ monocytes. Knockout of miR-21 in myeloid cells accelerates the progression of atherosclerosis. Absence of miR-21 in macrophages promotes macrophage/monocyte infiltration in the artery wall and increases the expression of inflammatory cytokines in macrophages. miR-21 deletion leads to cell death and attenuates the formation of plaque necrosis. Absence of miR-21 in macrophages results in ABCG1 degradation and increases formation of foam cells [24].

Treatment with rosuvastatin decreases the levels of plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and non-HDL-C, and reduces the mRNA levels of ABCA1 and ABCG1 in atherosclerotic plaques. ABCA1 is mainly expressed in macrophages. Treatment of high-dose (40 mg/day) rosuvastatin can improve the expression of ABCA1 in protein levels. The contradiction between mRNA and protein suggested a posttranscriptional regulation. The levels of miR-33b-5p are decreased in mice that were given a dose of rosuvastatin. What is more, transfection of a miR-33b-5p mimic into THP1 cells treated with rosuvastatin reduces the expression of ABCA1 [25].

Knockout of miR-143/145 prevents the formation of atherosclerosis compared with LDLR−/− mice fed with western diet. MiR-143/145 deficiency decreases the expression of actin alpha 2 (ACTA2) and smooth muscle 22 (SM22), and increases the expression of byglican (BGL) in smooth muscle cells, which proves that SMC turned into a synthetic phenotype. Moreover, the macrophages in plaque decrease significantly. Deletion of miR-143/145 reduces circulating cholesterol but not plasma TG levels. MiR-145

directly targets the 3′UTR of ABCA1. What is more, the levels of miR-145 in macrophages increase after treated with vascular smooth muscle cell (VSMC) conditional media. It proves miR-145 might be transmitted from VSMC to macrophages [26].

Furthermore, HDLs also can be a good vector that can carry several miRNAs. It has been reported that miRNAs carried by HDLs are important to inflammation response. Except for macrophages, HDLs also can change gene expression in endothelial cells. It is interesting to find that the effect of native HDL is different from its components, such as rHDL, apolipoprotein AI (ApoAI), and small unilamellar vesicles (SUV). Native HDL alters miRNAs in endothelia and the level of miR-223 increases significantly. HDL transfers miR-223 to endothelia and suppresses the expression of intercellular adhesion molecule-1 (ICAM-1) by targeting 3′UTR (814–816) to suppress inflammatory response [27].

MiR-126 highly expressed in endothelial cells is found in HDLs with ≈2800 copies/ug HDLs. Like miR-126, miR-92a, which is enriched in endothelial cells, is also detected in HDLs with almost 3400 copies/ug HDLs. Further, the number of miR-92a, miR-126, and miR-30c carried by HDLs is reduced obviously in patients with acute coronary syndrome (ACS) compared with that in healthy people. In smooth muscle cells (SMCs), treated with HDLs from ACS or coronary artery disease (CAD) suppresses the expression of miR-126 and miR-92a. Treatment with HDLs cannot influence the level of pri- and pre-miR-92a. In contrast, the expressions of pri- and pre-miR-126 are decreased after 1 h and increased 6 h later of HDLs stimulation [28].

In diabetic nephropathy (DN) patients, miR-132 carried by circulating HDLs is decreased significantly. To investigate how the HDL-miR-132 affects function of endothelia, researcher transferred fluorescently labeled HDLs with a miR-132 mimic sequence to human umbilical vein endothelial cells (HUVECs). The endothelial barrier function of HUVECs has not changed after treated with HDL-miR132. While after stimulation with HDL-miR-132, HUVECs show a higher total tube length. However, EV-miR-132 cannot change the capacity of angiogenic tube formation. Further, HUVECs in the presence of diabetes mellitus (DM) or DN patient serum coculture with HDL-miR-132 show an obviously increased tube formation capacity compared to without HDL-miR-132 [29]. What is more, the expression of miR-126, miR-21, and miR-222 is decreased in endothelial cells treated with HDLs separated from patients with chronic heart failure (CHF) compared with that from health people (miR-126: 0.69 ± 0.03 vs. 1.08 ± 0.17; miR-21: 0.79 ± 0.11 vs. 1.23 ± 0.06; miR-222: 1.25 ± 0.07 vs. 0.93 ± 0.05) [30]. Therefore, some kinds of miRNAs are involved in anti-inflammatory response, and HDLs-related miRNAs can be clinical biomarkers for some metabolic diseases (Fig. 12.1).

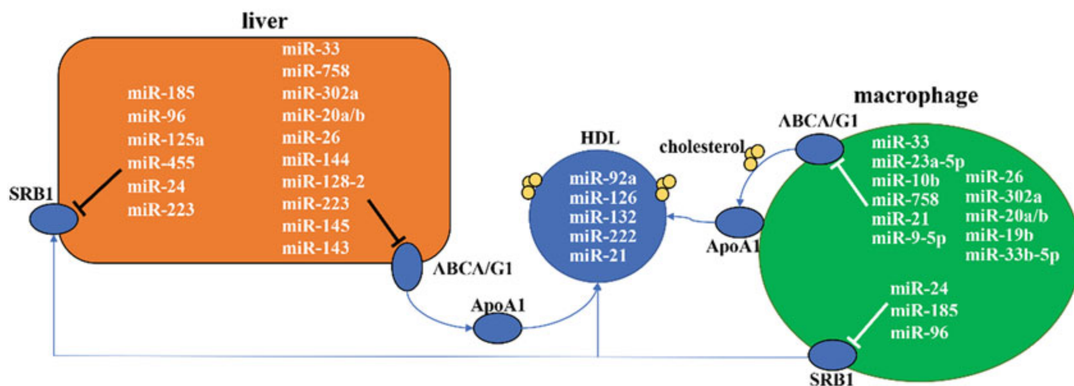

Fig. 12.1 miRNAs maintain HDL homeostasis

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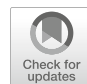

13 HDL and Kidney Diseases

Huanhuan Cao and Xia Meng

Abstract

Serum lipid profiles, as well as HDL can be altered in patients with kidney diseases. There are various types of kidney diseases, including nephrotic syndrome and chronic kidney disease. In patients with nephrotic syndrome, plasma levels of HDL cholesterol and ApoA-I were within or below the normal limits. The HDL cholesterol: total cholesterol ratio decreased compared to healthy individuals. In patients with chronic kidney disease (CKD), reverse cholesterol transport function of HDL is impaired, and CKD also affects the composition and function of HDL. Cardiovascular disease (CVD) is the severe complication of CKD. Furthermore, HDL might also be a potential target for the prevention of cardiovascular complications associated with CKD.

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Keywords

HDL · Nephrotic syndrome · Chronic kidney disease

13.1 Introduction

Patients with kidney diseases are often complicated with cardiovascular complications, and kidney diseases are significantly associated with cardiovascular diseases. A multitude of studies have suggested that lipid management is beneficial for both kidney diseases and cardiovascular complications [1], whereas little is known about HDL in kidney diseases. In this part, we will focus on HDL and kidney diseases and related cardiovascular complications.

There are various types of kidney diseases, including nephrotic syndrome and chronic kidney disease. Nephrotic syndrome (NS) is a common pediatric kidney disease, and it can cause huge burden. The incidence of childhood NS is reported as 4.7 per 100,000 children worldwide, and it varies by race and location [2]. Children with NS have higher cardiovascular burden, resulting in higher morbidities of cardiovascular diseases in the future [3]. Nephrotic syndrome refers to excessive proteinuria, with associated hypoalbuminemia, edema, and hyperlipidemia. There are many different causes of NS, including gene mutation, infections, drug, toxins, and glomerular hyperfiltration. Although causes are

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complex, they share the common pathophysiology, that is, the defect in the glomerular filtration barrier. Glomerular filtration barrier is essential for the function of the kidney, and defect in it results in massive loss of protein in the urine, following hypoalbuminemia, edema, and dyslipidemia [4]. In addition, immune dysregulation also contributes to the development of NS. Berg et al. found that exposure to allergens can cause NS, and they put forward the hypothesis that immune dysregulation plays a pathogenic role in disease development [5]. Audard et al. confirmed that Hodgkin's and other T-cell lymphomas may trigger NS, and chemotherapy can subsequently induce remission [6]. Other systemic circulating factors may also play a role in the pathogenesis of NS [7, 8]. The histological classification of NS is of great value to the treatment and prognosis of the disease. The most common form of NS in children is minimal change nephrotic syndrome (MCNS), while the most common form in adults is membranous nephropathy (MN). Focal segmental glomerulosclerosis (FSGS) can occur in both children and adult, which is more resistant to steroid, and more likely to progress to end-stage renal failure. The treatment of NS includes corticosteroids, levamisole, mycophenolate mofetil, and rituximab [9]. Among these suggested alternatives, oral corticosteroid is the first-line therapy. And 80% of patients with NS respond to corticosteroids [10, 11]. Hyperlipidemia is one of the clinical features of NS. Lipid-lowing drugs such as statins is recommended by the European Society of Cardiology and European Atherosclerosis Society guidelines in 2011 [12].

Chronic kidney disease (CKD) is defined as decreased kidney function shown by glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m^2, or markers of kidney damage, or both, of at least 3 months duration. When GFR is less than 15 mL/min per 1.73 m^2, CKD progresses to end-stage kidney disease (ESKD) [13]. CKD is a global health problem, and the burden of it is substantial. The prevalence of CKD is reported to be around 11% in developed countries, including Australia and the USA. The incidence and prevalence of CKD vary within countries, which have different economic conditions. Divided into four socioeconomic levels, those in the bottom quarter had a 60% higher risk of CKD than those in the top quarter. According to World Health Organization, the mortality of CKD is 12.2 deaths per 100,000 people, ranking fourteenth in the list of leading causes of death. And the mortality of CKD will continue to increase to reach 14 per 100,000 people by 2030 [14]. Chronic kidney disease originates from many heterogeneous diseases and eventually results in the changes of kidney structure and function. Therefore, the etiology of CKD is varied. Diabetes and hypertension are the two leading causes of CKD worldwide, no matter in high-income countries or many low-income countries. Diabetes accounts for 30–50% of all CKD, and the proportion is expected to increase in the future [15]. Hypertension also has a high incidence of disease worldwide, and studies have shown the relationship between increasing risk of developing CKD and worsening blood pressure control [16]. The histological change of CKD is renal fibrosis, characterized by glomerulosclerosis, tubular atrophy, and interstitial fibrosis. Glomerulosclerosis is attributed to endothelial dysfunction, proliferation of smooth-muscle cells, and destruction of podocytes. Furthermore, risk factors, including hypertension, dyslipidemia, and smoking, promoted the progression of glomerulosclerosis. Cardiovascular disease is one of the CKD complications. It is estimated that cardiovascular mortality is 57% higher in individuals with CKD compared to individuals without CKD. The risks of both myocardial infarction, stroke, and cardiovascular death increase as GFR declines [17, 18]. Antiplatelet treatment is recommended by clinical practice guidelines [19]. However, studies have suggested that although antiplatelet agents were effective at reducing the risk of myocardial infarction by 13% in CKD patients, the effects on cardiovascular and all-cause death were uncertain [20]. Dyslipidemia is also a risk factor for cardiovascular complication. A systematic review showed that statins were able to reduce all-cause mortality by 19%, cardiovascular mortality by 22%, and cardiovascular events by 24% in individuals with CKD [21].

Recent studies have also suggested that HDL is significantly associated with nephrotic syndrome and CKD, [22] and targeting HDL may provide a new insight into intervening the kidney diseases.

13.2 The Role of the Kidney Plays in HDL Metabolism

The protective role of HDL in many diseases is associated with the level, composition, and function of HDL. These are regulated by HDL metabolism [23]. As we all know that the primary sites of lipoprotein metabolism are in liver, skeletal muscles, adipose tissue, and some cells such as macrophages [24]. What is more, kidney is also a key modulator of HDL components, which in turn regulate HDL levels, composition, and function of HDL particles. Previously, kidney has not been believed to regulate lipid metabolism, for that glomerular filtration barrier blocks most of the molecules, allowing only the smallest ones to pass through. Recently, studies have shown that HDL is not an intact particle, and the metabolism of HDL is also not the removal of the whole intact particle, but the removal of component of HDL, such as apolipoprotein A-I, apoE. In addition, some transfer proteins and enzymes involved in HDL metabolism, such as cholesteryl ester transfer protein (CETP), lecithin cholesteryl ester acyltransferase (LCAT), phospholipid transfer protein (PLTP), could also be metabolized by the kidney [25].

The liver is the primary site of clearance of HDL-associated lipids and degradation of HDL-associated apolipoproteins. Hepatic SR-B1 plays an important role in it. SR-B1 binds HDL particles, and then HDL is hydrolyzed by hepatic lipase, releasing ApoA-I into circulation [26]. The kidney is another essential site of HDL metabolism. The normal function of kidney depends on the glomerular filtration barrier. The filtration barrier in the glomerular capillaries block passage of molecules >60–100 KD. Although HDL particles exceed this mass, components of HDL, including various lipid and proteins, could cross the normal glomerular filtration barrier. ApoA-I is the main component of HDL, and the molecular weight of ApoA-I is 28 KD [27]. It is obvious that ApoA-I could pass through the normal glomerular filtration barrier. In animal experiment, the majority of injected labeled human ApoA-I is cleared by the kidney [28]. The molecular weight of LCAT is 63kD, and it is predicted to pass through the glomerular filtration barrier. LCAT deficiency could impair the maturation of preβ--HDL. Except for the glomerular filtration barrier, renal tubule also plays roles in HDL metabolism. Take ApoA-I for an example, after being filtered by the glomerular filtration barrier, ApoA-I was absorbed by the proximal tubule, and proximal tubular reabsorption failure leads to increased urinary excretion of ApoA-I [29]. Therefore, the kidney through the filter of glomerular filtration barrier and uptake by proximal tubule regulates the plasma level of ApoA-I and HDL.

Taken together, the kidney plays a regulatory role in HDL metabolism. Changes in kidney structure and function will affect HDL level, composition, and function. Therefore, HDL can be a biomarker of kidney damage. For example, ApoA-I can cross the glomerular filtration barrier, and the damage of the renal barrier will inevitably increase the filtration of ApoAI; thus, apoAI in urine can be used as a marker of the disruption of the glomerular filtration barrier. Furthermore, this indicator is more sensitive than albumin. Injection of LMB2 to Nphs1-hCD25 transgenic mice could selectively damage podocyte, which is an important cell for the normal function of the glomerular filtration barrier. The experimental model induced by LMB2 could cause albuminuria and edema by 2 weeks, and recover by 6 weeks. Interestingly, ApoA-I in urine increased >tenfold in this model. Moreover, although urinary albumin returned to normal at week 6, urinary ApoA-I remained elevated, sevenfold higher than normal state. The data implied that urinary ApoA-I is more sensitive than albuminuria in face of the disruption of the glomerular filtration barrier [25].

13.3 The Abnormality of HDL Metabolism in Kidney Diseases

Serum lipid profiles, as well as HDL can be altered in patients with kidney diseases. The abnormality of HDL can, in turn, affect the development and progression of cardiovascular complications and various other comorbidities of kidney diseases, such as atherosclerosis and glomerulosclerosis.

13.3.1 The Abnormality of HDL Metabolism in Nephrotic Syndrome

Nephrotic syndrome is characterized by dyslipidemia, and the abnormalities include elevated plasma levels of cholesterol, triglycerides, and the apolipoprotein B–containing lipoproteins (including very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL) [30]. Moreover, the composition and function of lipoproteins are significantly altered: lipoprotein (a), ApoB, ApoC, and ApoE, and ApoC-III: ApoC-II ratio increase in patients with nephrotic syndrome [31]. However, in patients with nephrotic syndrome, plasma levels of HDL cholesterol and ApoA-I were within or below the normal limits. The HDL cholesterol: total cholesterol ratio decreased compared to healthy individuals [32]. Additionally, cholesterol-ester-poor HDL3 can convert to cholesterol-ester-rich HDL2, and the maturation process is impaired in NS patients, resulting in increased HDL3 and decreased HDL2 in plasma. These indicated that HDL-mediated reverse cholesterol transport was impaired in nephrotic syndrome [33].

The abnormalities of HDL metabolism in nephrotic syndrome involve various mechanisms. Acquired LCAT deficiency contributes to impaired HDL maturation. It is well known that LCAT is responsible for re-esterification of free cholesterol on the surface of HDL through the interaction with ApoA-I. The re-esterification is essential for the maturation of cholesterol ester-poor HDL3 to cholesterol-ester-rich HDL2. In order to study impaired maturation of HDL in nephrotic syndrome due to acquired LCAT deficiency, Vaziri et al. used Sprague-Dawley rats to make nephrotic syndrome models. Sprague-Dawley rats were given 130 mg puromycin aminonucleoside on day 1 and 60 mg ip on day 14 to induce nephrotic syndrome, and on day 30, hepatic LCAT mRNA abundance and plasma and urine LCAT activity were measured. The nephrotic syndrome rats showed a four- to five-fold rise in serum cholesterol and triglycerides, and a fourfold fall in the HDL: total cholesterol ratio. Interestingly, puromycin aminonucleoside-induced nephrotic syndrome models showed similar hepatic LCAT mRNA abundance with control rats, but significant increase in urinary LCAT was observed in nephrotic syndrome models. Therefore, nephrotic syndrome causes heavy LCAT losses, resulting in impaired HDL maturation [34]. Hypoalbuminemia is another factor contributing to increased cholesterol-ester-poor HDL in nephrotic syndrome. Hypoalbuminemia is one of the clinical features in nephrotic syndrome, because of massive losses of albumin from urine. Plasma albumin plays an important role in HDL metabolism. It can carry free cholesterol from the peripheral tissues to the plasma HDL3 [35]. Therefore, nephrotic syndrome causes hypoalbuminemia, which leads to abnormal HDL metabolism. In addition, the study has shown that plasma levels of CETP increased in patients with nephrotic syndrome [36]. CETP mediates the transfer of cholesteryl esters from HDL to VLDL and LDL. Many studies have shown that CETP inhibitor can increase HDL-C levels and decrease LDL-C levels, playing a protective role in cardiovascular diseases [37, 38]. The elevated CETP depletes the cholesterol esters and raises the triglyceride component of HDL, contributing to increased cholesterol-poor HDL3 and decreased cholesterol-rich HDL2 in nephrotic syndrome.

The HDL metabolism by the liver also changes in nephrotic syndrome. Hepatic SR-B1 deficiency, hepatic HDL endocytic receptor upregulation, and hepatic lipase deficiency were observed. SRB1 is the HDL receptor expressed

on the hepatocyte plasma membrane, and it is critical for HDL-mediated reverse cholesterol transport. Liang et al. found that hepatic tissue HDL receptor SR-B1 protein abundance markedly decreased in rats with puromycin-induced nephrotic syndrome, while hepatic SR-B1 mRNA abundance has no significant difference from control group [39]. The study suggested that there may be a posttranscriptional or posttranslational mechanism underlying the phenomenon. Subsequently, Ikemoto et al. identified a PDZ-domain-containing protein that interacted with C terminus of SR-B1 via its N-terminal first PDZ domain. The study suggested that the PDZ-domain-containing protein was the adaptor protein for SR-B1, and it regulated the stability of SR-B1 through preventing SRB-1 degradation at the posttranslational level [40]. In puromycin-induced nephrotic syndrome rats, the reduction in SR-B1 protein abundance was observed, significantly associated with parallel reductions in PDZ-containing kidney protein 1 (PDZK1) mRNA and protein abundance [41]. The β chain of ATP synthase is the ApoA-1 receptor and mediates endocytosis of the lipid-poor HDL. The receptor was shown to increase in puromycin-induced nephrotic syndrome rats. The findings suggested that hepatic HDL endocytic receptor upregulation played a role in nephrotic syndrome [41]. Hepatic lipase deficiency also affects HDL metabolism in nephrotic syndrome. Garber et al. found a significant downregulation of hepatic lipase expression and activity in experimental models [42]. The acquired hepatic lipase deficiency contributes to triglyceride enrichment of HDL in nephrotic syndrome.

13.3.2 The Abnormality of HDL Metabolism in Chronic Kidney Disease

Dyslipidemia is one of the clinical characterizations of CKD. The metabolism of lipids and Lipoproteins is abnormal, including increased levels of triglycerides, small dense and oxidized LDL (oxLDL), and decreased levels of HDL-cholesterol [43]. Furthermore, CKD also affects the composition and function of HDL, and reverse cholesterol transport function of HDL is impaired in CKD [44].

The mechanisms underlying the abnormalities of HDL composition, function, and metabolism in patients with CKD are complex. ApoA-I, the main protein component of HDL, was shown to markedly reduce in both human and animal CKD. ApoA-I is produced by the hepatocytes [45]. In vitro study, human uremic plasma could suppress the production of ApoA-I in cultured human hepatocytes [46], which suggested that uremic substances in CKD inhibited the ApoA-I biosynthesis. ApoA-I plays a critical role in the function of HDL: ApoA-I interacts with ABCA1 on the cell membrane to trigger an efflux of phospholipids and free cholesterol to the surface of HDL, which is important for removal of cholesterol from the peripheral tissues [47]. Therefore, ApoA-I deficiency in CKD contributes to structural and functional abnormalities of HDL. Hypoalbuminemia is common in CKD because of impaired glomerular filtration barrier, resulting in massive losses of albumin from urine. It is well known that ABCA1 mediates uptake of surplus cholesterol from the peripheral tissues. Except for ABCA1, albumin also plays a role in reverse cholesterol transport of HDL, which carries free cholesterol from the peripheral tissues to plasma cholesterol-poor HDL3. Hypoalbuminemia in CKD may, therefore, contribute to decreased cholesterol-rich HDL [35]. Reduced levels of hepatic lipase are associated with the formation of triglyceride-rich HDL in CKD. Hepatic lipase catalyzes the hydrolysis and clearance of triglycerides from HDL. Therefore, hepatic lipase deficiency in CKD leads to triglyceride enrichment of HDL [48, 49].

In addition to decreased HDL levels and impaired function of reverse cholesterol transport, the anti-inflammatory activity of HDL is also impaired in CKD. Moradi et al. conducted a study, which included 32 stable hemodialysis-dependent CKD patients and 13 age-matched controls, and they found a significant reduction of antioxidant activity of HDL in patients with CKD. The reduction of antioxidant activity of

HDL is partly attributed to the reduction in paraoxonase-1 and glutathione peroxidase [50]. Moreover, reduced anti-inflammatory activity of HDL was shown in the majority of patients with end-stage kidney disease (ESKD) [51]. In the in vitro study, HDL isolated from healthy people showed the anti-inflammatory property, but HDL isolated from individuals with ESKD failed to inhibit the production of inflammatory cytokines by monocytes [52]. In ESKD, the impaired anti-inflammatory activity of HDL is caused by serum amyloid A (SAA). Thomas et al. used shotgun proteomics to identify HDL-associated proteins. Forty-nine HDL-associated proteins including SAA were found to enrich in HDL from patients with ESKD. SAA is similar to ESKD-HDL by promoting the production of inflammatory cytokines, and SAA levels in HDL are inversely associated with its anti-inflammatory properties in ESKD. As a result, the protein composition such as SAA contributed to impaired anti-inflammatory activity in patients with ESKD [52].

Previous studies have believed that the abnormalities of HDL metabolism are the consequences of CKD. However, recent studies have shown that altered HDL metabolism could also promote the development and progression of CKD. A prospective cohort study suggested that non-HDL-cholesterol to HDL-cholesterol ratio was an independent risk factor for the development of chronic kidney disease. The study included 1891 Chinese adults with normal or near-normal kidney function at baseline, and tracked them two years to observe new-onset CKD (the diagnostic criterion of glomerular filtration rate (eGFR) is <60 mL/min per 1.73 m^2). Individuals with non-HDL-c to HDL-c ratio in the highest tertile had a 1.45-fold higher risk of new-onset CKD than individuals in the lowest tertile [53]. A Global Case-Control Study showed that HDL was significantly associated with diabetic kidney disease. In the study, the case subjects included 1891 patients with type 2 diabetes mellitus and diabetic kidney disease, and the control subjects included 3683 type 2 diabetes mellitus patients without kidney disease. The odds ratio decreased by 0.86 (0.82–0.91) for every 0.2 mmol/L increase in high-density lipoprotein cholesterol. These findings suggested that HDL level was a significant predictor of the development and progression of diabetic kidney disease [54]. However, the mechanisms underlying it remain further investigations.

13.3.3 HDL and the Cardiovascular Risk in Kidney Diseases

Cardiovascular disease (CVD) is the leading cause of death in patients with CKD. According to the US Renal Data System, the prevalence of CVD in individuals with chronic kidney disease is 65.8%, more than twice the prevalence of CVD in people without CKD (31.9%). The cardiovascular complications in CKD include coronary artery disease, arteriosclerosis, cardiomyopathy, and an increased risk of sudden cardiac death resulting from arrhythmia [55]. CKD is characterized by dyslipidemia, which is a risk factor for the development of cardiovascular complications [56]. Cholesterol-lowering therapy with atorvastatins could reduce the risk of cardiovascular events in part of CKD patients [57]. Many clinical studies have shown little or no benefit of statins in the prevention of CVD in patients with CKD. These findings suggested that a substantial residual cardiovascular risk remained even after lipid-lowering therapy (statins) in most patients with CKD. Recently, evidence has shown that HDL might be a potential risk factor that contributed to the residual risk. A cross-sectional study showed that abnormalities in HDL were strongly and consistently associated with subclinical atherosclerosis in CKD. In the study, 6572 participants were involved, and subclinical atherosclerosis was measured by the common carotid intima-media thickness (CIMT). They found a stronger association between lower HDL-C and CIMT in patients with lower eGFR [58]. An observational cohort study showed that HDL could predict incident atherosclerotic CVD in CKD patients without history of CVD. In the study, 45,390 CKD patients without previous history of myocardial infarction (MI) were included, multivariate

logistic regression analysis showed that HDL was inversely associated with incident MI [59]. Moreover, lower HDL level is significantly associated with higher risk of death in patients with CKD [60]. In addition to plasma levels of HDL, Kathrin et al. showed that the composition and function of HDL could also predict future cardiovascular events in patients with CKD [61].

These findings implied that HDL might be a potential target for the prevention of cardiovascular complications associated with CKD. In the future, more studies are needed to explore the role of HDL in the treatment of CKD and CKD-related cardiovascular complications.

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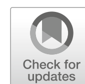

14 HDL and Therapy

Ke Li, Xianwei Xie, and Yansong Guo

Abstract

A wealth of evidence indicates that high-density lipoprotein assumes the unique antiatherosclerosis and other cardioprotective properties. Based on that, HDL-C has been considered as a promising therapy target to reduce the cardiovascular diseases. Recombinant HDL (rHDL) and apolipoprotein mimetic peptides emerge in recent years and have great potential in the future. Here we discussed the pleiotropic therapeutic effect of rHDL based on the effects of atherogenic, angiogenesis, platelet, vascular, and Alzheimer's disease. On the other hand, rHDL not only plays the key role as the major protein component of HDL, it is also used as a nanovector in antiatherosclerotic, antitumor, cardiovascular diagnosing and other therapeutic areas. Synthetic apolipoprotein mimetic peptides like apoA-I and and apoE mimetics have undergone clinical assessment, and we have also reviewed the advances of clinical trials and gave an outlook for the therapy of rHDL and mimetic peptides.

Keywords

High-density lipoprotein · Mimetic peptides · Apolipoprotein A-I · Recombinant HDL therapy

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Results of the Framingham study first revealed the role of high-density lipoprotein cholesterol (HDL-C) in cardiovascular diseases (CVD); the epidemiology data suggested HDL-C levels increased by approximately 1 mg/dL and was associated with 2–3% decrease in CVD risk. Therefore, HDL-C was recognized as a potential protective factor. But increasing the amount of circulating HDL did not exert protection against

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CVD or total death [1]. A series of clinical trials found that the amount of HDL has a closer association with the clinical outcome than HDL-C. Human hypothesized the real protagonist protects against vascular disease and is HDL other than HDL-C, which explained supplementation of HDL-C does not improve the CVD outcome [2].

As present in the various study, HDL displayed plentiful biological activities, which contribute to atheroprotective effects. Thus, HDL was recognized as a new therapeutic target for cardiovascular disease. Exogenous supplement of HDL could improve both amount and quality of circulating HDL. HDL was reconstituted in vitro with the major components, apo A-I and phospholipid. The reconstituted high-density lipoprotein (rHDL) possessed several enhanced functionalities, and underlying mechanisms of rHDL particles were studied. With the deepening of scientific research, it was found that the efficacy of rHDL relies not only on the amount, but also on the composition (the relative abundance of apolipoprotein and phospholipids) and structure of rHDL. Phospholipid species-mediated different signal activation, thereby different kinds of phospholipids or different kinds of apolipoproteins incorporated into rHDL could be considered as a novel therapeutic strategy [3]. Here we summarize biological functions and therapeutic action of different Recombinant HDL and apolipoproteins (Fig. 14.1).

14.1 Pleiotropic Therapeutic Effect of rHDL Antiatherogenic Effects of rHDL

The vascular inflammation inhibition properties of rHDL have been shown in a routine of fields. In rabbit models subjected to inserting a periarterial carotid collar, rabbits administrated with the infusion of rHDL (lipid-free apoA-I) increased DHCR24 and heme oxygenase-1 (HO-1) expression, attenuated the intima/media neutrophil infiltration. Preincubation of rHDL with human coronary artery endothelial cells activated phosphatidylinositol 3-kinase/Akt and showed less inflammation [4].

In the double-blind randomized trial, 29 patients with recent ACS were recruited. Subjects were injected with rHDL or albumin, and then the marks of vascular inflammation like oxidized LDL and vascular vasodilatation response to endothelium-dependent and endothelium–independent were measured. It exhibits no apparent conflict between the two groups. ($P = 0.11$;14.9 ± 9.2 vs. 14.5 ± 12.4, $P = 0.93$ and 12.8 ± 7.1 vs. 13.2 ± 9.6, $P = 0.27$, respectively). The negative results presented the challenge of rHDL further interventions [5].

CER-001 was an rHDL product constructed of wild-type ApoA-I and diphosphatidylglycerol and sphingomyelin. Based on preliminary studies of CER-001 influence on vascular inflammation. A double-blind, randomized, multicenter trial about CER-001 was constructed, patients with ACS received infusions of CER-001 (3 mg/kg, $n = 135$) or placebo ($n = 137$) for 10 weeks in addition to citations. The regression of percent atheroma volume demonstrated no difference (57.7% vs. 53.3%; $P = 0.49$) [6]. The apoA-I structure of ETC-642 was a 22 L-amino acid amphipathic apoA-I mimetic, the apoA-I mimetic combined with sphingomyelin and DPPC. Current data from animal experiment present the potential in treating atherosclerosis by inhibiting vascular inflammation. New Zealand white rabbits with chronic vascular inflammation were divided into 3 groups: placebo, rHDL (8 mg/kg), ETC-642 (30 mg/kg peptide). ETC-642 significantly inhibited intracellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) the same as rHDL, the protection from chronic vascular inflammation that similar to rHDL provided a treatment of inflammatory disease [7].

The progression of atherosclerosis was inseparable from the massive accumulation of intracellular cholesterol. In several studies, rHDL's role of accelerating cholesterol efflux had been confirmed [8]. Patients receiving rHDL infusion had increased capacity of cholesterol efflux [9, 10].

Sanjay Patel carried out a randomized crossover design study administrate thirteen male patients with type 2 diabetes mellitus with rHDL (80 mg/kg of apolipoprotein A-I) or saline, and then measured the anti-inflammatory properties

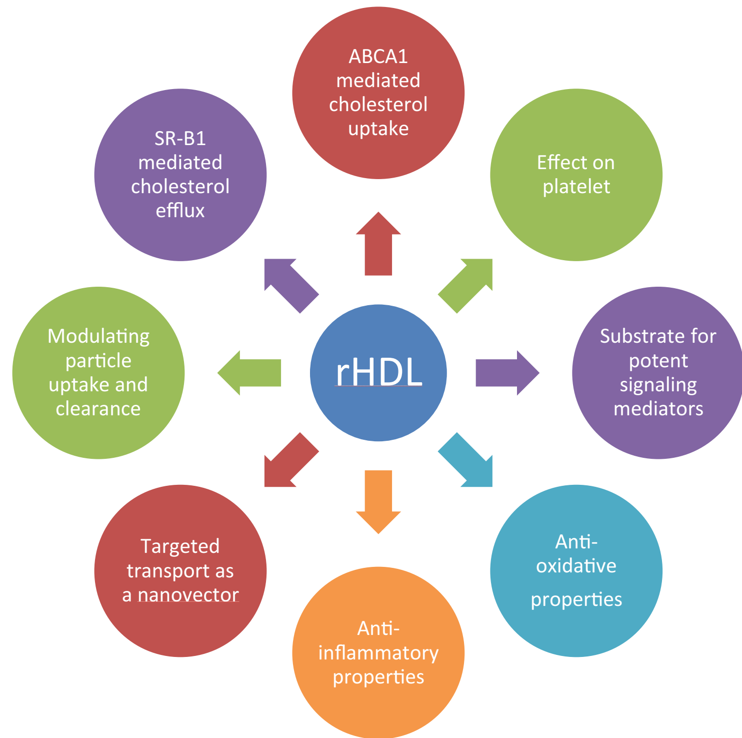

Fig. 14.1 rHDL possess multiple biological functions

of isolated HDL, they found that Participants' peripheral blood monocyte CD11b expression and neutrophil adhesion to a fibrinogen matrix was reduced 72 h post-rHDL. The capacity of plasma to receive cholesterol from THP-1 macrophages increased after rHDL infusion [11].

14.2 Promotion of Angiogenesis Based on rHDL

Given the inverse correlation between the HDL and ischemic cardiomyopathy, it has been surveyed that injection of rHDL effected endothelial progenitor cells differentiate and blood flow recovery in murine models. rHDL activated phosphatidylinositol 3-kinase/Akt pathway and rescued ischemia-induced injury through stimulating angiogenesis [12]. Increasing evidence verified the property of enhancing angiogenesis of rHDL, preincubation of human coronary artery endothelial cells with rHDL, the pathway of HIF-1α was activated. Inhibition of SRB1, as well as upstream PI3K/Akt pathway, attenuated the enhancement of angiogenesis of rHDL [13]. In diabetic mice, the blood flow recovery and angiogenesis were impaired. rHDL had the potential for effecting vascular biological including rescued tubulogenesis and increased endothelial nitric oxide synthase activity, which was immediately by influencing hypoxia-inducible factor-1α (HIF-1α) stabilization and expression levels of VEGF [14]. Evidence on the other hand proved rHDL restored angiogenesis. In the postischemic animal models, after injection of rHDL, the angiogenic miRNA, miR-181c-5p was significantly reduced, indicating rHDL-regulated angiogenesis by specifically targeting mmu-miR-181c-5p at the ischemic site [15].

14.3 Protection against Alzheimer's Disease of rHDL

Alzheimer's disease is characterized by the pathological accumulation of Amyloid-beta(Aβ). In 1998, Koudinov AR reported that Aβ is bound

to many apolipoproteins including apoA-I, apoA-II,apoE, and apoJ, which implied that HDL may play an important role in Alzheimer's disease [16]. Apolipoprotein E3-reconstituted high-density lipoprotein (ApoE3-rHDL) was constructed and served as a novel nanomedicine bind to Aβ, facilitated the Aβ degradation in microglial, astroglia. In animal models, after 4 weeks of injection of apoE3-rHDL, the neurologic changes were reduced and the memory retrogradation was rescued [17].

In recent years, rHDL-apoJ was constructed by assembling recombinant human apoJ with phospholipids. Data shows that Aβ fibrillization was reduced and mediated higher cholesterol efflux along with rHDL accumulation in the cerebral [18].

Accordingly, rHDL assembled with drugs like donepezil was proposed. On the one hand, the complex targeted to Aβ clearance; on the other hand, the complex could execute the inhibition of acetylcholinesterase. In vivo and in vitro studies, the treatment of rHDL-Do nano drugs could prompt Aβ degradation and ameliorate neurologic changes [19].

14.4 Improvement of the Vascular Complication of rHDL

In neonatal rat ventricular cardiomyocytes, rHDL could activate Stat 3 and stimulate the ERK1/2 pathway, finally modulate ventricular cardiomyocytes adapt to stress [20]. To assess the benefit of rHDL to improve cardiac dysfunction and insulin resistance, researchers injected obese insulin-resistant rats with rHDL. They found that rHDL infusion rescued performance of the cardiac function and myocardial lesions in IR rats, the short term infusion of rHDL may be beneficial to improving the vascular complication of metabolic syndrome [21]. With the use of patch-clamp technique and electrocardiograms, cardiac repolarization was studied before and 24 h after injection with rHDL. The data suggested that rHDL shortened repolarization in rat models and shortened the heart rate corrected QT interval in subjects [22].

A similar study suggested the Ant arrhythmogenic Effect of rHDL, the Wistar rats were pretreated with placebo or rHDL, then the rats underwent temporary occlusion of the left main coronary artery, the infusion of rHDL promoted NO production in endothelial cells and reduced the incidence of reperfusion-induced arrhythmias. The probable mechanism may be activation of ABCA1 or ABCG1 through an Akt/ERK/NO pathway [23].

14.5 Effect on Platelet of rHDL

rHDL could bind lipopolysaccharides and affect platelet function: In an experimental study, 8 healthy volunteers were recruited and injected with LPS + placebo or LPS + rHDL. Net result: rHDL treatment significantly reduced platelet aggregation [24].

There have been researches surveyed that individuals with increased risk of cardiovascular disease due to type 2 diabetes, patients with diabetes mellitus were always resistant to antithrombotic treatment and showed increased risks of cardiovascular disease. In the clinical trial, patients with type 2 diabetic received rHDL (CSL-111, 20 mg/kg/h) treatment, 4 h later, platelet function reduced in the ex vivo test, and thrombus formation was inhibited up to 50%. These findings indicated its effective therapeutic property for reducing the risk of cardiovascular disease [25]. In the poststenting murine models, the mice infused with apoA-I displayed preserved smooth muscle cell phenotype and inhibition of platelet activation. These findings implicated the rHDL may have a potential therapeutic effect of improving stent biocompatibility [26].

14.6 rHDL Modulate Metabolism

The rHDL modulation role of fatty acid metabolism was investigated in thirteen patients with type 2 diabetes, the subjects were infused with rHDL or placebo, then measured the plasma lipids using mass spectrometry. Results suggested

that rHDL inhibited lipolysis through reducing the AMPK-mediated phospholipase hydrolysis [27].

As for glucose metabolism, By incubating Min6 cells and primary islets with rHDL, it was found that rHDL increased insulin secretion depended on ABCG1. The promotion of beta-cell insulin secretion capacity provides evidence of rHDL may be beneficial to treat type 2 diabetes [28]. rHDL increased myocardial glucose uptake via activating the Akt pathway, the increasing utilization of glucose in cardiomyocytes improved postischemic cardiac recovery. The study supported the rHDL infusion at the early stage after an acute coronary syndrome contributed to cardiac recovery [29].

14.7 Clinical Evaluation of rHDL in Humans

On account of the pleiotropic property of reconstituted HDL, various recombinant HDL have made steps from bench-testing, it has been tested in a number of clinical trials for stabilization of acute coronary syndrome (ACS) based on the mobilization of excess cholesterol from cells.

ApoA-I Milano is the first identified apolipoprotein gene mutations in individuals leading to an Arg → Cys substitution in the 173 positions in rural Italy who exhibit very low levels of HDL [30]. ETC-216 was developed by Eserion based on apoA-Imilano, but it was abandoned because of the incidence of host cell protein contamination during production. MDCO-216, a new formulation of apoA-Imilano, was a complex of dimeric recombinant apoA-IM and POPC. By contrast to its predecessor, ETC-216, MDCO-216 presented reduced adverse immunostimulant [31]. MDCO-216 was examined in a double-blind randomized clinical trial included 22 hospitals in Canada and Europe. Patients with ACS received an intravenous infusion of MDCO-216 (n = 59) for 5 weeks or placebo (n = 67), other than HDL-C levels, the regression of percent atheroma volume and total atheroma volume showed similar changes (67.2% vs. 55.8%; P = 0.21; 68.9% vs. 71.2%; P = 0.79) [32].

A similar randomized multicenter trial investigated the rHDL effect on the protection of atherosclerosis was conducted. Five hundred and seven patients were enrolled, patients were injected with placebo,3 mg/kg,6 mg/kg,12 mg/kg CER-001 for 3 weeks randomly, the change in the total atheroma volume showed no difference among groups(0.02, −0.02, 0.01, and 0.19%; nominal P = 0.53 for 12 mg/kg vs. placebo), as well as the secondary outcome, Nominal changes in percent atheroma volume on intravascular ultrasonography(IVUS)and coronary scores on quantitative coronary angiography(QCA)showed no difference [33].

ERASE trial included 33 subjects with recent ACS, the patients were a randomized received infusion of CSL-111 40 mg/kg or placebo, patients displayed increased circulating CD34+ cells after the fourth infusion, the endothelial progenitor cell was protected against apoptosis [30].

CSL-112 was characterized as a native human apoA-I combined with phosphatidylcholine, CSL-112 possessed a reduced lipid/protein ratio compared to CSL-111. It was conceived of as a promising reconstituted HDL due to the manifestation of anti-inflammatory, antioxidative activity and enhanced efflux of cholesterol. To assess the safety and tolerability, patients with moderate chronic kidney disease following an ACS within 7 days received weekly infusions of 6 g CSL-112 or placebo, there was no deterioration observed after 4 weeks [34]. A phase 2a random, multicentre clinical study assessed the antithrombotic property of CSL-112 in patients with atherosclerosis. Patients enrolled in the trial received 1.7 g, 3.4 g, 6.8 g CSL-112 randomly, in addition to receiving dual antiplatelet therapy. No significant influence on platelet aggregation was observed [35, 36]. Clinical use for the effective treatment of cardiovascular disease still has a long way to go.

Rabbits with Watanabe-heritable hyperlipidemic (WHHL) were injected with various doses of ETC-642 (15 mg/kg or 50 mg/kg) or placebo. The regression of aortic atherosclerosis assessed by intravascular ultrasound was significantly different compared to placebo. The highest

dose of the group implicated stronger efficacy than the low dose group [7].

14.8 rHDL: A Lipoprotein-Derived Nanovector

rHDL featured as a biocompatible, nonimmunogenic reconstituted particle, the component of rHDL was endogenous, so rHDL can completely biodegradable and did not trigger immunological responses. Besides, rHDL possessed innate ligands binding to a cellular receptor, as well known as scavenger receptor class B type 1 (SR-B1) and ATP binding cassette transporter G1. These receptors were involved in atherosclerosis, so the intrinsic capability of rHDL delivering system made it ideal for targeting delivery. According to a different characterized receptor, rHDL could possess different penetrating activity. For instance, rHDL could transport across plasma membranes and reach subcellular sites, apoE-rHDL could transport across the blood-brain barrier abound of apoE40. The schematic of rHDL featured as a nanoplatform was showed below [37] (Fig. 14.2).

14.9 rHDL with the Contrast Agent

Human combined optical probes with rHDL to make the second generation contrast agent for the more specific and selective property. The new nanoparticle made the magnetic resonance imaging of the animals show clear enhancement for atherosclerotic plaques [38]. In view of intraplaque macrophage uptake of rHDL, Assembling a carboxyfluorescein-labeled apolipoprotein E-derived lipopeptide, P2fA2, with rHDL enhanced MRI imaging of macrophages in atherosclerosis [39].

14.10 rHDL with Membrane Proteins

The membrane proteins could also be incorporated into rHDL to exert their influence as a drug, for instance, the mechanistic study of cytochrome P450 3A4 in rHDL was advantageous due to the homotropic cooperativity [40]. Human purified glucagon-like peptide-1 (GLP-1) receptor (GLP1R) and incorporated it into rHDL. The complex enabled GLP1R to prolong stabilization in lipid bilayers, which contributed to treat type 2 diabetes [41].

14.11 rHDL with siRNA

Various siRNA delivery methods had been developed, such as viruses, liposomes, aptamers. On account of the target-specific therapy property, siRNA was encapsulated within rHDL. In addition, rHDL-siRNA increased the stability of RNA. It was confirmed that rHDL/Chol-siRNA translated into the cytoplasm by FACS and confocal measurement. The siRNA was targeting VEGF gene and promoted the degradation of VEGF mRNA, followed by the entrance of the rHDL nanoparticles, vascular endothelial growth gene was down-regulated and tumor angiogenesis was inhibited. So as to achieve the propose to inhibit breast cancer [42]. A paradigm shift contained rHDL, SR-A siRNA and pita vastatin, the dual-targeting nanoparticles established a positive feedback loop, accumulated in the atherosclerotic plaques and enhanced cholesterol deposition [43]. rHDL carried cholesterol-conjugated siRNA for Poekmon gene exhibited high stability sustained release characteristics in serum, the non-viral nanoparticle inhibited Pokemon and Bcl-2 expression and effectively treated hepatocellular carcinoma in the tumor of nude mice [44]. The other dual-targeting nanoplatform integrated siRNA against lectin-like oxidized low-density lipoprotein receptor-1 and atorvastatin. The co-delivery system acted on both endothelial cell and macrophages, so the bio functions of antiatherosclerotic were outstanding, the plaque size reduced 39%, CD68 macrophage content reduced 68% [45]. Similarly, rHDL loaded with miR-204-5p-inhibitor was targeted at inhibiting angiogenesis in ovarian tumors [46].

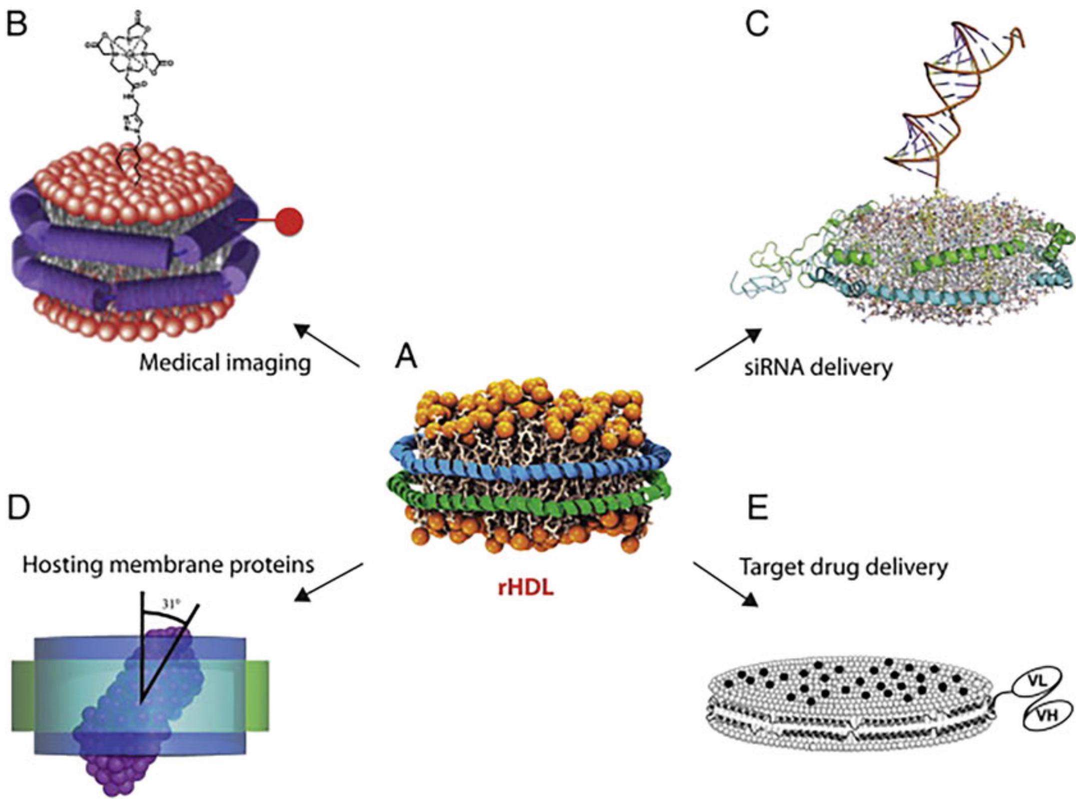

Fig. 14.2 Schematic illustrations of rHDL particles comprised of phospholipids and two apoA-I proteins with and without incorporating compounds [37]. (**a**) The canonical discoidal model of the 9.6 nm rHDL. (**b**) The medical imaging model—phospholipids are labeled with dyes such as gadolinium. The red moiety represents a red-fluorophore incorporated into ΔN-apoA-I(43-residue truncation variants of human apoA-I). (**c**) The yellow unit corresponds to a cholesterol-conjugated siRNA, which was embedded in rHDL. (**d**) Bacteriorhodopsin (purple unit) is incorporated into two ΔN-apoA-I proteins (green unit), the model showed how rHDL enabled novel studies of hosting membrane proteins. (**e**) Black dot represents drugs like curcumin, rHDL was modified by a single-chain variable fragment (comprised of the variable heavy (VH) and light (VL) chains from an antibody) to achieve target drug delivery

14.12 rHDL with Drugs

So far, there have many rHDL complexes served as drug delivery vehicles. Three molecules of taxol incorporated with rHDL showed fewer toxic side effects when treated with several cancer cell lines compared to control. rHDL-ACM consisted of rHDL and actinomycin, the binding affinity of rHDL-ACM was significantly higher than free ACM. Data shows a preferential uptake for SMMC-7721 hepatoma cells of rHDL-ACM [47].

Some anticancer drug was limited by poor absorption, uncontrolled tissue distribution, such as 10-Hydroxycamptothecin.rHDL-HCPT nanoparticles was reconstituted. Compared to native HCPT, rHDL-HCPT presented increased concentrations in target organs and sustained release pattern [48]. Miaomiao Gong et al. constructed an rHDL from mimetic peptide 5A contains anticancer drug docetaxel (DTX) against breast cancer. The survey demonstrated that rHDL is a promising drug vehicle, DTX-rHDL enhanced the uptake of DTX, increased the cytotoxicity against MCF-7 cells, and reduced the

off-target side-effects to normal cells. It was derived that rHDL-DTX nanoparticles were superior to free DTX no matter what sustained drug release properties or the antitumor efficacy [49]. The rHDL-CD-PEI-p53 complexes were incorporated with rHDL and p53 plasmid, the complexes efficiently transferred into the cytoplasm by specific targeted SRB1, in vivo, and in vitro investigation, the complexes inhibited the tumor tissue angiogenesis by disrupting VEGF expression. It suggested a promising therapy for bladder cancer using the rHDL delivery system [49].

14.13 rHDL for Cocktail and Tandem Therapy

Wenli et al. constructed Tanshinone IIA discoidal and spherical recombinant HDL, next characterized the physicochemical and biomimetic properties of them in vitro, in addition, they studied in vivo performances of rHDL with Tanshinone IIA, such as pharmacokinetics, antiatherogenic efficacies. The study considered the rHDL could be an effective vehicle of Tanshinone IIA on account of a higher affinity for atherosclerotic lesions [50].

siRNA and chemotherapy agents integrated into rHDL, for instance, siVEGF and antitumor drugs paclitaxel coencapsulated into rHDL, the complex possessed specific tumor targe, siVEGF inhibited neovascularization, the collaborative effect resulted in enhanced anticancer efficacy [50]. In 2012, Yang Ding constructed rHDL nanoparticles loaded with Chol-siRNA, rHDL/Chol-siRNA-Pokemon complexes specific transported into hepatocellular carcinoma cell, decreased expression of Pokemon and Bcl-2 protein, as a consequence, the growth of the hepatocellular carcinoma was inhibited [44]. In 2014, they combined siRNA with rHDL for target therapy of tumor angiogenesis. They established rHDL nanoparticles loaded with cholesterol-conjugated siRNA. Because of the lipophilic, rHDL offered a highly effective approach to directly transfer into the cytoplasm, and promoted siRNA accumulation within tumors and down-regulated VEGF mRNA and protein expression. As a consequence, the growth of breast cancer was inhibited through the target-specific antiangiogenic therapy [42]. Lisha Liu utilized hyaluronic acid (HA) to decorate rHDL loaded with lovastatin, which improved the antiatherogenic efficacy through targeting atherosclerotic lesions and increasing drug accumulated in the atherosclerotic lesions via strong HA affinity to inflammatory sites [51]. RGD-rHDL/So/antimiRNA21 was formulated by anti-miRNA21, Sorafenib, and rHDL, Sorafenib exerted inhibition of tumor proliferation and angiogenesis,antimiRNA21 improved tumor sensibility to chemotherapy and that rHDL drive the complexes to target site precisely to enhance the efficacy [52].

14.14 Pleiotropic Therapeutic Effects of an Apolipoprotein Mimetic

14.14.1 apoA-I Mimetic

Through proteomics dataset analyses, it was revealed that the protein component of HDL has diversity up to more than 100 kinds. These kinds of proteins were divided into 4 categories: the apolipoprotein, enzyme, Lipid transporters, minor proteins. Many kinds of apolipoprotein appeared in HDL, for example, apoA-I, apoA-II, apoA-IV, apoA-V, apoC-I, apoC-II, apoC-III, apoC-IV, apoD, apoE, apoF, apoH, apoJ, apoL-I, and apoM. apoA-I accounted for almost 70% and predominated in HDL and apoA-II accounted for 20% less than apoA-I. ApoA-I had 243 amino acids, the secondary structure of apoA-I contained 10 amphipathic α-helices. The amphipathic alpha-helices are the main function structure of apoA-I. Now apoA-I mimetics are synthetic peptides that have similar amphipathic alpha-helices to apoA-I. An 18-amino-acid sequence DWLKAFYDKVAEKLKEAF could mimic apoA-I biological functionality [53]. Despite the complexity and diversity of protein components, many treatments had been tested and reported based on imitating apoA-I, such as full-length apoA-I, mutate apoA-I gene,

construct apoA-I mimetic. ApoA-I mimetic peptides were formulated as a cost-effective approach among apoA-I based therapies.

Ananth Ramaiah et al. Synthesized the first apoA-I mimetic peptide, 18A [54], subsequently, human modulated 18A to enhance its function, Yancey et al. created a peptide call 2F by blocking the ends of 18A with an amide group and an acetyl group, which has a stronger effect in inducing cholesterol efflux [55]. The function of apoA-I mimetic peptides was dependent on the structure. The underlying mechanism of mimetic peptides remained obscure. There has been researching showed that apoA-I mimetic peptides may act in the small intestine more than plasma HDL, mimetic peptides affected unsaturated LPA homeostasis, and decreased intestinal inflammation gene expression [56].

Previous research demonstrated that apoE produced by astrocytes bound to lipids, the lipidation of apoE promoted Aβ aggregation and progression of Alzheimer's disease, HDL mimetic peptide, 4F, increased apoE secretion and reduced accumulated apoE's detrimental effects on Aβ [57]. The latest publication revealed 4F interaction with Aβ to constrain its structural plasticity through the NMR experiment [58]. D4F was designed as an enantiomer of 4F consisting of D-amino acids. In Handattu et al. study, mice were divided into groups to received D-4F and pravastatin, D4F, pravastatin, and water alone respectively, D4F + statin group showed the most reduction of Aβ load(1.6 ± 0.1%, $p < 0.05$ vs. the other groups) and the best performance in the Morris Water Maze test [59]. Apolipoprotein A-1 mimetic 4F treatment significantly increased SRB1 expression, Akt phosphorylation downstream was activated, leading to increased endothelial nitric oxide synthase (eNOS) activity and stimulated endothelial cell migration and repairment. These findings confirmed the potential therapeutic function of cardiovascular disease [60].

In Moreira et al. experiments, male Wistar rats were induced to myocardial infarction, then these rats were injected with Apolipoprotein A-I mimetic 4For control, the researcher found that 4F-treated rats displayed decreased the ischemic myocardial area and minimized renal apoptosis and signs of cardiac neovascularization, which implied 4F could protect against MI-induced cardiac dysfunction and 4F have the potent of renoprotection [61].

There has been a researcher reviewed that the emerging roles for apolipoprotein in lung disease development and treatment because of the latent correlations between apolipoproteins and inflammation, oxidative stress, lipid homeostasis [62].

Asthma is characterized by labored breathing, abnormally rapid breathing, audible wheezing, low systolic blood pressure arises from airway inflammation, neutrophilic aggravation, etc. Some studies showed that OVA-induced neutrophilic airway inflammation was inhibited by reducing G-CSF production dependent on apoA-I which suggested apoA-I is a potent target of asthma treatment [63, 64] administration of apoA-I mimetic peptides to experimental allergic asthma murine models decreased inflammatory cells (e.g., neutrophils, eosinophils) and inflammatory cytokines(IL-25, TGF-β, IL-33) [65, 66]. In the clinical survey, it also has been found apoA-I levels were positively correlated with less severe asthma symptoms in a cohort of 159 subjects [67]. The survey considered the negative correlation between apoA-I and chronic pulmonary disease such as COPD (chronic obstructive pulmonary disease, emphysema) [68].

In the LPS-induced acute lung injury rodent models, administration of aopA-I to mice could protect the renal, liver function and reduced mortality [69, 70]. The study suggested that apolipoprotein AI mimetic peptides have similar protective functions due to direct inhibition of inflammation induced by endotoxin and preservation of HDL antioxidant activity [71]. The other study draws a similar conclusion that 4F protect against acute lung injury in endotoxemic rats. The underlying mechanism was associated with stimulation of Akt and inhibited NF-κB pathway, suppressed expression of a pro-inflammatory mediator like adhesion molecules [72]. In LPS-induced acute lung injury mice model, the mice were injected with Rev-D4F, then the differentiation of LPS- injured pulmonary capillary endothelial cells was stimulated, by activating

PI3kinase-dependent pathway, the function of endothelial progenitor cell was restored [73].

Serum apoA-I has been reported to be significantly associated with the development and prognosis of lung cancer [74, 75]. apoA-I now were known as a novel positive factor in solid tumors [76]. Chattopadhyay et al. study demonstrated that oral apoA-I mimetic 6Falter Notch pathways. Increased Notch1 protein levels and decreased Dll4 protein levels leading to reduced tumor burden and improving the outcome of metastatic lung cancer in mouse models [77].

Pulmonary arterial hypertension is a chronic lung disease associated with the out-of-balance of oxidized lipids. 4F improved cardiac and pulmonary function and rescued pulmonary hypertension by decreasing plasma oxidized lipid levels, 4F treatment restored lung miR193 levels resulting in inhibition of HPASMCs [78]. Overexpression of apoA-I decreased lung fibrosis and protected against silica-induced lung inflammation [79]. The above effection indicated the protect potent of apoA-I against pulmonary fibrosis. D-4F suppressed IL-4 induced alternative activation, increased TNF-α transcription and translation, and reduced TGF-β1 transcription and translation [80].

The virus has been regarded as participating in the development of atherogenesis, Van Lenten BJ thought influenza infection caused the alterations of paraoxonase, platelet-activating factor acetylhydrolase, ceruloplasmin leading to loss of HDL anti-inflammatory function [81]. And then they concluded the injection of female mice with D-4F prevents the macrophage from transporting into the innominate arteries by detecting the Sry gene [82]. Furthermore, the research group revealed that type II pneumocytes respond to influenza by activating caspases and secreting oxidized phospholipids. D-4F inhibited the inflammatory response induced by influenza A infection of human type II pneumocytes. The results supported D-4F have therapeutic potential for influenza [83].

Different apoA-I mimetic peptides have different antiatherogenic abilities. The female apoE null mice administrated with peptide 3F-2 were prone to inhibit atherosclerosis compared to the 3F-14 group. In contrast, only 3F-14 affected the terminal methyl group of the acyl chain, these results could be explained by differentials between two peptides resulting from different interactions with membrane bilayers [84]. 5A-mimetic peptide, as a bihelical amphipathic peptide, increased ATP-binding cassette transporter(ABCA1) expression, stimulated ABCA1-mediated cholesterol efflux, and reduced atherosclerosis in apoE-KO mice [84]. Garber et al. gave a detailed account on mice fed with an atherogenic diet having mimetic peptide 5F, the mice injected with peptide 5F significantly decreased peptide 5F. This is the first survey that demonstrated that peptide 5F has a similar antiatherosclerotic function to HDL [85]. Similar results gave evidence that mimetic peptides 5A reduced the expression levels of TNF-α induced adhesion molecules vascular cell adhesion molecule-1 and the activity of NADPH oxidase, thereby protecting against acute inflammation and oxidative stress [86]. Lídia Cedóet al. explored that overexpressed human apoA-I does not impede tumor growth in PyMT mice but treated PyMT mice with D-4F, the tumor development was inhibited by reducing serum oxLDL levels and restraining oxLDL-mediated proliferation [87].

Iwata et al. constructed a new apoA-I mimetic peptide ETC-642, they tested the antiatherogenic properties in Watanabe-heritable hyperlipidemic (WHHL) rabbits.ETC-642 enhance the cholesterol efflux and inhibited the progression of the aortic plaque [88].

In high-fat diet mice models, mice were administered with D4F (enantiomer of 4F consisting of D-amino acids, the D-4F peptide with reverse order of amino acids)via drinking water, after 5 weeks, the blood glucose clearance improved macrophage infiltration and inflammatory response reduced, the glucose and lipid synthesis was suppressed [89]. Du Lin studied the therapeutic role of D-4F in vitro, D4F(1 mg/kg) and R-D4F(1 mg/kg) treatment inhibited intimal hyperplasia (−42%), and facilitates prevention of neointimal formation, in vitro study, D4F and R-D4F inhibited expression of the proinflammatory mediator [90]. High-fat diet

significantly impaired function and number of endothelial progenitor cells. In atherosclerosis mice model, R-D4F inhibited endothelial oxidative stress and decreased atherosclerotic lesion area independent on plasma total and HDL-cholesterol levels [91]. ApoA-I mimetic peptide reverse-D-4F restored TNF-α induced dysfunction of endothelial progenitor cell by increasing stromal cell derived factor 1α (SDF-1α) level. PI3K inhibitor and Akt inhibitor blocked the recovery of Rev-D4F [92].

14.14.2 apoA-E Mimetic

In most cases, the apolipoprotein component of rHDL was apoA-I. Additionally, the other far less abundant apolipoprotein, apoE, commonly constructed of rHDL. apoE mimetic had been shown to mimic various properties on account of the similar dual-domain structure of the native apoE, such as lowering cholesterol [93], enhancing macrophage cholesterol efflux [94], anti-inflammatory, antioxidative .these comprehensive properties supported the therapeutic potential in reducing atherosclerosis [95].

Ac-hE18A-NH2 was an Arg-rich receptor-binding domain of apoE linked to prominent domains 18A of apoA-I. After a single intravenous injection(15 mg/kg) of Ac-hE-18A-NH2, the plasma cholesterol levels and arterial endothelial function in the Watanabe heritable hyperlipidemic rabbit model were improved [54]. Ac-hE18A-NH2 demonstrated stronger anti-inflammatory effects more than 4F in mice infected with E. coli 055:B5, In THP-1 cells, isolated human primary leukocytes, and whole human blood, Ac-hE18A-NH2 showed remarkable anti-inflammatory effects. As a member of the apoE family, Ac-hE18A-NH2 displayed a potential therapeutic effect on Alzheimer's disease. It relieved amyloid plaque deposition and astrocytes activation in APP/PS1ΔE9 mice administrated with 50 μg Ac-hE18A-NH2 three times a week [96].

apoE mimetic peptide presented anti-inflammatory property in various studies. 6KApoEp was composed of apoE mimetic and 6 lysines added at the N-terminus. 6KApoEp reduced pro-inflammatory tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) secretion intermediated neuroinflammation by interacting with LDL receptor [96]. The mimetic peptide of the Apolipoprotein E(apoE) COG1410 increased the viability, migration of 5-fluorouracil (5-FU)-injured intestinal cells IEC-18, the apoptosis induced cell death was suppressed, which suggested the protective role of COG1410 in intestinal mucositis [97]. The other study surveyed the apoE peptide COG 133 triggered the activation of the Wnt/b-catenin pathway, decreased AXIN and APC transcripts, and increased cellular viability. This study confirmed the protective effect of apoE mimetic in intestinal cells [98].

apoE mimetic peptides also had a protective function on brain injury.in experimental subarachnoid hemorrhage models. After subarachnoid hemorrhage, the increasing degradation of BBB permeability caused evolutional neuron apoptosis, apoE mimetic attenuated the blood-brain barrier disruption, thereby secondary brain injury was ameliorated [99].

ATI-5261 was a novel, single-helix, 26-mer peptide similar to native apoE, ATI-5261 stimulated cholesterol efflux effectively withα--helical structure. In the mouse model with arterial stiffness, ATI-5261 reversed earl postpartum and arterial stiffness by increasing ABCA1 expression and cholesterol efflux capacity [100].

In AICD- overexpressing transgenic mice, after subcutaneous administration of COG112 three times per week for 3 months, the researcher measured neuroinflammation and phosphorylated tau. Results showed apoE-mimetic COG112 suppressed accumulation of tau and protected against neuroinflammation in AICD-induced AD-like pathologies [94].

14.15 The Other Apolipoprotein Mimetic

Some other kinds of apolipoprotein mimetic like apoJ mimetic were constructed and studied. For instance, D-apoJ characterized as a 10-residue

class G* peptide from apoJ [73, 101–107], inhibited both sphingomyelinase-induced and spontaneous LDL aggregation [108]. Some studies investigated the effect of apoJ mimetic compared to apoE mimetic [109]. Here, we have given a summary of common mimetic peptides in Table 14.1.

14.16 Conclusion and Outlook for rHDL and Apolipoprotein

A number of rHDL and apolipoprotein analog have shown beneficial effects in some studies. By now, many types of rHDL and apoA-I mimetic peptides have been used in clinical trials. These synthetic apolipoproteins displayed favorable safety and tolerability [34, 114]. As current status stands, present rHDL and mimetic peptides have not reached the adequate efficacy. Despite the failure of clinical trials of rHDL and mimetic peptides in antiatherosclerosis, the diverse efficacy of apolipoprotein mimetic in different disease models indicated rHDL and apolipoprotein mimetic are still promising therapeutic target.

There are still many questions remaining to be solved. Such as the intravenous injection of administration limit rHDL chronic and long-term treatment. Also, the high cost of rHDL production restricts the spread of clinical use. Furthermore, the narrow treatment efficacy window restricted to its short half-life was adverse to treating acute cardiovascular disease, generally speaking, the half-life of rHDL-associated apoA-I was 48–72 h, to reach the effective plasma concentration usually need high dose and frequently infusion of rHDL.

Moreover, because of a lack of consensus about the best method for administration, we usually used apolipoprotein mimetic peptide and rHDL based on experience. The research revealed a different method for administration significantly affect the pharmacokinetics and pharmacodynamics [115].

Table 14.1 Characteristics and therapeutic effects of ApoA-I mimetic peptides [7, 32, 36, 73, 88, 101–107, 110–113]

Mimetic peptide type	Description	Therapeutic effect
MDCO-216	apoA-I Milano, a naturally mutant in apoA-I protein	Regression of arteriosclerosis Plaque
ETC-642	PVLDLFRELLNELLEALKQKLK	Inhibits vascular inflammation; Regression of arteriosclerosis Plaque
CER-001	Recombinant human apoA-I and produced in mammalian cell expression	Inhibits vascular inflammation; Regression of arteriosclerosis Plaque
CSL-111	apoA-I isolated from human HDL and reconstituted phospholipids	Inhibits platelet function; Restores endothelial cell function
CSL-112	apoA-I isolated from human HDL with reduced lipid/protein ratio	Inhibits platelet function; Restores endothelial cell function
4F	Ac-DWFKAFYDKVAEKFKEAF-NH2	Reduces apoE accumulation; Restores endothelial cell function; Decreases apoptosis; improved cardiac and pulmonary function reported
D4F	Also called APP018, an oral apoA-I mimetic peptide	Anti-inflammation; antioxidative stress; reduces pulmonary fibrosis; inhibits tumor development
Ac-hE18A-NH2	LRKLRKRLLR apoE mimetic	Anti-inflammatory

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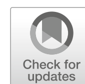

15 HDL and Surgery

Yue-Ming Peng and Jing-Song Ou

Abstract

In addition to the well-known functions, plasma HDL also plays an important role in postsurgery periods. In this chapter, we summarized the changes of HDL after surgery like bariatric surgery and cardiac surgery. Not only the amount of HDL changed, the HDL components or functions have also been altered after various surgeries. Furthermore, a few HDL-related indexes have been recognized as important clinical predictors after surgery, such as HDL cholesterol efflux capacity, HDL pro-inflammatory index, HDL cholesterol (HDL-C) concentration, and monocyte count to HDL ratio (MHR).

Keywords

HDL · Bariatric surgery · Cardiac surgery · Small HDL subfractions

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15.1 Surgery Alter Plasma High-Density Lipoprotein Cholesterol Level (HDL-C)

15.1.1 Bariatric Surgery Increase Plasma HDL-C

There are consistent data to prove that bariatric surgical procedures increase HDL cholesterol (HDL-C) level [1–8]. Two biologically weight loss stages (acute weight loss and weight maintenance) after bariatric surgical procedures occur and the plasma HDL levels change during the course of weight loss. Firstly, during acute weight loss, the concentration of lipoprotein lipase reduces by 50% [2]. Because the lipoprotein lipase catalyzes the lipolysis of TG-rich lipoproteins and provides lipid and protein constituents of HDL, the decreased levels of lipoprotein lipase during acute weight loss block the transfer of lipid and protein to HDL and therefore decrease the levels of plasma HDL [2]. In the contrast, lipoprotein lipase activity was increased during weight maintenance time and the lipids and apolipoproteins were transferred to HDL obviously and therefore increase plasma HDL [2, 3]. However, different bariatric surgical procedures have different effects on HDL levels. Clinical studies have showed a hierarchy on the impact of bariatric surgery on HDL [2].

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15.1.2 Cardiac Surgery Reduce Plasma HDL-C Level

A study reported that plasma HDL-C levels in children were significantly decreased 24 h after the Fontan operation (a type of cardiac surgery) [9]. Same result was obtained in the adult: HDL-C levels were reduced in patients undergoing cardiovascular surgery 1 day postoperatively. The decrease of cholesteryl ester transfer protein (CETP) mass and activity was consistent with the decrease of HDL-C levels [10].

Even more noteworthily, HDL-C concentration declines in both studies occurred in 1-day postoperation, still in the acute phase response time. There is an explanation for decreased HDL levels during acute phase response: the proinflammatory cytokines induce group IIA secretory phospholipase A2 and serum amyloid A during acute phase response. The IIA secretory phospholipase A2 can modify HDL and induces scavenger receptor class B type I interaction and promotes HDL catabolism [11, 12]. Meanwhile, some study reported that serum amyloid A increases after cardiac surgery [10, 13] and induces sPLA2 activity [14].

15.2 Surgery Modifies the Composition of High-Density Lipoprotein

15.2.1 Bariatric Surgery Change HDL Subspecies Profile Characterized

Davidson reported that bariatric surgery in severely obese adolescent males with vertical sleeve gastrectomy (VSG) raises the large size HDL levels [4]. In this study, ten 14–20 year-old male patients were recruited. A low large size HDL levels and a high small size HDL levels compared to lean adolescents were showed in adolescents with severe obesity before VSG [4]. The large size HDL levels were significantly increased to the levels similar to the lean adolescents after 1 year of VSG [4]. However, the VSG surgery did not affect the small size HDL levels [4]. There was no change in small size HDL levels after VSG. Furthermore, they identify the change of large HDL subspecies-associated proteins after VSG, and found that apolipoprotein A-I (ApoA-I) and apolipoprotein E (ApoE) were increased, while plasma kallikrein was decreased in large HDL subspecies. ApoA-I is a major functional apolipoprotein of HDL. ApoE associated with large size HDL can compete with LDL for heparin-sulfate proteoglycans in the vessel wall to inhibit atherosclerosis [15]. Plasma kallikrein, a serine protease, might associate with pathogenesis of inflammation, thrombosis, and blood pressure regulation [16]. Laparoscopic sleeve gastrectomyin obese patients also enhance large size HDL levels and reduce small size HDL levels 1 day after surgery compared with before surgery [17].

Similar studies had been done in other kinds of bariatric surgery. Coimbra et al. [1] conducted a study in twenty obese adult patients who have obesity-related comorbidities and undergo laparoscopic adjustable gastric banding (AGB) surgery, and showed an enhanced large size HDL and middle size HDL levels, and a reduced small size HDL levels 13 months after AGB. Using a nondenaturing, linear, polyacrylamide gel electrophoresis system, they separated HDL into 10 subfractions. 1–3 subfractions, 4–7 subfractions, and 8–10 subfractions were classified as large HDL subfractions, middle HDL subfractions, and small HDL subfractions respectively [1]. Before AGB, less large HDL subfractions and cholesterol content were showed in patient compared with control group; but the levels of middle HDL subfractions and small HDL subfractions are similar, while their cholesterol content was lower [1]. Thirteen-months after AGB, there was a significant increase in large HDL subfractions and its cholesterol content, and indeed, there was no difference between patients who had undergone AGB and controls group. Meanwhile, the levels of middle HDL subfractions remained unchanged, but the cholesterol content increased, though remarkably less than the control value. Small HDL subfractions

decreased significantly and its cholesterol content enhanced, and had no difference compares with those of the control group [1].

In Judith et al. study [18], they enrolled 34 morbidly obese women who underwent laparoscopic Roux-en-Y gastric bypass (RYGB) surgery and isolated HDL subfractions using isopycnic density gradient ultracentrifugation. Differently, their data showed that RYGB surgery had no impact on plasma HDL-C levels, but raised plasma ApoA-I levels at 6 months after surgery compared to baseline. As for HDL subfractions, the levels of large HDL2 subfraction increased at 6 months after RYGB surgery compared to baseline, but those of small HDL3 subfraction persisted.

In conclusion, large size HDL levels increase and small size HDL levels decrease or persisted after bariatric surgery. Weight loss, subsequent decrease in insulin resistance and hypertriglyceridemia, increased peripheral insulin sensitivity, diminished CETP, and lecithin–cholesterol acyltransferase protein level and activity may contribute to the changes of HDL subfraction profile [2].

15.2.2 Small HDL Subfractions Increase in Patients with Renal Transplant

HDL subspecies change is different in pediatric patients with renal transplant. Plasma HDL-C levels were no different between renal transplant during childhood group and control group [19]. Plasma CETP activity inversely was obviously increased in patients with renal transplant compared with controls. They separated HDL subfractions using composite polyacrylamide gradient gels electrophoresis and showed that the patients with renal transplant contained less large HDL 2b subclass and more small HDL 3a and 3b subclasses [19].

15.2.3 Cardiac Surgery Change HDL Composition

The HDL composition was also changed after cardiac surgery. Hacquebard et al. [13] showed that cardiac surgery with cardiopulmonary bypass changed the composition of HDL during acute phase response. They collected blood samples before and day 2 after surgery, and then separated HDL from plasma and identified the composition of HDL, respectively. Their data indicated that HDL ApoA-I concentration decreased on day 2 after surgery, while the HDL apoSAA levels increased substantially after surgery. As for the lipids, HDL total cholesterol and cholesteryl ester (CE) decreased, but, free cholesterol (FC), phospholipid and triglycerides(TG) levels in the HDL fraction did not change postoperatively [13]. Therefore, the ratio of HDL CE/ApoA-I was not significantly modified while TG/ApoA-I ratios increased after surgery. This indicates a raise in core lipid molecules and volume in postoperative HDL particles. Accordingly, both FC/ApoA-I and phospholipid/ApoA-I ratios enhanced in HDL after surgery [13]. In conclusion, all these suggest that HDL area and of HDL radius had increased after cardiac surgery. Meanwhile, the hydroperoxide level was not obviously change, but alpha-tocopherol concentration had decreased after surgery in the HDL fraction. The reduction of alpha-tocopherol may influence the cells and organs obtained alpha-tocopherol in a condition (ischemia–reperfusion) characterized by oxidative stress [13].

15.3 Surgery Affects High-Density Lipoprotein Functions

Surgery did not only modify HDL subfractions, but also their functions.

15.3.1 Bariatric Surgery Improve HDL Functions

In Davidson et al. [4] study, we discussed above, they also determine HDL function both before and after VSG surgery. The data showed that VSG surgery increased 12% of cholesterol efflux capacity and 25% of HDL antioxidative capacity, decreased 30% of HDL lipid peroxidation potential. In addition, there are no correlation between the degree of alteration and BMI loss or HDL-C levels [4]. Therefore, the improvements in HDL function are likely dedicated by combination of metabolic risk factors. Other studies also proved that bariatric surgical can improve HDL functions in cholesterol efflux capacity(RCT) [18] and antioxidant potential [20].

In Osto [21] research, they isolated HDL from rat and patient by sequential density ultracentrifugation before and after RYGB surgery and measured the properties of HDL. Their data demonstrated that RYGB surgery rapidly reversed HDL dysfunction and ameliorated HDL functions in patients and rats, including endothelial cell apoptosis, nitric oxide (NO) production, endothelial nitric oxide synthase (eNOS) activity, anti-inflammatory properties, antioxidant effects, and RCT [21]. Glucagon-like peptide-1(GLP-1) analog liraglutide can mimic the effects of RYGB in rat. However, treatment with exendin9-39(a GLP-1 receptor antagonist) after RYGB did not affect the properties of HDL. Therefore, these improvements of HDL protective effects were GLP-1 dependent, but were independent of GLP-1 receptor activation. In addition, weight matched to RYGB group even impaired HDL function compared with post-RYGB surgery group both in rats and patients; therefore, RYGB-surgery-induced HDL functions improvements are not weight loss dependent [21].

In conclusion, HDL functions are improved after bariatric surgical, but the underlying mechanisms are associated with metabolic risk factors and GLP-1, instead of weight loss.

15.3.2 Cardiac Surgery Impaired HDL Function

We previously also found that HDL functions were changed in valvular heart disease (VHD) patients with cardiac surgery [22]. We used a cell-free assay to determine the pro-inflammatory HDL index. The data showed that the pro-inflammatory HDL index was dramatically enhanced in VHD patients before surgery and significantly increased 6 h after cardiac surgery compared with before operation [22]. The pro-inflammatory HDL index returned to the value of preoperation 12 h after surgery, and is lower than preoperation 48 h after surgery [22]. We further found that endothelium-dependent relaxation was significantly impaired by HDL from preoperation VHD patients compared to HDL from control subjects. The endothelium-dependent relaxation was impaired even more in HDL from patients with cardiac surgery compared with HDL from preoperation VHD patients [22]. Further research showed that HDL from VHD patients before and after surgery can inhibit eNOS associated with heat shock protein 90. As a result, eNOS uncoupled to generate superoxide anion instead of NO to impair endothelium-dependent relaxation [22]. Interestingly, we found that perioperative simvastatin therapy can reverse this impaired HDL function [22].

In Singh et al. [23] study, they evaluated HDL function by RCT and endothelial progenitor cell migration assay and found that these two highly distinct dimensions of HDL function are impaired in patients with heart transplant. Furthermore, HDL function in patients was not influenced by corticosteroid use and cardiac allograft vasculopathy (CAV) status and prior ischemic cardiomyopathy.

15.4 High-Density Lipoprotein as a Predictor in Surgery

15.4.1 Monocyte Count to HDL Ratio (MHR)

MHR was recently suggested as an indicator of inflammation and oxidative stress.

Karatas et al. [24] analyzed 513 patients with acute ST-segment elevation myocardial infarction (STEMI) and treated with primary percutaneous coronary intervention (PCI) in his retrospective study. They found that preoperative MHR levels can independently predict in hospital mortality and major adverse cardiac events including ventricular arrhythmia, reinfarction, cardiopulmonary resuscitation, target vessel revascularization, and death during index hospitalization using multivariate regression analysis [24].

Similar studies had done by Saskin et al. [25] in 662 patients with sinus rhythm preoperatively and underwent isolated coronary artery bypass grafting. Using multivariate regression analysis, they also found that preoperative MHR was an independent risk factor for the occurrence of atrial fibrillation in the postoperative period and mortality in the early postoperative period [25].

In a word, the MHR value is a predictor of in-hospital mortality and major adverse cardiac events in STEMI patients treated with primary PCI, and a predictor of postoperative atrial fibrillation and mortality in patients underwent isolated coronary artery bypass grafting.

15.4.2 High-Density Lipoprotein(HDL) Cholesterol Efflux Capacity

A prospective study executed by Annema et al. [26] showed that HDL RCT is not relative with cardiovascular or all-cause mortality but is a strong predictor of graft failure in patients with renal transplant, the incidence of graft failure decreased with increasing RCT. In this study, backward multiple linear regression analysis found that RCT had a strong, independent relationship with plasma ApoA-I and HDL-C levels [26]. Cox proportional hazard analyses showed that RCT at baseline was a significant predictor of graft failure, even after additional adjustments for ApoA-I and HDL-C levels [26]. Although univariate analyses showed that HDL-C levels and ApoA-I levels had association with graft failure, there is no statistical significance after adjusting for RCT [26]. All data combined suggest that HDL RCT is a strong independent risk marker of graft failure in patients with renal transplant. More importantly, this clinical association of RCT was independent of plasma HDL-C and ApoA-I levels.

Javaheri et al. [27] study demonstrated that reduced RCT is associated with disease progression and mortality in cardiac transplant recipients (CAV). They determined the relationship between RCT and survival in patients with CAV, verified by blinded review of angiography by an cardiologist, multivariable cox proportional hazard models demonstrated that RCT was associated with survival even after further controlling for HDL-C and LDL-C levels [27]. Furthermore, they demonstrated CAV progression by measuring the changes in maximal intimal thickness on intravenous ultrasound (IVUS) before transplant and 1-year posttransplant in another cardiac transplant recipient cohort, and the data also proved that pretransplant basal RCT was independently associated with CAV [27].

15.4.3 High-Density Lipoprotein Cholesterol (HDL-C) Concentration

In Li et al. [28] study, they enrolled 2529 consecutive patients with normal levels of cardiac troponin I (cTnI) and creatine kinase-MB and without acute myocardial infarction in the past 4 weeks who underwent elective PCI and tried to evaluate the association between preprocedural HDL-C concentration and periprocedural myocardial injury. Periprocedural myocardial injury was defined as postprocedural cTnI $> 1 \times$ limit of normal (ULN), periprocedural myocardial

infarction was defined as postprocedural cTnI $> 3 \times$ ULN, and postprocedural cTnI $> 5 \times$ ULN, which was a requirement in the arbitrarily revised diagnosis criteria [28]. They found out that only in patients with LDL-C < 70 mg/dL, the preprocedural HDL-C concentration was a predictor of periprocedural myocardial injury, higher HDL-C concentration was associated with reduced risk of periprocedural myocardial injury [28].

A study [29] enrolled 391patients who underwent cardiac surgery(including elective coronary artery bypass grafting, valve surgery, or ascending aortic surgery requiring thoracotomy or sternotomy) and found that an elevated preprocedural HDL-C concentration was independently associated with a lower ΔSCr (the maximum change in serum creatinine level from baseline to 48 h post-operation), ΔSCr used to define acute kidney injury (AKI) after cardiac surgery. The magnitude of the association between increased HDL-C concentration and reduced postoperative ΔSCr was further increased by long-term statin therapy and perioperative atorvastatin exposure [29]. Besides, raising dosage of long-term statin therapy further potentiated the relationship between high preoperative HDL-C level and reduced postoperative ΔSCr [29]. In conclusion, a higher preoperative HDL-C level was independently related with a reduction of risk of AKI after cardiac surgery, and preoperative and perioperative statin therapy enhance this relationship between elevated HDL-C level and the reduction of AKI [29].

In summary, as in other diseases states, not only the HDL level was changed in surgery, but the composition and function of HDL were altered after surgery. However, the implication of HDL change in surgery is not fully understood. This needs to be studied in the future.

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