Signaling by the High-Affinity HDL Receptor Scavenger Receptor B Type I

Sonika Saddar; Chieko Mineo; Philip W. Shaul

Abstract—Scavenger receptor B type I (SR-BI) plays an important role in mediating cholesterol exchange between cells, high-density lipoprotein (HDL) cholesterol, and other lipoproteins. SR-BI in hepatocytes is essential for reverse cholesterol transport and biliary secretion of HDL cholesterol; thus, it is atheroprotective. More recently, it has been discovered that the HDL–SR-BI tandem serves other functions that also likely contribute to HDL-related cardiovascular protection. A number of the latter mechanisms, particularly in endothelial cells, involve unique direct signal initiation by SR-BI that leads to the activation of diverse kinase cascades. SR-BI signaling occurs in response to plasma membrane cholesterol flux. It requires the C-terminal PDZ-interacting domain of the receptor, which mediates direct interaction with the adaptor molecule PDZK1; and the C-terminal transmembrane domain, which directly binds membrane cholesterol. In endothelium, direct SR-BI signaling in response to HDL results in enhanced production of the antiatherogenic molecule nitric oxide; in a nitric oxide–independent manner, it serves to maintain endothelial monolayer integrity. The role of SR-BI signaling in the numerous other cellular targets of HDL, including hepatocytes, macrophages, and platelets, and the basis by which SR-BI senses plasma membrane cholesterol movement to modify cell behavior are unknown. Further understanding of signaling by SR-BI will optimize the capacity to harness the mechanisms of action of HDL–SR-BI for cardiovascular benefit. (Arterioscler Thromb Vasc Biol. 2010;30:144-150.)

Key Words: HDL ■ SR-BI ■ endothelium ■ signaling ■ Src kinase ■ cholesterol

Several epidemiological studies1–3 have established that the risk of coronary heart disease is inversely related to the plasma concentration of high-density lipoprotein (HDL) cholesterol. In addition, a number of clinical studies using pharmacological agents that increase the level of HDL cholesterol have demonstrated beneficial effects on atherosclerosis progression and clinical outcomes.4 HDL plays an essential role in reverse cholesterol transport, transferring cholesterol from peripheral tissues to the liver for excretion into the gut and delivering cholesterol to steroidogenic tissues to serve as a substrate for steroid hormone synthesis.5–9 The cardiovascular protection afforded by HDL was previously attributed to the process of reverse cholesterol transport. However, multiple lines of evidence indicate that other effects of HDL, mediated by novel signal initiation by the high-affinity HDL scavenger receptor B type I (SR-BI), contribute to the cardiovascular benefits of the lipoprotein. In particular, HDL–SR-BI signaling modifies the behavior of vascular endothelial cells in a favorable manner.10–13 This review will highlight recent advances in our understanding of cell signaling initiated by the HDL–SR-BI tandem, with a focus on the structure-function requirements of the SR-BI protein and partner molecules of SR-BI participating in signal transduction events of relevance to cardiovascular health and disease.

Cellular and Systemic Roles of SR-BI

SR-BI is a cell surface glycoprotein first identified by its homology to the scavenger receptor CD36, a class B scavenger receptor.14,15 Class B scavenger receptor family members share similar hairpin-like membrane topologies, with the large midportion of the protein that resides extracellularly anchored to the plasma membrane by transmembrane domains adjacent to short N- and C-terminal cytoplasmic domains.14,16 SR-BI proteins from different mammalian species share 70% to 80% sequence identity over their 509 amino acid lengths. An alternatively spliced variant of SR-BI messenger RNA also exists (ie, SR-BII), which differs from SR-BI in its C-terminal cytoplasmic domain.17 In cultured cells, both SR-BI and SR-BII are localized to cholesterol and...
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The systemic in vivo role of SR-BI in cholesterol metabolism has been addressed by manipulations of the level of its expression in mice. SR-BI−/− mice have an elevated plasma total cholesterol level that consists primarily of large, heterogeneous, HDL-like particles; decreased cholesteryl ester stores in steroiogenic tissues; and reduced biliary cholesterol secretion.21 Conversely, hepatic overexpression of SR-BI by either adenovirus injection or transgenic strategies in mice afforded by either apolipoprotein E or LDL receptor deletion and the provision of an atherogenic diet. In these models, atherosclerosis is exacerbated by the global absence of SR-BI and attenuated by hepatic overexpression of SR-BI; under most conditions, its presence in bone marrow–derived cells lessens atherosclerotic lesion development.21,34–36 The latter observations are consistent with an overall protective role of SR-BI in macrophages. To provide conditional deletion of SR-BI for cell-selective studies in vivo, loxP sites were inserted into the mouse SR-BI gene. This yielded a hypomorphic allele and floxed SR-BI mice with markedly attenuated SR-BI expression and a 2-fold elevation in plasma total cholesterol. Hypomorphic SR-BI mice given an atherogenic diet had more atherosclerosis than control mice, and Cre-mediated SR-BI gene inactivation of the hypomorphic SR-BI allele in hepatocytes yielded even greater atherosclerosis when the atherogenic diet was implemented. The latter finding of worse disease with liver-specific loss of function for SR-BI is consistent with the attenuation in atherosclerosis observed with liver-specific gain of function for SR-BI.21,34–36 However, the degree of atherosclerosis with hepatocyte-specific inactivation of the hypomorphic SR-BI allele was exceeded by the amount of disease observed in atherogenic diet-fed global SR-BI−/− mice with a similar atherogenic lipoprotein profile.37 Thus, the atheroprotective role of SR-BI is not sufficiently explained by its function in the liver.

Evidence of cardiovascular protective functions of SR-BI in sites other than the liver or macrophages is accumulating. It has been reported that HDL inhibits platelet activation; more recent work has suggested that oxidative modification of HDL results in markedly more potent antithrombotic activity. The inhibition of platelet activation by oxidized HDL requires SR-BI, which is expressed in platelets. The interpretation of findings in platelets from SR-BI−/− mice is complicated by the indirect impact of the high plasma unesterified to total cholesterol ratio in the null mouse.38,39

Our laboratory demonstrated that HDL binding to SR-BI in vascular endothelium causes direct activation of endothelial nitric oxide synthase (eNOS), thereby generating the potent antithromogenic molecule NO. In endothelium, SR-BI is colocalized with eNOS in caveolae; and HDL causes robust eNOS activation in isolated endothelial cell caveolae membranes, revealing that the receptor and enzyme are mechanistically coupled in the microdomain. In addition, the increase in eNOS enzymatic activity in cultured endothelial cells is accompanied by an increase in eNOS serine 1177 phosphorylation, indicating that there is kinase activation by HDL–SR-BI.40,41 HDL also stimulates endothelial cell migration in vitro in an NO-independent manner via SR-BI–mediated activation of Rac guanosine triphosphatase. Paralleling the in vitro findings, carotid artery reendothelialization after perivascular electric injury is markedly diminished in apolipoprotein A-I−/− mice; these mice have HDL cholesterol levels that are more than 80% lower than wild-type mice, and reconstitution of apolipoprotein A-I expression in apolipoprotein A-I−/− mice rescues normal reendothelialization. Furthermore, reendothelialization is dramatically impaired in SR-BI−/− mice. Thus, in addition to its actions on eNOS, HDL stimulates endothelial cell migration via SR-BI–initiated signaling; these mechanisms promote endothelial monolayer integrity in vivo.42 In mice, there is also evidence of a key role for SR-BI–induced signaling in endothelial progenitor cells in the capacity of human apolipoprotein A-I to blunt allograft vasculopathy.43
which binds to the extracellular domain of the receptor and prevents SR-BI–mediated cholesterol efflux.\textsuperscript{45} Taken together, these findings indicate that cholesterol flux is required for SR-BI–initiated intracellular signaling. Because the capacity to invoke cholesterol efflux varies between different HDL species and with chemical modifications of the lipoprotein,\textsuperscript{46} it is likely that HDL subpopulations have disparate abilities to activate intracellular signaling via SR-BI. Further investigation is needed in this area.

**Requirement for the C-Terminal Cytoplasmic Domain**

In the initial studies of eNOS activation by HDL in plasma membranes isolated from endothelial cells, signaling was abrogated by the addition of antibody against the C-terminal cytoplasmic tail of SR-BI.\textsuperscript{44} In eNOS-expressing COS-M6 cells, SR-BI, the splice variant of SR-BI that differs from it only in its C-terminal cytoplasmic domain and that binds HDL with the same affinity as SR-BI, is unable to mediate HDL-induced eNOS activation.\textsuperscript{20,44} In addition, deletion of the extreme C-terminal lysine of SR-BI, which is a PDZ-interacting domain necessary to bind the PDZ domain–containing SR-BI adaptor protein PDZK1, yields a receptor that is incapable of mediating HDL-induced eNOS phosphorylation or activation.\textsuperscript{44} Thus, residues within the C-terminal cytoplasmic domain of SR-BI play an essential role in signal transduction by the receptor.

**Requirement for the CTTM**

The structural domains of SR-BI required for signal initiation were further investigated in experiments using SR-BI and CD36 chimeric proteins coexpressed with eNOS in COS-M6 cells. This strategy was chosen because, in contrast to SR-BI, CD36 does not invoke signaling in this model system; CD36 and SR-BI share 30% identity over their primary sequence and have a similar secondary structure, and CD36 binds HDL with comparable affinity as SR-BI. A chimera consisting primarily of CD36, to which the SR-BI C-terminal cytoplasmic domain has been added at the C-terminus, does not invoke eNOS phosphorylation or enzyme activation; this observation indicates that other domains of SR-BI in addition to the C-terminal cytoplasmic tail are required to initiate signal transduction. The inclusion of the SR-BI CTTM, along with the SR-BI C-terminal cytoplasmic domain, yields a chimera that is as capable of activating eNOS phosphorylation and enzyme activity as wild-type SR-BI. Thus, residues within the CTTM of SR-BI also play an essential role in signal transduction by the receptor. Prompted by the prior work indicating that sterol sensing by 3-hydroxy-3-methylglutaryl–coenzyme A reductase, SREBP cleavage–activating protein, and Niemann-Pick C1 entails cholesterol binding to transmembrane domains within these proteins, photocholesterol binding studies further revealed that the CTTM of SR-BI binds cellular cholesterol. However, the SR-BI CTTM sequence lacks homology with any known cholesterol-binding domain. The molecular basis and functional signifi-
Signaling Molecule Partners of SR-BI

Proteins That Interact With SR-BI

The PDZ domain–containing protein PDZK1 (also called CLAMP) was first identified as an SR-BI–associated protein in affinity chromatography studies of rat liver membrane extracts. PDZK1 contains four PDZ domains, and the N-terminal PDZ domain interacts with the extreme C-terminal residues of SR-BI (Fig. 2). In both humans and mice, PDZK1 is highly expressed in the liver, kidney, and intestines (places where SR-BI is prevalent). In humans, in addition, PDZK1 is highly expressed in the adrenal gland, ovary, and testis (places where SR-BI is also abundant); in contrast, in mice, PDZK1 is minimally expressed in these tissues. In the liver, PDZK1 is essential for the stability of the SR-BI protein in the plasma membrane. As a result, PDZK1−/− mice have a greater than 95% decrease in hepatic SR-BI protein and resulting abnormally high plasma cholesterol levels and large HDL particles. Studies of hepatic overexpression of portions of PDZK1 indicate that the various PDZ domains play differential roles in SR-BI stability and function in the liver. In contrast to the liver, in the aorta and in cultured bovine endothelial cells where PDZK1 is also found, steady-state levels of SR-BI are unchanged in the absence of the adaptor molecule. In endothelial cells, PDZK1 also does not influence the targeting of SR-BI to the plasma membrane, SR-BI binding by HDL particles, or cell uptake of cholesteryl esters. As such, there are disparate mechanisms of regulation of SR-BI in different cell types.

Although not critical to SR-BI abundance or subcellular localization in endothelial cells, PDZK1 is essential for SR-BI–mediated signal initiation in endothelium. In cultured endothelial cells, PDZK1 depletion prevents HDL activation of both eNOS and cell migration. Endothelial cell responses to other stimuli, including vascular endothelial growth factor, are PDZK1 independent, indicating that PDZK1 is uniquely required for HDL–SR-BI signaling in the endothelium. Parallel to the in vitro findings, carotid artery reendothelialization after perivascular electric injury is absent in PDZK1−/− mice; this phenotype persists in PDZK1−/− with genetic reconstitution of PDZK1 expression in the liver, whereas PDZK1 modifies SR-BI abundance and thereby influences plasma lipids. Thus, PDZK1−/− mice phenocopy apolipoprotein A-I−/− and SR-BI−/− mice regarding impaired reendothelialization. Because PDZK1 specifically modulates HDL-mediated endothelial cell migration, these collective observations reveal that, in the context of all other factors regulating endothelial cell phenotype, the molecular signaling pathway composed of HDL–apolipoprotein A-I, SR-BI, and PDZK1 is a major promoter of endothelial monolayer integrity in vivo.

Additional studies in bovine aortic endothelial cells have revealed that Src family kinase(s) participate in the proximal signaling events activated by HDL–SR-BI in the endothelium. Coimmunoprecipitation experiments have further indicated that c-Src interacts with SR-BI (Fig. 2). It is predicted that c-Src interacts with the cytoplasmic C-terminus of SR-BI, but the specific SR-BI domain participating in the interaction is yet to be identified. Although the activation of c-Src and downstream signaling is PDZK-1 dependent, the interaction between c-Src and SR-BI is not PDZK-1 dependent. These findings suggest that PDZK-1 modifies the localization or function of kinase(s) required for c-Src phosphorylation induced by HDL–SR-BI, and that either another adaptor molecule(s) mediates c-Src binding to SR-BI or there is another mechanism.
is direct protein-protein interaction between c-Src and the receptor. Such possibilities warrant further investigation.

Ligand-Receptor Pairs Approximated by SR-BI
In addition to the direct signal initiation by SR-BI that is caused by cholesterol flux, there is evidence that the receptor provides spatial proximity of HDL particle constituents and their cell surface receptors. This may be the case for three lysophospholipids present in HDL (ie, sphingosylphosphorylcholine, sphingosine-1-phosphate, and lysosulfatide) and the lysophospholipid receptor S1P3, which is a G protein-coupled receptor that activates eNOS in endothelial cells.63,64 Other HDL-associated molecules, including estradiol, that activate nongenomic cell responses via a subpopulation of estrogen receptors in endothelial cell caveolae65 have also been implicated in signaling initiated indirectly by HDL. However, the role of estradiol in HDL activation of eNOS is disputed and is currently unclear.64

Signaling Molecules Downstream of SR-BI
By using both pharmacologic and genetic strategies, the signaling molecules mediating the coupling of Src activation to eNOS activation by HDL–SR-BI in endothelial cells have been identified (Fig. 2). Src activation leads to the activation of phosphatidylinositol 3-kinase (PI3K), and PI3K then induces the independent activation of both Akt kinase and mitogen-activated protein (MAP) kinase pathways. Akt phosphorylates serine 1177 of eNOS, and the basis for MAP kinase–mediated activation of eNOS is unknown. More important, the concerted effects of both the Akt and MAP kinase cascades are required for HDL-induced stimulation of eNOS enzymatic activity.40 In human endothelial cells, the activation of eNOS by HDL that occurs through these processes inhibits S1P-induced adhesion molecule expression.66 As previously mentioned, the activation of endothelial cell migration by HDL–SR-BI is an NO-independent process. However, the proximal signaling events are identical; Src-dependent activation of PI3K activates Akt and MAP kinases, and these events lead to increased Rac activity, lamellipodia formation, and cell migration. Studies with pertussis toxin further indicated that endothelial cell migration stimulated by sphingosine-1-phosphate is G protein dependent as a result of the participation of S1P3, whereas HDL-induced migration is not affected in the same way.42

In addition to the previously mentioned signaling molecules activated by HDL via SR-BI in the endothelium, other signaling events have been reported in HDL-treated cells. These include the activation of phosphatidylinositol- and phosphatidylcholine-specific phospholipases C and D (ie, PI-PLC, PC-PLC, and PC-PLD), protein kinase C (PKC), and heterotrimeric G-proteins; the production of 3′-5′-cyclic adenosine monophosphate and ceramide; and the stimulation of intracellular calcium release. These signaling events have been shown to modify cell behavior in a number of contexts, and greater understanding of the specific role of SR-BI in these processes is warranted. For example, studies in Chinese Hamster Ovary (CHO) cells have provided evidence that the selective lipid uptake activity of SR-BI is regulated by the PKC and PI3K signaling pathways; PKC activation and PI3K inhibition increase the efficiency of SR-BI–mediated selective lipid uptake, whereas PKC inhibition and PI3K activation reduce its efficiency.67 As such, the cholesterol trafficking activities of SR-BI may be modulated by kinase signaling in a variety of cell types, including hepatocytes, steroidogenic cells, macrophages, platelets, and endothelial cells.

Conclusions
SR-BI was originally recognized as an important high-affinity receptor for HDL, critical to the exchange of cholesterol between cells and the lipoprotein. Pioneering studies in genetically modified mice revealed that SR-BI in the liver is particularly essential for reverse cholesterol transport and biliary cholesterol secretion and, therefore, that SR-BI has considerable antiatherogenic capacity. Evidence continues to accumulate that SR-BI also serves other functions that likely contribute to the cardiovascular protection provided by HDL. A number of the latter processes, particularly in endothelial cells, have been demonstrated to involve unique direct signal initiation by SR-BI that leads to the activation of diverse kinase cascades. SR-BI signaling occurs in response to cholesterol flux. It requires the C-terminal PDZ-interacting domain of the receptor, which mediates direct interaction with the adaptor molecule PDZK1; and the CTTM, which directly binds cholesterol. In endothelium, SR-BI signaling underlies potent activation of NO production by HDL and the maintenance of intimal layer integrity. The role of SR-BI signaling in the numerous other cellular targets of HDL and the underlying mechanisms by which the sensing of cholesterol movement modifies cell behavior are yet to be elucidated.

There continues to be great interest in the development of strategies to elevate HDL levels in humans. This includes the use of a variety of agents, including statins, fibrates, nicotinic acid, apolipoprotein A-I Milano–phospholipid complexes, apolipoprotein A-I mimetic peptides, peroxisome proliferator–activated receptor (PPAR) γ agonists, and cholesteryl ester transfer protein inhibitors.68,69 As these efforts proceed to increase bioavailable HDL, the activities of the HDL–SR-BI tandem that lie beyond its cholesterol-transporting properties deserve further interrogation. By doing so, we will ultimately determine how to take optimal advantage of the potent cardiovascular protective properties of HDL for prophylactic and therapeutic benefit.

Disclosures
None.

References


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In the article, “Signaling by the High-Affinity HDL Receptor Scavenger Receptor B Type I” by Saddar et al, which appeared in the February 2010 issue of the journal (Arterioscler Thromb Vasc Biol. 2010;30:144–150; DOI: 10.1161/ATVBAHA.109.196170), the publisher omitted several important corrections from the final published version:

1. Page 146, 1st column, 8th line from bottom, “Lp2A-I” should have been defined in the text, for the final version to read “. . . containing 2 molecules of apolipoprotein A-I (Lp2A-I). . .”
2. Page 148, 1st column, 6th line from bottom, “5'-H11270- monophosphate” should have appeared as “3'-5'-cyclic adenosine monophosphate.”

The online version has been corrected.

The publisher sincerely regrets the errors.

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