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Novel Functions of Endothelial Scavenger Receptor Class B Type I

Liming Yu[1], **Yao Dai**[1], **Chieko Mineo**[1]

[1]Center for Pulmonary and Vascular Biology, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Abstract

Purpose of Review—Scavenger receptor class B type I (SR-BI) serves a key role in the reverse cholesterol transport in the liver as the high-affinity receptor for HDL. SR-BI is abundantly expressed in endothelium, and earlier works indicate that the receptor mediates anti-atherogenic actions of HDL. However, more recent studies uncovered novel functions of endothelial SR-BI as a lipoprotein transporter, which regulates transcellular transport process of both LDL and HDL. This brief review focuses on the unique functions of endothelial SR-BI and how they influence atherogenesis.

Recent Findings—Earlier studies indicate that SR-BI facilitates anti-atherogenic actions of HDL through modulation of intracellular signaling to stimulate endothelial nitric oxide synthase. In vivo studies in global SR-BI knockout mice also showed a strong atheroprotective role of the receptor; however, a contribution of endothelial SR-BI to atherosclerosis process in vivo has not been fully appreciated. Recent studies using cultured endothelial cells and in mice with endothelial-specific deletion of the receptor revealed previously unappreciated pro-atherogenic actions of SR-BI, which relates to its ability to deliver LDL into arteries. On the other hand, SR-BI has also been implicated in transport of HDL to the sub-intimal space as a part of reverse cholesterol transport.

Summary—SR-BI mediates internalization and transcellular transport of both HDL and LDL, and the cellular and molecular mechanism of the process has just begun to emerge. Harnessing these dual transport functions of the endothelial SR-BI may provide a novel, effective intervention to atherosclerosis.

Keywords

Endothelium; Scavenger receptor type B class I; Transcytosis; Atherosclerosis

✉Chieko Mineo, Chieko.Mineo@utsouthwestern.edu.

Compliance with Ethical Standards

Conflict of Interest All authors declare no conflict of interest.

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Introduction

Scavenger receptor class B type I (SR-BI, SR-B1, or SCARB1) was initially discovered as a scavenger protein that binds to a series of native and modified lipoproteins [1]. The receptor was subsequently identified as the high-affinity receptor for high-density lipoprotein (HDL), which mediates the selective uptake of HDL-associated cholesterol esters in the hepatocytes [2]. The receptor is composed of two cytoplasmic domains and a large highly glycosylated extracellular domain, where the ligands interact [3, 4]. Global deletion of SR-BI in mice results in abnormal lipid profiles and severe atherosclerotic phenotypes [5–9]. This strong atheroprotective role of SR-BI has been mainly assigned to its function in the liver, in which SR-BI mediates HDL-cholesterol uptake as a crucial part of reverse cholesterol transport (RCT) [5–8, 10]. In addition to the liver, SR-BI is abundantly expressed in macrophages and endothelial cells, and accumulating evidence indicates that the receptor has extrahepatic functions to influence the progression of atherosclerosis [10–14]. Although atheroprotective function of macrophage SR-BI was demonstrated in vivo through bone marrow transplantation experiments [12, 15, 16], an in vivo role of endothelial SR-BI in atherogenesis has not been well understood until recently [14••]. A number of studies in cultured endothelial cells have shown that SR-BI mediates upregulation of nitric oxide (NO) production induced by HDL through activation of endothelial NO synthase, which plays a pivotal role in maintaining atheroprotective endothelial integrity [17–19]. In addition to being a key mediator of anti-atherogenic actions of HDL, more recent studies in cultured cells and in vivo have revealed that SR-BI is involved in both HDL and low-density lipoprotein (LDL) transport across the endothelium [20–22]. During the initial step of the RCT pathway, HDL need to cross the endothelial barrier to subendothelial spaces, where HDL removes excess cholesterol deposited in macrophages in atherosclerotic plaques [23]. Multiple studies in cultured endothelial cells indicate SR-BI is involved in the HDL transport process. In contrast, the subendothelial accumulation of pro-atherogenic LDL particles represents a pivotal step in the early stage of atherosclerosis [24, 25]. Most recently, using a hyperlipidemic mouse model lacking the receptor selectively in endothelium, Huang and his colleagues discovered a novel pro-atherogenic role of endothelial SR-BI, which relates to its function as an LDL transporter [14••]. In this review, we summarize the function of the endothelial SR-BI in the regulation of HDL and LDL transport, and how it influences the progression of atherosclerosis. More comprehensive recent reviews on endothelial transcytosis are available [20, 26].

Transcytosis of Lipoproteins Across Endothelium

The endothelium forms a cellular monolayer which selectively regulates the passage of molecules between the bloodstream and tissues [27]. The transport of macromolecules, including lipoproteins, across the endothelium is actively controlled by endothelial cells via the transcellular pathway, termed transcytosis [28]. The process of transcytosis involves ligand uptake by receptor-mediated endocytosis or fluid-phase pinocytosis, trafficking of the cargo through the cytoplasm, and exocytotic release of the cargo at the sub-intimal side [26]. The transcytosis is initiated by the interaction of molecules with their receptors or transporters on the luminal surface of endothelium. During the initial stages of atherosclerosis, LDL particles are transported across the endothelial barrier and accumulate

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in the subendothelial space. These LDL particles are then oxidized to form oxidized LDL (oxLDL), which promotes the formation of macrophage foam cells within atherosclerotic lesions [24, 25, 29]. Early studies examining LDL transport across the endothelium in vivo in rats using transmission electron microscopy clearly visualized the process of LDL transcytosis via a vesicular trafficking pathway [30]. Despite the relevance of LDL transport across the endothelium during atherogenesis, the molecular mechanism that controls this process has not been fully understood. Recent studies have identified several key molecules that participate in transcytosis, which include SR-BI, caveolin-1 and activin receptor-like kinase 1 (ALK1) [14, 20, 31, 32]. In contrast to atherogenic LDL, the atheroprotective effect of HDL is attributed to its ability to facilitate RCT through which cholesterol is delivered from the peripheral macrophages to the liver for biliary excretion [24, 33–36]. To achieve the removal of excess cholesterol deposited in the atherosclerotic lesions, circulating HDL need to cross the endothelial barrier to get access to lipid-laden macrophages in atherosclerotic plaques [23]. Previous studies found that the endothelial transcytosis of mature HDL requires SR-BI and an ATP-binding cassette transporter, ABCG1 [37, 38]. Thus, these studies imply that SR-BI may have a dual role in endothelial transport of both HDL and LDL. Here, we will highlight recent findings on the role of SR-BI in endothelial lipoprotein transcytosis.

Endothelial SR-BI and HDL Transport

Blood Endothelial SR-BI

Earlier studies established that the transport of lipids from HDL to SR-BI occurs through a selective cholesteryl ester uptake, in which the lipid content of HDL is transferred without concomitant internalization of the HDL particle itself [39–41]. On the other hand, the study by Silver et al. first showed in polarized hepatocytes in culture that SR-BI mediates HDL internalization and transport to the endosomal recycling compartment and apical membrane regions [42]. Pagler et al. also demonstrated concomitant endocytosis of SR-BI and HDL, which was followed by HDL particle resecretion [43, 44]. Although an exact itinerary of HDL-SR-BI in hepatocytes is yet to be determined, these studies and others suggest that HDL is likely routed to two possible intracellular pools after internalization, which include endosomal recycling compartment and multivesicular bodies [42, 43, 45–48]. In contrast to HDL internalization and secretion process in hepatocytes, HDL transport pathway in endothelium is less defined. HDL is considered to be actively transported through endothelial cells by transcytosis, which involves apical endocytosis, intracellular trafficking, and basolateral exocytosis. Previous studies indicate that in arteries, HDL transcytosis only occurs from the luminal surface to the basolateral intima and not in the reverse direction [20••], and that HDL leaves the intima mainly via the lymphatic system [49]. Endothelial SR-BI has been implicated in transport of HDL from circulation to the intima, as well as the removal of HDL from intimal plaques via lymphatics [50, 51]. The study by von Eckardstein's group first revealed that both SR-BI and ABCG1 mediate HDL transcytosis in polarized aortic endothelial cells [37]. Using cell surface biotinylation experiments, they showed that approximately 30% of the total cell-associated [^{125}I]-HDL was recovered in intracellular compartments. Internalization of HDL by endothelial cells was further investigated by confocal fluorescence microscopy in endothelial cells incubated with FITC-

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conjugated HDL and Alexa 594-transferrin. After 10 min of incubation, vesicles containing HDL were partially co-localized with transferrin, indicating that HDL is internalized into early endosomes. In the polarized endothelial cells, HDL translocated from the apical to the basolateral compartment, but no HDL transport was detected from basolateral to apical compartment. Furthermore, HDL binding was partially reduced by silencing of SR-BI or ABCG1, but not after reducing ABCA1 expression. However, co-silencing of SR-BI and ABCG1 did not further decrease HDL transcytosis, indicating existence of additional pathways. More recently, using a combination of spinning-disc confocal and total internal reflection fluorescence microscopy, Fung et al. examined the uptake and transcytosis of HDL by human primary brain microvascular endothelial cell monolayers [52•]. They found that internalized HDL partially co-localized with SR-BI and that knockdown of SR-BI attenuated HDL internalization. Interestingly, Velagapudi et al. reported that vascular endothelial growth factor (VEGF)-A regulates cellular localization of SR-BI to influence transendothelial transport of HDL [38•]. In search of kinases that may be required for HDL uptake in endothelial cells, they performed a microscopy-based high-throughput screening by incubating human aortic endothelial cells with 141 kinase inhibitors and fluorescent-labeled LDL or HDL. They identified the inhibitors of VEGFR as suppressors of HDL uptake, but not LDL, and further confirmed that silencing of VEGFR2 decreased cellular binding, association, and transendothelial transport of HDL. HDL transport was reduced without VEGF-A in cell culture medium, and it was restored by an addition of VEGF-A. The effect of VEGF-A on HDL transport was dependent on activation of phosphoinositide 3-kinase-Akt pathway or p38 mitogen-activated protein kinase, as well as the presence of SR-BI. They further found that VEGF-A is required for the localization of SR-BI in the plasma membrane of endothelial cells. The identification of VEGF as a regulatory factor of transendothelial transport of HDL but not LDL supports the concept that the endothelium is a highly specific barrier for the entry of lipoproteins into the vascular wall, and that this unique function can be a target of pharmaceutical interventions. Although HDL transport across endothelium is largely considered unidirectional from plasma to the intima, additional role for SR-BI in free cholesterol transport from basolateral to apical side of endothelial layer has been reported [53]. Using cultured mouse aortic endothelial cells, Miao et al. observed that unesterified cholesterol can be transported across the endothelial cell monolayer from the basolateral to the apical compartment. Administration of HDL or apolipoprotein AI (apoAI) to the apical compartment enhanced transendothelial cholesterol transport in a concentration-dependent manner. Knockdown of ABCG1 or SR-BI, or inhibition of SR-BI by BLT-1 diminished HDL-induced transendothelial cholesterol transport. SR-BI-mediated transport of cholesterol from the subendothelial intima back to the circulating blood may contribute to atheroprotection, but whether the process occurs in vivo is yet to be determined.

Lymphatic Endothelial SR-BI

Delivery of excess cholesterol from peripheral tissues and macrophages to the bloodstream by HDL is one of the early steps in the RCT process [33, 34]. The lymphatic system is considered to be the primary location for the return of lipoproteins from the interstitial space to circulation [50, 54–56]. It has been shown that lymphatic transport of cholesterol by HDL is mediated by SR-BI expressed on lymphatic endothelium [50]. Lim et al. [50]

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demonstrated for the first time that SR-BI is expressed in lymphatic endothelial cells both in culture and in vivo, and that treatment of wild-type mice with SR-BI blocking antibody suppressed the transport of HDL via lymphatic vessels. They further confirmed that in vivo reverse cholesterol transport was also impaired in wild-type mice treated with the SR-BI neutralizing antibody or in global SR-BI$^{-/-}$ mice. Their findings indicate that lymphatic vessels play a key role in HDL-mediated RCT through a mechanism that involves the ability of lymphatic endothelial SR-BI to facilitate HDL transcytosis. Using a mouse strain in which endothelial SR-BI is overexpressed by the Tie2 promoter-driven SR-BI transgene, Vaisman et al. have shown that endothelial cell-specific overexpression of SR-BI in high-fat, high-cholesterol diet-fed C57BL/6 mice results in an increase in plasma HDL-cholesterol levels and a decrease in total cholesterol levels, thereby contributing to a reduction in atherosclerosis [57]. The mechanism by which endothelial SR-BI overexpression alters plasma lipid profiles is not clear; however, because Tie2 is expressed both in blood and lymphatic endothelium [58], the changes in plasma lipid profiles observed in these mice may be consistent with the finding that lymphatic endothelial SR-BI participates in the removal of cholesterol from peripheral tissues by lymphatic vessels through the uptake and transcytosis of HDL [56].

Collectively, accumulating evidence suggests that HDL transport across endothelial cells via transcytosis plays a major role in atheroprotective RCT and that both blood and lymphatic endothelial SR-BI are a crucial participant in the process (Fig. 1a).

Endothelial SR-BI and LDL Transport

As a number of studies established SR-BI as a mediator of HDL uptake and intracellular transport in endothelial cells, it is relatively recent that studies have started to emerge testing whether SR-BI mediates transcytosis of LDL to deliver the circulating atherogenic lipoproteins into the subendothelial space, where they are engulfed by macrophages that become foam cells to promote the formation of atherosclerotic lesions. Warren L. Lee’s group initially uncovered an unexpected role for endothelial SR-BI in LDL transcytosis [21]. They first perfused mouse aortas ex vivo with LDL and small molecular weight dextran to monitor transendothelial and paracellular transport, respectively. They detected LDL accumulation in the subendothelial space, indicating that LDL transcytosis occurs in intact vessels. No intimal dextran was observed despite its smaller size. They further confirmed that LDL transcytosis occurs in polarized human coronary artery endothelial cells in culture. An assay was then developed to quantify transcytosis of DiI-LDL in real time using total internal reflection fluorescence microscopy. DiI-LDL transcytosis was inhibited by excess unlabeled LDL, whereas degradation of the LDL receptor by PCSK9 had no effect, indicating that the LDL receptor plays a minimum role in LDL transcytosis. Instead, LDL co-localized partially with SR-BI and overexpression of SR-BI increased LDL transcytosis, whereas knockdown by siRNA significantly reduced it. Furthermore, excess HDL, the canonical SR-BI ligand, decreased LDL transcytosis, consistent with a receptor-mediated process rather than non-specific diffusion across paracellular routes. Incubation with Dyngo4A, a specific chemical inhibitor of the GTPase dynamin, abrogated the process. More recently, the same group demonstrated that estrogen suppresses LDL transcytosis in cultured human endothelial cells [59•]. LDL transcytosis was significantly higher in cells

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derived from men compared with those from premenopausal women. Estrogen treatment attenuated LDL transcytosis in endothelial cells from male but not female donors. They found that estrogen caused downregulation of endothelial SR-BI, and overexpression of SR-BI was sufficient to restore LDL transcytosis. Compared to endothelial cells, treatment with estrogen had no impact on SR-BI expression in hepatocarcinoma-derived HepG2 cells. Inhibition of estrogen receptor α or β had no effect on estrogen-mediated attenuation of LDL transcytosis. Instead, estrogen's effect on LDL transcytosis was blocked by depletion of the G protein– coupled estrogen receptor 1 (GPER, also known as G protein–coupled receptor 30). GPER was found to be enriched in endothelial cells compared with hepatocytes and it has been known to signal via transactivation of the epidermal growth factor receptors (EGFR) [60]; inhibition of EGFR prevented the effect of estrogen on SR-BI and LDL transcytosis. The study also found that SR-BI protein expression was significantly higher in human coronary artery endothelial cells from male compared with premenopausal female donors.

More recently, Huang and his colleagues demonstrated that SR-BI in endothelial cells mediates the delivery of LDL into arteries and its accumulation by artery wall macrophages, contributing to promotion atherosclerosis [14••]. Using mice lacking SR-BI selectively in the endothelium (SR-BI$^{\Delta EC}$), they found that, compared with SR-BI$^{fl/fl}$ controls, both male and female SR-BI$^{\Delta EC}$ mice displayed markedly decreased formation of hyperlipidemia-induced atherosclerosis. The endothelial deletion of SR-BI did not alter circulating total cholesterol, triglyceride, or HDL levels and it also did not change vascular inflammation. Instead, they discovered that DiI-labeled native LDL and oxLDL incorporation into the aorta in vivo was diminished in SR-BI$^{\Delta EC}$ mice, indicating that the previous finding [21] that there is less LDL accumulation in ex vivo perfused arteries from global SR-BI$^{-/-}$ mice is likely to have been related to loss of the receptor in the endothelium. Furthermore, using cultured human aortic endothelial cells, they identified the molecular basis for LDL trafficking by SR-BI. Studies using RNAi-mediated silencing and neutralizing antibodies indicated that SR-BI, LDL receptor, and cluster of differentiation 36 (CD36), the known LDL binding receptors in endothelial cells, all promote LDL uptake by human endothelial cells; however, they found that only SR-BI is required for LDL transcytosis. In contrast to these classical receptors for LDL, downregulation of ALK1, to which LDL binding and transport has been previously described [61], reduced LDL transcytosis to a similar degree by the selective loss of SR-BI, and there was a further decline with their concurrent knockdown. Thus, both SR-BI and ALK1 mediate LDL transcytosis in endothelial cells likely through different mechanisms. Moreover, using a series of deletion and point mutants of SR-BI, they showed that the ability of SR-BI to facilitate LDL transcytosis requires eight amino acids (487–494, IQAYSESL) in the C-terminal cytoplasmic domain of the receptor. Through analysis of the proteins in endothelial cells that were pulled down with wild-type SR-BI using liquid chromatography and tandem mass spectrometry, they identified DOCK4 (guanine nucleotide exchange factor dedicator of cytokinesis 4) as a necessary partner of SR-BI for LDL transcytosis. DOCK4 is a membrane-associated cytoplasmic protein that functions as a guanine nucleotide exchange factor and a participant in actin cytoskeleton regulation, and it has been shown to mediate PDGF receptor internalization in fibroblasts [62–64]. Further studies in cultured endothelial cells showed that LDL binding to SR-BI increases DOCK4 recruitment to the cytoplasmic

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domain and that DOCK4 promotes internalization of SR-BI through activation of the small GTPase Rac1. Interestingly, in mice compared with the atherosclerosis-resistant greater curvature of the aortic arch, mRNA levels of both SR-BI and DOCK4 were increased in the atherosclerosis-prone lesser curvature before lesion formation. To seek human relevance, they compared gene expression levels of SR-BI and DOCK4 in human atherosclerotic versus normal arteries in three publicly available independent human cohorts (Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/)). The analysis of the datasets revealed that expression of both SR-BI and DOCK4 transcripts was greater in atherosclerotic arteries than normal arteries.

Collectively, these recent studies provide evidence that LDL transcytosis is a key mechanism that drives atherosclerosis, and that this process is actively regulated by SR-BI in the endothelium (Fig. 1b).

Conclusions

The recent findings that are summarized in this review highlight the importance of endothelial SR-BI in regulating lipoprotein transcytosis to influence atherosclerosis (Fig. 1). Although these studies have identified several molecules in addition to SR-BI that participate in the transcytosis process, there are a multitude of questions that remain to be addressed.

The study by Huang et al. has implicated DOCK4 and Rac1 in the initial step of LDL internalization. However, the exact route of LDL-SR-BI in transcytotic pathway is unknown. Several early studies have suggested the involvement of both clathrin-mediated endocytosis and non-coated caveolae (also called plasmalemmal vesicle)-mediated endocytosis in the initial step of transcytosis across the endothelium [30, 65]. It was reported that LDL endocytosis in endothelial cells devoid of caveolin-1 (Cav-1), a structural component of caveolae, was lower than in wild-type cells, supporting a role of caveolae in LDL transcytosis [66, 67]. Furthermore, global Cav-1 knockout mice exhibited reduced LDL infiltration into the artery wall and a drastic reduction of atherosclerotic plaques, whereas endothelial cell-specific re-expressing Cav-1 in these mice reversed both effects [31, 68]. SR-BI has been shown to be localized within caveolae in cultured endothelial cells [69]; however, other studies dispute the involvement of caveolae in LDL transport [52, 70]. Furthermore, in addition to dynamin and DOCK4, what molecules are involved in budding and fusion of transcytotic vesicles, as well as exocytotic events at the basolateral surface of endothelium? Distinctive members of Rab GTPase family members are known to regulate early, late, and recycling vesicular trafficking [71], but Rab proteins specifically involved in transcytosis are not yet identified. The recent finding that SR-BI co-localizes with LDL in trafficking vesicles in mouse aortic endothelial cells [14••] may provide a rationale to perform biochemical and proteomic analysis of purified “transcytotic vesicles” to further characterize them and to identify protein components.

Secondly, the cumulative studies demonstrated dichotomous functions of endothelial SR-BI in atherosclerosis; a mediator of anti-inflammatory, anti-atherogenic actions of HDL and a facilitator of LDL transport during an initial phase of atherogenesis. Because HDL

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and LDL shares binding sites on the extracellular domain of SR-BI [72] and that excess HDL competes transcytosis of LDL [21], circulating concentrations of the lipoproteins may influence which functions of SR-BI play a dominant role. Deciphering this enigma will be important to further consider therapeutic interventions targeting either endothelial SR-BI or the transcytosis process. Recently a considerable advancement in endothelial targeting of therapeutic cargos has been made, using monoclonal antibody or nanoparticles [73, 74]. It may be feasible to target VEGF inhibitors selectively to endothelium to evaluate its effect on LDL uptake and hyperlipidemia-induced atherosclerosis in rodent models. Also, targeted administration of SR-BI neutralizing antibodies or its inhibitor such as BLT-1 to inhibit selective function of SR-BI in endocytosis without influencing its main functions in the liver may provide an answer to the enigma, as well as possible novel therapeutic opportunities for atherosclerosis prevention.

Lastly, it is critically important to investigate whether endothelial SR-BI and its function as a lipoprotein transporter influence atherosclerosis process in humans. Genome-wide association studies identified human SNPs in the SR-BI (SCARB1) genes that are associated with HDL-cholesterol levels and cardiovascular disease risks [75–79]; however, these human phenotypes are likely due to the receptor's dominant function in the liver. The work by Huang et al. has found in multiple human cohorts that both SR-BI and DOCK4 gene expression are upregulated in atherosclerotic aortas compared to normal arteries [14••]. More studies are warranted to determine whether increased expressions of SR-BI and DOCK4 contribute to atherogenesis and to identify the transcriptional machinery responsible for the mRNA upregulation in endothelial cells.

Recent single-cell RNA sequencing studies have revealed that CD36, another member of scavenger receptor family which shares high structural homology with SR-BI, serves as a unique marker for a subpopulation of endothelial cells in mouse aortas [80–82]. One of the studies further demonstrated that the subpopulation of endothelial cells represented by the expression of CD36 preferentially located in the greater curvature of the aortic root. This is in contrast to the localization of SR-BI mRNA, which showed higher expression in the lesser curvature [14••]. It will be important to determine in human arteries whether SR-BI and other molecules implicated in transcytosis are component of a subpopulation of endothelial cells, and if so, whether the subset of endothelium is responsible for uptake of LDL or HDL by endothelial cells to cause or prevent formation of atherosclerotic plaques.

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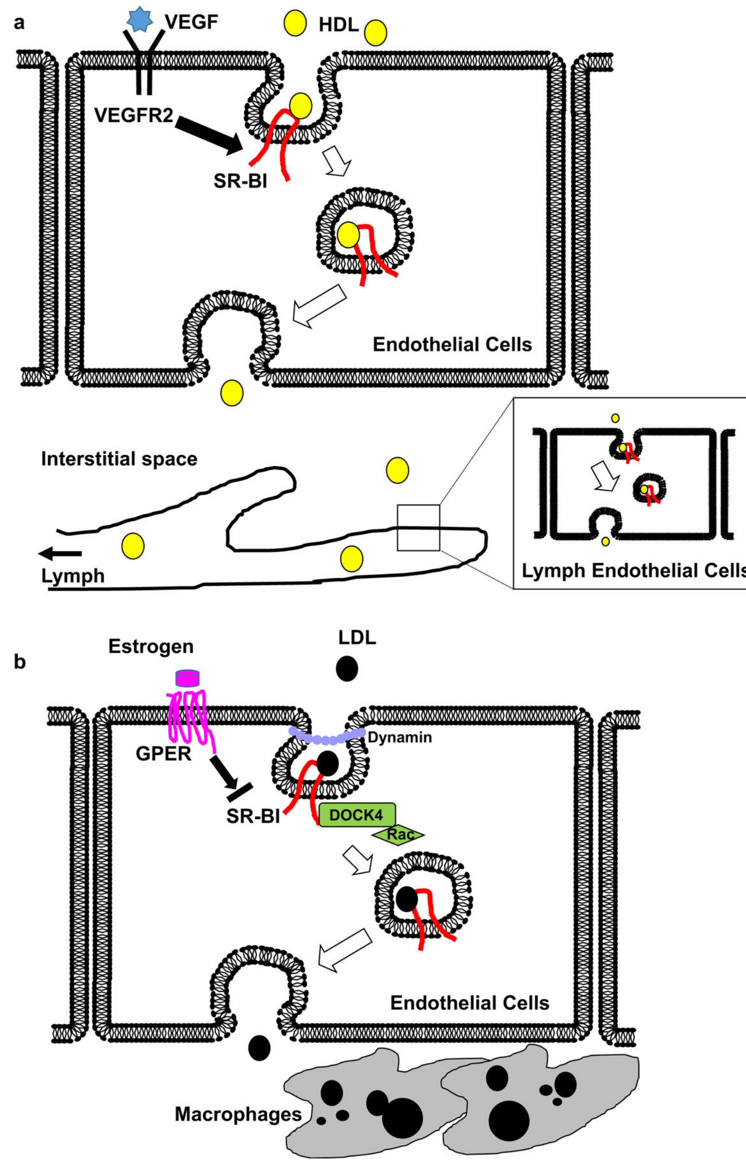

Fig. 1.

Dichotomous role of endothelial SR-BI as a transporter of both HDL and LDL. **a** Circulating HDL binds to SR-BI on the luminal surface of endothelial cells, and HDL-SR-BI complex is transported via a vesicular trafficking pathway. VEGF-VEGFR2 signaling promotes HDL transport by upregulating plasma membrane SR-BI. HDL is then released in the interstitial space, where it is further delivered to the lymphatic system through lymphatic endothelial cells in an SR-BI-dependent manner, as a critical step of the reverse cholesterol transport. **b** SR-BI facilitates internalization of LDL in a dynamin-dependent manner. LDL binding to SR-BI recruits DOCK4 to the C-terminal cytoplasmic domain of the receptor, which in turn promotes LDL internalization by Rac activation. Estrogen binding to GPER negatively regulates LDL transcytosis through downregulation of SR-BI. LDL-SR-BI complex is

transported across endothelium to be released in the sub-intimal space, where it is engulfed by macrophages to contribute to atherogenesis

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