Sterol-responsive Element-binding Protein (SREBP) 2
Down-regulates ATP-binding Cassette Transporter A1 in
Vascular Endothelial Cells

A NOVEL ROLE OF SREBP IN REGULATING CHOLESTEROL METABOLISM*

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Lingfang Zeng‡§, Hailing Liao‡§, Yi Liu‡, Tzong-Shyuan Lee‡, Minjia Zhu‡, Xian Wang‡, Michael B. Stemerman‡, Yi Zhu‡¶¶, and John Y.-J. Shyy‡

From the ‡Division of Biomedical Sciences, University of California, Riverside, Riverside, California 92521 and the ¶Department of Physiology, Key Laboratory of Molecular Cardiovascular Sciences of Education Ministry, Health Science Center, Peking University, Beijing, 100083, China

ATP-binding cassette transporter A1 (ABCA1) is a pivotal regulator of cholesterol efflux from cells to apolipoproteins, whereas sterol-responsive element-binding protein 2 (SREBP2) is the key protein regulating cholesterol synthesis and uptake. We investigated the regulation of ABCA1 by SREBP2 in vascular endothelial cells (ECs). Our results showed that sterol depletion activated SREBP2 and increased its target, low density lipoprotein receptor mRNA, with a concurrent decrease in the ABCA1 mRNA. Transient transfection analysis revealed that sterol depletion decreased the ABCA1 promoter activity by 50%, but low density lipoprotein receptor promoter- and the sterol-responsive element-driven luciferase activities were increased. Overexpression of the N terminus of SREBP2 (SREBP2(N)), an active form of SREBP2, also inhibited the ABCA1 promoter activity. Functionally adenovirus-mediated SREBP2(N) expression increased cholesterol accumulation and decreased apolipoprotein A-I-mediated cholesterol efflux. The conserved E-box motif was responsible for the SREBP2(N)-mediated inhibition since mutation of the E-box increased the basal activity of the ABCA1 promoter and abolished the inhibitory effect of SREBP2(N). Furthermore sterol depletion and SREBP2(N) overexpression induced the binding of SREBP2(N) to both consensus and ABCA1-specific E-box. Chromatin immunoprecipitation assay demonstrated that serum starvation enhanced the association of SREBP2 and the ABCA1 promoter in ECs. To correlate this mechanism pathophysiologically, we found that oscillatory flow caused the activation of SREBP2 and therefore attenuated ABCA1 promoter activity in ECs. Thus, this SREBP2-regulated mechanism may control the efflux of cholesterol, which is a newly defined function of SREBP2 in ECs in addition to its role in cholesterol uptake and biosynthesis.

Epidemiological studies have shown an inverse relationship between levels of high density lipoprotein-cholesterol and risk of coronary artery disease. High density lipoprotein promotes reverse cholesterol transport by facilitating the transfer of cholesterol from peripheral tissues to the liver for disposal (1). ATP-binding cassette transporter A1 (ABCA1),1 a 254-kDa cytoplasmic membrane protein, is a pivotal regulator of lipid efflux from cells to apolipoproteins (2). ABCA1 apparently plays an important role in reverse cholesterol transfer. Mutations in the ABCA1 gene, discovered in patients with Tangier disease, cause impaired efflux of lipids to apoA-I with a near absence of plasma high density lipoprotein (3–5). Study of ABCA1 heterozygotes provides direct evidence that the impaired cholesterol efflux is associated with reduced plasma high density lipoprotein-cholesterol and increased risk of coronary artery disease (6). Furthermore, under a high cholesterol diet, ABCA1 transgenic mice showed an atheroprotective lipoprotein profile with decreased atherosclerotic lesions, demonstrating the antiatherogenic effect of the ABCA1 transporter in vivo (7).

ABCA1 can be regulated at both the transcriptional and post-transcriptional levels (8). The most studied transcriptional regulation of the ABCA1 gene is the binding of liver X receptor (LXR)/retinoic X receptor (RXR) heterodimers to an imperfect direct repeat spaced by four nucleotides (DR4) to up-regulate the gene (9). Mutation of the DR4 element strongly reduced the LXR agonist-induced ABCA1 gene activation (9, 10). However, significant activity is still retained in DR4-deleted constructs, indicating the existence of regulation other than by LXR/RXR (11). Although a silencing regulatory element in ABCA1 promoter has been mapped to a region containing the E-box motif (12), the transcription factors responsible for the negative regulation of the ABCA1 promoter remain elusive.

Sterol-responsive element-binding proteins (SREBPs), including SREBP1a, -1c, and -2, modulate the transcription of a number of genes involved in the synthesis and receptor-mediated uptake of cholesterol and fatty acids (13–15). Results to date support the notion that SREBP1 primarily activates the

1 The abbreviations used are: ABCA1, ATP-binding cassette transporter A1; hABCA1, human ABCA1; SRE, sterol-responsive element; SREBP, sterol-responsive element-binding protein; SREBP2(N), N terminus of SREBP2; EC, endothelial cell; LDL, low density lipoprotein; LDLR, LDL receptor; LXR, liver X receptor; RXR, retinoic X receptor; DR4, direct repeat spaced by four nucleotides; USF, upstream stimulatory factor; HUVEC, human umbilical vein endothelial cell; FBS, fetal bovine serum; SFM, serum-free medium; RT, real time; HA, hemagglutinin; β-gal, β-galactosidase; Ad, adenovirus; ChIP, chromatin immunoprecipitation; SCAP, SREBP-cleavage-activating protein; luc, luciferase.

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§ Both authors contributed equally to this work.

¶ To whom correspondence should be addressed: Division of Biomedical Sciences, University of California, Riverside, CA 92521. Tel.: 909-827-7156; Fax: 909-787-5504; E-mail: yi.zhu@ucr.edu.

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fatty acid triglyceride and phospholipid pathways, whereas SREBP2 is the prominent isoform for cholesterol synthesis and uptake (13, 14, 16). Containing a basic-helix-loop-helix-leucine zipper motif, the N terminus of SREBP2 (SREBP(N)) mediates dimerization, nuclear entry, and DNA binding. In sterol-depleted cells, SREBP2s are cleaved by proteases in Golgi, releasing the N termini, which translocate into the nucleus and bind to SREs in the enhancers of multiple genes encoding enzymes and proteins involved in cholesterol biosynthesis, unsaturated fatty acid biosynthesis, triglyceride biosynthesis, and lipid uptake (for reviews, see Refs. 15–17). In addition to the SRE motif, SREBP2 recognizes several other sequences, including E-box in the promoters of certain genes (15, 18).

The vascular endothelium forms a barrier between the vessel wall and lipoproteins in the circulation. It plays an important role in maintaining vascular integrity. The disturbance or injury of endothelium can lead to cardiovascular impairments such as atherosclerosis. ABCA1 is expressed in vascular endothelial cells (ECs) and transcriptionally up-regulated by LDL and cholesterol, suggesting that ABCA1 in ECs plays an important role in cholesterol homeostasis in the vessel wall (19). However, the regulation of ABCA1 and its role in lipid trafficking in ECs remain largely unknown. We previously reported that shear stress activated SREBP2s and hence up-regulated their targeting genes in ECs (20, 21). Given the important role of SREBP2 and ABCA1 in cholesterol homeostasis and the significance of cholesterol traffic in the vessel wall, we investigated the regulation of ABCA1 by SREBP2 in ECs. Our results showed that, by binding to the E-box, SREBP2 could inhibit ABCA1 transcription. Importantly, oscillatory flow caused the activation of SREBP2 and inhibition of ABCA1 promoter activity in ECs. Thus, this SREBP2-regulated mechanism controlling cholesterol efflux is a newly defined function of SREBP2 in the vascular wall.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Cholesterol was purchased from Avanti, Inc. (Alabaster, AL), [γ-32P]ATP, [α-32P]dCTP, and [3H]cholesterol were from MP Biomedicals (Irvine, CA). The DECApriming II DNA labeling kit was from Ambion (Austin, TX). All of the DNA-modifying enzymes and PCR enzymes were obtained from Promega Corp. (Madison, WI). LXR agonist T9091317 was purchased from Cayman Chemical (Ann Arbor, MI). Anti-ABCA1 antibody was purchased from Novus Biologicals (Littleton, CO), and anti-SREBP2 upper 1 (USF1), USP2, c-Myc, Max, HA tag, and α-tubulin were from Santa Cruz Biotechnology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultured under standard technology (Santa Cruz, CA). LDL was isolated from non-frozen human plasma as described previously (19). Recombinant human fibroblast growth factor was a generous gift from Dr. J. A. Thompson at the University of Alabama. All other reagents, purchased from Invitrogen (Carlsbad, CA), were used. Bovine aortic endothelial cells were cultivated under standard conditions in the presence of a 32P-labeled ATP chimera. 

**Adenovirus Construction and Infection—** Recombinant adeno viruses encoding Ad-HA-SREBP2(N) were created, amplified, and titered as described previously (23). For adenovirus infection, the virus mixture was multiplicity of infection as indicated was added to confluent HUVECs in culture and incubated for 12 h. Ad-β-gal was used as a transfection control.

**Assessment of Cholesterol Efflux**—The cholesterol efflux was assayed as described previously (19) with modification. Briefly HUVECs in 12-well plates were infected with Ad-HA-SREBP2(N) or control virus for 12 h. The cells were then treated with LXR agonist T9091317 (10 μM) in EC medium or SFM for 18 h and labeled with [3H]cholesterol (0.2 μCi/mL) for 6 h. After washing with phosphate-buffered saline-bovine serum albumin solution, the cells were incubated with fresh SFM with or without apoA1 (10 μg/mL) for 2 h. The aliquots of medium and cell lysates were assayed by liquid scintillation.

**Electrophoretic Mobility Shift Assay**— Confluent HUVECs were treated with EC medium or SFM for 6 h or infected with Ad-HA-SREBP2(N) for 24 h. The cells were then lysed, and nuclear extracts were prepared. Double strand oligonucleotides containing the divergent (5′-CTAGAAACGAGGATCCCTGTTCATGAGC-3′) or consensus E-box oligos (Santa Cruz Biotechnology) were end-labeled with [γ-32P]ATP. Electrophoretic mobility shift assays were performed as described previously (24). To test the specificity of binding, a 100-fold molar excess of unlabeled ABCA1-E-box or irrelevant AP-1 and SP-1 probes were used for competition experiments. In supershift experiments, the nuclear extracts were incubated with anti-HA antibody for 3 h on ice before the addition of the labeled probes.

**Chromatin Immunoprecipitation (ChiP) Assay—** The ChiP assays were performed as described previously (23). In brief, cells were fixed with 1% formaldehyde and quenched prior to harvest and sonication. Goat anti-SREBP2 or normal goat IgG and single strand salmon sperm DNA saturated with Protein A-Sepharose 4B beads were added to sheared samples for immunoprecipitation. Immunoprecipitates were pelleted by centrifugation, and the supernatant of the control group was collected as an input control. The immunoprecipitates were eluted from Sepharose 4B beads, and proteinase K solution was added and incubated at 60 °C for 6 h. DNA was extracted, purified, and then used to amplify target sequences by PCR. The ABCA1 promoter containing the E-box element was amplified by use of primer set 5′-GCTGCGCGCGCGCGCTGCTGATC-3′ and 5′-TGGGCTGGTACCCAGGTCCAC-3′. The amplified products were visualized by agarose gel electrophoresis.

**Statistics**—Quantitative data were expressed as mean ± S.D. Statistical significance of the data was evaluated by analysis of variance or Student’s t test. p values less than 0.05 were considered significant. For nonquantitative data, results were representative from at least three independent experiments.

**Regulation of ABCA1**—Total RNA isolation and Northern blotting for hABCA1, LDL receptor (LDLR), and von Willebrand factor mRNA was carried out according to standard protocols. The cDNA probes for LDLR and hABCA1 were generated via RT-PCR, and the probes were labeled with [α-32P]dCTP by DECApriming (Ambion). For quantitative RT-PCR, total RNA was converted into cDNA by using reverse transcriptase with oligo(dT) as the primer. The obtained cDNAs were then used as the templates for quantitative RT-PCR with the use of Brilliant SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA). The relative amount of ABCA1 mRNAs was calculated using the comparative method with the β-actin mRNA as internal control. The nucleotide sequences of the primers were: hABCA1, 5′-GCTGCGCGCGCGCGCTGCTGATC-3′; β-actin, 5′-TGGGCTGGTACCCAGGTCCAC-3′. HUVECs were solubilized in a buffer containing detergent, and cellular proteins were separated by SDS-PAGE. The Western blotting analysis was performed with antibodies against ABCA1, SREBP2, SREBP1, USP1, USP2, c-Myc, Max, HA, or α-tubulin.
HUVECs were incubated with 180 mg/dl LDL, 10 μg/ml cholesterol (CHL) in 20% FBS medium or SFM for 24 h. RNA was isolated, and samples of 15 μg of total RNA were resolved by gel electrophoresis and then hybridized with α-32P-labeled ABCA1, LDLR, or von Willebrand factor (vWF) cDNA. B, HUVECs were incubated with LDL in 20% FBS medium or in SFM for different periods of time. Cells were lysed, and the proteins were then resolved by 6% SDS-PAGE, transferred to a nitrocellulose membrane, and visualized with anti-SREBP2 and anti-α-tubulin antibodies. C, HUVECs were transfected with hABCA1-luc (−928), LDLR-luc, or 4XSRE-luc for 24 h. Then cells were incubated with medium with or without serum (SFM) for 24 h. Promoter activities were measured by use of the reporter luciferase and normalized with β-galactosidase. Data are mean ± S.D. of the relative luciferase activities in three independent experiments, each performed in triplicate. Results are representative from three independent experiments.

RESULTS

The Reciprocal Response of ABCA1 and SREBP2 Transcripts to Serum Deprivation—To study the regulation of ABCA1 in ECs under serum-free condition, we cultured confluent HUVECs in SFM in the presence or absence of LDL for 24 h. Under this condition, HUVECs had normal morphology, and no cell death was detected. As shown in Fig. 1A, SFM greatly decreased ABCA1 mRNA in HUVECs, and the inclusion of LDL in SFM reversed such an inhibition. In control experiments, cells in EC medium containing 20% FBS were exposed to LDL or cholesterol. LDL and cholesterol increased the level of ABCA1 mRNA. The level of mRNA encoding LDLR, an SREBP2 target gene, was much lower in cells in FBS than those in SFM. Supplementing SFM with LDL inhibited the expression of LDLR mRNA. Consistent with the SFM-up-regulated LDLR, SREBP2 was activated as revealed by the increased SREBP2 cleavage at 6 h after exposure to SFM and the subsequent increase in both precursor and cleavage forms of SREBP2 (Fig. 1B). Furthermore results from transient transfection assays showed that SFM decreased the luciferase reporter driven by the ABCA1 promoter by 49 ± 6% compared with cells under 20% FBS (Fig. 1C). However, SFM increased the luciferase reporter driven by the LDLR promoter or SRE by 5–6 times. Thus, ABCA1 and SREBP2 transcripts in ECs responded reciprocally to SFM.

SREBP2 Inhibits the Expression of ABCA1—To ascertain whether SREBP2 up-regulation inhibits the expression of ABCA1, we compared the level of ABCA1 protein in HUVECs infected with recombinant Ad-HA-SREBP2(N) encoding the N terminus of SREBP2 with those infected with Ad-β-gal. Western blotting analysis revealed that ABCA1 in HUVECs was decreased by SREBP2(N) overexpression. This inhibitory effect was similar to that by SFM (Fig. 2A). Because ABCA1 is a pivotal regulator of cholesterol efflux from cells to apolipoproteins such as apoA-I, we explored cholesterol efflux to apoA-I as a functional consequence of the SREBP2-suppressed ABCA1. Compared with the Ad-β-gal-infected controls, the infection of Ad-HA-SREBP2(N) increased the [3H]cholesterol uptake and/or accumulation by 20 ± 8% (Fig. 2B). Furthermore cholesterol efflux in the presence of apoA-I was significantly decreased in Ad-HA-SREBP2(N)-infected cells (Fig. 2C). As an LXR target gene, ABCA1 is up-regulated by TO901317, a selective LXR agonist (26, 27). Treating Ad-β-gal-infected HUVECs with TO901317 promoted the apoA-I-mediated cholesterol efflux when the cholesterol accumulation was unchanged.

SREBP2 Down-regulates the ABCA1 Transcription via E-box Motif—To study whether the inhibition of ABCA1 by serum starvation or SREBP2 is at the level of transcription and to determine the involved transcriptional element within the ABCA1 promoter, we performed transient transfection assays using ABCA1 promoter-driven reporter constructs. Fig. 3A shows that overexpression of SREBP2(N) by the co-transfected pCMV5-HA-SREBP2(N) decreased the activities of promoter constructs pABCA1(−928)-luc and pABCA1(−156)-luc by 50% compared with controls transfected with vector plasmid pCMV5. Further deletion of a segment between −156 and −116 containing the E-box site, a silencer of the ABCA1 promoter (28, 29), abolished the inhibitory effect of SREBP2(N). Indeed mutation of the E-box in pABCA1(−928 E-boxmut)-luc not only increased the basal activity but also reversed the inhibition of ABCA1 promoter by SREBP2(N). Furthermore we studied the effect of serum starvation on the ABCA1 promoter by incubating the various transfected HUVECs with SFM or 20% FBS. As shown in Fig. 3B, SFM and SREBP2(N) overexpression exhibited a similar pattern of regulation on ABCA1 promoter constructs.

SREBP2(N) Binds to the E-box of the ABCA1 Promoter—Given the possibility that E-box is an SREBP-responsive element, which down-regulates the ABCA1 expression, we studied the binding of SREBP2(N) to E-box by electrophoretic mobility shift assay. As shown in Fig. 4A, SREBP2(N) overexpression in ECs because of Ad-HA-SREBP2(N) infection increased the binding of nuclear extracts to the ABCA1-specific E-box compared with control cells infected with Ad-β-gal. The supershifted band that resulted from the inclusion of anti-HA antibody revealed the specific binding of SREBP2 to the ABCA1-specific E-box (Fig. 4B). Addition of LDL or cholesterol to SFM attenuated such a binding. We also performed ChIP assay to ascertain whether SREBP2(N) binds to the ABCA1 promoter in vivo. As shown in Fig. 4C, although SREBP2(N) associated
with the ABCA1 promoter in cells in 20% FBS, SFM greatly enhanced the association. Thus, the inhibition of ABCA1 transcription by SREBP2(N) is most likely through the binding of SREBP2 to the E-box site in the ABCA1 promoter.

Serum Starvation Does Not Increase Other E-box-related Transcription Factors—In addition to SREBPs, E-box element also binds to several other nuclear proteins, including c-Myc, Max, and c-Myc-related regulatory factors such as USF1 and USF2. To explore the possible regulation of these E-box-binding proteins, the induction of Myc, Max, USF1, and USF2 by SFM was detected in the nuclear extract. Western blotting showed that SFM increased SREBP1 and SREBP2. However, levels of nuclear c-Myc and USF2 were decreased, and those of Max and USF1 remained unchanged (Fig. 5). This result suggested that Myc, Max, or USFs were unlikely to be involved in the binding of the E-box of ABCA1 promoter, leading to the suppression of the ABCA1 in response to SFM.

Flow Inhibits ABCA1 Promoter Activation in ECs—We previously reported that steady laminar flow caused a transient activation of the SREBP-regulated genes, whereas disturbed flow patterns caused a sustained activation of SREBPs and their targeting genes in ECs (20). To determine whether the oscillatory flow-activated SREBP2, like serum depletion, can down-regulate ABCA1, HUVECs were subjected to an oscillatory flow (0–1100 dyne/cm²), and the levels of ABCA1 mRNA were determined by quantitative RT-PCR. As shown in Fig. 6A, oscillatory flow indeed decreased the level of ABCA1 mRNA compared with static controls or cells subjected to laminar flow. Furthermore ECs were transiently transfected with pABCA1(-156)-luc, and the transfected cells were then subjected to different patterns of flow for luciferase induction assays. As shown in Fig. 6B, oscillatory flow decreased the...
ABCA1 promoter activity compared with static controls or laminar flow. The oscillatory flow-decreased ABCA1 promoter activity was reversed if E-box site was mutated or by co-transfection with SCAP-C, which encodes a truncated C terminus of SCAP and has been shown to block SREBP translocation from the endoplasmic reticulum to the Golgi (Fig. 6C) (30). These data suggest that oscillatory flow down-regulates ABCA1 in ECs, which is mediated through the up-regulation of SREBP2.

DISCUSSION

In the present study we reported that the binding of SREBP2(N) to the E-box of the ABCA1 promoter is responsible for the repressive effect of serum deprivation and oscillatory flow on the expression of ABCA1 in ECs. Evidence supporting such a thesis includes the following. 1) The adenovirus-mediated overexpression of SREBP2(N) could bind to the E-box motif of the ABCA1 promoter, hence inhibiting its activity, and the mutation of the E-box abolished the inhibitory effect of SREBP2(N). 2) Electrophoretic mobility shift and ChIP assays showed the association of SREBP2 and the ABCA1 promoter in ECs. 3) Functionally the SREBP2-down-regulated ABCA1 increased cholesterol accumulation and decreased the apoA-I-mediated cholesterol efflux. 4) Pathophysiologically disturbed flow patterns caused sustained activation of SREBP2 (21) with consequent decrease in ABCA1 expression in ECs. Thus, this SREBP-regulated mechanism controls the efflux of cholesterol, which is a newly defined function of SREBP2 in the vascular wall.

Apparently ABCA1 plays an important role in reverse cholesterol transfer and atherogenic protection (2). Most in vitro experiments involving ABCA1 function and regulation have been performed with the use of cultured macrophages, fibroblasts, and hepatocytes. Because it is exposed to the lipoproteins in the circulation, the vascular endothelium plays an important role in maintaining vascular integrity. We and others reported that ABCA1 is expressed in human aortic ECs, HUVECs, porcine brain capillary ECs, and rat liver ECs (19, 31, 32). Although the dysfunction of lipid metabolism and the ensuing oxidation and deposition in vascular cells impose important pathophysiological consequences, there have been only a few documented reports on ABCA1 regulation in ECs. The most studied transcriptional regulation of ABCA1 is the binding of the LXR/RXR heterodimer to the DR4 site of the ABCA1 promoter to up-regulate the expression of the ABCA1 gene (9). Similar regulation has also been observed in ECs (19). The E-box of the ABCA1 promoter has been reported to be a silencing regulatory element (12, 29) that binds to various transcrip-
In sterol-depleted cells, SREBP(N) is released from membranes and then translocates into the nucleus where it binds to SREs in the promoters of multiple genes encoding enzymes for cholesterol biosynthesis, unsaturated fatty acid biosynthesis, triglyceride biosynthesis, and lipid uptake (for reviews, see Refs. 15–17). SREBP2 can bind both the SRE sequence and E-box motif in the promoters of several genes (15). SREBP1 primarily activates the fatty acid triglyceride and phospholipid pathways, whereas SREBP2 is responsible for cholesterol synthesis and uptake. Since the major function of ABCA1 is to mediate intracellular free cholesterol efflux to apolipoproteins, we therefore focused on the regulation of ABCA1 by SREBP2. It was reported that LDL incubation decreases the expression of SREBP2 and its target genes in cultured ECs (33). We found that sterol depletion activated SREBP2 with the concurrent increase in LDLR and decrease in ABCA1 at the transcription level. This regulation was mimicked by the SREBP2(N) over-expression, which inhibited ABCA1 at the protein level with ensuing increase in cholesterol accumulation and decrease in apoA-I-mediated cholesterol efflux.

In peripheral cells, intracellular cholesterol homeostasis is exquisitely regulated and depends on the balance between cholesterol synthesis, degradation, cholesterol ester formation, influx, and efflux (34, 35). In principle, the sterol deprivation-activated SREBP(s) serve as transcriptional factors for lipid/cholesterol synthesis, uptake, and storage. But whether SREBP(s) also regulate genes governing cholesterol efflux is not clear. It was postulated that caveolin functions as a regulator of cellular free cholesterol homeostasis in quiescent peripheral cells in which caveolin mediated free cholesterol efflux. Previous study by others showed that SREBP(s) inhibited the expression of the caveolin gene, which is contradictory to its stimulating effect on other promoters (36). Our result indicates that SREBP2 binds directly to the E-box of the ABCA1 promoter. Whether SREBP2 forms a complex with other transcriptional factors or the binding of SREBP2 recruits other repressors deserves further studies. Recently we found that endoplasmic reticulum stress-induced activating transcription factor 6 suppressed the SREBP-mediated transcription in glucose-deprived HepG2 cells. The attenuated transcriptional activity of SREBP was due to the recruitment of a repressor, histone deacetylase 1, to the ATF6-SREBP complex (23, 24). Nevertheless ABCA1 down-regulation by SREBP2 is another example of the coordinated regulation of cholesterol synthesis, influx, and efflux.

In *in vitro* study suggested that SREBP(s) in peripheral cells were suppressed by high levels of serum or cholesterol (17). The physiological implications of the SREBP2 activation and ABCA1 down-regulation in ECs by SFM would be plausible since endothelium is exposed to full blood *in vivo*. We observed that oscillatory flow activated SREBP(s) and upregulated their targeting genes in ECs in the presence of high levels of LDL and 25-hydroxycholesterol, an agent that is commonly used to suppress the SREBP-mediated transcription in glucose-deprived HepG2 cells. The attenuated transcriptional activity of SREBP was due to the recruitment of a repressor, histone deacetylase 1, to the ATF6-SREBP complex (23, 24). Nevertheless ABCA1 down-regulation by SREBP2 is another example of the coordinated regulation of cholesterol synthesis, influx, and efflux.

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