Characterization of the Human Gene Encoding the Scavenger Receptor Expressed by Endothelial Cell and Its Regulation by a Novel Transcription Factor, Endothelial Zinc Finger Protein-2*

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The scavenger receptor expressed by endothelial cell (SREC), mediates the selective uptake of modified low density lipoprotein (LDL), such as acetylated LDL and oxidized LDL, into endothelial cells. The SREC gene spans 12 kilobase pairs and contains 11 exons. Analysis of full-length cDNA clones of SREC from a peripheral blood leukocyte cDNA library revealed that at least five alternatively spliced cDNAs were present, and two of them encoded soluble forms of SREC. The transcription start site of the SREC gene was mapped, and DNA sequence analysis revealed an Sp1 binding site in its proximal region. Deletion analysis of the 5'-flanking sequence revealed that sequence between base pairs −108 and −98 was critical for the promoter activity. This region contained half of an inverted repeat (IR) sequence with a triple nucleotide spacer (IR-3). A protected sequence between base pairs −268 and +17 was defined by in vitro DNase I footprinting analysis using human umbilical vein endothelial cell (HUVEC) nuclear extract. A novel transcription factor, endothelial zinc finger protein-2 (EZF-2), that binds to the 5'-flanking critical region of the SREC promoter activity was cloned from a HUVEC cDNA library employing a one-hybrid system. Whereas purified recombinant Sp1 alone produced similar protection in in vitro DNase I footprinting analysis, EZF-2 also bound to the 5'-flanking region SREC promoter. Co-transfection of SREC promoter and Sp1 or EZF-2 expression plasmids in HUVEC revealed that EZF-2 but not Sp1 increased SREC promoter activity. On the other hand, the mutation of either the Sp1 motif or IR-3 motif resulted in a decrease in the promoter activity. These results suggest that whereas Sp1 is the major nuclear protein bound to the regulatory region of the promoter, both EZF-2 and Sp1 are responsible for its regulation.

Scavenger receptors mediate the endocytosis of chemically modified lipoproteins, such as acetylated low density lipoprotein (LDL) and oxidized LDL, and have been implicated in the pathogenesis of atherosclerosis. The scavenger receptor gene family is composed of a series of unlinked genes that encode membrane proteins that bind multiple ligands (1–5), and we have cloned a subgroup of this family, the class F receptor, scavenger receptor expressed by endothelial cell (SREC), from a human umbilical vein endothelial cell (HUVEC) cDNA library (6, 7). SREC mediates the binding and degradation of acetylated LDL and oxidized LDL in endothelial cells. The primary structure of the molecule has no significant homology to other types of scavenger receptors. SREC has several characteristic domain structures, including an N-terminal extracellular domain with seven epidermal growth factor (EGF)-like cysteine pattern signatures and an unusually long C-terminal cytoplasmic domain (391 amino acids) composed of a Ser/Pro-rich region followed by a Gly-rich region. SREC is expressed at high levels in endothelial cells but not in smooth muscle cells.

Regulation of gene expression at the transcriptional level is mediated by the interaction of trans-acting factors with cis-acting DNA sequences in genes (8). However, the molecular mechanism underlying the transcriptional regulation of the SREC gene has not been clarified. Therefore, identification of basal and regulatory DNA elements in the 5'-flanking region of the human SREC gene will provide important insight into the molecular mechanisms underlying the regulation of expression of this gene. In this paper, we report the isolation and functional analysis of the promoter of the human SREC gene. To examine the regulation of the human SREC gene promoter, chimeric constructs containing serial deletions of the 5'-flanking region of the human SREC gene ligated to the luciferase reporter gene were prepared and transfected into HUVEC. We have identified the region that plays an important role in determining the basal promoter activity of the gene. This region contained an inverted repeat (IR) sequence with a triple nucleotide spacer (IR-3) and Sp1 motif. Employing a one-hybrid system, we cloned a novel transcription factor, endothelial zinc finger protein-2 (EZF-2), that bound to the 5'-flanking region, IR-3 motif of the SREC gene from a HUVEC cDNA library. Co-transfection of the SREC promoter and Sp1 or EZF-2 expression plasmids in HUVEC revealed that EZF-2 but not Sp1 increased SREC promoter activity. On the other hand, the

* This work was supported in part by Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science, and Technology of Japan as well as grants from the ONO Medical Research Foundation, Uehara Medical Foundation, and Naito Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequences reported in this paper have been submitted to the DDBJ/GenBank™/EBI Data Bank with accession numbers AB052946, AB052947, AB052948, AB052949, AB052950, AB052954, and AB052955.

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1 The abbreviations used are: LDL, low density lipoprotein; DiI, 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate; SREC, scavenger receptor expressed by endothelial cells; HUVEC, human umbilical vein endothelial cells; CASM, coronary arterial smooth muscle cells; EZF-2, endothelial zinc finger protein-2; EGF, epidermal growth factor; IR, inverted repeat; IL, interleukin; TNF, tumor necrosis factor; RLM-RACE, RNA ligase-mediated rapid amplification of cDNA ends.
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mutation of either the Sp1 motif or IR-3 motif resulted in a decrease in promoter activity. It was also found that inflammatory cytokines such as IL-1α, IL-1β, and TNF-α inhibited SREC promoter activity. Our results suggest that whereas Sp1 is the major nuclear protein bound to the regulatory region of the SREC promoter and can regulate the gene expression, EZF-2, a novel transcription factor, is also a major regulator of the promoter activity.

EXPERIMENTAL PROCEDURES

Isolation of Human SREC Gene—A human genomic DNA library (CLONTECH) was combined by a combination of PCR and hybridization (9) using two oppositely oriented oligonucleotides from the 5’ region of the SREC cDNA (5’-CCCGCTGCTGCCTGCTGTG-3’ and 5’-TGGCTG-GCTGCGACTG-3’) that corresponded to nucleotides 47–64 and 354–370 of SREC cDNA, respectively (7), causing the amplification of 324 bp. Once a human genomic DNA library was plated at a density of 5000 plaque-forming units/plate on LB agar plates using Escherichia coli XL1-Blue MRA as a host. The crude phage solution of each plate was plaque-purified and screened by PCR essentially as described above. After 16 h of plating, cells were washed once with serum-free OPTI-MEM. Either 1.6 µg of pG3-Promoter plasmid or an equivalent molar amount of test plasmid was co-transfected into HUVEC along with 0.032 µg of pRL-CMV plasmid using the synthetic cationic lipid component, Lipofectin reagent, according to the manufacturer’s instructions (Invitrogen). The pRL-CMV vector containing the Renilla luciferase gene under control of the herpes simplex virus thymidine kinase promoter (Promega) was used as an internal control of differences in transfection efficiency and cell number. For functional analysis of the basal promoter region of the SREC gene, the transfected cells were maintained for 48 h in serum-supplemented medium before harvesting. For analysis of regulation of the SREC promoter by various stimulators, the transfected cells were re cultured in a serum-free medium and incubated for 24 h in serum-supplemented medium supplemented with or without stimulants before harvesting. At the end of the culture period, the transfec- tants were lysed and the luciferase activity in the cell lysates was measured by a dual luciferase reporter assay system (Promega).

Preparation of Nuclear Extracts—All steps were carried out at 4 °C or on ice. The cells were grown in 10-cm dishes to ~80% confluence, washed twice with ice-cold phosphate-buffered saline, and harvested by scraping into 1 ml of ice-cold phosphate-buffered saline containing 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, and 2 µg/ml aprotinin. The cells were pelleted by centrifugation at 500 × g for 5 min, gently resuspended in 1 ml of hypotonic buffer (10 mM HEPES-NaOH, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.1 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, and 2 µg/ml aprotinin), and incubated on ice for 10 min. The cells were then lysed by homogenization, and the nuclei were collected by centrifugation for 5 min at 1000 × g. Nuclear extracts were prepared by a modification of the method of Dignam et al. (11). The nuclei were resuspended in 150 ml of ice-cold extraction buffer (10 mM HEPES-NaOH, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, and 1 mM phenylmethylsulfonyl fluoride). After slow mixing for 30 min at 4 °C, the suspensions were centrifuged at 13,000 × g for 15 min. The supernatant was dialyzed against dialysis buffer (20 mM HEPES-NaOH, pH 7.9, 75 mM NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, and 1 mM phenylmethylsulfonyl fluoride) for 2 h. The dialysate was then centrifuged at 13,000 × g for 15 min, divided into aliquots, and stored at –80 °C. Protein concentration was determined by the method of Bradford (12) with a kit from Bio-Rad using bovine serum albumin as a standard.

In Vitro DNase I Footprinting—In vitro DNase I footprinting was performed as described by Sandaltzopoulos and Becker (13), employing a Sure Track footprinting kit (Amersham Biosciences). DNA fragments containing bp 268 to +17 (for noncoding strand analysis) of the human SREC gene were labeled with biotin using the biotin-labeled 5’-biotin-GCGCTCGGTTCTGCTG-3’ and 5’-AGTGGAAAGCC-GTGGTCCCA by PCR. Samples were amplified for 15 cycles under the following conditions: denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, and extension for 1 min at 72 °C employing AmpliTaq Gold. Samples were then purified using a QIAquick PCR Purification Kit (Qiagen). About 50 µg of the labeled DNA fragment was incubated with 25 µg of nuclear extract or bovine serum albumin and 1 µg of poly(dI-dC)poly(dI-dC) for 25 min on ice and then 2 min at room temperature. In some experiments, recombinant human Sp1 (1 foot- printing unit; Promega) or ERF-2 (as described below) was used in addition to bovine serum albumin. Samples were treated with increases of 500 units of DNase I 0.02–1.0 units with bovine serum albumin and 0.5–2 Kunitz units with nuclear extract) at room temperature. Samples were analyzed as described (13, 14) using a 6% denaturing polyacrylamide/urea gel and an SEQ-Light™ DNA sequencing system. For comparison, DNA-sequence reactions of the genomic DNA around the transcription-initiation site of the SREC gene were run in parallel (G, A, C, and T) on the gel using a primer (5’-biotin-ATGG-AAAGCCGTTGCTGCA-3’).
Mutagenesis—The "megaprimer" method (15) was used to mutagenize bp -128 to +17 of the 5'-flanking sequence of SREC promoter cloned in pGL3-Basic. In the first step, PCRs were carried out using an internal mutagenic primer with an external vector-targeted primer (RVprimer3, 5'-CTAGCGAAATAGGCTGTCC-3'). The internal reverse primers (mutated bases underlined) were IR3-mut (5'-GAGAGGGAAT-AGGTGGCTGG-3') and Sp1-mut (5'-CCGGAGGATAGGGCTGCCAC-3'). The PCR product from each set of first step PCRs (e.g. RVprimer3/IR3-mut and RVprimer3/Sp1-mut) were purified and used in the second step PCR with an external vector-targeted primer (GLprimer2, 5'-CTTTATGTTTTTGGCGTCTTCC-3') primer. Products from the second-step PCRs were digested with KpnI and XhoI and cloned into pGL3-Basic and sequenced to confirm the presence of the desired mutations.

To construct the Sp1/IR3 double mutant, the IR3 site mutant cloned in pGL3-Basic was used as template. The mutagenic primer was Sp1-mut. The second step PCR product was cloned and sequenced as described above.

Cloning of Novel Transcription Factor from HUVEC cDNA Library—A target reporter construct and a reporter yeast strain were prepared by using a matchmaker one-hybrid system (CLONTECH). Three tandem copies of the nucleotide sequence between 110 and 89 of the 5'-flanking sequence of the SREC gene (5'-CAGGGGAAT-AGGTGGCTGG-3') were prepared by annealing two complementary strands of synthetic oligonucleotides, which include the cohesive ends of EcoRI and XhoI, and inserted upstream of the reporter gene HIS3 in pHISi. The resulting target-reporter construct was integrated at the HIS3 locus of yeast strain YM4271, yielding a reporter yeast strain. The second step PCR product was cloned and sequenced as described above.

RESULTS

Cloning of SREC Gene and Alternatively Spliced cDNAs—A human genomic DNA library (CLONTECH) was screened by PCR using two oppositely oriented oligonucleotides from the 5' region and then the 3' region of the human SREC cDNA (7). Two overlapping clones that covered the entire coding region of SREC were identified. A restriction map of the insert of these clones and the location of SREC exons and introns is shown in Fig. 1. The SREC gene spans 12 kb and contains 11 exons and 10 introns. The exon-intron boundaries of the SREC gene were determined by sequencing the SREC gene entirely, and it was found that all donor and acceptor splicing sequences contained consensus GT and AG dinucleotides, respectively. Most of the exons were small, with the exception of exon 11 (-2 kb), which contained a 3'-untranslated sequence.

As shown in Fig. 2, various alternatively spliced forms of SREC cDNAs were cloned from the peripheral blood leukocyte...
cDNA library. The nucleotide sequences of these clones contained exon-intron boundaries on the SREC gene, indicating that they were alternatively spliced transcripts for SREC. Among them, two transcripts, SREC-2 and SREC-4, encoded soluble forms of SREC. By alternative splicing, a stop codon was introduced before the transmembrane region of the SRECs. Whereas SREC-2 encoded 342 amino acids, SREC-4 encoded 337 amino acids and was five amino acids shorter than SREC-2 in domain 8 of the EGF-like repeat. Three transcripts, including the previously reported SREC-1 (7), encoded membrane-bound forms of SREC. SREC-3 encoded a different intracellular domain from SREC-1 by skipping the last 37 bp of exon 9. SREC-5 encoded a shorter extracellular domain by skipping the last 34 bp of exon 5, the entire exon 6, and the first 47 bp of exon 7. When these three membrane-bound forms were expressed transiently in COS-1 cells, they all showed receptor activity toward DiI-acetylated LDL (data not shown).

Mapping of the Transcriptional Start Site—As the first step to characterize the human SREC gene promoter, the transcriptional start site was mapped by RLM-RACE employing poly(A) RNA from HUVEC. In the first amplification, no specific PCR product was detectable using "outer RNA adaptor primer" as the sense primer complementary to "RNA adaptor" and SREC outer primer as the gene-specific antisense primer. Potential regulatory cis-elements are enclosed in boxes. Two rectangles indicate an IR. The nucleotide sequence has been deposited in the GenBank<sup>TM</sup>/DDBJ/EBI Data Bank under accession number AB052946.

**Fig. 3.** Nucleotide sequence of the 5'-flanking region of the human SREC gene. The transcriptional initiation site determined by RLM-RACE is assigned to be +1. The positions of gene-specific antisense primers used for RLM-RACE are underlined. The translation initiator ATG codon is marked by a double underline. Arrows indicate the deletion sites of the constructs used in the reporter assays. Potential regulatory cis-elements are enclosed in boxes. Two rectangles indicate an IR. The nucleotide sequence has been deposited in the GenBank<sup>TM</sup>/DDBJ/EBI Data Bank under accession number AB052946.
Fig. 4. Functional analysis of 5′-deletion constructs of the human SREC gene. A series of DNA fragments with different 5′-ends (nucleotides −2358, −2031, −1657, −1140, −686, −468, −268, −168, −148, −128, −108, −98, −88, and −68) and a common 3′-end (nucleotide +17) were ligated into the pGL3-Basic promoterless plasmid. HUVEC were transiently co-transfected with test plasmid and pRL-CMV internal control plasmid. 5′-Deletion constructs are schematically shown on the left. Luciferase activity of each construct is graphically shown in the right panel. Results are expressed as means ± S.D. from 4–7 independent experiments.

primer. Products of −120 bp were then cloned and sequenced. The results of sequencing analysis demonstrated that all selected clones terminated at the same nucleotide (designated as +1) located 35 bp upstream of the translation site (Fig. 3), indicating that this nucleotide is the major transcriptional initiation site of the human SREC gene. The 5′-CA-3′ nucleotide pair at this position is the most common transcription initiation site (17). The 5′-flanking sequence of the human SREC gene lacks TATA and CCAAT boxes near the transcription initiation site (17). The 5′-flanking sequence with sequences in the transcription factor data base revealed several putative DNA-binding elements that may play roles in basal and regulated SREC transcriptional activity (Fig. 3). Putative binding sites of MZF1, GATA-1, GATA-2, AML-1a, Lyf-1, and Sp1 are shown in triangles. Rectangles denote consensus sequences for the nuclear factor and the essential region for SREC promoter activity identified. Numbers represent positions of nucleotides in relation to the transcription initiation site. For comparison, DNA sequence reactions of the genomic DNA around the transcription initiation site of the SREC gene were run in parallel (G, A, C, and T) on the gel using a primer (5′-GTTATGGAAAAGCCTGCTCCCA-3′). To compare findings with the in vitro DNase I footprint produced by HUVEC nuclear extract, Sp1 and EZF-2 were also run in the same gel. The −268 to +17 bp DNA fragment of the SREC promoter was incubated with HUVEC nuclear extract, recombinant Sp1, or recombinant EZF-2 and digested with DNase I in increasing concentrations (triangles).

Fig. 5. Interactions of nuclear proteins with the human SREC promoter defined by in vitro DNase I footprinting. The −268 to +17 bp DNA fragment of the SREC promoter was used to assess DNA-protein interactions on the noncoding strand by in vitro DNase I footprinting analysis. The fragments were incubated with bovine serum albumin (control), HUVEC, or CASM nuclear extract and were incubated with increasing concentrations of DNase I represented by triangles. Rectangles denote consensus sequences for the nuclear factor and the essential region for SREC promoter activity identified. Numbers represent positions of nucleotides in relation to the transcription initiation site. For comparison, DNA sequence reactions of the genomic DNA around the transcription initiation site of the SREC gene were run in parallel (G, A, C, and T) on the gel using a primer (5′-GTTATGGAAAAGCCTGCTCCCA-3′). To compare findings with the in vitro DNase I footprint produced by HUVEC nuclear extract, Sp1 and EZF-2 were also run in the same gel. The −268 to +17 bp DNA fragment of the SREC promoter was incubated with HUVEC nuclear extract, recombinant Sp1, or recombinant EZF-2 and digested with DNase I in increasing concentrations (triangles).
densely protected nucleoprotein complex was formed in the presence of HUVEC nuclear extract but not of control and CASM nuclear extract on nucleotide sequence from −108 to −65 (Fig. 5).

Cloning of a Transcription Factor Bound to the 5'-Flanking Region of the Human SREC Gene—Since the nucleotide sequence between −108 to −98 was important for the regulation of SREC gene expression, we next attempted to clone a transcription factor bound to the 5'-flanking region of the SREC gene by employing a one-hybrid system. After screening of the HUVEC cDNA library, one candidate cDNA that interacted with the nucleotide sequence from −110 to −89 of the SREC promoter was cloned, and the nucleotide sequence was determined. The database search revealed a match with an unknown human placental cDNA (AK001999); sequences from the human genome sequencing projects, unknown cDNAs (AF074985 and BC021282), and chromosome 19 (AC024530); and highly similar sequence from unknown cDNA (XM_063584) and chromosome 15 at 15q21.3 (AC090515 and AC025918). The deduced amino acid sequence and the schematic structure of the cDNA are shown in Fig. 6. The deduced amino acid sequence contained leucine-rich region/SCAN (SRE-ZBP, Cfp51, AW-1, and number 18 cDNA) domain (20) and the C2H2-type zinc finger domain. We temporarily termed it as EZF-2, since another Kruppel-like zinc finger protein termed EZF was already cloned from the HUVEC cDNA library (21). Recombinant EZF-2 was expressed in E. coli W3110 as an RGS-His epitope-tagged form and purified by chelating Sepharose and DEAE-Sepharose column chromatographies.

Sp1 and EZF-2 Bind to the SREC Promoter—To identify the nuclear protein(s) present in HUVEC nuclear extract that contribute to the nucleoprotein complex, we compared the binding activity of HUVEC nuclear extract with that of recombinant Sp1 in in vitro footprinting assays using the human SREC fragment from −268 to +17 as a probe. To our surprise, recombinant Sp1 had essentially the same binding activity as HUVEC nuclear extract (Fig. 5), thereby presenting the possibility that Sp1 was attributable mainly to the binding activity of HUVEC nuclear extract to the fragment. As expected, EZF-2 also bound to the region between −122 and −88 bp, immediately upstream of the Sp1 binding site (Fig. 5).

**EZF-2 Modulates the SREC Promoter Activity**—To examine if elevated Sp1 or EZF-2 expression was sufficient to increase SREC promoter activity, HUVEC were transiently transfected with the −2358/+17 Luc, human SREC promoter luciferase reporter construct, and expression plasmids for either Sp1 or EZF-2 (Fig. 7A). Overexpression of EZF-2 caused a nearly 2.5-fold increase in SREC promoter activity in HUVEC. On the other hand, expression of Sp1 showed little effect on the basal SREC promoter activity in HUVEC. Transfection of reporter construct together with Sp1 and EZF-2 expression plasmids showed nearly the same promoter activity as that of reporter construct with EZF-2 expression plasmid.

To confirm the significance of these transcription factors further, we constructed mutant plasmids having mutated Sp1 and/or IR-3 motifs. As shown in Fig. 7B, mutation of either Sp1 motif or IR-3 motif caused a decrease in promoter activity of nearly 50% in HUVEC. Moreover, mutant plasmid having a mutation in both motifs had little promoter-enhancing activity. These results suggest that both Sp1 and EZF-2 play roles in the induction of SREC gene expression.

**Regulation of the SREC Promoter by Cytokines**—To determine whether or not the expression of the SREC gene was physiologically and/or pathologically regulated, HUVEC transfected with −2358/+17 Luc construct were treated with various biologically active substances. Whereas treatment with phorbol 12-myristate 13-acetate or platelet-derived growth factor-AB slightly increased luciferase activity in HUVEC transfected with the −2358/+17 Luc construct, treatment with inflammatory cytokines such as IL-1α, IL-1β, or TNF-α inhibited the expression of luciferase activity (Fig. 8). These results suggested that the expression of the SREC gene could be regulated by these inflammatory cytokines.

**DISCUSSION**

In this study, we have characterized the human gene encoding SREC. The gene is ~12 kb in length and contains 11 exons and 10 introns. Only a single SREC transcription start site was
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To define DNA-protein interactions in this region and determine the proteins binding to the SREC promoter, we performed in vitro DNase I footprinting assays. A densely protected nucleoprotein complex was formed on the nucleotide sequence from −108 to −65 bp in the presence of HUVEC nuclear extract but not of control and CASM (Fig. 5), suggesting that this region is critical for the transcriptional regulation of the SREC gene. Furthermore, we have cloned a novel transcription factor, EZF-2, that bound to this critical region, by employing a one-hybrid system. The domain structure and the amino acid sequence of EZF-2 are similar to those of several zinc finger transcription factors such as Krüppel-like ZNF191 (26) or ZNF24. The leucine-rich region/SCAM domain has been shown to be able to mediate homo- and hetero-oligomerization (27). The C2H2 zinc finger is composed of two short β-strands followed by an α-helix and the conserved cysteines and histidines that coordinate with a zinc ion. Since many members of the C2H2 zinc finger gene family regulate differentiation processes and genetic mutation in some zinc finger genes has been associated with specific human diseases (28, 29), it is interesting to elucidate the role of this novel transcription factor. Further work is required.

Recombinant Sp1 had essentially the same binding activity as HUVEC nuclear extract (Fig. 5), suggesting that the major binding protein in HUVEC nuclear extract was attributable to Sp1. On the other hand, recombinant EZF-2 was found to bind to the sequence between −122 and −88, immediately upstream of the Sp1 binding site. Overexpression of EZF-2 increased SREC promoter activity by nearly 2.5-fold in HUVEC, while expression of Sp1 alone showed no significant effect on basal SREC promoter activity in HUVEC. On the other hand, mutation analysis of reporter constructs revealed that the intact Sp1 and IR-3 motifs were required for the maximal expression of the SREC gene. These results suggested that Sp1 and EZF-2 can induce SREC gene expression independently. It is conceivable that in the overexpression experiments, Sp1 had little effect because of the high and saturation level of endogenous Sp1 expression in HUVEC. The precise mechanism of the endothelial cell-specific expression of the SREC gene remains to be elucidated.

Employing the 5′-flanking region of SREC, the regulation of SREC expression in HUVEC in vitro by various stimuli was determined at the transcriptional level. Among them, inflammatory cytokines such as IL-1α, IL-1β, and TNF-α inhibited promoter activity. A parallel decrease in DiI-acetylated LDL uptake was observed after treatment with these stimuli (data not shown). These results suggested the possibility that cytokines regulated the expression level of SREC and thus modified the inflammatory process. Pathophysiological implications of these regulations should be elucidated by analyzing the physiological function of the SREC gene. In summary, we have characterized the human SREC gene promoter in detail and found that a novel transcription factor, EZF-2, regulated the gene expression. The gene expression was regulated by several cytokines, such as TNF-α, IL-1α, and IL-1β, suggesting that SREC plays a role in the course of inflammation. Since several investigators have reported that the stimulation of scavenger receptors in endothelial cells induces the expression of proteins related to vascular functions, the elucidation of the underlying mechanism for SREC gene regulation will provide new insight into the pathological function of the SREC.

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Characterization of the Human Gene Encoding the Scavenger Receptor Expressed by Endothelial Cell and Its Regulation by a Novel Transcription Factor, Endothelial Zinc Finger Protein-2
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J. Biol. Chem. 2002, 277:24014-24021.
doi: 10.1074/jbc.M201854200 originally published online April 26, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M201854200

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