Type F Scavenger Receptor SREC-I Interacts with Advillin, a Member of the Gelsolin/Villin Family, and Induces Neurite-like Outgrowth*

Received for publication, April 7, 2004, and in revised form, June 30, 2004
Published, JBC Papers in Press, July 9, 2004, DOI 10.1074/jbc.M403844200

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The scavenger receptor expressed by endothelial cells (SREC) was isolated from a human endothelial cell line and consists of two isoforms named SREC-I and -II. Both isoforms have no significant homology to other types of scavenger receptors. They contain 10 repeats of epidermal growth factor-like cysteine-rich motifs in the extracellular domains and have unusually long C-terminal cytoplasmic domains with Ser/Pro-rich regions. The extracellular domain of SREC-I binds modified low density lipoprotein and mediates a homophilic SREC-I/SREC-I or heterophilic SREC-I/SREC-II trans-interaction. However, the significance of large Ser/Pro-rich cytoplasmic domains of SRECs is not clear. Here, we found that when SREC-I was overexpressed in murine fibroblastic L cells, neurite-like outgrowth was induced, indicating that the receptor can lead to changes in cell morphology. The SREC-I-mediated morphological change required the cytoplasmic domain of the protein, and we identified advillin, a member of the gelsolin/villin family of actin regulatory proteins, as a protein binding to this domain. Reduction of advillin expression in L cells by RNAi led to the absence of the described SREC-I-induced morphological changes, indicating that advillin is a prerequisite for the change. Finally, we demonstrated that SREC-I and advillin were co-expressed and interacted with each other in dorsal root ganglion neurons during embryonic development and that overexpression of both SREC-I and advillin in cultured Neuro-2a cells induced long process formation. These results suggest that the interaction of SREC-I and advillin is involved in the development of dorsal root ganglion neurons by inducing the described morphological changes.

Scavenger receptors are defined by their ability to bind and metabolize modified low density lipoproteins (LDLs), such as acetylated LDL (AcLDL) and oxidized LDL (OxLDL), and have been regarded as relevant in the pathogenesis of atherosclerosis (1, 2). Mammalian cells have several different classes of scavenger receptors, and their relative contributions to lipid metabolism in pathophysiological conditions, such as atherosclerosis, are the subject of intense investigation (2).

Endothelial cells express several distinct scavenger receptors, such as SR-BI (3–5), LOX-1 (6), and FEEI-1/stabilin-1 (7). We cloned a novel scavenger receptor from a DNA library prepared from human umbilical vein endothelial cells employing expression cloning and termed it SREC (scavenger receptor expressed by endothelial cells)-I (8). Subsequently, we also succeeded in cloning a homologous protein, SREC-II, by a database search (9). These two receptors are now classified as type F scavenger receptors (2, 9).

Both SREC-I and -II have no significant homology to other types of scavenger receptors. They contain 10 repeats of epidermal growth factor-like cysteine-rich motifs in their extracellular domains and unusually long C-terminal cytoplasmic domains with Ser/Pro-rich regions (8, 9). SREC-I mediates the binding and degradation of AcLDL and OxLDL in endothelial cells, whereas SREC-II has little scavenger receptor activity, making it likely that these type F scavenger receptors have biological functions not linked to scavenger receptor activity. We showed previously (9) that SREC-I and -II display respective homophilic interaction through their extracellular domains between separate cells (trans-interaction) and strong SREC-I/SREC-II heterophilic trans-interaction. The homophilic and heterophilic trans-interactions of SREC-I and -II were effectively suppressed by the presence of scavenger receptor ligands, such as AcLDL and OxLDL.

The cytoplasmic domains of SREC-I and -II consisting of ~400 amino acids contain several potential phosphorylation sites for kinases A, C, and G, suggesting that these domains transduce intracellular signals generated by the receptors. SREC-I and -II may transduce different signals because of low sequence similarity and different potential phosphorylation sites. However, the biological role of the cytoplasmic domain of SRECs is so far totally unknown.

In this study, we focused on the function of the SREC-I cytoplasmic domain. We previously employed murine L cells to elucidate the receptor/receptor trans-interaction (9), because this cell type is commonly used in experiments demonstrating trans-interaction of various cell adhesion molecules (10–13). In the experiments, we had noticed that prolonged culture of SREC-I-transfected L cells induced striking morphological cell changes. Based on these preliminary observations, we show here that SREC-I, but not SREC-II, induces neurite-like long processes after overexpression in murine fibroblastic L cells and that the cytoplasmic domain of SREC-I is a prerequisite for
this activity. Moreover, we demonstrated that advillin, a member of the gelsolin/villin family of actin regulatory proteins, binds specifically to the cytoplasmic domain of SREC-I and is required for this morphological cell change. The biological implications of this activity are discussed.

EXPERIMENTAL PROCEDURES

Cell Culture—Murine L cells (CCL-1, American Type Culture Collection, Manassas, VA) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine. Chinese hamster ovary cells were maintained in Ham’s F-12 medium supplemented with 50 mg/ml penicillin, 50 mg/ml streptomycin, 2 mM L-glutamine, and 10% fetal bovine serum. The murine neuroblastoma line Neuro-2a cells (CCL-131, American Type Culture Collection) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine.

Plasmid Construction—The EcoRI-XhoI fragment of the mouse cDNA for SREC-I, SREC-I cytoplasmic domain deletion mutant that lacks amino acid residues 451–820 (SREC-I-ΔC370), SREC-II, SR-BI, and advillin were subcloned into the mammalian expression vector pcDNA3 (Invitrogen), and expression plasmids were termed pcDNA3-SREC-I, pcDNA3-SREC-I-ΔC370, pcDNA3-SREC-II, pcDNA3-SR-BI, or pcDNA3-advillin, respectively. We noted that a hemagglutinin tag was added at the C terminus of advillin in pcDNA3-Advillin.

Uptake of DiI-AcLDL—L cells (1 × 10⁴ cells/well) in 24-well plates were mock transfected or transfected with either pcDNA3-SREC-I, pcDNA3-SREC-I-ΔC370, pcDNA3-SREC-II, pcDNA3-SR-BI, or pcDNA3-advillin using LipofectAMINE reagent (Invitrogen) according to the manufacturer’s instructions. The cells were incubated for 72 h, incubated again in the presence of 2 μg/ml DiI-AcLDL (Biomedical Technologies Inc.) for 2 h, washed, and then fixed with 3.7% formaldehyde in PBS for 15 min at room temperature. The presence of fluorescent DiI in the fixed cells was determined by visual inspection using fluorescence microscopy.

GST Fusion Proteins—The EcoRI-Sall fragment encoding the first half (C1, amino acid residues 451–643), the central part (C2, amino acid residues 561–752), or the last half (C3, amino acid residues 643–820) of the cytoplasmic domain of mouse SREC-I was subcloned into a multicloning site downstream of the sequence for GST in pGEX-4T-1 (Pharmacia Corporation). This plasmid was transformed into the JM109 strain of Escherichia coli and induced with isopropyl-1-thio-β-D-galactopyranoside to produce GST fusion proteins. The bacteria were suspended in PBS, and vigorous sonication was performed before centrifugation at 10,000 × g for 20 min. The resulting supernatants were applied to a glutathione-Sepharose column and then eluted with an elution buffer (50 mM Tris-HCl, pH 9.6, 120 mM NaCl, 10 mM glutathione) and then eluted with the elution buffer. The eluted fractions of affinity purifying C1- or C2-binding protein(s). L cell cytosolic extracts were loaded onto the GST-C1 or -C2 fusion proteins, bound to the glutathione-Sepharose column, were used to affinity-purify C1- or C2-binding protein(s). L cell cytosolic extracts were loaded onto the GST-C1 or -C2 glutathione-Sepharose column and then eluted with the elution buffer. The eluted fractions of affinity chromatography were collected, precipitated by 10% trichloroacetic acid, and subjected to SDS-PAGE. A Coomassie Brilliant Blue-stained band of 60 kDa was cut out and digested with Acpronobacter protease I (API; a gift from Dr. Masaki, Ibaraki University) (14). The resulting peptides were separated by reverse phase high pressure liquid chromatography on tandemly connected DEAE-SPW (1 × 20 mm; Tosoh, Tokyo, Japan) and Capcell Pak C18, UG120 (1 × 50 mm; Shiseido, Tokyo, Japan) columns with a 0–50% gradient in 0.1% trifluoroacetic acid. Isolated peptides were analyzed by automated Edman degradation on an Applied Biosystems protein sequencer model 477A (PerkinElmer Life Sciences) connected on line to a PTH Analyzer model 120A (PerkinElmer Life Sciences) using an in-house-generated gas phase program and were also examined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry with a Reflex MALDI-TOF (Bruker-Franzen Analytik, Bremen, Germany) in linear mode, with 2-mercaptobenzothiazole used as a matrix.

Antibodies—The polyclonal antibodies against SREC-I and advillin were prepared as follows. Peptides corresponding to the C-terminal domain of mouse SREC-I (NH2-KEQEEPKEYNVPMVSYFPQH-COOH) and mouse advillin (NH2-DGEKPYYPVEVLLKGNQEL-COOH) were synthesized. The synthesized peptides were conjugated with keyhole limpet hemocyanin using an Imject sulphydryl-reactive antibody production Kit ( Pierce). The keyhole limpet hemocyanin peptides were gel-purified and emulsified with an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, MI). Female Wistar rats were immunized with the emulsions. These rat sera were collected and purified using an affinity column (Sulfolink Coupling Gel, Pierce) to which the corresponding antigen peptide was coupled.

Small Interference RNA—The mammalian expression vector pSUPER was used for expression of siRNA in the L cells. Three parts of the gene-specific targeting sequence (19-nucleotide sequences: 1, 5′-CCGCAGCAGAAAGACGTCG-3′; 2, 5′-CCGCCAGCAGAGACGTCG-3′; and 3, 5′-CAGAAGGGATCAGAGATG-3′) from the target transcript were separated by a 9-nucleotide noncomplementary spacer (TTCAGAGA) from the reverse complement of the same 19-nucleotide sequence were inserted in pSUPER. These vectors were referred to as pSUPER-advillin/RNAi-1, -2, and -3, respectively. L cells were transfected with either pSUPER-advillin/RNAi, control vector (pSUPER), pcDNA3-SREC-I plus pSUPER, pcDNA3-SREC-I plus pSUPER-advillin/RNAi, or...
pcDNA3-GFP plus pSUPER-advillin/RNAi as described above, and the cells were cultured for 72 h. Total cell lysates were prepared in lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% (w/v) Triton X-100, 0.5% (w/v) Nonidet P-40, 1 mM EDTA, protease inhibitor mixture (Sigma), and 1 mM phenylmethylsulfonyl fluoride). The lysates were cleared by centrifugation at 18,600 × g for 20 min at 4 °C, were harvested and homogenized in 1 ml of PBS, and then centrifuged at 100,000 × g for 1 h at 4 °C. The resultant supernatants were analyzed by Western blotting with anti-advillin or anti-SREC-I antibodies.

Immunofluorescence Microscopy—L-cells were transfected with pcDNA3-SREC-I and pSUPER-advillin/RNAi vector or control vector (pSUPER), incubated for 72 h at 37 °C, fixed with 3.7% formaldehyde in PBS for 20 min at room temperature, permeabilized with 0.1% Triton X-100 for 5 min, and then blocked with 3% bovine serum albumin in PBS for 1 h at room temperature. The cells were then incubated with the anti-SREC-I antibody for 2 h at room temperature, washed 4 times with PBS, incubated again with an Alexa Fluor 594 goat anti-rat IgG(H+L) antibody for 1 h at room temperature, washed thoroughly with PBS, embedded, and then visualized using a fluorescence microscope. Neuro-2a cells were transfected with pcDNA3-SREC-I and pcDNA3-advillin, and incubated for 72 h at 37 °C. The cells were then fixed with 3.7% formaldehyde in PBS for 20 min at room temperature, permeabilized with 0.1% Triton X-100 for 5 min, blocked with 3% bovine serum albumin in PBS for 1 h at room temperature, and incubated with the anti-SREC-I antibody and anti-hemagglutinin antibody for 2 h at room temperature. After that, the cells were washed four times with PBS, incubated again with an Alexa Fluor 594 goat anti- rat IgG(H+L) antibody for 1 h at room temperature, washed thoroughly with PBS, embedded, and then visualized using a fluorescence microscope.

Western Blot Analysis—C57/BL6 mice (adults or 18-day embryos) were perfused with ice-cold SET buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) containing protease inhibitor mixture and 1 mM phenylmethylsulfonyl fluoride. Thereafter, the brain and spinal cord were homogenized in 4 volumes (w/v) of SET buffer and then centrifuged at 1,000 × g for 10 min at 4 °C. The resultant supernatants were used as the brain and spinal cord total protein lysates. The DRG was homogenized in SET buffer and then centrifuged at 1,000 × g for 10 min at 4 °C. The resultant supernatant was concentrated by 10% trichloroacetic acid precipitation. The resultant pellets were suspended in SET buffer and used as the DRG total protein lysate.

The protein concentrations of samples were determined by BCA assay (Pierce). Each total protein lysate (100 μg/lane) was separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% (w/v) skim milk (Wako, Osaka, Japan) in TTBS buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% (w/v) Tween 20) and incubated with anti-advillin or anti-SREC-I antibodies in TTBS. The levels of protein were analyzed with an ECL kit (Amersham Biosciences) according to the manufacturer’s instructions.

Immunoprecipitation—DRG extracts were prepared in lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% (w/v) Triton X-100, 0.5% (w/v) Nonidet P-40, 1 mM EDTA, protease inhibitor mixture (Sigma), 1 mM phenylmethylsulfonyl fluoride). The DRG extracts were preclariﬁed for 2 h with protein G-agarose beads (Amersham Biosciences) and then incubated overnight with anti-SREC-I antibody at 4 °C. Immunocomplexes were precipitated with protein G-agarose beads for 45 min, washed three times with lysis buffer, and boiled in SDS sample buffer containing 2-mercaptoethanol. The supernatants were subjected to SDS-PAGE and Western blotting.

RESULTS

SREC-I-induced Morphological Change of L Cells—First, L cells were transfected with vectors for various scavenger receptors, and morphological changes were monitored. As shown in Fig. 1, transfection of the expression vector for SREC-I into the cells caused significant morphological changes with generation of neurite-like long processes. On the other hand, no change in cell morphology was observed when other scavenger receptors, such as SR-A, SR-BI, or even SREC-II were overexpressed in these cells. These results suggested that SREC-I is the specific scavenger receptor that can induce neurite-like outgrowth when overexpressed in L cells. When SREC-I was expressed in Chinese hamster ovary cells, no morphological cell change was observed (data not shown).

SREC-I contains a large cytoplasmic domain consisting of ~400 amino acids. To elucidate the role of this cytoplasmic domain, deletion mutants lacking C-terminal fragments of 370 amino acids were created, and their effects on cell shape were examined. We have shown previously (9) that this truncated receptor was expressed in L-cells to a degree similar to the full-length receptor and showed a comparable level of AcLDL uptake activity. In contrast to these findings, the mutant protein did not induce a change in cell morphology (Fig. 2B). These results indicated that the cytoplasmic domain is required for the induction of neurite-like outgrowth, and we hypothesized...
that proteins interacting with this domain are required to mediate the effects of this receptor.

**Identification of Binding Proteins to the Cytoplasmic Domain of SREC-I**—To identify the proteins that bind to the cytoplasmic domain of SREC-I, we prepared several expression vectors for GST-fused C-terminal fragments of the SREC-I protein (Fig. 3A, C1–C3). Among others, we could successfully express GST-C1 and -C2 fragments in E. coli and analyze their binding activities. The cytoplasmic fraction prepared from L cells was loaded onto either a GST-C1 or -C2 affinity column, and the proteins bound to the respective column were co-eluted with GST-fused peptide by the addition of glutathione. As shown in Fig. 3B, we could not detect any proteins specifically binding to the C1 fragment. On the other hand, a protein with a molecular mass of ~90 kDa was specifically detected in the glutathione eluate from a GST-C2 column onto which the cytoplasmic fraction of L cells was loaded, although the column loaded with control solution yielded no such protein. These results indicated that the identified 90-kDa protein could bind to the amino acid sequence between residues 643 and 752 of the SREC-I cytoplasmic domain.

The 90-kDa protein was then subjected to amino acid sequencing. Seven peptides derived from the protein were determined, and all were the partial sequences of advillin, an actin regulatory protein belonging to the gelsolin/villin family (16).

**Advillin Is Required for SREC-I-mediated Morphological Cell Change**—To examine whether advillin is involved in SREC-I-mediated morphological cell change, the RNAi technique was applied (17–20). L cells were treated with several constructs (RNAi-1 to -3; see “Experimental Procedures”) of advillin siRNA. First, we confirmed by Western blotting that murine fibroblastic L cells intrinsically expressed advillin (Fig. 4A, lanes 1 of (a) and (b), Advillin). Moreover, we found that the RNAi-1 vector was most efficient in decreasing the expression of advillin (Fig. 4A). Thereafter, L cells were co-transfected with SREC-I and advillin siRNA (RNAi-1) vectors, and morphological cell change was monitored. The expression vector for SREC-I was transfected into the cells, and the expressed protein was analyzed by Western blotting. Two bands with Mₚ of 141,000 and 147,000 were observed. These two bands were not detected when the green fluorescent protein expression vector was transfected, indicating the heterogeneity of the expressed SREC-I, most probably because of the varying glycosylation of the protein. Co-transfection of RNAi-1 caused a significant decrease in the expression of endogenous advillin without affecting the expression pattern of SREC-I (Fig. 4B). As shown in Fig. 4C, transfection of the SREC-I vector alone induced neurite-like outgrowth, whereas this phenomenon was impaired significantly by the co-transfection of the RNAi-1 vector. When counting the cells showing long processes, it was apparent that co-transfection of the RNAi-1 vector together with that of SREC-I caused a dramatic decrease in cell number. These results indicate that advillin is required for the SREC-I-mediated induction of neurite-like outgrowth in L cells. It should be noted that unlike in the L cells, advillin was undetectable in Chinese hamster ovary cells in which SREC-I had no apparent ability to induce the described morphological cell change (data not shown).

**SREC-I Is Expressed in Peripheral Nerve Neurons**—Because it was reported that advillin is expressed in the peripheral nervous system in areas such as the DRG and the superior cervical ganglion and plays a role in the neurite outgrowth of neuronal cells (21), we examined whether SREC-I is also expressed in peripheral nerve neurons. We focused on the embryonic expression of the protein, because it was reported that advillin is expressed in peripheral nerve neurons, especially during embryonic development (16). SREC-I protein was barely detectable in the brain, spinal cord, or DRG of the adult mouse but was clearly detectable in each of these tissues in 18-day embryonic mice (Fig. 5A). These results suggested that SREC-I is expressed transiently in the nervous system during fetal development. On the other hand, both in adult and embryonic mice, advillin was detectable in DRG but not in brain or spinal cord.

To determine whether SREC-I interacts with advillin in the DRG of 18-day embryonic mice, we performed immunoprecipitation studies with the anti-SREC-I antibody. Immunoblot analysis revealed that the immunoprecipitates contained advillin in addition to SREC-I (Fig. 5B). No anti-SREC-I or anti-advillin signals were detected in the immunoprecipitates treated with normal rabbit serum (data not shown). These results demonstrated that SREC-I interacts with advillin in the DRG of 18-day embryonic mice.

Next, we examined whether the interaction of SREC-I and advillin induces neurite outgrowth in neuronal cells. Because most of the neuronal cell lines intrinsically expressed neither SREC-I nor advillin, we used a murine neuroblastoma cell line,
Neuro-2a, to perform transfection experiments. The cells were transfected with the SREC-I and/or advillin vectors, and morphological cell change was monitored. As shown in Fig. 6, the cells co-expressing SREC-I and advillin showed neurite outgrowth, whereas the cells expressing either SREC-I or advillin did not show this phenomenon, indicating that the interaction of SREC-I and advillin induces neurite formation in cultured neuronal cells.

DISCUSSION

The present results demonstrate that SREC-I is capable of interacting with advillin through its large Ser/Pro-rich cytoplasmic domain and thereby is capable of inducing neurite-like outgrowth. Members of this actin regulatory protein family are capable of capping and severing actin filaments (16). Neuro-2a, to perform transfection experiments. The cells were transfected with the SREC-I and/or advillin vectors, and morphological cell change was monitored. As shown in Fig. 6, the cells co-expressing SREC-I and advillin showed neurite outgrowth, whereas the cells expressing either SREC-I or advillin did not show this phenomenon, indicating that the interaction of SREC-I and advillin induces neurite formation in cultured neuronal cells.

Fig. 4. Advillin is required for the SREC-I-induced morphological change. A, suppression of advillin expression by pSUPER-advillin/RNAi. (a), pSUPER (lane 1) or pSUPER-advillin/RNAi-1 to -3 (lanes 2–4) were transfected into L cells as described under “Experimental Procedures.” Total cell lysates were subjected to SDS-PAGE and immunoblotted to detect advillin proteins. (b), pcDNA3-SREC-I and pSUPER (lane 1) or pcDNA3-SREC-I and pSUPER-advillin/RNAi-1 (lane 2) or pcDNA3-GFP and pSUPER-advillin/RNAi-1 (lane 3) were transfected into L cells as described under “Experimental Procedures.” Total cell lysates were subjected to SDS-PAGE and immunoblotted to detect SREC-I (upper panel) and advillin (lower panel) proteins. B, L cells transfected with pcDNA3-SREC-I and pSUPER (left panels) or pcDNA3-SREC-I and pSUPER-advillin/RNAi-1 (right panels) were immunostained with polyclonal antibody against SREC-I. Upper panels, fluorescence images. Lower panels, phase-contrast light micrographs of the same fields as in the upper panels. Bar, 10 μm. C, percent of >40-μm long processed L cells.

Fig. 5. Western blot analysis of SREC-I and advillin in murine brain and DRG. A, immunoblot analysis of lysates from mouse brain, spinal cord, and DRG with polyclonal antibody against SREC-I (upper panel) and advillin (lower panel). Each total protein lysate (100 μg/lane) was separated by SDS-PAGE and subjected to Western blotting. B, mouse DRG lysates were immunoprecipitated with polyclonal antibody against SREC-I. Immunoblot analysis of the immunoprecipitates and the lysates with polyclonal antibody against SREC-I (upper panels) and advillin (lower panels).
Among these proteins, advillin is most closely related to villin in its domain structure, including the C-terminal F-actin-binding headpiece domain (16). Villin is expressed mainly in differentiated epithelial tissues possessing a brush border, such as intestinal villi or proximal renal tubules (22, 23), and most likely plays an important role in the morphogenesis of microvilli (24–26). Advillin, however, is highly expressed in the dorsal root and trigeminal ganglia during embryonic development and only at low levels in adult uterine and intestinal epithelial cells (16). Transfection of the advillin expression vector to primary cultures of rat DRG sensory neurons resulted in increased neurite outgrowth (21), indicating that the protein plays a significant role in the morphogenesis of peripheral neurons through an actin-bundling domain. It is therefore reasonable that the cytoplasmic domain of SREC-I binds to advillin and regulates the intracellular cytoskeletal organization, resulting in the generation of neurite-like long processes.

Unexpectedly, murine fibroblastic L cells were found to intrinsically express advillin (Fig. 4A). The parent L strain was derived from normal subcutaneous areolar and adipose tissue of male C57BL/6J mice according to the CCL-1 catalogue. Intrinsically expressed advillin made it possible to elucidate the function of SREC-I in advillin-mediated morphological changes in L cells. Depletion of endogenous advillin protein in L cells by RNAi inhibited the generation of long processes, indicating that advillin is indispensable for this change. Chinese hamster ovary cells, in which advillin was undetectable, displayed no morphological changes upon SREC-I overexpression. This provides further evidence for the necessity of advillin.

Our results are the first to show that SREC-I is expressed not only in endothelial cells but also in neuronal cells. Furthermore, SREC-I and advillin are co-expressed and interact with each other in DRG neurons, especially during embryonic development. We also demonstrated that overexpression of both SREC-I and advillin in cultured Neuro-2a cells induces the formation of long processes. These results suggest that the interaction of SREC-I and advillin are involved in the development of DRG neurons by inducing the described morphological changes.

SREC-I is characterized by its extremely large cytoplasmic domain. The present data indicate that this domain is a prerequisite for the receptor-mediated morphological change of L cells, which shows that the cytoplasmic domain of SREC-I plays a role in transducing intracellular signals of SREC-I. We showed that advillin binds to the C2 peptide (amino acid residues 562–752) but not to the C1 peptide (amino acid residues 452–643) of the cytoplasmic domain. This suggests that the region within 643–752 is responsible for advillin binding. This region is also rich in Ser and Pro, but it is not similar to the known domains that transduce signals into the cell interior. Interestingly, although ~20% homology was observed in the entire cytoplasmic domains of SREC-I and -II, this region is less homologous (<10%), which can explain why SREC-II exerted no morphogenetic activity on L cells.

It remains unclear how the SREC-I signal is transduced into the cell interior. We have demonstrated previously that SREC-I shows a homophilic trans-interaction between separate cells through its extracellular domain (9). This homophilic trans-interaction of SREC-I may serve as a signal for the induction of neurite-like long processes. However, this may not be the case, because the L cells that display long processes did not necessarily show contact with neighboring SREC-I-expressing L cells (Figs. 1, 2, and 4B). Moreover, although homophilic trans-interaction of SREC-I is effectively disrupted by the addition of AcLDL or OxLDL (9), these ligands had little effect on the formation of long processes (data not shown), which supports the idea that trans-interaction of SREC-I between cells is not obligatory for the transduction of the signal into the cells. In our preliminary study, we observed that SREC-I forms an oligomer in the membrane (homophilic cis-interaction), possibly a dimer when overexpressed in L cells. The extracellular domain may be indispensable for the oligomerization, because upon co-transfection of native full-length SREC-I and the ΔC370 deletion mutant, which lacks most of the cytoplasmic domain into L cells, both receptors could be co-immunoprecipitated. Interestingly, in most of these cells, long process formation was greatly diminished, indicating that the ΔC370 deletion mutant may function as a dominant

![Image](http://www.jbc.org/)

**Fig. 6. Co-expression of SREC-I and advillin induces neurite outgrowth in Neuro-2a cells.** Neuro-2a cells were transfected with pcDNA3-SREC-I and pcDNA3-advillin (A), pcDNA3-SREC-I (B), or pcDNA3-advillin (C). The cells were immunostained with rat polyclonal antibody against SREC-I (red) and mouse monoclonal antibody against hemagglutinin-tagged (green). Note the long processes with the presence of SREC-I and advillin (arrowheads). Bar, 20 μm.
negative effector for SREC-I activity. These observations suggest that close association of the SREC-I cytoplasmic domain themselves may send a signal to L cells that induces the formation of long processes. In in vivo situations, some ligands might stimulate SREC-I oligomerization producing a signal in the cells like other growth hormone receptors. SREC-II, which shows a strong heterophilic trans-interaction with SREC-I, is a possible natural ligand for SREC-I activity. These observations suggest that other factor(s) serve as ligands for SREC-I extracellular domains. Further studies are needed to identify the natural ligand in these tissues and to elucidate the mechanism of advillin activation through the SREC-I cytoplasmic domain.

SREC-I was originally identified from a human endothelial cell line (9). Endothelial cells play important roles in vasculogenesis, angiogenesis, and the repair of injuries along the endothelium (27). Under these situations, endothelial cells actively migrate along the substratum in a coordinated and polarized fashion. This process involves extension of filopodia and lamellipodia, both of which have specific actin-based architectures. SREC-I might be involved in the formation and disruption of actin bundles through the gelsolin/villin family in endothelial cells.

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J. Biol. Chem. 2004, 279:40084-40090.
doi: 10.1074/jbc.M403844200 originally published online July 9, 2004

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