LR11, an LDL Receptor Gene Family Member, Is a Novel Regulator of Smooth Muscle Cell Migration

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Abstract—LR11, a member of the LDL receptor family, is highly expressed in vascular smooth muscle cells (SMCs) of the hyperplastic intima, and induces enhanced migration of SMCs in vitro via its upregulation of urokinase-type plasminogen activator receptor (uPAR) expression. In this study, we have delineated the mechanism by which LR11 elevates the expression levels of uPAR in SMCs. Secretion of soluble LR11 is induced in SMCs during the rapidly proliferating phase, and the secreted LR11 induces the migration activities of SMCs. Both the cell-anchored and secreted forms of LR11 have the capacity to bind to and form complexes with uPAR. LR11-overexpressing cells show significantly enhanced uPAR binding, but decreased uPAR internalization. LR11 colocalizes with uPAR on the cell surface and inhibits the LDL receptor–related protein (LRP)-mediated binding and internalization of uPAR. Thus, LR11 mediates the uPAR localization to the plasma membrane. LR11 is highly expressed in the atheromatous plaque areas of apoE knockout mice, particularly in the intimal SMCs at the border between intima and media. The neutralization of LR11 function with anti-LR11 antibody reduced cuff-induced intimal thickness in mice. The novel mechanism of regulation of uPAR localization in SMCs accompanied with enhanced migration activity possibly constitutes an important factor in the process of atherosclerosis and arterial remodeling. (Circ Res. 2004;94:752-758.)

Key Words: atherosclerosis ■ smooth muscle cells ■ receptors ■ urokinase-type plasminogen activator receptor ■ LDL receptor–related protein

Receptors belonging to the family of LDL receptor relatives (LRs) play key roles in the catabolism of lipoproteins and of complexes between proteases and their receptors.1,2 Histocenial studies have revealed that LRs are markedly induced during formation of atherosclerotic lesions.3,4 Certain LRs are expressed in the arterial wall, implying possible roles in the progression of atherosclerotic plaques. For instance, LDL receptor–related protein (LRP) has been demonstrated in lesion macrophages and vascular smooth muscle cells (SMCs),5,6 the very low-density lipoprotein receptor (VLDLR/LR8) in endothelial cells, macrophages, and SMCs; in contrast, the LDL receptor is not abundant in arterial walls.6 However, so far the full physiological significance of LR expression in the arterial wall has not been fully elucidated.

Recent functional studies using receptor-deficient or -overexpressing animals and cells have suggested that certain LRs are important regulators of the normal migration of neurons and of the migration (and proliferation) of various cells, such as fibroblasts, neurons, and SMCs.7-14 In murine embryonic fibroblasts and fibrosarcoma cells, loss of LRP expression is associated with increased cell surface urokinase-type plasminogen activator receptor (uPAR) and with increased cell migration in vitro.7,8 Similar changes were reported to occur when VLDLR/LR8 activity was neutralized in cultured breast cancer cells.9 LR-mediated regulation of migration appears to be caused by modulation of the uPAR/ uPA/plasminogen system for degradation of extracellular matrix and/or of uPAR-mediated intracellular signaling through activation of extracellular signal-regulated kinase (ERK) and Rac1.13 Furthermore, LRP causes suppression of platelet-derived growth factor (PDGF)–mediated SMC migration in vitro.14 Smooth muscle cell–specific LRP inactivation in mice shows that LRP forms a complex with the PDGF receptor, and results in marked susceptibility of cholesterol-induced atherosclerosis in mice.14

An unusually complex and highly conserved LR with, as of yet, poorly defined function, LR11, has been discovered and molecularly characterized by us and others.15,16 In two experimental models of atherogenesis, we observed significant induction of LR11 in SMCs during intimal thickening.17 Moreover, overexpression of LR11 in SMCs enhances their migration via elevated levels of uPAR, and appears to thereby increase the activation of the uPA system.11
In this article, we studied the mechanism by which LR11 elevates the levels of uPAR in SMCs, which leads to their enhanced migration in vitro. As evaluated by biochemical and immunohistochemical methods, LR11 causes uPAR localization to the plasma membrane, because both the membrane-spanning and the secreted soluble forms of LR11 bind to and colocalize with uPAR on the cell surface. LR11 is highly expressed in the intimal SMCs at the border between intima and media in the plaque area of apoE knockout mice. We propose that the novel regulatory mechanism of SMC migration mediated by the interaction among LR1s constitutes an important factor in the process of atherosclerosis and arterial modeling.

Materials and Methods

Antibodies, Cells, and Plasmids

A monoclonal antibody against human LR11, 5-4-30-19-2, was described previously.18 Rabbit polyclonal antibodies against human and mouse LR11, ph23 and pm11, were produced by immunizing rabbits with the recombinant GST-LR11 fusion protein containing residues 287 to 679 of human LR1119 and the synthetic peptide GIVQCRGSDEDAFAACOS (residues 1298 to 1316 of murine LR11),20 respectively. IMR32 and COS-7 cells were obtained from the ATCC. The recombinant expression plasmid pBKCMVLR11, carrying a fragment encoding the entire protein coding sequence of the rabbit LR11 cDNA in the vector pBKCMV, was generated as described.11 COS-7 cells were transfected with pBKCMVLR11 or pBKCMV using a proprietary nonliposomal lipid (Efectana, Fecteana, Gigien). The serum-free medium collected for 12 or 4 hours before the indicated time was used for immunoblot analysis. The LR11-overexpressing ldl-A7 cells (an LDLR-deficient CHO cell line), KT38, and its control KT2, were established as described.21 Primary cultures of SMCs were prepared from rabbit aortas as described17 and used at passages 3 and 4.

Immunoblotting and Immunoprecipitation

Cultured cells were washed three times with phosphate-buffered saline (PBS) and harvested in PBS containing 0.5 mM/L phenylmethane-sulfonyl fluoride (PMSF) and 2.5 μmol/L leupeptin. Pellet and supernatant after ultracentrifugation at 100 000g for 1 hour were used as membrane and cytosol fractions, respectively. The pellet was resuspended in solubilization buffer (200 μmol/L Tris-maleate, pH 6.5, 2 μmol/L CaCl2, 0.5 μmol/L PMFS, 2.5 μmol/L leupeptin and 1% Triton X-100) as previously described.15 The supernatant was concentrated 5-fold using Centricon-100 (Millipore). Conditioned media were concentrated 20-fold using Centricon-100, or purified using a RAP-GST affinity column.11 For immunoblotting, equal amounts of membrane protein or conditioned media were subjected to SDS-PAGE under reducing or nonreducing conditions.18 The immunoreactive signals were detected by anti-human LR11 monoclonal antibody (5-4-30-19-2), goat polyclonal antibody against mouse uPAR (M-17, Santa Cruz Biotechnology, Santa Cruz), or mouse monoclonal antibody against human LR5 (5A6, Research Diagnostics), followed by peroxidase-conjugated anti-mouse or anti-goat IgG. For immunoprecipitation, 100 μg of membrane protein or of the protein in concentrated medium was mixed with 1 μg recombinant human uPAR (R&D systems) at 4°C for 3 hours in the presence or absence of α-uPA/PAI-1 complex (0.5 mM/L, American Diagnostica), apoE (5 or 50 μg, Cosmo Bio), or RAP (10 μg). Then 3 μg of anti-human uPAR goat IgG (AF807, R&D systems) or anti-LR11 antibody (ph23) was added, and rotated overnight at 4°C. The LR11:uPAR-antibody complex was precipitated by protein A Sepharose. The proteins were released into 25 μL SDS sample buffer by heating to 95°C for 10 minutes. For immunodetection, ph23 and AF807 was used followed by peroxidase-conjugated anti-rabbit and anti-goat IgG, respectively. Development was performed with the ECL detection reagents (Amersham Pharmacia). The signals were quantified by densitometric scanning using NIH Image software.

Migration and Invasion

Cell migration and invasion were measured in a 96-well micro-Bouyden chamber (its surface was coated with type I collagen) and Transwell (Corning Incorporated) 24-well plates coated with collagen I, respectively, as described.21 The lower or outer chamber contained 1%-FBS-DMEM with or without 5 ng/mL PDGF-BB (R&D systems). After incubation for 4 hours or the indicated times at 37°C in the presence or absence of conditioned media of KT38 or KT2 cells, the cells on the upper surfaces were washed, fixed, and stained by Diff-Quik (International Reagents). The number of cells that migrated to the lower surface of the filters was determined microscopically by counting.

Immunofluorescence

Cells were grown to 90% confluence on Laboratory-Tek chamber slides (Nune Inc.). The cells were chilled on ice for 1 hour, washed three times with PBS, and then incubated with or without 6 μg/mL recombinant uPAR in binding buffer (2% BSA, 20 mM/L HEPES in HAM F-12 medium) at 4°C for 2 hours. Unbound uPAR was removed, cells were fixed in 4% paraformaldehyde in PBS at 4°C for 15 minutes, and quenched in PBS containing 0.1% BSA and 10 mM/L glycine for 30 minutes as described.21 For intracellular analysis, cells were permeabilized with 0.05% Triton X-100 in PBS at 4°C for 3 minutes. Cells were then incubated with primary antibody (ph23, M-17, or 5A6) at 4°C overnight, followed by incubation with Alexa 594-conjugated IgG for 1 hour. Cells were washed with PBS three times, followed by incubation with Alexa 488-conjugated IgG for 1 hour. Slides were examined with a Zeiss LSM410 (Carl Zeiss) confocal laser scanning microscope using a C-Apochromat 63×, 100× with Zeiss immersion oil. The 594-nm line of the helium-neon laser or the 488-nm line of the argon laser was used for excitation of Alexa 594 and Alexa 488, respectively. Each fluorophore was scanned independently, and saved as a 1024×1024-pixel image at 8-bit resolution before merging of channels and import into Adobe Photoshop (Adobe Systems) for compilation and direct printing.

Ligand Blot Analysis and Binding and Internalization Assays

Human recombinant uPAR was iodinated using chloramine T (specific activities 6000 to 8000 cpm/ng). Ligand blot analysis using 125I-labeled ligands was performed under nonreducing conditions as described.21 For the cell surface binding assay, cells were grown to confluence in 24-well plates. The cell monolayers were prechilled on ice for 1 hour, washed with binding buffer twice, followed by incubation with 125I-labeled uPAR. The cells were incubated for 1 hour at 4°C (binding) or for 30 minutes at 37°C (cell binding and internalization). To measure internalized ligand, the cells were washed twice with binding buffer and then incubated with 50 mM/L glycine, 150 mM/L NaCl (pH 3.0) at 4°C for 15 minutes to dissociate cell surface-bound ligands following incubation for 30 minutes at 37°C. The cells were dissolved by adding 0.1N NaOH for 1 hour, and the extract was counted. For the analysis of secreted LR11 activity, KT2 cells were incubated in the presence or absence of concentrated conditioned media of KT38 cells at 1:10 dilution.

Immunohistochemistry

Serial paraffin-embedded sections (10 μm) were used for immunohisto- staining as described.17 Deparaffinized sections were pretreated with 3% H2O2 to inactivate endogenous peroxidase. Slides were stained in the presence of 0.1% BSA with anti-LR11 polyclonal antibody (ph23) at 1:50 dilution, or anti-smooth muscle actin HRP (Yamasa) at 23°C for 1 hour. Biotinylated anti-rabbit and goat IgG secondary antibodies (Santa Cruz) were diluted 1:100. The slides were counterstained with hematoxylin. Controls with nonimmune rabbit IgG were conducted in parallel with each immunohassay procedure.

Animal Experiments

Male C57BL/6J mice (Takasugi, Kasukabe, Japan), aged 20 weeks, were anesthetized, and the left femoral artery was detached from surrounding tissues, loosely sheathed with a nonconstrictive poly-
ethylene cuff made of PE90 tubing (length, 2 mm; inner diameter, 0.86 mm; outer diameter, 1.27 mm; Becton Dickinson) and tied in place with a 7-0 suture. The mice were injected intraperitoneally with 100\(\mu\)L anti-LR11 polyclonal antibody (ph23) at 1:10 dilution or PBS (control) each 2 to 3 days after cuff treatment. Twenty-five days after cuff placement, the left femoral artery was fixed in 10\% formalin and embedded in paraffin. Paraffin sections (2\(\mu\)m) were stained with hematoxylin-eosin and elastica van Gieson. Image analysis software, WinROOF (Mitani Co), was used to measure the areas of the intima and media.

Statistics
The results are shown as mean±SD for each index. Comparison of data was performed using the Student’s t test. A value of P<0.05 was considered significant.

Results

LR11 Is Secreted From Cultured SMCs During Proliferation
COS-7 cells transfected with LR11 cDNA generated the membrane-anchored as well as the secreted forms of LR11; the latter was recovered in the culture medium (Figure 1A). Analysis of the time course of LR11 production and secretion showed that the amount of soluble form increased for 24 hours after transfection, whereas that of the membrane-anchored form peaked at 12 hours after transfection (Figure 1B). The soluble form was clearly detected in the medium of rabbit SMCs, as well as of human IMR32 cells\(^{22}\); there was no difference in the sizes of the immunoreactive proteins between the two species (Figure 1C). The smaller size of the secreted protein compared with that of the membrane form is consistent with previous observations.\(^{23,24}\) Temporal analysis of the formation of the secreted form by SMCs revealed that the highest levels were produced 3 to 6 days after serum addition (Figure 1D). These kinetics suggest that LR11 first localizes to the plasma membrane, from where the extracellular domain is released into the medium, and secretion is induced in SMCs during the rapidly proliferating phase.

Soluble Form of LR11 Induces Migration in SMCs
We have shown that LR11 overexpression enhances activities required for migration and invasion of SMCs in vitro.\(^{11}\) In order to test whether the secreted form of LR11 might mediate these activities, the effects of conditioned medium of KT38 cells,\(^{18}\) CHO cells stably expressing human LR11, on SMCs were analyzed. The conditioned medium caused a 3.2-fold increase in SMC migration in the presence of PDGF, compared with the medium from mock-transfected cells (KT2; Figure 2A). Importantly, secreted LR11 protein could be isolated from the conditioned medium of KT38 cells, but not of KT2 cells, by affinity purification using RAP-GST (Figure 2A, inset). Furthermore, the medium of KT38 cells, but not of KT2 cells, stimulated the invasion activity of SMCs in time-dependent fashion (Figure 2B). Thus, increased migration and invasion activities of SMCs appear to be correlated with the presence of secreted LR11 in conditioned media of LR11-expressing cells.

![Figure 1](image1.png)

**Figure 1.** Immunodetection of secreted soluble form of LR11. Membrane extracts and 12 hour-conditioned media (A), and the membrane extracts and media collected for 4 hours before the indicated times (B) after transfection of pBKCMVR11 into COS-7 cells, membrane extracts, and media of IMR32 and rabbit SMCs (C), and the media of SMCs collected for 24 hours before the indicated days (D) were subjected to immunoblot analysis using 5-4-30-19-2. In D, Relative amounts of the signals were determined by densitometric scanning as described in Materials and Methods. Amounts of LR11 immunoreactive protein were normalized relative to the cell numbers in triplicate wells. Open circles, cell numbers; open squares, signal intensity/cell (\(\times 10^3\)).

![Figure 2](image2.png)

**Figure 2.** Effect of secreted soluble LR11 on migration and invasion activities of smooth muscle cells. A, Stimulation of migration activities of SMCs by PDGF-BB for 4 hours in the presence or absence of conditioned medium of KT38 cells,\(^{18}\) CHO cells stably expressing human LR11, on SMCs were analyzed. The conditioned medium caused a 3.2-fold increase in SMC migration in the presence of PDGF, compared with the medium from mock-transfected cells (KT2; Figure 2A). Importantly, secreted LR11 protein could be isolated from the conditioned medium of KT38 cells, but not of KT2 cells, by affinity purification using RAP-GST (Figure 2A, inset). Furthermore, the medium of KT38 cells, but not of KT2 cells, stimulated the invasion activity of SMCs in time-dependent fashion (Figure 2B). Thus, increased migration and invasion activities of SMCs appear to be correlated with the presence of secreted LR11 in conditioned media of LR11-expressing cells.
Whereas the specific surface binding of $^{125}$I-uPAR to KT38 cells showed high affinity and saturability in a dose-dependent manner. We next investigated the ability of LR11 to bind and internalize $^{125}$I-uPAR on the cell surface. We therefore tested how LR11 might cause the decreased cellular uPAR levels, as shown in Figure 6A. Ligand blot analysis with $^{125}$I-uPAR using KT38 cells showed that addition of the secreted form of LR11 (open circles) incubated with the indicated concentrations of $^{125}$I-uPAR. Data are the average of duplicate determinations and represent the difference between activities in the presence or absence of 1 nmol/L of unlabeled uPAR.

### Both Secreted Soluble and Membrane-Anchored LR11 Interact With uPAR
Enhanced migration and invasion of LR11-overexpressing SMCs have been suggested to be mediated by the upregulation of LR11. Therefore, we investigated whether LR11 might cause the decreased cellular uPAR levels, by using Western blot analysis and immunoprecipitation experiments. Binding and immunoprecipitation with anti-uPAR antibody showed that neither the level nor the localization of uPAR were different in KT2 cells in comparison to KT38 cells. Immunofluorescence showed that LR11 localized to the cell membrane and was colocalized with uPAR on the cell surface of KT38 cells, but not KT2 cells (Figure 5B). In agreement with the results of Figure 5A, uPAR staining in the cytoplasm was clearly less in KT38 cells than in KT2 cells. In fact, anti-uPAR antibody stained the molecule in the cytoplasm of the perinuclear region in the KT2 cells, where it was not colocalized with LR11. These imaging data are in good agreement with the biochemical results of Figure 4. Taken together, these data provide evidence for our notion that LR11 binds uPAR on the cell surface and thereby plays a protective role against internalization of the uPA receptor into the cell.

#### Different Effects of LR11 on uPAR Binding and Internalization
We next investigated the ability of LR11 to bind and internalize uPAR, using the KT11-overexpressing cells (Figure 4). Both cell-surface binding and internalization of $^{125}$I-uPAR showed high affinity and saturability in a dose-dependent manner. Whereas the specific surface binding of $^{125}$I-uPAR to KT38 cells was 3.1-fold higher than to KT2 cells at 150 nmol/L ligand, the internalization activity of KT38 cells was 35% lower than that of KT2 cells. This strongly suggests that LR11 has significantly different effects on uPAR binding and internalization, respectively, ie, enhancing the former, but inhibiting the latter.

### LR11 Increases the Extent of uPAR Localization to the Membrane Fraction
In order to elucidate the mechanism for the different effects on uPAR binding and internalization of LR11, we next analyzed the distribution of uPAR between membrane and cytosolic fractions. Figure 5A showed that uPAR protein in KT38 cells is predominantly localized to the membrane (as is LR11), whereas it is evenly distributed between the two fractions in KT2 cells. We further investigated by immunofluorescence the localization of LR11 and uPAR on the cell surface using LR11-overexpressing cells. Analysis by confocal microscopy showed that LR11 colocalized with uPAR on the cell surface of KT38 cells, but not KT2 cells (Figure 5B). In agreement with the results of Figure 5A, uPAR staining in the cytoplasm was clearly less in KT38 cells than in KT2 cells. In fact, anti-uPAR antibody stained the molecule in the cytoplasm of the perinuclear region in the KT2 cells, where it was not colocalized with LR11. These imaging data are in good agreement with the biochemical results of Figure 4. Taken together, these data provide evidence for our notion that LR11 binds uPAR on the cell surface and thereby plays a protective role against internalization of the uPA receptor into the cell.

#### uPAR Becomes Predominantly Localized to the Cell Surface via Inhibition of Its Binding to LRP by LR11
uPAR degradation is dependent on its internalization via LRP, a member of the LDLR family. Immunochemistry analysis showed that neither the level nor the localization of LRP were different in KT2 cells in comparison to KT38 cells (Figure 6A). Ligand blot analysis with $^{125}$I-uPAR using KT38 cells showed that addition of the secreted form of LR11 inhibited the binding of uPAR to LRP as well as to LR11 itself (Figure 6B). Addition of conditioned medium of KT38 cells reduced the surface binding of $^{125}$I-uPAR to KT2 cells by 76%, and $^{125}$I-uPAR internalization by 72% (Figure 7). These results show that the secreted form of LR11 inhibits the LRP-mediated binding and internalization of uPAR. Consistent with the inhibitory effects of LR11 on uPAR-LRP
interaction, the extent of localization of uPAR on the cytoplasm of KT2 cells, after incubation with exogenous recombinant uPAR, was drastically decreased in the presence of conditioned medium of KT38 cells (Figure 6C).

**LR11 Is Highly Expressed in SMCs Localized in Plaques, and Anti-LR11 Antibody Reduces the Intimal Thickening in Cuff-Injured Model**

LR11 is highly expressed in SMCs in aortic plaques from rabbits fed a high-cholesterol diet. Figure 8A shows that LR11 is highly expressed in the plaque area of apoE-knockout mice, particularly in the intimal SMCs at the border between intima and media. Significantly, staining for α-actin was highest in the cells showing low staining for LR11, in agreement with the finding that cultured SMCs from the intimal layer show increased migration activity compared with those from the medial layer. These results suggest that the functional neutralization of LR11 reduces the intimal thickening in vivo. We therefore analyzed the effect of anti-LR11 antibody on the intimal thickness caused by the migration and proliferation of SMCs in cuff-injured mouse model. The treatment of cuff-injured mice with anti-LR11 antibody for 25 days indicated the inhibition of intimal thickening by 49% compared with control injured mice. Histological analysis showed that the number of intimal SMCs decreased obviously in the antibody-treated mice. These results suggest that LR11, which is expressed in the intimal SMCs, is important in the process of the migration and proliferation of SMCs in vivo.

**Figure 5.** Enhanced localization of uPAR to the cell surface in LR11-overexpressing cells. A, Membrane and cytosol fractions of KT2 or KT38 cells were subjected to immunoblot analysis for the presence of LR11 or uPAR using the antibodies ph23 or M-17, respectively. *P<0.05; n=4. B, Surface and intracellular localization of LR11 and uPAR was analyzed in KT38 and KT2 cells by immunofluorescence using the anti-LR11 and uPAR antibodies, ph23 and M-17, respectively.

**Figure 6.** Inhibition of uPAR binding to LRP by membrane-anchored and secreted soluble forms of LR11. A, Surface localization of LRP was analyzed in KT2 and KT38 cells by immunofluorescence with anti-LRP antibody 5A6. B, Membrane extracts from KT38 cells were subjected to ligand blot analysis using 125I-uPAR in the presence (lane 4) or absence (lane 3) of conditioned medium of KT38 cells. Positions of LR11 and LRP were determined by comparison of immunoreactive signals (lanes 1 and 2, nonreducing condition) using the anti-LR11 and LRP antibodies ph23 and 5A6, respectively. C, Cytoplasmic localization of uPAR (red) and LRP (green) was analyzed in KT2 cells by immunofluorescence following incubation with 1 μg/mL recombinant uPAR in the presence or absence of conditioned media of KT38 cells using anti-LRP and anti-uPAR antibodies (5A6 and AF807, respectively).

**Figure 7.** Surface binding (A), binding and internalization (B), and internalization (C) activities of KT2 cells with the indicated concentrations of 125I-uPAR in the presence (open circles) or absence (filled circles) of conditioned media of KT38 cells. Data are the average of duplicate determinations and represent the difference between activities in the presence or absence of 1 mmol/L of unlabeled uPAR.
Discussion

In this study, we have proposed a novel LR11-mediated mechanism for uPAR localization in the plasma membrane. Both the membrane-spanning and the secreted soluble forms of LR11 bind to and colocalize with uPAR on the cell surface; this de-facto immobilization effectively stabilizes the receptor-protease complex by inhibiting its degradation via LRP. We propose that the resulting enhanced uPA-mediated cell migration constitutes an important factor in the process of atherosclerosis and arterial modeling.

LR11, a membrane-spanning protein, is highly expressed in proliferating cultured cells, including SMCs, IMR32, and PC12 cells. The extracellular domains of LR11 protein and of a homologue in hydra are facultatively shed from the cell surface membrane as soluble secreted forms. Of particular relevance is the high level of LR11 expression in SMCs in intimal layers of atheromatous lesions, whereas in medial layers, the receptor was not detectable. This study further clarified that LR11 is highly expressed in the plaque area, particularly in the intimal SMCs at the border between intima and media in plaques of apoE-knockout mice. These LR11-expressing cells seem to be rather active in their migration and proliferation. The experiments in cultured cells showed that LR11 first localizes to the plasma membrane in SMCs, from where the extracellular domain is released into the medium, and that the secretion is induced during the rapidly proliferating phase. Neutralization of LR11 function in vivo reduced the intimal thickness in cuff-injured mouse model, although the further minute analyses are needed to know the specific function of LR11 on the inhibition of migration of SMCs in vivo.

LR11-overexpressing cells showed upregulation of uPAR expression and increased activities of migration and invasion, and anti-uPA and anti-uPAR antibodies inhibited these increases. The catabolism of uPAR was delayed in these cells. The increased migration and invasion activities of SMCs appear to be correlated with the presence of secreted LR11 in conditioned media of LR11-expressing cells. The binding and coimmuno-precipitation experiments show that both the cell-anchored and secreted forms of LR11 have the capacity to bind to and to form complexes with uPAR. Further functional analysis revealed that, to our surprise, LR11 has different effects on uPAR binding and internalization, respectively, ie, enhancing the former, but inhibiting the latter. These data suggested to us that LR11 binds uPAR on the cell surface, and thereby decreases the internalization of uPAR into the cell. In fact, the secreted form of LR11 inhibits the LRP-mediated binding and internalization of uPAR, as shown by biochemical and immunohistochemical analysis. uPAR, a glycosyl-phosphatidyl-inositol (GPI)-anchored membrane protein, mediates cell migration and invasion by activation of the uPA:PAI-1 system and/or by interaction with vitronectin/integrin on the cell surface. This implies that the synthesis of uPAR, its localization on the cell surface, and its degradation are important parameters influencing the activation of these systems. It has been recently demonstrated that uPAR internalization is a consequence of its direct binding to LRP. In summary, we suggest a novel mechanism to explain the observed LR11-mediated regulation of uPAR degradation, which in turn may exert control over SMC migration. SMCs in plaques produce LR11 localized both on the cell surface and secreted into the surrounding areas of the cells. The LR11 forms bind to and interact with the uPAR on the surface of the same and/or neighboring cells. This complex formation inhibits the internalization of uPAR via LRP, and thereby its degradation and relocation, resulting in the enhanced localization of uPAR on the cell surface. Finally, SMCs expressing LR11 cause the increased migration mediated by activation of the uPA:PAI-1 system and/or by interaction with vitronectin/integrin on the cell surface. The modulation of such a signaling pathway might have an impact on the regulation of cell migration, as well as on the degradation of extracellular matrices by the activation of proteinases via the uPA:PAI-1 system. This possibility is supported by our previous results that aprotinin, a plasmin inhibitor, significantly inhibited the increased invasion activity in LR11-overexpressing cells; however, the inhibition was only half as much as in the presence of anti LR11 antibody or uPAR antibody.

Enhanced expression of LRP and other LRs is observed in atherosclerotic lesions. These receptors may influence the cellular catabolism of uPAR in competitive or cooperative manner. An example for cooperation of LRs, albeit along a different line, is provided by VLDL receptor and ApoE.
receptor-type2. Also, a secreted soluble form of ApoE receptor-type2 can act as a dominant-negative receptor by inhibiting Reelin signaling. Furthermore, dramatically altered levels of LRs, as well as of uPAR, are observed in cells whose migration activity is abnormally regulated, such as malignant cells. One of the family members, LR1P1B, has actually been identified as a tumor suppressor and is frequently inactivated in lung cancer cells. A recent study using LRP-deficient mice revealed that LRP has a pivotal role in protecting vascular wall integrity and preventing atherosclerosis by controlling PDGF receptor–dependent signaling pathways and other mechanisms that increase SMC proliferation and migration. As revealed by the present study, uPAR-mediated SMC migration is likely regulated by LRs, in particular LR11 and LRP. The disturbance of this regulatory system may lead to the processes of atherosclerosis, neural diseases, and cancer invasion.

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References

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