The low density lipoprotein receptor-related protein-deleted in tumor (LRP1B, initially referred to as LRP-DIT) was cloned and characterized as a candidate tumor suppressor. It is a new member of the low density lipoprotein receptor gene family. Its overall domain structure and large size (~600 kDa) are similar to LRP and suggest that it is a multifunctional cell surface receptor. Herein, we characterize a series of ligands for the receptor using cell lines that stably express it as a domain IV minireceptor (mLRP1B). Ligands of LRP including receptor-associated protein, urokinase plasminogen activator, tissue-type plasminogen activator, and plasminogen activator inhibitor type-1 each demonstrate binding, internalization, and degradation via mLRP1B. Interestingly, the kinetics of ligand endocytosis is distinctly different from that of LRP, with LRP1B exhibiting a markedly diminished internalization rate. In addition, tissue expression analysis reveals that the LRP1B gene is expressed in brain, thyroid, and salivary gland. These studies thus extend the physiological roles of members of the LDL receptor family.

Members of the low density lipoprotein receptor gene family play a wide variety of roles in normal cell function and development. For example, mutations of the low density lipoprotein receptor gene, the prototypic family member, result in the genetic disease familial hypercholesterolemia (1). Much attention has focused on other members of this gene family (including LRP, megalin, apolipoprotein E receptor-2, very low density lipoprotein receptor) because of their recently recognized roles in development, cell signaling, and pathogenesis (2, 3).

The low density lipoprotein receptor-related protein LRP1B (initially referred to as LRP-DIT (deleted in tumor)), a candidate tumor suppressor, is a new member of the giant receptor subgroup of this gene family. It is located at chromosome 2q21.2 and was isolated by positional cloning based on homozygous deletions detected in human cancer cell lines (4–6). Mutation analysis revealed that the gene is frequently (45%) inactivated in human non-small cell lung cancer cell lines by intragenic homozygous deletions, point mutations, and aberrant transcripts missing internal portions. Loss of heterozygosity analysis also detected high allelic loss within the locus using two independent microsatellite polymorphism markers, suggesting that the gene is a candidate tumor suppressor (4).

The low density lipoprotein receptor gene family previously contained two very large members, LRP (LRP1), a dimer of 515 and 85 kDa, and its closely related homolog, megalin (LRP2), a single species of ~600 kDa (2, 3). LRP1B is more closely related to LRP (with similarity of 59 and 52% identity at the cDNA and predicted amino acid levels, respectively) than to LRP2 (4). The cDNA of LRP1B is 16.5 kilobases, with an open reading frame of 13,797 base pairs, which encodes a protein of 4599 amino acids. Characterization of exon-intron boundaries determined that the genomic DNA of LRP1B contains 91 exons, 89 of which are nearly identical in size and location to those of the 89 exons in the LRP gene (4, 5). The two extra exons in LRP1B, exon 68 and exon 90, have no counterparts in LRP. However, the average size of the introns in LRP1B is at least 10 times larger than that of LRP (5). Protein domain structure comparison of the two genes reveals that the overall domain structural organization of the two proteins is almost identical to one another, except for the fragments encoded by the two extra exons in LRP1B (4; see Fig. 1). Similar to LRP, LRP1B protein has four putative ligand-binding domains (I, II, III, and IV from the amino terminus) that consist of 2, 8, 10, and 12 cysteine-rich ligand-binding repeats, respectively. As in LRP, these domain clusters are separated from one another by three clusters of epidermal growth factor precursor repeats (F/Y)WXD spacer repeats. The number and arrangement of the two types of repeats are the same as those found in LRP, except for the one additional ligand-binding repeat in domain IV, which is encoded by exon 68. LRP1B also contains a putative furin endopeptidase processing site (REKR) at positions 3954–3957 (4), similar to that in LRP (7, 8). This post-translationally processing event results in the formation of mature LRP as a noncovalently associated heterodimer, consisting of an extra-
LRP1B Binds RAP, uPA, tPA, and PAI-1

Cellular 515-kDa subunit and a transmembrane 85-kDa subunit (7, 8). The transmembrane domain of LRPIB is similarly separated from domain IV and by a cassette of six epidermal growth factor-like precursor repeats. The cytoplasmic tail of LRPIB contains two NPXY motifs. Between these two is a unique insertion of 33 amino acid residues (contributed by exon 90) not present in LRP (4).

LRP is widely expressed and plays important roles in lipoprotein catabolism, blood coagulation, cell adhesion and migration, neuronal process outgrowth, and pathogenesis of Alzheimer’s disease (2, 3, 9). The LR gene is also essential for early embryonic development (10, 11). This diversity in biological processes is mediated by the interactions of LRP with multiple ligands, including proteases, proteinase-inhibitor complexes, and lipoprotein particles (9). LRP is also involved in signal transduction (12–14).

The close similarity in protein domain structure between LRPIB and LRP strongly suggests that the two receptors may share similarity in ligand interaction; i.e. many ligands for LRP may also be ligands for LRPIB. This notion is supported by the observation that LRP2 binds some ligands for LRP, such as receptor-associated protein (RAP) and urokinase plasminogen activator (uPA) complexed with plasminogen activator inhibitor type-1 (PAI-1), although their homology is not as high as that between LRP and LRPIB. Furthermore, some of the ligands, such as apolipoprotein E and RAP, bind most of the receptors of the gene family (9). RAP is a 39-kDa protein that binds to LRP and inhibits the binding of all other ligands of LRP (22). Physiologically, RAP serves as a folding chaperone for LRP during receptor biosynthesis (22). In this study we analyzed the biogenesis and endocytic functions of a minireceptor of LRPIB and demonstrated its ability to bind and endocytose several LR ligands including RAP, uPA, PAI-1, and tissue-type plasminogen activator (tPA).

EXPERIMENTAL PROCEDURES

Materials—Human recombinant RAP was expressed in a glutathione S-transferase expression system and isolated as described previously (23). scuPA was kindly provided by G. F. Vovis of Collaborative Research (24). uPA was from Genentech. PAI-1 was from American Diagnostica. All tissue culture media, serum, and plastic ware were from Life Technologies, Inc. Non-enzymatic cell dissociation solution was from Sigma. Monoclonal anti-HA antibody has been described before (25). Goat anti-mouse IgG FITC was from Becton Dickinson. Cy3-goat anti-mouse IgG was from Sigma. Quantum Simply Cellular micro bead standard was from Flow Cytometry Standards Corp. (San Juan, Puerto Rico). Peroxidase-labeled anti-mouse antibody and the ECL system were from Amersham Pharmacia Biotech. The Immobilon-P transfer membrane was from Millipore. Rainbow molecular weight markers were from Bio-Rad. Carrier-free Na125I was purchased from PerkinElmer Life Sciences.

DNA Dot Blot Hybridization—To examine the expression level of LRPIB in human tissues, Multiple Tissue Expression Array was purchased from CLONTECH and hybridized with 32P labeled cDNA probe following the manufacturer’s instructions. The cDNA fragment (612 base pairs covering 1435–2847) for hybridization was prepared by PCR amplification from a subcloned cDNA plasmid and labeled with [32P]dCTP and the MegaPrim DNA random labeling kit (Amersham Pharmacia Biotech), after which the free [32P]dCTP was removed as described.

Cell Culture and Transfection—The LRP-null Chinese hamster ovary (CHO) cell was cultured in Ham’s F-12 medium as described (26). Stable transfection into LRPIB-null CHO cells was achieved by transfection of 30 μg of plasmid DNA in 10-cm dishes via a calcium phosphate precipitation method (27). Stable transfection was performed according to previously published methods (29). Briefly, CHO cells were detached by incubation with non-enzymatic cell dissociation solution. Successful transfections were evaluated from the standardized Quantum Simply Cellular bead calibration plot as described.

Protein Iodination—RAP, scuPA, PAI-1, and tPA (50 μg each) were radioiodinated using the IODO-GEN method as described (30).

Kinetic Analysis of Endocytosis—Kinetic analysis of endocytosis was performed according to previously published methods (29). Stably transfected CHO cells were plated in 12-well plates at a density of 2 × 105 cells/well and used after overnight culture. Cells were rinsed twice in ice-cold ligand binding buffer (minimal Eagle’s medium containing 0.6% bovine serum albumin), and 125I-RAP was added at a 5 nM final concentration in cold ligand binding buffer (0.5 ml/well). The binding of 125I-RAP was carried out at 4 °C for 30 min with gentle rocking. Binding of 125I-RAP was specific, i.e. the addition of 100-fold excess unlabeled RAP inhibited binding by 90–95%. Unbound ligand was removed by washing cell surface with cold binding buffer three times with cold binding buffer. Ice-cold stop/treatment solution (0.2 M acetic acid, pH 2.6, 0.1 M NaCl) was added to one set of plates without warming and kept on ice. The remaining plates were then placed in a 37 °C water bath, and 0.5 ml of ligand binding buffer prewarmed to 37 °C was quickly added to the well monolayers to initiate internalization. After each time point, the plates were quickly placed on ice, and the ligand binding buffer was replaced with ice-cold stop/treatment solution. Ligand was then stripped by incubation of cell monolayers with ice-cold stop/treatment solution for a total of 20 min (0.75 ml for 10 min, twice) and counted. Cell monolayers were then solubilized with low SDS lysis buffer (62.5 mM Tris-HCl, pH 6.8, 0.2% SDS, and 10% (v/v) glycerol) and counted. The sum of ligand that was internalized plus that which remained on the cell surface after each assay was used as the maximum potential internalization. The fraction of internalized ligand after each time point was calculated and plotted.

Analyses of LRPIB Ligand Binding Activity and Ligand Degradation Efficiency—For ligand binding, cells (2 × 105) were seeded into 12-well dishes 1 day prior to assays. Assay buffer (minimal Eagle’s medium containing 0.6% bovine serum albumin with 5 mM radioligand; 0.6 ml/well) was added to cell monolayers in the absence or presence of unlabeled 500 nM RAP, followed by incubation for 1 h at 4 °C. Thereafter, overlying buffer containing unbound ligand was removed, and cell monolayers were washed, lysed in low SDS lysis buffer, and counted. Ligand degradation efficiency was measured using the methods described (29). Briefly, 2 × 105 cells were seeded into 12-well dishes 1 day prior to assays. Pre-warmed assay buffer was added to cell monolayers in the absence or presence of unlabeled 500 nM RAP, followed by incubation for 4 h at 37 °C. Thereafter, the medium overlying the cell monolayers was removed, and proteins were precipitated by addition of bovine serum albumin to 10 mg/ml and trichloroacetic acid to 20%. Degradation of radioligand was defined as the appearance of radioactive fragments in the overlying medium that were soluble in 20% trichloroacetic acid. The protein concentration of each cell lysate was reverse transcription-PCR. Briefly, total RNA was isolated from human mammary gland tissues using Trizol (Life Technologies, Inc.); first strand cDNA synthesis was performed using Life Technologies, Inc); and reverse transcription-PCR was used with Advantage cDNA amplification kit (CLONTECH). The PCR products were sequenced by the sequencing method (21). The combination of the following primers was used for the reverse transcription-PCR amplification of the cDNA fragment are mLRP1B4F (5’-CTCTGGATGATAAATAATGGTGGTTGC-3’) and mLRP1B4R (5’-CAAGGACGCTGATCTGAACCTTGAGACC-3’). The final construct was verified by DNA sequencing.

Pulse-and Western Blot Analyses of LRP and LRPIB Minireceptors—Pulse-and-chase analysis of mLRP4 or mLRP1B4 stably expressed in LRPIB-null CHO cells was performed essentially as described (25). For Western blotting, pcDNA3, mLRP4, or mLRP1B4 stably transfected LRPIB-null CHO cells were lysed with lysis buffer (phosphate-buffered saline containing 1% Triton X-100 and 1 mM ethylenediaminefluoride) at 4 °C for 30 min. Equal quantities of protein were subjected to SDS-polyacrylamide gel electrophoresis (6%) under reducing conditions. Following transfer to a polyvinylidene difluoride membrane, successive incubations with anti-HA antibody and horseradish peroxidase-conjugated goat anti-mouse IgG were carried out for 60 min at room temperature. The immunoreactive proteins were detected using the ECL system.

Flow Cytometric Analysis of Cell Surface LRP and LRPIB Minireceptors—Cell surface LRPIB minireceptor expression was determined as described (29). Human recombinant RAP was expressed in a glutathione S-transferase expression system and isolated as described previously (23). scwPA was kindly provided by G. F. Vovis of Collaborative Research (24). uPA was from Genentech. PAI-1 was from American Diagnostica. All tissue culture media, serum, and plastic ware were from Life Technologies, Inc. Non-enzymatic cell dissociation solution was from Sigma. Monoclonal anti-HA antibody has been described before (25).

Antibodies—Anti-mouse IgG was from Sigma. Quantum Simply Cellular micro bead calibration plot as described.
measured in parallel dishes that did not contain LRP ligands. The ligand degradation efficiency is the value of degraded ligand (cpm/mg of cell protein) divided by the number of cell surface LRP minireceptors per mg of cell protein (as determined by flow cytometry).

Immunofluorescence and Confocal Microscopy—Stably transfected CHO cells expressing various proteins were grown on glass coverslips, fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min, and permeabilized with 0.2% Triton X-100 for 10 min at room temperature. The cells were then incubated for 30 min with anti-HA antibody followed by a 30-min labeling with Cy3 goat anti-mouse IgG. Confocal microscopy was performed on a Bio-Rad MRC 1024 model using a Zeiss 363 (nominal aperture, 1.4) oil immersion lens.

RESULTS

Construction of LRP1B Minireceptor mLRP1B4—To study the biochemistry of LRP1B, we initially attempted to develop a cell line that stably expresses full-length LRP1B cDNA. However, this effort was unsuccessful due to apparent instability of the full-length LRP1B cDNA. Because minireceptors of several large cell surface receptors, such as LRP and insulin-like growth factor receptor II, have been used successfully for functional analyses (25, 27, 31, 32), especially of individual domains, we generated a minireceptor of LRP1B containing domain IV, the transmembrane domain, and the cytoplasmic tail (Fig. 1). This construct is similar to LRP minireceptors that we developed earlier to study receptor trafficking and endocytic function (25, 27, 32). The LRP1B minireceptor contains residues from amino acids 3276 to 4599 of the full-length LRP1B. This corresponds to the single cysteine-rich epidermal growth factor precursor repeat before the fourth cluster of ligand-binding repeats (domain IV) through the cytoplasmic carboxyl terminus of the receptor. This minireceptor is referred to as mLRP1B4, with “m” representing “membrane-containing minireceptor” and “4” representing “domain IV.” An HA epitope was inserted near the amino terminus to facilitate immunological detection of the minireceptor protein in transfected cells.
has been done with the LRP minireceptors. Thus, the structure of this minireceptor is very similar to that of LRP domain IV minireceptor (mLRP4), which undergoes similar trafficking with the full-length LRP (29, 32, 33). The subcloned DNA sequence was confirmed by DNA sequencing and in vitro translation before it was used for cell transfection. The LRP1B minireceptor includes the two inserted sequences (Fig. 1, C–F) that are not present within LRP and that represent the major structural differences between the two receptors. We have recently developed LRP-null CHO cells expressing mLRP4 and characterized the internalization and degradation of several LRP ligands, such as RAP, scuPA, tPA, and PAI-1 (32). Thus, the LRP1B minireceptor construct allows for direct comparative analysis with mLRP4.

**mLRP1B is Processed and Expressed on the Cell Surface**—To avoid the complexities inherent in examining cells expressing both LRP1B and LRP, we transfected LRP-null CHO cells (26) with mLRP1B4 minireceptor DNA to develop stable cell lines. Cell lines with stable expression of the minireceptor were initially identified via Western blotting with anti-HA antibody, further purified by subcloning, and confirmed by flow cytometry (Fig. 2). As seen in Fig. 2A, immunoblot analysis of stably transfected cells expressing mLRP4 reveals two distinct bands, an upper band of ~200 kDa (1257 residues) representing the full-length endoplasmic reticulum precursor form that lacks complex sugar modification and a smaller, ~120-kDa (662 residues) species representing the larger extracellular subunit of the mature form following furin processing (27, 29, 32). The 85-kDa small subunit containing the transmembrane domain and cytoplasmic tail is not detected in the immunoblot because the HA epitope is attached near the amino terminus of the minireceptor. As shown in the same immunoblot (Fig. 2A), two bands of similar mobility are seen in extracts from the mLRP1B4-expressing cells. Here, the upper band is ~210 kDa (1341 residues), a bit larger than that seen for the non-processed endoplasmic reticulum form of mLRP4; the lower band is ~120 kDa, similar to that of the larger subunit following furin processing of mLRP4 (27, 29, 32). These two species of mLRP1B4 are precisely those expected following furin cleavage at the predicted recognition sequence REKR (residues 3954–3957). Flow cytometric analysis of non-permeabilized cells demonstrated abundant mLRP1B4 expression at the cell surface, as seen in Fig. 2B, similar to that observed for mLRP4-expressing cells (Fig. 2B).
cell surface expression of mLRP1B was further confirmed using both confocal immunofluorescent analyses and cell surface ligand binding assays (see below).

To compare the kinetics of biosynthesis and processing of mLRP4 and mLRP1B4, we performed pulse-chase analysis with cells stably expressing these minireceptors. Thus, these cells were metabolically labeled with [35S]cysteine for 30 min and chased for 0, 30, 60, or 120 min. After each chase, cell lysates were immunoprecipitated with anti-HA antibody and analyzed via SDS-polyacrylamide gel electrophoresis. As seen in Fig. 3, at the beginning of the chase, both mLRP4 and mLRP1B4 were seen as single polypeptide precursor forms. Following chase, two new bands are seen that probably represent furin-cleaved processed forms of these minireceptors. The 120-kDa forms are identical to those seen from Western blot analysis (see Fig. 2) and represent the ligand-binding region of these minireceptors. The 85-kDa bands represent the transmembrane domain and are coimmunoprecipitated with the 120-kDa subunits following anti-HA immunoprecipitation. Thus, these results together confirm that LRP1B is probably processed by furin with kinetics similar to those of LRP.

LRP1B Binds, Internalizes, and Degrades RAP—RAP, the 39-kDa intracellular molecular chaperone for LRP family members, was examined for its ability to bind to mLRP1B4. RAP binds domain IV of LRP with high affinity (Kd > 2 nM) and is efficiently internalized and degraded via the LRP minireceptor mLRP4 (25, 27–29). Thus, we compared the binding, internalization, and degradation of 125I-RAP with CHO cells expressing mLRP1B4, mLRP4, or neither (i.e. pcDNA 3.1 vector alone). As seen in Fig. 4, 125I-RAP binding, internalization, and degradation were seen in cells expressing mLRP4 or mLRP1B4. The approximately 3-fold increase in specific 125I-RAP binding in mLRP1B4 cells compared with mLRP4 cells is consistent with the amount of the receptor species present at the cell surface (see Fig. 2). However, the rate of 125I-RAP uptake and degradation (see Fig. 4B) was similar for cells expressing mLRP1B4 and mLRP4. To determine whether this was a result of different affinities of RAP for the two receptors, we assessed the saturation binding of 125I-RAP to mLRP1B4. As seen in Fig. 4, C and D, the Kd of ~3.3 nM is similar to that seen for mLRP4 (33). Thus, we next examined the internalization/endocytic rates for 125I-RAP in mLRP1B4- and mLRP4-expressing cells.

As seen in Fig. 5, the rate and extent of 125I-RAP internalization is more rapid and more complete in cells expressing mLRP4. This observation is consistent with the greater cell surface receptor expression and cell surface 125I-RAP binding by mLRP1B4, yet similar 125I-RAP uptake and degradation kinetics by mLRP4 and mLRP1B4 (Figs. 2 and 4).

To examine whether a slower endocytosis rate of mLRP1B results in a relative cell surface accumulation of this receptor, we performed immunofluorescent staining of mLRP4 and mLRP1B4 stably transfected CHO cells with or without cell permeabilization, using anti-HA antibody and analyzed via laser-scanning confocal microscopy. As seen in Fig. 6, permeabilized mLRP4 cells exhibited abundant staining of both cell surface and intracellular organelles (i.e. endosomes), whereas mLRP1B4 cells predominantly demonstrated cell surface staining, with little intracellular staining. These results are consistent with a slower endocytosis rate mediated by mLRP1B and suggest a prolonged residence time for this receptor at the cell surface.

mLRP1B4 Degrades scuPA, PAI-1, and tPA—Domain IV of
LRP represents one of the two major ligand-binding regions of LRP (32, 33). Indeed, several ligands can bind to either domain IV or domain II (34). Thus, we examined the interactions of mLRP1B4 with several ligands that interact with mLRP4. As seen in Fig. 7, scuPA, tPA, and PAI-1 are each avidly taken up and degraded via mLRP1B4-expressing cells. The rates of uptake and degradation, however, vary. scuPA and tPA have similar rates in both cell lines, whereas PAI-1 degradation appears to be greater in mLRP1B4-expressing cells. This greater ability of LRP1B4 in degrading PAI-1 may reflect a high affinity of this minireceptor to PAI-1 when compared with that of mLRP4. However, because the overall affinities of PAI-1 to these minireceptors are very low, kinetic analyses of ligand-receptor affinity (via saturation binding) are not conclusive with this ligand (data not shown). We believe that such experiments in comparing ligand binding affinities and degradation efficiency by LRP and LRP1B are important and should be performed in the future when a cell line expressing either LRP1B naturally or the full-length LRP1B cDNA becomes available.

**DISCUSSION**

Based on its predicted amino acid sequence, LRP1B is a new member of the low density lipoprotein receptor gene family with a size (~600 kDa) similar to that of LRP and megalin. LRP1B is more closely related to LRP than to megalin and LRP2 both at the amino acid sequence level and in its domain structural organization (4, 5; Fig. 1). These structural similarities suggest that the ligands of the receptors as well as the function of the receptors may well overlap. Our studies have utilized minireceptors of LRP1B and LRP. We have shown that mLRP1B4 is abundantly expressed at the cell surface and is probably processed by the endoprotease furin within the trans-Golgi compartments. At the cell surface, mLRP1B4 binds and internalizes each of the four LRP ligands examined (scuPA, tPA, PAI-1, and RAP). Furthermore, RAP inhibits the binding of each of these ligands to mLRP1B4, as it does to mLRP4.

Despite these similarities, LRP1B and LRP differ in two important aspects. First, the kinetics of ligand endocytosis (as determined with RAP) are distinctly different between these two receptors. Although mLRP1B4 and mLRP4 display similar affinity for ligand (i.e. $K_d$) and rates of ligand degradation, the internalization rate and efficiency is substantially less for
mLRP1B4. The rate of internalization is more than 15-fold slower for mLRP1B4, which results in increased time spent by the ligand/receptor at the cell surface. The physiological significance of this difference is not yet clear but may limit the effectiveness of LRP1B as a protein clearance system. Alternatively, slower receptor internalization may provide for enhanced cell surface signaling capacity, should LRP1B be found to play a role in intracellular signaling. This appears likely, because several other members of this receptor family have been found to play a role in intracellular signaling (2). Furthermore, the major difference within the cytoplasmic tails of LRP and LRP1B is the 33-amino acid insert in the tail of LRP1B. This insert may promote interaction with adapter proteins and thus provide a mechanistic explanation for the slower internalization rate of LRP1B. Future studies similar to those that have dissected the signals within the cytoplasmic tails of LRP and apolipoprotein E receptor-2 (35, 36) will address this issue.

Second, the expression patterns of LRP, LRP1B, and other family members differ. LRP is highly expressed in liver, brain, and lung. In liver LRP is a major receptor for clearance of chylomicron particles and proteases (37), whereas in brain LRP plays an important role in neurite growth (38, 39) and is involved in the pathological processes of Alzheimer’s disease (3) and long term potentiation (14). Megalin is mainly expressed in kidney, with limited expression in lung and brain. In kidney it serves as an endocytic receptor regulating homeostasis of vitamin D and other nutrients (40). LRP1B is most abundantly expressed in brain, thyroid, and salivary gland, clearly a distinct pattern from those of the other receptors of the gene family. A more precise definition of LRP1B expression requires further examination via either in situ hybridization with an antisense riboprobe or immunohistochemical staining with the antibody when it becomes available.

The identification of uPA and PAI-1 as ligands for LRP1B deserves comment because they are key components of the uPA system, one of the major extracellular matrix-degrading protease systems (41, 42). The uPA system plays a central role in cancer invasion and metastasis as well as other physiological and pathological processes involved in tissue remodeling. Accumulating evidence supports the idea that a major role of the uPA system in promoting cell invasion and metastasis independent from its protease activities is dependent upon the interactions between uPA, uPAR, PAI-1, and other cell surface and extracellular components (41, 42). LRP is well recognized as one important regulator of uPA, uPAR, and PAI-1 function (15–21). Pro-uPA first binds to cell surface uPAR with high affinity and is converted into active uPA. Receptor-bound uPA catalyzes the conversion of the inactive plasminogen to active plasmin, which in turn mediates the degradation of extracellular components including collagens. Active uPA can be inhibited by PAI-1 with the formation of a uPAR-uPA complex. The trimolecular complex (uPAR-uPA-PAI-1) binds to LRP with high affinity and is internalized with the ligand complex subsequently directed to lysosomes for degradation. uPAR and PAI-1 recycle back to the cell surface (17, 18). A similar sequence of events may involve LRP1B and may link its cellular physiology to altered cellular invasion/metastasis. LRP1B was identified via its homozygous deletions in human cancer cell lines and thus represents a candidate tumor suppressor. Preliminary mutation analysis showed that it was frequently (~50%) inactivated in cell lines of human lung cancer (4). Thus, our present results, which show that LRP1B interacts with uPA and PAI-1, warrant further studies on the potential function of LRP1B in mediating the function of the uPA/uPAR system and in tumor metastasis.

In summary, using LRP1B minireceptor (LRP1B4)-expressing cells, we identified that RAP, uPA, tPA, and PAI-1 are ligands for LRP1B. However, LRP1B differs from LRP in both endocytic kinetics and in its tissue expression pattern. Future studies will focus on its physiological and pathophysiological roles as well as the molecular bases thereof.
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