The Low Density Lipoprotein Receptor-related Protein LRP Is Regulated by Membrane Type-1 Matrix Metalloproteinase (MT1-MMP) Proteolysis in Malignant Cells*

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We demonstrate that the presentation of LRP and the subsequent uptake of its ligands by malignant cells are both strongly regulated by MT1-MMP. Because LRP is essential for the clearance of multiple ligands, these findings have important implications for many pathophysiological processes including the pericellular proteolysis in neoplastic cells as well as the fate of the soluble matrix-degrading proteases such as MMP-2. MT1-MMP is a key protease in cell invasion and a physiological activator of MMP-2. Cellular LRP consists of a non-covalently associated 515-kDa extracellular α-chain (LRP-515) and an 85-kDa membrane-spanning β-chain, and plays a dual role as a multifunctional endocytic receptor and a signaling molecule. Through the capture and uptake of several soluble proteases, LRP is involved in the regulation of matrix proteolysis. LRP-515 associates with the MT1-MMP catalytic domain and is highly susceptible to MT1-MMP proteolysis in vitro. Similar to MT1-MMP, the metalloproteinases MT2-MMP, MT3-MMP and MT4-MMP also degrade LRP. The N-terminal and C-terminal parts of the LRP-515 subunit are resistant and susceptible, respectively, to MT1-MMP proteolysis. In cells co-expressing LRP and MT1-MMP, the proteolytically competent protease decreases the levels of cellular LRP and releases its N-terminal portion in the extracellular milieu while the catalytically inert protease co-precipitates with LRP. These events implicate MT1-MMP, not only in the activation of MMP-2, but also in the mechanisms that control the subsequent fate of MMP-2 in cells and tissues.

The low density lipoprotein receptor (LDLR) family consists of at least six members; the LDLR-related protein (LRP), LRP1b, the very low density lipoprotein receptor (VLDLR), apolipoprotein E receptor 2 (apoER2), megalin and LDLR itself (1). LRP, LRP1b, and megalin are significantly larger (~600 kDa) than the other members of the LDLR family. There are several common extracellular modules in the structure of all LDLRs such as the epidermal growth factor (EGF)-like repeats and the cysteine-rich complement-like ligand binding repeats.

Each LDLR family member shows unique features such as the number of ligand-binding and EGF-like repeats suggesting differences in their binding specificities and function. All of the members of the LDLR family have been known as cell surface-associated endocytic receptors which, following binding to the ligand, internalize the bound ligand and target it to the lysosomal compartment for degradation (2).

LRP (CD91) is an important, albeit incompletely studied, member of the LDLR family (3–6). In addition to the ectodomain modules, LRP contains a single transmembrane domain that spans the plasma membrane and a 100 amino acid cytoplasmic domain that plays a role in signal transduction by interacting with the cytoplasmic scaffold and adaptor proteins (3, 7–9). LRP consists of an 85-kDa membrane-spanning light β-chain (LRP-85) that is non-covalently associated with a 515-kDa large extracellular α-chain (LRP-515).

Ligand interactions with LRP are antagonized by a receptor-associated 39–40-kDa glycoprotein (RAP) (10). Endogenous RAP operates as an LRP chaperone to facilitate receptor folding and to diminish the aberrant associations with the improper intracellular ligands during receptor trafficking. Because of its ability to antagonize the binding of ligands to LRP, exogenous RAP has been used in many studies to analyze LRP-mediated processes. LRP functions as a scavenger receptor mediating the uptake of multiple ligands including anionic liposomes, thrombospondins 1 and 2, α2-macroglobulin-protease complexes, fibronectin, lipoprotein lipase, urokinase- and tissue-type plasminogen activators, and matrix metalloproteinases MMP-13, MMP-2 (gelatinase A), and MMP-9 (gelatinase B) (7, 11, 12).

Evidence exists indicating that LRP plays a crucial role in regulating the pericellular proteolysis by removing the matrix degrading urokinase- and tissue-type plasminogen activator, MMP-2, MMP-9, and MMP-13 proteinases from the extracellular milieu, and moving them into the cell compartment for subsequent lysosomal degradation (3, 11, 12). Both MMP-2 and MMP-9 are members of the MMP family of zinc-enzymes and share a number of common structural and functional features (13, 14). MMPs degrade the extracellular matrix and cell surface molecules, and are strongly associated with neoplasms. The multifunctional MMP-9 is the largest and most complex member of the MMP family. Functionally, MMP-9 is a pure effector molecule, which acts as a switch and catalyst in angio-
Membrane-tethered MT1-MMP is the most common member of the membrane type MMP subfamily which, in addition to MT1-MMP, includes MT2-MMP, MT3-MMP, MT4-MMP, MT5-MMP, and MT6-MMP. The invasion-promoting MT1-MMP activity is strongly associated with many types of malignancies (19). In addition to its role in the activation of soluble secretory MMPs, MT1-MMP is also directly involved in cell locomotion and matrix degradation (20). MT1-MMP has been shown to primarily localize the leading edge of migrating cells where the protease cleaves matrix substrata and cell surface-associated adhesion receptors such as tissue transglutaminase, CD44, and the precursors of certain protease cleaves matrix substrata and cell surface-associated MMPs, MT1-MMP is also directly involved in cell locomotion (19). In addition to its role in the activation of soluble secretory activity is strongly associated with many types of malignancies (19). Following the induction of the AOX1 promoter, MT1-PEX was efficiently expressed by yeast cells and secreted in medium for 5 days. MT1-PEX was precipitated from medium (200 ml) with ammonium sulfate at 85% saturation. The precipitate was dissolved in 20 mM MES, pH 6.0 (2 ml) and dialyzed against the same buffer. The dialyzed samples were purified by FPLC on a Mono S HR 5/5 column equilibrated with 20 mM MES, pH 6.0. After washing of the unbound material, MT1-PEX was eluted by a 0–1 M NaCl gradient. Elution of protease was monitored by absorbance measurement at 280 nm. The 1-ml fractions were tested for MT1-PEX by 15% SDS-PAGE. The positive fractions were pooled and dialyzed against 5 mM Tris-HCl buffer, pH 7.0, containing 100 mM NaCl and 0.01% Brij 35.

**Cleavage of LRP in Vitro**—The purified LRP-515 and α1-antitrypsin were each digested for 2 h at 37 °C by the proteases in 15 μl of the cleavage buffer (50 mM HEPES, pH 6.8, containing 20 mM CaCl2, 0.5 mM MgCl2, 1 mM ZnCl2, 4 mM CaEGTA, 200 mM D-glucopyranoside, 1 mM CaCl2, 1 mM MgCl2, 1 μg/ml leupeptin, 1 μg/ml pepstatin, and 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride for 1 h at 4 °C. Insoluble material was removed by centrifugation. Supernatants were preclarified for 3 h on ice with protein G-agarose (Calbiochem, La Jolla, CA). Aliquots (0.6 mg of total protein) of preclarified supernatants were then incubated overnight at 4 °C with the control rabbit IgG (antibody control), the rabbit anti-LRP 2629 antibody or the rabbit anti-MT1-MMP A8515 antibody (1 μg each) and 30 μl of a 50% protein G-agarose slurry. Following washes, the beads were incubated at 100 °C for 3 min in 2× SDS-PAGE loading buffer (20 μl) containing with 25 mM β-mercaptoethanol. The samples were separated by SDS-PAGE and further transferred onto an Immobilon-P membrane (Millipore, Bedford, MA). Biotin-labeled proteins were visualized by using ExtrAvidin-HRP and a TMB/M substrate (Chemicon).
LRP protein (1 μg/ml in phosphate-buffered saline) was allowed to bind the ligands for 1 h at 37 °C. Bound LRP was detected by incubating plates with an anti-LRP rabbit polyclonal antibody 2629 (1 μg/ml) followed by a goat anti-rabbit antibody conjugated with HRP (1:5000 dilution) and a TMB/E substrate (both from Chemicon), phosphate-buffered saline, 0.05% Tween 20 was used to wash the wells between each change of the reagents. The data from the one representative experiment performed in triplicate were shown.

ELISA of Cell Surface-associated LRP—MCF-zeo, MCF-MT-WT, and MCF-MT-E240A cells were seeded at 10^4 cells each per well of a 48-well plate in DMEM, 10% FCS. In 24 h, 100% confluent cells were fixed in 1% glutaraldehyde for 1 h at room temperature. Following extensive washing to remove excess glutaraldehyde, cells were co-incubated with a rabbit antibody 2629 to LRP or the rabbit IgG control (1 μg/ml each). After removing excess antibody, the cell-associated immune complexes were detected by incubating cells with goat anti-rabbit IgG conjugated with HRP and a TMB/E substrate.

Antibody Iodination and Cellular Internalization Assays—Monoclonal LRP-specific antibodies 8G1 and 5A6 were each labeled with 125I using Iodogen (Pierce Chemical Co.). Specific activity of labeled antibodies was ranging from 2 to 10 Ci/μg protein. HT-neo and HT-MT1 cells were seeded in wells of a 6-well plate (1 × 10^5 cells/well) and grown for 48 h in DMEM containing 0.2 mg/ml G418 and 10% fetal bovine serum. The cells were then washed twice with assay medium (DMEM containing 20% HEPES, pH 7.4 and 1.5% BSA), and additionally incubated in this medium for 1 h at 37 °C. To fully saturate cellular LRP, the cells were then incubated for 1 h at 37 °C in the same medium containing 125I-labeled antibody (10 nm). Following incubation, the cells were washed twice with PBS. To detect the internalized 125I-labeled antibody, the residual LRP-125I-antibody complexes were detached from the cell surfaces by treating the cells for 3–5 min with a trypsin-proteinase K solution (50 μg/ml each) (30). As judged by SDS-PAGE of the digest samples, LRP was detected with the LRP monoclonal antibody 8G1. MT1-PEX and MMP-9/TIMP-1 complexes were identified with the LRP-specific rabbit antibody 2629 following the removal of the excess LRP, the LRP-protease complexes were identified with the LRP-specific antibody 8G1 followed by a secondary antibody conjugated to alkaline phosphatase. Con-

RESULTS

The Catalytic Domain of MT1-MMP Interacts Strongly with LRP—Previous studies implicate LRP in the catabolism of MMP-9 and MMP-2 (11, 12). While the binding of MMP-9 to LRP occurs directly with the proteinase (12), the binding of MMP-2 to LRP requires thrombospondin-2 for the formation of a trimeric LRP-thrombospondin-MMP-2 complex (11). We compared the binding of the full-length MMP-9 and MMP-2 enzymes to the binding of individual domains of MT1-MMP, MT1-PEx, and MT1-CAT, to LRP. To prepare the soluble PEX of MT1-MMP, the cDNA fragment coding for the Leu282→Ser288 was expressed in P. pastoris yeast. FPLC on a Mono S column was employed to isolate the purified MT1-PEX construct from the extracellular medium (Fig. 1A). MT1-CAT was expressed and purified from E. coli, and refolded to restore the catalytic activity (26).

To assess the binding efficiency of LRP, we used two different ELISA test systems. First, MMP-9/TIMP-1 complexes and MT1-PEX were each coated on wells of a 96-well plate and then allowed to associate with the serially diluted samples of the purified LRP. The binding of LRP to the immobilized ligands was detected with the LRP-specific antibody 8G1 followed by a secondary antibody conjugated to alkaline phosphatase. Con-

![Fig. 1. The catalytic, but not hemopexin domain of MT1-MMP, associates with LRP. A. Purification of the individual hemopexin-like domain (MT1-PEX) of MT1-MMP. The cDNA fragment coding for the PEX domain of MT1-MMP was expressed in P. pastoris. MT1-PEX was purified from medium by ammonium sulfate precipitation and FPLC. The fractions containing MT1-PEX are shown with hatching. Inset, 15% SDS-PAGE of the ammonium sulfate fraction and purified MT1-PEX. B. MMP-9 but not MT1-MMP binds LRP. LRP was allowed to bind MT1-PEX and MMP-9/TIMP-1 complexes were identified with the LRP-specific antibody 2629. C. MT1-CAT construct binds LRP. MT1-CAT, proMMP-2 and BSA were each coated on wells of a 96-well plate. LRP (1 μg/ml) was added into each well for 1 h. The binding of LRP was detected with the LRP antibody 2629.

Proteolysis of Purified LRP in Vitro—To confirm our hypothesis that LRP is susceptible to MT1-MMP proteolysis, we co-incubated purified LRP with catalytic amounts of the soluble form of MT1-MMP (MT1-ATM/CT) lacking the transmembrane and cytoplasmic domains and isolated from P. pastoris yeast (30). As judged by SDS-PAGE of the digest samples, LRP was
Fig. 2. **MT1-MMP cleaves LRP.** A, soluble MT1-MMP construct cleaves LRP in vitro. MT1-MMP-ΔTM/CT (100 ng; 2 pmol) was incubated LRP and α1-antitrypsin (1 μg each; upper and lower panels, respectively). Where indicated, a hydroxamate inhibitor GM6001 (1 μM), TIMP-2 (200 ng; 10 pmol) and TIMP-1 (500 ng; 10 pmol) were added to the reactions. B, LRP is highly sensitive to MT1-MMP in vitro. LRP (2 pmol; 1 μg) and α1-antitrypsin (16 pmol; 1 μg) were each digested by the indicated amounts of MT1-CAT (upper and bottom panels, respectively). C, MT1-MMP-ΔTM/CT and MT1-CAT are both efficient in cleaving LRP. MT1-MMP-ΔTM/CT (100 ng; 2 pmol) and MT1-CAT (50 ng; 2 pmol) were each incubated with LRP and α1-antitrypsin (1 μg each; upper and lower panels, respectively). D, both LRP and RAP are susceptible to MT1-MMP proteolysis in vitro. LRP and RAP (1 μg each; upper and lower panels, respectively) were co-incubated with MT1-CAT (50 ng; 2 pmol). In A–D reactions were examined by 4–20% gradient SDS-PAGE followed by Coomassie staining. E, several individual MT-MMP species are similarly efficient in the proteolysis of LRP. The MT1-, MT2-, MT3-, and MT4-CAT constructs (10 ng each; ~0.4 pmol) were co-incubated with LRP (200 ng; upper panel) and α1-antitrypsin (1 μg; lower panel). Reactions were examined by 4% SDS-PAGE for LRP and by 10% SDS-PAGE for α1-antitrypsin followed by immunoblotting with the LRP antibody 2629 (upper panel) and Coomassie staining (lower panel). F, N-terminal portion of LRP is most resistant to MT1-MMP cleavage. The left lane represents untreated LRP (1 μg). LRP (10 and 1 μg in the middle and right lanes, respectively) was cleaved with MT1-MMP-ΔTM/CT (100 and 10 ng in the middle and right lanes, respectively). Reactions were separated by 8% SDS-PAGE. The gels were stained with Coomassie (left and middle lanes) or with the monoclonal antibody 8G1 specific to the N-terminal portion of LRP-515 (right lane). Because the 8G1 antibody does not recognize reduced LRP, the non-reduced LRP sample was analyzed in lane 3. Arrows indicate the main cleavage products which are recognized by the 8G1 antibody and represent the N-terminal part of the LRP-515.
fully proteolyzed by MT1-ΔTM/CT and converted to several major proteolytic fragments (Fig. 2A). A potent, wide-range hydroxamate inhibitor of MMP activity GM6001 (1 μM) fully blocked LRP proteolysis. If added at a 3-fold molar excess relative to MT1-CAT, TIMP-1, a poor MT1-MMP inhibitor, had no effect on LRP proteolysis by the protease, while highly similar concentrations of TIMP-2, an inhibitor known to be highly active against MT1-MMP (37), fully suppressed the cleavage of LRP. Similarly, the MT1-ΔTM/CT enzyme was capable of efficiently cleaving α1-antitrypsin, a protein substrate susceptible to MMPs, including MMP-2 and MT1-MMP (38). The cleavage of α1-antitrypsin by MT1-ΔTM/CT was also inhibited by TIMP-2 and GM6001 but not by TIMP-1 (Fig. 2A).

To corroborate these data and to demonstrate that the MT1-MMP catalytic domain alone is sufficient to degrade LRP, we analyzed the efficiency of LRP cleavage by the individual, highly purified and refolded MT1-CAT. Fig. 2B shows that LRP is highly sensitive to MT1-CAT proteolysis. Thus, efficient degradation of LRP was observed at a 1:50 ratio of MT1-CAT to LRP. The sensitivity of LRP to MT1-CAT proteolysis is comparable to that of α1-antitrypsin which is well-known as a highly sensitive proteolytic target of MMPs including MT1-MMP (38).

To support these data further, we evaluated the efficiency of LRP cleavage by the highly similar molar concentrations of the MT1-CAT and MT1-ΔTM/CT constructs. Fig. 2C shows the similarity of the cleavage products of LRP-515 generated by MT1-CAT and MT1-ΔTM/CT. These data support our opinion that the catalytic domain itself is sufficient for the binding and the cleavage of LRP.

It is well established that RAP directly associates with and regulates the functionality of LRP (3). To test if RAP is susceptible to MT1-MMP in a manner similar to that of LRP, LRP, and RAP were co-incubated with the MT1-CAT construct and analyzed by SDS-PAGE. Following MT1-MMP proteolysis, the major RAP digestion products of 33, 30, and 14 kDa were observed in gels (Fig. 2D). There was no difference in the pattern of RAP cleavage in samples where RAP alone or jointly with LRP was co-incubated with MT1-CAT. We conclude therefore that the presence of LRP in the samples does not affect MT1-MMP proteolysis of RAP.

To support our findings further, we evaluated if LRP is susceptible to cleavage by other individual membrane-type proteinases from the MT-MMP subfamily such as MT2-MMP, MT3-MMP, and MT4-MMP. For these purposes, LRP and α1-antitrypsin were each co-incubated with the catalytic domain of MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP and analyzed by SDS-PAGE and Western blotting. Similar to MT1-MMP, other tested MT-MMPs efficiently and specifically degraded LRP in vitro. The cleavage fragments of LRP generated in vitro by MT1-CAT were highly similar to those resulting from the cleavage of LRP by MT2-MMP, MT3-MMP and MT4-MMP (Fig. 2E). These data provide key evidence that the activity of the membrane-tethered MT-MMPs is almost surely an important factor in regulating the concentrations and the activity of cell surface-associated LRP in vivo.

To identify the relative position of the cleavage fragments in the LRP peptide sequence, we used the monoclonal antibody 8G1 specific to the N-terminal portion of the LRP-515 subunit (39). Because the antibody 8G1 does not recognize reduced LRP,2 the digest samples were separated by non-reducing SDS-PAGE, transferred to a membrane support and probed with the 8G1 antibody. In parallel, following the transfer to a membrane, untreated LRP and the resulting cleavage fragments were each visualized by Coomassie staining. The major bands representing the intact LRP-515 and the cleavage species were subjected to N-terminal amino acid sequencing. Fig. 2F demonstrates that the 8G1 antibody recognizes both the intact LRP protein and the major digest species of LRP. These data suggest that the cleavage products of MT1-MMP proteolysis represent the N-terminal portion of LRP-515. The N-terminal sequencing, however, failed to identify the N-terminal sequence of both the intact LRP-515 and the cleavage fragments. These negative results suggest that the N-terminal amino acid residue of LRP-515 is modified post-translationally and indirectly support the results of the antibody mapping. The existence of the N-terminal modifications of LRP is consistent with our earlier data (32). It appears that the MT1-MMP cleavage sites are localized in the C-terminal, membrane-adjacent portion of the LRP-515 molecule.

MT1-MMP and LRP Interact in Breast Carcinoma MCF7 Cells—To confirm the direct interactions of MT1-MMP with LRP in vivo, we used breast carcinoma MCF7 cells that are deficient in MT1-MMP (35) and naturally synthesize low levels of LRP. These cells were stably transfected with the wild-type protease (MCF-MT-WT cells) and the catalytically inert MT1-MMP-E240A mutant (MCF-MT-E240A cells). Flow cytometry analysis employing an antibody to MT1-MMP confirmed similar levels of expression of the protease in the stably transfected MCF-MT-WT and MCF-MT-E240A cells (data not shown). MCF-zeo cells transfected with the original vector without the MT1-MMP insert were used as a control. Several major forms of MT1-MMP have been described in cells, including a 63-kDazymogen, a 55–60-kDa active species and a 38–45-kDa catalytically inactive truncated fragments (30, 40, 41). To evaluate the cell surface-associated molecules of MT1-MMP, the cells were surface labeled with membrane impermeable biotin. The cell lysates were immunoprecipitated with an anti-MT1-MMP antibody. The precipitated samples were analyzed by Western blotting to identify the biotinylated, cell surface pool of MT1-MMP (Fig. 3A, upper panel). No specific bands were detected by using control rabbit IgG (data not shown). Consistent with our earlier observations in breast carcinoma MCF7 cells, the wild type MT1-MMP on the cell surface was predominantly represented by the 39- and 42-kDa stable, catalytically inactive ectodomain forms (35, 42). The cells expressing the MT-E240A construct mainly exhibited the 60-kDa species because self-proteolysis of the enzyme leading to its conversion into the catalytically inactive 39- and 42-kDa ectodomain forms was largely suppressed (Fig. 3A, upper panel). Co-incubation of MCF-MT-WT cells for 24 h with a hydroxamate inhibitor GM6001 (25 μM) blocked self-proteolysis and stabilized the full-length form of MT1-MMP.

To evaluate the status of cell surface-associated LRP, MCF7 cells were surface-biotinylated and lysed. LRP was precipitated from cell lysates with the rabbit LRP-specific polyclonal antibody and then analyzed by Western blotting. The anti-LRP antibody co-precipitated a 60-kDa biotin-labeled protein from the lysates of MCF-MT-E240A cells (Fig. 3A, lower panel). This protein band was similar in its mobility to the MT1-MMP-E240A mutant. No similar bands were observed in mock cells and MCF-MT-WT cells. These results are not surprising because MCF-zeo cells are deficient in MT1-MMP while MT1-MMP in MCF-MT-WT cells is proteolyzed and predominantly represented by the inactive ectodomain forms lacking the catalytic domain. In turn, the inert MCF-MT-E240A mutant escapes self-proteolysis and preserves the catalytic domain, which is available for binding to LRP.

The use of cell biotinylation allowed us to increase the sensitivity of LRP detection and to unambiguously identify the
surface-associated LRP-515 and LRP-85 subunits in MCF-7 cells (Fig. 3B). Both subunits were readily detectable in MCF-MT-WT cells. Consistent with the minor functional activity of MT1-MMP, co-incubation with GM6001 failed to modify the concentrations of surface-associated LRP in HT-neo cells. Conversely, GM6001 significantly enhanced the levels of LRP associated with HT-MT1 cells (Fig. 4A, left panel) by blocking MT1-MMP activity and consequently MMP-2 activation (Fig. 4A, right panel). These data suggest that LRP is a target for MT1-MMP in HT1080 cells.

To confirm the cleavage of LRP by naturally produced MT1-MMP and the release of the proteolytic fragments of LRP in the extracellular milieu, the parental HT1080 cells were either left untreated or stimulated for 18 h with phorbol 12-myristate 13-acetate (PMA) in order to stimulate MT1-MMP protein synthesis. Aliquots of conditioned medium were then subjected to Western blot analysis. Fig. 4B demonstrates that PMA-treated cells released the soluble LRP fragment which was highly similar to the digest product observed in the in vitro cleavage reaction, thereby indicating a role for naturally produced MT1-MMP in the proteolysis of LRP.

**MT1-MMP Proteolysis of LRP Inhibits Internalization of LRP Ligands**—To demonstrate that the MT1-MMP proteolysis diminishes the endocytic activity of the LRP receptor, we evaluated the uptake and internalization of the radiiodinated LRP-specific monoclonal antibodies 8G1 and 5A6 by HT-neo and HT-MT cells (Fig. 4C). The antibody 8G1 recognizes the N-terminal portion of the 515-kDa heavy chain of LRP and blocks the LRP-mediated internalization of activated α2-macroglobulin (39). In turn, the epitope of the monoclonal antibody 5A6 resides in the LRP-85 subunit. This antibody does not inhibit LRP ligand internalization (39). Our studies showed that the internalization of the $^{125}$I-8G1 by HT-MT1 cells was significantly less than by the control cells ($p < 0.01$), whereas $^{125}$I-5A6 internalization was similar for both cell types. These results are in agreement with our in vitro and cellular cleavage experiments presented in Figs. 2, 3, and 4, A and B and demonstrate that LRP-515 is predominantly cleaved by MT1-MMP and that the cleavage products are released into the media. In addition, these data clearly indicate that while LRP endocytosis itself is not altered by MT1-MMP, the cleavage of LRP by MT1-MMP impairs ligand internalization by shedding and thereby inactivating the ligand-binding domain of LRP.

Overall, our data suggest that either overexpressed or naturally produced MT1-MMP strongly contributes to the proteolysis and presentation of cell surface-associated LRP in malignant cells. The MT1-MMP proteolysis inactivates the ligand-binding domain of LRP and affects the subsequent up-
take of LRP ligands by malignant cells. Because LRP is essential for the clearance of multiple ligands, these findings are likely to have important implications for many physiological and, especially, pathophysiological processes. These data cannot, however, exclude the presence of the additional MT1-MMP-unrelated proteases mediating shedding of LRP in malignant cells.

DISCUSSION

A cell surface-associated receptor LRP is directly involved in the capture, internalization and clearance of MMP-2 and MMP-9 from the extracellular milieu (11, 12). These events regulate the extracellular concentrations of MMPs and the proteolysis of the extracellular matrix. We suspect that MT1-MMP plays a dual role in the regulation of the matrix-degrading proteolytic activity, including the functional activity of MMP-2: in malignant cells MT1-MMP initiates the activation pathway of MMP-2, and, then, by cleaving LRP, protects the active MMP-2 enzyme from the uptake and clearance. LRP functions as a scavenger receptor to mediate the uptake of urokinase- and tissue-type plasminogen activators and
matrix metalloproteinases (7, 11, 12). LRP proteolysis by MT1-MMP contributes to maintaining high levels of the soluble secretory proteinases such as MMP-2 in the extracellular milieu and the extensive matrix degradation by aggressive migrating cells. Our hypothesis is schematically represented in Fig. 5.

Our experimental results agree well with this hypothesis. We demonstrated that the catalytic domain rather than the hemopexin domain of MT1-MMP associates with LRP, thus forming a conventional enzyme-substrate complex. Both the individual catalytic domain and the full-length species of MT1-MMP are efficient in cleaving the heavy 515-kDa chain of LRP in vitro, thereby destroying the functional activity of the receptor and releasing its N-terminal ligand binding portion into the extracellular milieu. In addition to MT1-MMP, three other members of the MT1-MMP subfamily including MT2-MMP, MT3-MMP, and MT4-MMP are efficient in cleaving LRP in vitro. Altogether, these findings suggest that LRP is likely to be susceptible to proteolysis by several individual MT-MMPs in many cancer cell types. On the other hand, we infer from these data that the functionally inert MT1-MMP mutant (MT-E240A) rather than the ectodomain forms of the protease, that are lacking the catalytic domain, or the active species of MT1-MMP, are likely to co-precipitate with LRP. In agreement, co-precipitation of LRP with the cell surface-associated protein with a molecular mass highly similar to that of the full-length MT1-MMP, was observed only in the cells co-expressing LRP with the proteolytically inactive MT1-MMP-E240A construct.

In turn, proteolysis of LRP by MT1-MMP was most evident in cells co-expressing the receptor with the functionally active wild type protease. GM6001, a hydroxamate inhibitor of MT1-MMP activity, if co-incubated with MCF-MT-WT cells, restored the levels of cell surface-associated LRP to those characteristic to the MT1-MMP-deficient MCF-zeo cells or MCF-MT-E240 cells which express the inert protease. The data on MT1-MMP’s role in the cleavage of LRP acquired on MCF7 cells were confirmed further by the analyses of HT1080 cells. Stimulation of MT1-MMP activity with PMA enhanced the release of LRP-immunoreactive material by HT1080 cells confirming that naturally expressed proteinase is potent in cleaving the cell surface-associated LRP.

Recent evidence suggests that MT1-MMP functions in multiple ways and is, in fact, a pleiotropic regulator of many face-associated LRP.

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The Low Density Lipoprotein Receptor-related Protein LRP Is Regulated by Membrane Type-1 Matrix Metalloproteinase (MT1-MMP) Proteolysis in Malignant Cells

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