The Role of Grp 78 in α2-Macroglobulin-induced Signal Transduction

EVIDENCE FROM RNA INTERFERENCE THAT THE LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN IS ASSOCIATED WITH, BUT NOT NECESSARY FOR, GRP 78-MEDIATED SIGNAL TRANSDUCTION*

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The low density lipoprotein receptor-related protein (LRP) is a scavenger receptor that binds to many proteins, some of which trigger signal transduction. Receptor-recognized forms of α2-Macroglobulin (α2M) bind to LRP, but the pattern of signal transduction differs significantly from that observed with other LRP ligands. For example, neither Ni2+ nor the receptor-associated protein, which blocks binding of all known ligands to LRP, blocks α2M*-induced signal transduction. In the current study, we employed α2-macroglobulin (α2M)-agarose column chromatography to purify cell surface membrane binding proteins from 1-LN human prostate cancer cells and murine macrophages. The predominant binding protein purified from 1-LN prostate cancer cells was Grp 78 with small amounts of LRP, a fact that is consistent with our previous observations that there is little LRP present on the surface of these cells. The ratio of LRP:Grp 78 is much higher in macrophages. Flowcytometry was employed to demonstrate the presence of Grp 78 on the cell surface of 1-LN cells. Purified Grp 78 binds to α2M with high affinity (Kd ~ 150 pM). A monoclonal antibody directed against Grp 78 both abolished α2M*-induced signal transduction and co-precipitated LRP. Ligand blotting with α2M showed binding to both Grp 78 and LRP heavy chains in these preparations. Use of RNA interference to silence LRP expression had no effect on α2M*-mediated signaling. We conclude that Grp 78 is essential for α2M*-induced signal transduction and that a “co-receptor” relationship exists with LRP like that seen with several other ligands and receptors such as the uPA/uPAR (urinary type plasminogen activator or urokinase/uPA receptor) system.

α2-Macroglobulin (α2M) is a plasma proteinase inhibitor with broad specificity. Upon binding to proteinases, it undergoes a major conformational change that exposes receptor recognition sites on the molecule (1). This activated form of α2M is designated α2M*. Though difficult to reconcile, accumulating evidence has demonstrated that α2M*-induced signaling occurs via a receptor that is functionally unique from the previously characterized α2M* receptor, the low density lipoprotein receptor-related protein (LRP). LRP is a scavenger receptor (1) that binds to a variety of proteins, many of which trigger signal transduction (2–5). This pathway requires the activation of a pertussis toxin-sensitive G protein (2–5). The receptor-associated protein (RAP) that blocks the binding of all known ligands to LRP is also an antagonist for this signaling pathway (2–3). Whereas α2M* binds to LRP, when cells are exposed to α2M*, a distinct set of signaling events is observed that differ from those induced by other ligands for this receptor (2–6). These include activation of a different G protein and the lack of antagonism by RAP or Ni2+ (2–7). In addition, binding studies with a variety of cells in culture demonstrate two classes of binding sites, one of very high affinity (KD ~ 100 pM and 1600 sites/cell) and one of lower affinity (KD ~ 2–5 nM and ~70,000 sites/cell). The high affinity binding site is responsible for α2M*-induced signal transduction and has been termed the α2M* signaling receptor (α2MSR), whereas the lower affinity binding site has been identified as LRP (8–12). Numerous studies have suggested that high affinity binding sites alone are involved in α2M*-induced signal transduction (6–13).

In this report we demonstrate by flow analysis that Grp 78 is on the surface of 1-LN human prostate cancer cells and that cell surface Grp 78 is essential for α2M*-induced signal transduction in both 1-LN human prostate cancer cells and murine macrophages. A monoclonal antibody directed against Grp 78 completely abolished α2M*-induced signal transduction in both 1-LN cells and murine macrophages as judged by the effects of this antibody on the ability of α2M* to induce a rise in [Ca2+]i. This response is always seen when signal transduction is activated by α2M* in these cells (8–12). This monoclonal antibody co-precipitates Grp 78 and LRP in both 1-LN cells and macrophages. But in the former cells the ratio of Grp 78:LRP is much greater than in macrophages. We also show by ligand binding assays to membranes that α2M* binds to Grp 78 and the heavy, but not light, chain of LRP. The latter observation is consistent with the known receptor-binding properties of LRP (1). Using the technique of RNA interference, we find that LRP is not required for α2M*-dependent signal transduction. Finally, we demonstrate that highly purified Grp 78 binds to α2M* with very high affinity (KD ~ 150 pM).

EXPERIMENTAL PROCEDURES

Materials—The sources of thiangocytate, cell culture material, and endotoxin-free α2M* have been described previously (2–4, 6). α2M* was prepared by reaction of human α2M with methylamine as in previous
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studies (2–4, 6). 1H]Mycosol was from ABC, St. Louis, MO. Antibodies against the heavy (515 kDa) and light (85 kDa) chains of LRP were procured from American Diagnostica Inc. (Greenwich, CT). Antibodies against Grp 78 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bacitracin and β-ocetylglucoside were procured from Sigma. Polyclonal anti-human antibodies against Grp 78 and normal goat IgG employed for flow cytometry were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Chromomix (500 μg/ml) and fluorescein isothiocyanate (FITC)-conjugated Rabbit anti-goat IgG, F(ab′)2 Fragment Specific, and Rabbit Gamma Globulins were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). Purified anti-mouse H-2K® (AF6–88.5) was purchased from Pharmin- gen. Affinity chromatography materials and ECM kits were from Am- ersham Biosciences. All other reagents used were procured locally and were of the highest available grade. Murine macrophages and human prostate cancer cells were cultured as previously described (2–4, 6, 13). The 1-LN cell line was a kind gift from Dr. Philip Walther (Duke University Medical Center, Durham, NC).

Receptor Purification—Cell membranes from murine macrophages and 1-LN prostate cancer cells were obtained and solubilized in β-oc-etylglucoside and chromatographed on an αM®-agarose affinity column as previously described (14). By this procedure both LRP and Grp 78 were isolated as demonstrated by several techniques (see below). Grp 78 was also purified from the cell membranes of 1-LN prostate cancer cells by suspending the membranes in 20 mM Tris-HCl, pH 8.0 containing 1% Triton X-100 and chromatography on anti-Grp 78-agarose. These precipitates were subjected to SDS-PAGE (10%) and blotted with Grp 78 from cell lysates to immobilized αM®. These precipitates were subjected to SDS-PAGE (10%) and blotted with Grp 78 was also purified from the cell membranes of 1-LN prostate cancer cells by suspending the membranes in 20 mM Tris-HCl, pH 8.0 containing 1% Triton X-100 and chromatography on anti-Grp 78-agarose. This precipitation of αM® bound to immobilized αM®. The use of these techniques is described in detail elsewhere (9).

Binding of αM® to Immunobilized Grp 78—To study the specific bind- ing of αM® to immobilized Grp 78, 96-well culture plates were coated with Grp 78 (1 μg/ml in 0.1 M sodium carbonate, pH 9.6, 200 μl/well at 37 °C for 2 h). After coating, plates were rinsed with 10 mM sodium phosphate, 100 mM NaCl, pH 7.4, containing 0.05% Tween 80 (PBS-Tween) to remove unbound protein. Non-specific sites were blocked by incubation with PBS-Tween containing 2% (w/v) bovine serum albumin at room temperature for 1 h. Plates were rinsed twice with PBS-Tween, air-dried, and stored at 4 °C. For assays, increasing concentrations of αM® were added to triplicate wells and incubated at 37 °C for 1 h. An ELISA procedure was used to calculate the amount of bound ligand to Grp 78. This procedure involves the construction of calibration curves in 96-well culture plates coated with αM® as described above and then titration with a specific rabbit anti-αM®-IgG followed by detection with a secondary anti-rabbit IgG conjugated to alkaline phosphatase. Values from this curve were used to determine the amount of αM® bound to immobilized Grp 78.

Binding of LRP and Grp 78 to Immunobilized αM®—Binding of LRP and Grp 78 from cell lysates to immobilized αM® was carried out by an ELISA on 96-well culture plates. Plates were first coated with αM® (5 μg/ml) in 0.1 M sodium carbonate, pH 9.3, 0.01% sodium azide. The plates were rinsed with 10 mM sodium phosphate, pH 7.3, containing 0.15 M sodium chloride and 0.05% (w/v) Tween 80 (PBS-Tween) and incubated at room temperature for 3 h with a 0.3 mg/ml rabbit gamma globulins, 0.4 mg/ml goat IgG Fc fragments, 0.01% NaN3 at a concentration of 1 x 10^6 cells/ml. Aliquots (100 μl) of these cell suspensions were incubated at 4 °C for 30 min with an appropriate dilution of goat polyclonal anti-Grp 78 or normal goat IgG (control). Cells were washed three times with ice-cold staining buffer, pelleted, and resuspended in 100 μl of ice-cold staining buffer. These cell suspensions were then incubated in the dark with a FITC-conju- gated rabbit anti-goat IgG, F(ab′)2 for 30 min. Cells were then washed three times with ice-cold staining buffer, resuspended in ice-cold 1% paraformaldehyde, and stored in the dark at 4 °C until analysis by flow cytometry. The mean relative fluorescence was measured at 486 nm with a FACS Vantage SE flow cytometer. The mean relative fluorescence after excitation at 486 nm was determined for each sample on a FACS Vantage SE flow cytom- eter, and data was analyzed using CellQuest® software (BD Pharmingen).

Measurement of [Ca2+]i, in αM® Treated with Macrophages— Changes in [Ca2+]i, were measured in macrophages and 1-LN prostate cancer cells preloaded with Fura-2/AM digital imaging microscopy as previously described (2–4, 6). For some studies, either bacitracin (3 mg/ml) or anti Grp 78 (5 μg/ml) was added 20 min prior to αM® (100 pg) and determination of [Ca2+]i.

Chemical Synthesis of dsRNA Homologous in Sequence to the Target LRP-1 Gene Sequence—The chemical synthesis of dsRNA homologous to the target LRP gene amino acid sequence 146–152 nucleotide base length was performed by Operon Technologies (Huntsville, AL). A >98% (by primary accession number Q0754) was performed by Ambion sequence ID 285, Austin, TX. For making dsrna, the sense (5′-AACAUUGAAG- GACUUUGAUtt3′) and antisense (5′-AUAAAGUCUCAUUCAU-GUtt3′) oligonucleotides were annealed according to the manufacturer’s instructions. Throughout the entire period of experiment handling of reagents was performed in a RNase-free environment. Briefly, equal amounts of sense and antisense oligonucleotides were mixed and heated at 90 °C for 1 min and then for 1 h at 37 °C in an incubator. The dsRNA preparation was stored at −20 °C before use.

Transfection of Murine Peritoneal Macrophages with dsRNA Homologous in Sequence to the Target LRP-1 Gene Sequence—This procedure has been described in detail elsewhere (16). In brief, thioglycolate- elicted murine peritoneal (1 x 10^6 cells/well in a 6-well plate) were lavaged as above and allowed to adhere to 2 h in RPMI 1640 medium containing 10% FBS, penicillin (12.5 units/ml), streptomycin (6.5 μg/ml), and 2 μg streptomycin at 37 °C in a CO2 (5%) humidified incubator at 37 °C. The non-adherent cells were aspirated, monolayers were washed twice with Hank’s balanced salt solution containing HEPES (HBSS), 2 ml of DMEM medium containing 10% FBS and above antibiotics added, and cells incubated as above for 16 h. For each transfection, 2 μg of dsRNA was diluted into 100 μl of serum-free DMEM in a tube. In another tube, 10 μl of LipofectAMINE was diluted into 100 μl of serum-free medium. The two solutions were combined, mixed gently, and incubated for 45 min at room temperature followed by the addition of 800 μl of serum-free and antibiotic-free medium to each tube. The monolayers were washed twice with serum-free DMEM medium, layer- ered in each well with 1 ml of LipofectAMINE-DMEM (10 μl/ml) or lipid-dsRNA mixtures, dsRNA (26 μg), gently mixed, and incubated for 5 h at 37 °C in a humidified CO2 incubator. At the end of the incubation, the medium were aspirated, 10% antibiotic-free DMEM medium containing 10% FBS, penicillin, streptomycin, and 2 μg/mL at 37 °C with 5% CO2 was added and the well cells were incubated for 16 h as above. Microscopic observation of the monolayers did not show obvious evidence of toxicity. The cells did not take up Trypan Blue, and their morphology was not altered by the procedure (data not shown). The medium was replaced with DMEM medium containing antibiotics and 10% FBS 24 h after the start of incubation. The monolayers were washed with 2 ml of DMEM medium once, and Western blotting of LRP and Grp 78 in cell lysates was performed. Equal amounts of proteins from both groups of lysates were electrophoresed and transferred to membranes. LRP and Grp 78 were detected by ELISA as described above.

Flow Analysis of 1-LN Human Prostate Cancer Cells for Cell Surface- associated LRP—Grp 78—1-LN cells were grown at 37 °C and 5% CO2 in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 35 nM human insulin. Cells were detached by incubation for 5 min at 37 °C with Ca2+- and Mg2+-free PBS containing 4 mM EDTA and then pelleted. Cells were washed once with PBS before being resuspended in ice-cold staining buffer (Phenol Red- neutralized) and 1-LN cell surface was determined for each suspension by a FACS Vantage SE flow cytom- eter, and data was analyzed using CellQuest® software (BD Pharmingen).
The blot demonstrates that LRP co-precipitated with Grp 78.

Grp 78 purified as described under Experimental Procedures.

Purification of α₂M* binding proteins from 1-LN human prostate cancer cells membranes were prepared, solubilized in β-octylglucoside, and applied to an α₂M*-agarose affinity column. The eluates were subjected to SDS-PAGE and transferred to Hybond-P membranes for immunoblotting and visualization employing ECF and phosphorimaging (Storm®).

**FIG. 1. Purification of α₂M* binding proteins.** 1-LN human prostate cancer cell membranes were prepared, solubilized in β-octylglucoside, and applied to an α₂M*-agarose affinity column. The eluates were subjected to SDS-PAGE and transferred to Hybond-P membranes for immunoblotting and visualization employing ECF and phosphorimaging (Storm®). A, lane 1, protein bands in the eluate were localized by Coomassie Brilliant Blue staining; lane 2, the 515-kDa heavy chain of LRP; lane 3, the 85-kDa light chain of LRP; and lane 4, Grp 78 purified as described under Experimental Procedures. The proteins in lanes 2–4 were identified by Western blotting/ECF. Lanes 5 and 6, Coomassie Brilliant Blue staining of Grp 78 immunoprecipitated with anti-Grp 78 antibody from 1-LN cell and macrophage membranes. The blot demonstrates that LRP co-precipitated with Grp 78. B, the binding of α₂M* to the proteins in the membrane preparation from 1-LN prostate cancer cells and macrophages. Immunoprecipitation was performed with anti-Grp 78 as in panel A, lanes 5 and 6. After membrane transfer, ligand binding of α₂M* to the proteins on the membrane was performed as previously described (15). Lane 1 is from 1-LN prostate cancer cell membranes, whereas lane 2 is from macrophages. Lane 3 is purified Grp 78 obtained as described under Experimental Procedures for comparison. C, in a separate experiment performed as in A and B, the immunoprecipitated Grp 78 was probed for MHC class I 45-kDa protein as previously described (17).

Quantification of [³H]IP₃ Formation in Macrophages after LRP Gene Silencing—The quantification of [³H]inositol (1, 4, 5)trisphosphate [³H]IP₃ formed from [³H]myoinositol-labeled, dsRNA-transfected macrophages upon stimulation with α₂M* (100 pm) was performed as described earlier (2, 6). Briefly, 2-h adhered macrophages in 6-well plates (1 × 10⁶ cells/well) were transfected with dsRNA homologous in sequence to target the LRP gene as above. The transfected cells in DMEM medium containing 10% FBS and antibiotics were incubated with [³H]myoinositol (10 μCi/ml) overnight. The cells were washed with HHBSS containing 10 mM LiCl, 1 mM CaCl₂, and 1 mM MgCl₂ thrice. A volume of the wash buffer was added and cells were incubated at 37 °C for 5 min for temperature equilibration. The cells were stimulated with α₂M* (100 pm) for varying periods of time. The reaction was stopped by aspirating the medium and adding a volume of 6.25% HClO₄. The cells were scraped into respective glass tubes, 1 ml of freon:octylamine (1:1, v/v) was added, and the mixture was vortexed for 10 s and centrifuged at 1500 rpm for 10 min. The aqueous phase was applied onto a 1 ml Dowex resin (AG 1-X8-formate), and an inositol phosphates batch was eluted as described previously (6). The untransfected cells were processed similarly and were the controls. In experiments where the effect of pertussis toxin was studied on the formation of [³H]IP₃ upon stimulation with α₂M, the cells were incubated with pertussis-toxin (1 μg/ml/
both 1-LN cells and macrophages (Fig. 1A). In these studies, LRP heavy and light chains were co-precipitated with Grp 78. 

Ligand blot analysis of solubilized membranes from either 1-LN cells or macrophages immunoprecipitated with anti Grp 78 showed H9251 2M* binding to Grp 78 and the heavy, but not light, chain of LRP (Fig. 1B). The latter result is expected because H9251 2M* binds only to the heavy chain of LRP (1). Previous studies have demonstrated that Grp 78 is a co-receptor with MHC class I histocompatibility antigens (17). We were able to demonstrate that the Grp 78 immunoprecipitate from murine membranes also contained MHC class I 45-kDa protein (Fig. 1C) consistent with this report (17). Based on these observations, we next determined whether Grp 78 is expressed on the surface of 1-LN cells. After staining the cells with an antibody against Grp 78, cell-surface expression was confirmed by flow cytometry (Fig. 2A). In light of these observations, we then purified Grp 78 free of LRP from 1-LN cell membranes as described under "Experimental Procedures." H9251 2M* binding to the purified preparation was detected by ELISA. The data fit a simple hyperbolic curve, indicating a single class of binding sites, $K_d \sim 150$ pM (Fig. 2B). These results are consistent with previous observations of an H9251 2M* high affinity binding site on these cells, $K_d \sim 100$ pM (13).

**Fig. 2.** Calcium ([Ca$^{2+}$]) changes in H9251 2M*-stimulated Fura-2/AM loaded cells. A, macrophages were preloaded with Fura-2/AM (4 μg/25 min/25°C) prior to stimulation with H9251 2M* (100 pM). ○, H9251 2M*; □, bacitracin (3 mg/ml/20 min/25°C) pretreatment prior to H9251 2M*; ▲, anti Grp 78 antibody (5 μg/ml/20 min/25°C) prior to H9251 2M*. B, 1-LN cells were preloaded with Fura-2/AM as above prior to treatment with H9251 2M (100 pM). ●, H9251 2M*; ○, bacitracin prior to H9251 2M*; □, anti Grp 78 antibody prior to H9251 2M*.

**Fig. 3.** Effect of silencing the LRP gene with dsRNA homologous in sequence to the target gene on the expression of LRP and Grp 78. See Experimental Procedures for details. A, detection of LRP heavy chains by ELISA. ▲, cells treated with LipofectAMINE; ○, cells transfected with dsRNA plus LipofectAMINE. B, detection of Grp 78 by ELISA. ▲, cells treated with LipofectAMINE; ○, cells transfected with dsRNA plus LipofectAMINE. Values are the average of two experiments.
to silence LRP expression. An ELISA was then utilized to demonstrate that LRP expression was completely silenced in these cells, whereas Grp 78 expression was unchanged (Fig. 4).

Having established that LRP expression was ablated, we next studied signal transduction in these macrophages in comparison to the untreated cells. For these studies, IP₃ synthesis was directly measured (Fig. 5A). α₂M⁺ treatment of macrophages causes a significant and transient rise in IP₃, which then causes Ca²⁺ mobilization from the endoplasmic reticulum (3, 4, 6–12). We chose to directly measure IP₃ rather than [Ca²⁺], for two reasons. First, the rise in [Ca²⁺] is directly attributable to IP₃ synthesis (3–6), and second, it would be very difficult to perform the technique of RNA interference with macrophages plated on coverslips as is required for digital imaging microscopy. As can be seen in Fig. 5A, exposure of either macrophages treated with dsRNA homologous to LRP or the control cells resulted in a comparable increase in IP₃, although the duration of the increase appears prolonged in macrophages where LRP is not produced. This increase was pertussis toxin-insensitive (Fig. 5B), as is a characteristic of α₂M⁺-induced synthesis of IP₃ (2–4, 6–12).

FIG. 5. α₂M⁺-dependent [³H]IP₃ synthesis in macrophages after silencing LRP expression. See “Experimental Procedures” for details. A, generation of [³H]IP₃. ●, cells transfected with dsRNA; ○, cells transfected with dsRNA plus LipofectAMINE then stimulated with α₂M⁺ (100 pM) for varying periods of time; △, cells transfected with dsRNA plus LipofectAMINE then stimulated with α₂M⁺ (100 pM) for 60 s; 3, cells transfected with dsRNA then stimulated with α₂M⁺ (100 pM) for 60 s. The values are the mean ± S.E. from two separate experiments performed in triplicate and are expressed as percent change over basal (100%).

FIG. 6. A model representing the binding of α₂M* to LRP and Grp 78. The activation of the p21⁰⁶⁺, dependent MAP kinase pathway is shown.

DISCUSSION

Studies by Pastan and co-workers (20–22) first reported isolation of an α₂M⁺ receptor by classical purification methods in the 1980’s. They demonstrated that a protein of 85,000 molecular weight bound to ¹²⁵I-α₂M⁺ (20–22). Moreover, they observed two classes of binding sites on cells: one of high affinity (Kᵦ = 200 pM and 10,000 sites/cell) and one of lower affinity (Kᵦ = 100 nM and 600,000 sites/cell) (20–22). They also reported that bacitracin blocked binding to the high, but not low, affinity sites. The studies were performed at 4 °C, thus ruling out the possibility that these results represented the well known effect of bacitracin on endocytosis at 37 °C (20–22).

These observations were not pursued, and this protein was never identified. LRP was then identified by Strickland et al. (18) in 1990 as the α₂M⁺ receptor. Subsequent studies indicated that this receptor is a multidomain, multiligand receptor that is capable of interacting with a large variety of proteins (1, 18, 19). The receptor consists of a 515-kDa heavy chain and an 85-kDa light chain. Many subsequent reports have supported the identification of LRP as the α₂M⁺ receptor (for example, Refs. 1 and 18).

In 1993 and 1994 we observed that exposure of macrophages to α₂M⁺ triggered a typical IP₃-dependent signaling pathway (2, 3, 6). We then demonstrated that a number of other ligands for LRP also activated signal transduction (2, 3). Whereas we initially believed that α₂M⁺-induced signal transduction resulted from ligation of LRP, more detailed studies suggested that a unique α₂M⁺ signaling receptor must exist (2–4, 6–12). The basis for this hypothesis has been extensively discussed elsewhere (2–4, 6–12). In brief, α₂M⁺-dependent signal transduction requires activation of a different G protein than seen with other ligands for LRP, and signaling is not blocked by either Ni²⁺ or a very high molar excess of RAP, which is an antagonist for LRP-dependent signal transduction (2, 3, 7).

In contrast to other ligands for LRP, α₂M⁺ selectively activates phospholipase D, increases cytosolic pH, and activates p21⁰⁶⁺ and PI 3-kinase (23–26).

Bacskaı et al. (27) reported that α₂M⁺ binding to LRP on the surface of neurons mediates signaling by activation of N-methyl-D-aspartate receptors, which presumably represent a “coreceptor.” More recently, Qiu et al. (28) have suggested that α₂M⁺ binding to LRP...
negative relationship may exist between \( \alpha_2M \) binding to neurons and the signal induced by N-methyl-d-aspartate. Other recent studies have shown that the cell-free (Wnt/\( \beta \)-catenin) signaling pathway requires that Wnt simultaneously bind to both Frz and LRP to induce signal transduction (29–32).

A co-receptor relationship also exists between the uPA/uPAR system and LRP (1). This relationship may represent a model for the Grp 78/LRP relationship. In this case uPA signaling depends exclusively on uPAR binding; however, the association of the uPA/uPAR complex, which also probably includes the inhibitor of uPA, binds to LRP and is internalized, thus regulating the signaling of uPAR (1).

We chose to purify \( \alpha_2M \) from the membranes of 1-LN human prostate cancer cells, because these cells express very little LRP but significant amounts of \( \alpha_2M \). Human prostate cancer cells, because these cells express very little LRP but significant amounts of \( \alpha_2M \) negative relationship may exist between \( \alpha_2M \) binding to LRP and is internalized, thus regulating the signaling of uPAR. In this study, we purified Grp 78 from 1-LN cells (17).

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\( \alpha_2M \) Signaling Receptor