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The Role of MTJ-1 in Cell Surface Translocation of GRP78, a Receptor for α_2-Macroglobulin-Dependent Signaling[1]

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MTJ-1 associates with a glucose-regulated protein of M_r ~78,000(GRP78) in the endoplasmic reticulum and modulates GRP78 activity as a chaperone. GRP78 also exists on the cell surface membrane, where it is associated with a number of functions. MHC class I Ags on the cell surface are complexed to GRP78. GRP78 also serves as the receptor for α_2-macroglobulin-dependent signaling and for uptake of certain pathogenic viruses. The means by which GRP78, lacking a transmembrane domain, can fulfill such functions is unclear. In this study we have examined the question of whether MTJ-1, a transmembrane protein, is involved in the translocation of GRP78 to the cell surface. MTJ-1 and GRP78 coimmunoprecipitated from macrophage plasma membrane lysates. Silencing of MTJ-1 gene expression greatly reduced MTJ-1 mRNA and protein levels, but also abolished cell surface localization of GRP78. Consequently, binding of the activated and receptor-recognized form of α_2-macroglobulin to macrophages was greatly reduced, and activated and receptor-recognized form of α_2-macroglobulin-induced calcium signaling was abolished in these cells. In conclusion, we show that in addition to assisting the chaperone GRP78 in protein quality control in the endoplasmic reticulum, MTJ-1 is essential for transport of GRP78 to the cell surface, which serves a number of functions in immune regulation and signal transduction. *The Journal of Immunology,* **2005, 174: 2092–2097.**

The molecular chaperone glucose-regulated protein of M_r ~78,000 (GRP78)[3] is a member of the heat shock protein 70 (HSP70) family. It is involved in many cellular processes, including translocation of newly synthesized polypeptides across the endoplasmic reticulum (ER) membrane and their subsequent folding, maturation, transport, or retrotranslocation (1–5). GRP78 binds transiently to newly synthesized proteins in the endoplasmic reticulum (ER) and with higher affinity to misfolded, underglycosylated, or unassembled proteins whose transport from the ER is blocked (6, 7). GRP78, like other HSP proteins, consists of two domains; namely, the 44-kDa NH_2-terminal domain, which is responsible for its ATPase activity, and a 20-kDa COOH-terminal polypeptide binding domain. In addition, it contains a highly helical and variable 10-kDa COOH-terminal tail of unknown function (8). DnaJ-like proteins collaborate with members of the HSP70 family in directing protein conformation and oligomerization in yeast (6–14). Multiple DnaJ-like proteins are present in subcellular compartments and are often concentrated in different locations, which enables HSP70 family members to catalyze localized protein reactions (15). DnaJ family members contain different combination of four conserved domains, which includes the so-called J domain (15–17). Murine tumor cells express a murine tumor cell DnaJ-like protein 1 (MTJ-1) (8, 18). MTJ-1 is a transmembrane protein, and it is an accessory protein for GRP78 catalytic activity. The J domain of MTJ-1 interacts with GRP78, stimulates its ATPase activity, and functions as its cochaperone in protein folding and translocation of newly synthesized polypeptides across ER membranes (8, 14).

A number of studies demonstrate that GRP78 is also located on the cell surface. It is coexpressed with MHC class I Ags, it serves as a receptor for the uptake of certain viruses, and it functions as the activated and receptor-recognized form of α_2-macroglobulin (α_2M*) signaling receptor (19–33). α_2M* binds not only to low density lipoprotein receptor-like protein (LRP), but also to an α_2-macroglobulin signaling receptor (α_2MSR) with vastly different binding affinities and signaling characteristics from those of LRP (25–30). We have isolated and purified α_2MSR from macrophages and 1-LN prostate cancer cells and identified it as GRP78 (29, 30). α_2M* binds to GRP78 on the macrophage cell surface with high affinity (K_d = 50–100 pM). Treatment of cells with Abs against GRP78 abolishes α_2M*-induced calcium signaling. Silencing of the expression of GRP78 gene expression also greatly attenuates α_2M*-induced calcium signaling, IP_3 generation, and [^{3}H]thymidine incorporation (30). Cell surface-associated chaperones function in receptor signaling involving the glucocorticoid, androgen, and progesterone receptors (34, 35). In view of the role of MTJ-1 in regulating the ER functions of GRP78 and the role of GRP78 in receptor biology, we hypothesized that in addition to being a partner with GRP78 in ER protein quality control, MTJ-1 may also be involved in the translocation and anchoring of GRP78 to the cell surface for its functioning as a signaling receptor. We report here that MTJ-1 and GRP78 are coimmunoprecipitated in plasma membranes. Silencing of MTJ-1 gene expression by RNA interference greatly attenuates the expression of MTJ-1 protein and GRP78 protein localization in the cell membrane fraction. Concomitantly,

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[3] Abbreviations used in this paper: GRP78, glucose-regulated protein of M_r ~78,000; $[Ca^{2+}]_i$, cytosolic free Ca^{2+}; CELISA, cell-based ELISA; ECF, enhanced chemifluorescence; ER, endoplasmic reticulum; HSP, heat shock protein; IP_3, inositol 1,4,5-trisphosphate; LRP, low density lipoprotein receptor-like protein; α_2M*, activated and receptor-recognized form of α_2-macroglobulin; α_2MSR, α_2M signaling receptor identified as GRP78; MTJ-1, murine tumor cell DnaJ-like protein 1.

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cell surface binding of α_2M* and α_2M*-induced calcium signaling are abolished.

Materials and Methods

Culture media were purchased from Invitrogen Life Technologies. Polyclonal Abs against GRP78 were purchased from Stressgen. Anti-actin Abs were purchased from Sigma-Aldrich. Abs against MTJ-1 were raised against the sequence beginning at residue 105, NH_2-LVAIYEVLKVDER RQRYVDVL, of MTJ-1 (SWISS-PROT, primary accession no. Q61712) in rabbits (Genemed Synthesis). α_2M* was prepared as described previously (25–30). Other reagents of the highest grade available were procured locally.

Cell culture

The use of mice for these studies was approved by the institutional animal use committee in accordance with relevant federal regulations. Thioglycolate-elicited peritoneal macrophages were obtained from pathogen-free, 6-wk-old C57BL/6 mice (National Cancer Institute) in HBSS containing 10 mM HEPES (pH 7.4) and 3.5 mM $NaHCO_3$ (HHBSS). The cells were washed with HHBSS and suspended in RPMI 1640 medium containing 2 mM glutamine, penicillin (12.5 U/ml), streptomycin (6 μg/ml), and 5% FBS; plated in six-well plates (3×10^6 cells/well); and incubated for 2 h at 37°C in a humidified CO_2 (5%) incubator. The monolayers were washed with HHBSS three times to remove nonadherent cell, and the monolayers were incubated overnight at 37°C in the above RPMI 1640 medium before study.

Immunoprecipitation of GRP78 and MTJ-1 in plasma membranes

Macrophages (3×10^6 cells/well) incubated overnight were washed twice with HHBSS, and a volume of the medium was added. The medium was aspirated, and to the monolayers was added a volume of chilled HHBSS buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 1 mM PMSF, 10 μM benzamidine, and 10 μM leupeptin. The cells were scraped into chilled glass homogenizing tubes and plasma membranes were isolated as described previously (28, 36). Briefly, the cells were homogenized by 30 up-down strokes with a Teflon pestle at 4°C. The homogenate was centrifuged at $600 \times g$ for 5 min at 4°C, and the pellet was discarded. The homogenate was layered onto a sucrose step gradient of 50 and 30% (3 ml each) and centrifuged at $200{,}000 \times g$ for 75 min in a Beckman Coulter ultracentrifuge (model Optma LE 80) at 4°C. The plasma membrane fraction at the interface between the sucrose layers was removed and suspended in a volume of incubation buffer containing 25 mM HEPES (pH 7.4), 10 mM KCl, 3 mM NaCl, 5 mM $MgCl_2$, 2 mM leupeptin, 1 mM PMSF, and 1 mM Ca^{2+}. The suspension was centrifuged at $400{,}000 \times g$ for 90 min as described above. The pellet was suspended in a volume of incubation buffer. The purity of the plasma membrane (92–95%) was assessed as described previously, including electron microscopy (28, 36). The plasma membrane pellet was lysed in lysis buffer containing 20 mM Tris-HCl (pH 8.6), 0.1 mM NaCl, 1 mM EDTA, 50 mM sodium fluoride, 30 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, 20 μg/ml leupeptin, and 0.5% Nonidet P-40 for 10 min on ice. The DNA strands were broken by passing the lysates through a 27-gauge needle and syringe several times. The lysates were centrifuged at $800 \times g$ for 5 min at 4°C to remove cell debris. The supernatant was transferred to a clean tube, and protein contents were determined (37). An equal amount of lysate protein was immunoprecipitated with buffer, GRP78 Abs, or Abs diverted against MTJ-1, respectively, according to the manufacturer's instructions. As a control, nonimmune rabbit serum was used in similar studies. No GRP78 or MTJ-1 was precipitated under these conditions (data not shown). The respective immunoprecipitates were washed three times with lysis buffer, a volume of sample buffer was added, and the samples were boiled and centrifuged. The preparations were then electrophoresed on gels, transferred to membranes, immunoblotted with Abs against GRP78 and MTJ-1, and visualized and quantified by ECF in a Storm 800 phosphorimager (Amersham Biosciences). The GRP78 immunoblot was reprobed for MTJ-1, and the mMTJ-1 immunoblot was reprobed for GRP78 according to the manufacturer's instructions.

Detection of cell surface GRP78 using a cellular ELISA (CELISA)

This was essentially performed as previously described (38). Macrophages incubated overnight in RPMI 1640 medium containing penicillin (12.5 U/ml) and streptomycin (6.5 μg/ml) were scraped into 15-ml polypropylene tubes and centrifuged at 400 rpm for 5 min at 4°C to remove broken cells. The pellet was suspended in PBS, pipetted into 96-well culture plates (5×10^4 cells/well), and centrifuged at $100 \times g$ for 5 min. Without disturbing the cell layer, the glutaraldehyde solution was added in PBS at 4°C. After 5 min the plate was removed, and glutaraldehyde was washed away by immersing the plates in three successive tanks containing PBS and discarding PBS with 2% BSA. The cells were kept in this solution for 1 h at room temperature to saturate protein-binding sites on the plastic. Wells in triplicate were then incubated with specific rabbit anti GRP78 IgG (Santa Cruz Biotechnology) or nonimmune rabbit IgG (100 ng/well) at 22°C for 90 min, followed by three rinses with PBS. The cells were incubated with an alkaline phosphatase-conjugated anti-rabbit IgG (0.2 ml of PBS containing 50 ng of secondary Ab) at 22°C for 90 min. Bound IgG was monitored by the hydrolysis of the alkaline phosphatase substrate-*p*-nitrophenyl phosphate at a wavelength of 405 nm using an Anthos Labtec kinetic plate reader. Cell surface GRP78 was expressed as the change in $A_{405\ nm/min}$.

Chemical synthesis of dsRNA homologous in sequence to the target MTJ-1 gene

The chemical synthesis of dsRNA homologous in sequence to the target MTJ-1 K_{321} RQAPEW$_{327}$ peptide (mRNA sequence 5′-AAAAGACAAG CACCAGAATGG-3′; Swiss-Pror, primary accession no. Q61712) was performed by Ambion. For making dsRNA, the sense (5′-AAGACAAG CACCAGAAUGGtt-3′) and antisense (5′-CCAUUCUGGUGCUUGU CUUTT-3′) oligonucleotides were annealed according to the manufacturer's instructions. Throughout the entire period of experimentation, handling of reagents was performed in an RNase-free environment. Briefly, equal amounts of sense and antisense oligonucleotides were mixed in annealing buffer and heated at 90°C for 1 min, then maintained for 1 h at 37°C in an incubator. The dsRNA preparation was stored at −20°C before use.

Determination of mRNA levels of MTJ-1 by RT-PCR

Macrophages incubated overnight in RPMI 1640 medium containing 10% FBS, penicillin (12.5 U/ml), streptomycin (6.5 μg/ml), and 2 mM glutamine were washed twice with serum-free and antibiotic-free DMEM. The monolayers in the respective wells were layered with 1 ml of Lipofectamine and DMEM or Lipofectamine and MTJ-1 dsRNA (25 μg/ml). The contents were gently mixed, and monolayers were incubated for 5 h at 37°C in a humidified CO_2 incubator. At the end of the incubation, 1 ml of antibiotic-free medium containing 10% FBS was added to each well, and cells were incubated for 16 h as described above. The medium was replaced with DMEM containing FBS and antibiotics, and cells incubated overnight. The incubations were terminated by aspirating the medium and adding a volume of DMEM to each well. Total RNA from respective monolayers was extracted by a single-step method using an RneasyR minikit (Qiagen) according to the manufacturer's instructions. Total RNA was reverse transcribed with 1 μg of RNA in a 20-μl reaction mixture, using Maloney murine leukemia virus transcriptase (200 U) and oligo(dt) as the primer for 1 h at 42°C. The resulting cDNA (5 μl) was used as a template, and a 350-bp segment of cDNA was amplified using a 21-mer upstream primer (5′-AAGTATGGTTAAGTTCCCAGG-3′) identical with positions corresponding to aa 339–349 and a 21-mer down primer (5′-CTGG AATCGTTTTGTTCTGAT-3′) complimentary to positions 472–481 aa encoded by MTJ-1 mRNA. A 302-bp segment of mouse β-actin (constitutive internal control) cDNA was coamplified using a set of PCR printers provided in an R&D Systems kit. Amplification was conducted in a Techne Thermal Cycler PHC for two cycles (one cycle: 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s). PCR products were analyzed on a 1.2% (w/v) agarose-ethidium bromide gel. The gels were photographed, and the intensities of the MTJ-1 and β-actin mRNA bands were quantified using a 860 PhosphorImager (Molecular Dynamics, marketed by Amersham Biosciences).

Measurement of MTJ-1 protein levels in MTJ-1 gene-silenced cells by Western blotting

Macrophages incubated overnight in RPMI 1640 medium containing 10% FBS, penicillin (12.5 U/ml), streptomycin (6.5 μg/ml), and 2 mM glutamine were washed twice with serum-free and antibiotic-free DMEM, and monolayers in respective wells were layered with 1 ml of Lipofectamine and DMEM, Lipofectamine and MTJ-1 dsRNA (25 μg/ml), or Lipofectamine and scrambled RNA (25 μg/ml). The contents were gently mixed, and monolayers were incubated for 5 h at 37°C in a humidified CO_2 incubator. At the end of incubation, 1 ml of antibiotic-free medium containing 10% FBS was added to each well, and cells were incubated for 16 h as described above. The medium was replaced with DMEM containing

FBS and antibiotic, and cells were incubated overnight. The incubations were terminated by aspirating the medium, and cells were lysed in lysis buffer and processed for electrophoresis, immunoblotting, and quantitation of immunoblots as described above.

Measurement of GRP78 and MTJ-1 protein levels in plasma membranes from MTJ-1 gene-silenced cells by Western blotting

The details of MTJ-1 dsRNA transfection, plasma membrane preparation, immunoprecipitation, Western blot detection, and visualization of GRP78 and MTJ-1 were identical with those described in previous sections.

Measurements of intracellular calcium levels in macrophages after silencing MTJ-1 gene expression

Changes in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) in MTJ-1 dsRNA-transfected macrophages upon stimulation with α_2M* (100 pM) were measured as described previously (27–30). Briefly, macrophages (1.5×10^5 cells/coverslip) incubated overnight on glass coverslips were transfected with MTJ-1 dsRNA (25 μg/ml) and incubated in DMEM containing 10% FBS and antibiotics as described above. Forty-eight hours post-transfection, the cells were loaded with fura-2/AM (4 μM/30 min) in the dark at room temperature, washed with HHBSS, and then placed on the stage of a digital microscope to determine $[Ca^{2+}]_i$. Basal $[Ca^{2+}]_i$ levels in the fura-2/AM-preloaded cells were measured for 3 min before the addition of α_2M* (100 pM).

Results

GRP78 and MTJ-1 are coimmunoprecipitated in plasma membrane lysates

Recently, we have shown that GRP78, in addition to its predominant ER location, is a cell surface receptor for α_2M* in macrophages and 1-LN cells (29, 30). MTJ-1, a J domain-containing cochaperone, is a partner of intracellular GRP78 in many of its functions during maintenance of protein quality control in the ER. We hypothesized that MTJ-1 may also play a role in the translocation of its partner GRP78 to the cell surface. If this were so, then one would expect the presence of both MTJ-1, and GRP78 in plasma membranes of the cell. We have addressed this by isolating plasma membranes from macrophages and probing for GRP78 (Fig. 1*A*) and MTJ-1 (Fig. 1*B*) in the GRP78 immunoprecipitate and for MTJ-1 (Fig. 1*A*) and GRP78 (Fig. 1*B*) in the MTJ-1 immunoprecipitate from plasma membrane lysates by Western blotting. The studies demonstrate that immunoprecipitation with GRP78 and MTJ-1 polyclonal Abs, respectively, contained GRP78 and MTJ-1, which suggests that both GRP78 and MTJ-1 are colocalized in the plasma membranes of murine peritoneal macrophages.

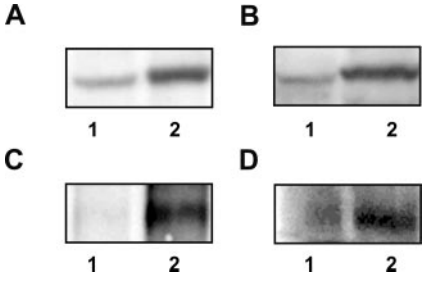

FIGURE 1. MTJ-1 and GRP78 are coimmunoprecipitated in plasma membranes lysates. Experimental details are described in *Materials and Methods*. The presence of GRP78 and MTJ-1 in GRP78 and MTJ-1 immunoprecipitates from plasma membrane lysates was determined by Western blotting. *A*, GRP78 protein in GRP78 immunoprecipitate (*lane 1*) and MTJ-1 immunoprecipitate (*lane 2*); *B*, MTJ-1 protein in GRP78 immunoprecipitate (*lane 1*) and MTJ-1 immunoprecipitate (*lane 2*). *C*, GRP78 protein in MTJ-1 immunoprecipitate in plasma membrane lysates from cells transfected with MTJ-1 dsRNA; *D*, MTJ-1 protein in MTJ-1 immunoprecipitate of plasma membranes from MTJ-1 dsRNA-transfected cells. The lanes in both panels are Lipofectamine and MTJ-1 dsRNA (*lane 1*) and Lipofectamine (*lane 2*). The immunoblots shown are representative of two independent experiments.

Silencing of MTJ-1 gene expression by RNA interference blocks the association of GRP78 with the plasma membrane

In the next series of experiments we evaluated the role of MTJ-1 in cell surface translocation of GRP78 by silencing the expression of the gene using RNA interference. The levels of both MTJ-1 mRNA (Fig. 2*A*) and protein (Fig. 2*B*) were greatly reduced in cells transfected with MTJ-1 dsRNA, but it had no effect on GRP78 mRNA levels (Fig. 2*A*). Transfection of cells with scrambled dsRNA showed negligible effects on MTJ-1 protein levels (Fig. 2*B*), demonstrating the specificity of gene silencing with MTJ-1 dsRNA (Fig. 2). Silencing of MTJ-1 gene expression, however, caused loss of GRP78 protein (Fig. 1*C*) and MTJ-1 protein (Fig. 1*D*) from the plasma membrane. These studies demonstrate that MTJ-1 is required for cell surface trafficking of GRP78.

*Silencing of MTJ-1 gene expression suppresses cell surface binding of α_2M**

We have previously shown that α_2M* binds to cell surface α_2MSR/GRP78 with high affinity (25–30). We reasoned that if MTJ-1 is indeed involved in the translocation of GRP78 to the cell surface, then silencing of the MTJ-1 gene would limit the availability of cochaperone MTJ-1 for translocation of GRP78 to the cell surface, causing a reduction in the level of cell surface GRP78, resulting in reduced cell surface binding of GRP78. Indeed, this was found to be the case (Fig. 3). Silencing of MTJ-1 gene expression also reduced the binding of α_2M* by ~80% (Fig. 3). In cells transfected with scrambled RNA there was a negligible effect

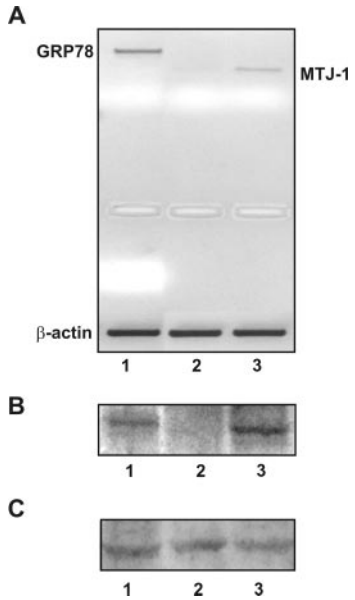

FIGURE 2. Silencing of the expression of the MTJ-1 gene by RNA interference suppresses mRNA and protein levels of MTJ-1. *A*, mRNA levels of MTJ-1 and GRP78 in cells transfected with MTJ-1 dsRNA. *Lane 1*, GRP78 in cells treated with Lipofectamine and MTJ-1 dsRNA; *lane 2*, MTJ-1 in cells treated with Lipofectamine and MTJ-1 dsRNA; *lane 3*, Lipofectamine. *B*, Protein levels of MTJ-1 in cells transfected with MTJ-1 dsRNA. *Lane 1*, Lipofectamine; *lane 2*, Lipofectamine and MTJ-1 dsRNA; *lane 3*, Lipofectamine and scrambled dsRNA. *C*, Protein levels of GRP78 in cells transfected with MTJ-1 dsRNA. *Lane 1*, Lipofectamine; *lane 2*, Lipofectamine and MTJ-1 dsRNA; *lane 3*, Lipofectamine and scrambled dsRNA.

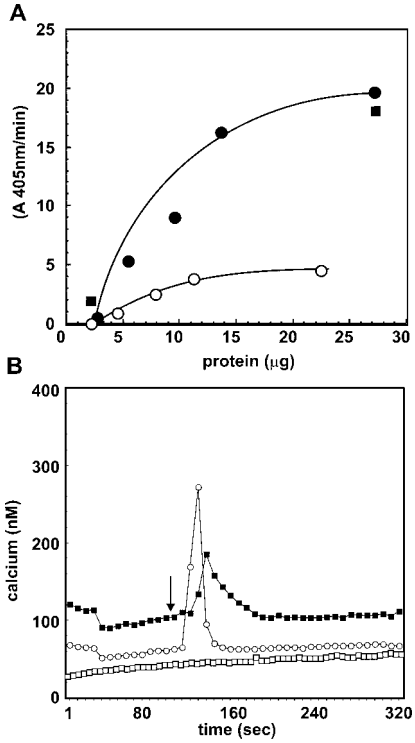

FIGURE 3. Silencing of MTJ-1 gene expression by RNA interference suppresses cell surface binding of α_2M* and α_2M*-induced calcium signaling. *A*, Cell surface binding of α_2M* to macrophages. ●, Cells treated with Lipofectamine and α_2M*; ○, cells transfected with MTJ-1 dsRNA and then exposed to α_2M*; ■, cells transfected with Lipofectamine and scrambled dsRNA. The values are expressed as an average of two experiments. *B*, Changes in intracellular levels of calcium in cells on exposure to α_2M*. Cells were treated with Lipofectamine and stimulated with α_2M* (100 pM; ○), transfected with MTJ-1 dsRNA and then exposed to α_2M* (100 pM; □), or treated with Lipofectamine and scrambled dsRNA (■). The arrow indicates the time of addition of α_2M*. The data shown are representative of two experiments using 25–30 cells/experiment.

on α_2M* binding (Fig. 3). Because silencing of MTJ-1 gene expression did not affect mRNA levels of GRP78, but did affect its cell surface expression and α_2M* binding, it was suggested that limitations imposed on MTJ-1 availability affected the cell surface expression of GRP78.

Suppression of α_2M-induced calcium signaling in macrophages transfected with MTJ-1 dsRNA*

Binding of α_2M* to GRP78 on the surface of cells such as macrophages activates signaling cascades and elevates the levels of $[Ca^{2+}]_i$ by several-fold within 30 s (25–30, 39). Therefore, calcium signaling can be used as a parameter of cell surface expression of α_2MSR/GRP78. To further affirm the role of MTJ-1 in cell surface translocation of GRP78, we examined α_2M*-dependent calcium signaling patterns in cells transfected with MTJ-1 dsRNA (Fig. 3). Silencing of MTJ-1 gene expression reduced the increase in $[Ca^{2+}]_i$ upon exposure of these cells to α_2M* compared with that in cells exposed to Lipofectamine and α_2M* (Fig. 3). In an earlier report we showed that the silencing of GRP78 gene expression also suppressed α_2M*-induced calcium signaling (29, 30). Taken together, these results demonstrate that MTJ-1, a modulator of GRP78 catalytic activity, is also involved in the translocation of GRP78 to the cell surface.

Discussion

The principal findings of this study are that GRP78 and MTJ-1 are coimmunoprecipitated in plasma membranes, and silencing of MTJ-1 gene expression greatly suppresses its mRNA and protein levels, the occurrence of GRP78 in the plasma membrane fraction, cellular binding of α_2M*, and α_2M*-induced calcium signaling. These results unequivocally show that the cochaperone MTJ-1 is functionally involved in the transport of the chaperone GRP78 to the plasma membrane, where MTJ-1/GRP78 complexes behave as a signaling complex.

In eukaryotic cells, most polypeptides destined to become membrane protein are initially integrated into the membrane of ER before being sorted to the location at which they function. In most cases, proteins are integrated into the bilayer cotranslationally. Regulated surface expression of plasma membrane receptors is key to normal cellular physiology (40, 41). Membrane trafficking proceeds under tight control to establish steady state expression levels, respond to acute stimuli, and monitor the quality of secreted multimeric products (40, 41). MTJ-1 is a membrane DnaJ-like protein, is an accessory protein for GRP78 catalytic activity, and functions as a cochaperone of GRP78 in protein folding and translocation of newly synthesized polypeptides across the ER membrane (8–18). Multiple DnaJ-like proteins are present in subcellular compartments and are often concentrated to different locations, which enables HSP70 family members to catalyze localized protein reactions (15). MTJ-1 contains a lumenal J domain, a single membrane domain, and a cytosolic domain in close contact with translating ribosomes in microsomes (14). MTJ-1 mediates transmembrane recruitment of DnaK-like chaperones to ribosomes and transmembrane signaling between ribosomes and DnaK-like chaperones of the ER (14). Cochaperones can also show chaperone-like activity. They, too, can bind to unfolded or partially folded polypeptides, prevent them from aggregation, and maintain them in a folding-competent conformation. DnaJ-like cochaperones can be highly divergent in their functions. For example, HSP90 and HSP70 chaperones and Hop, HSP40, and p23 cochaperones are required for the activation of steroid binding activity and assembly of glucocorticoid receptor (39, 40). Depletion of endogenous DnaJ-like proteins from rabbit reticulocyte lysates inhibited the association between glucocorticoid receptor and HSP90, and addition of purified Ydji protein restored binding (41).

Chaperones are retained in the ER by cognate receptors, which constantly retrieve escaped chaperones from a dynamic intermediate compartment between the ER and the Golgi. ER receptors responsible for protein retention recognize motifs encoded in the primary amino acid sequences, such as the KDEL tetrapeptide sequence for lumenal chaperones (42), and dilysine motifs for membrane-bound chaperones (43) at their COOH terminal. ER-resident chaperones containing KDEL motifs include GRP78, GRP94, calreticulin, and protein disulfide isomerase. However, there is growing evidence that some of these ER chaperones are found on the cell surface of certain cells and have functions in cellular physiology. In human fibroblasts, surface calreticulin functions as a receptor for fibrinogen and is essential for mitogenic effects (44). In melanoma cells, surface calreticulin is responsible for the initiation of cell spreading. Protein disulfide isomerase is present on the platelet plasma membrane and is involved in pathological functions (45). Immature $CD4^-/CD8^-$ thymocytes demonstrate cell surface expression of the molecular chaperone calnexin (46). The expression of GRP78 on the cell surface has been reported in a number of studies (19–33). GRP78 is a cell surface-expressed receptor that is involved in mitogenesis and cellular proliferation (29, 30). In addition to its function as a signaling receptor, cell

surface-expressed GRP78 is associated with MHC class I Ags (31) and has been identified as a receptor for both Dengue virus (32) and coxsackievirus A9 (33). These observations show that ER-resident GRP78 escapes ER retention and translocates to the cell surface. The escape of resident protein from the ER is not the property of all cells and proteins, but, rather, appears to be somewhat selective (23). Also, the escape of proteins from the ER requires ongoing protein synthesis, which suggests that newly synthesized proteins are able to escape, whereas preformed ER proteins are retained quite efficiently (23). The association of cell surface-appearing chaperones with other proteins, including co-chaperones, during the processes of folding and maturation may be involved in their escape from ER retention. Such chaperone-protein complexes may block the domains that are critical for ER retention (23, 47–50). Misfolded or resident proteins that escape the ER are retrieved into coat protein 1 vesicles via interaction with cytoplasmic dibasic retrieval motifs (51). Cytoplasmic protein 14-3-3 acts in a protein kinase A phosphorylation-dependent manner to inhibit the association of coat protein 1 with dibasic sites and allow forward transport (51). Surface expression of the potassium channels, $KCNK_3$ or $KCNK_9$, α_4-nicotinic acetylcholine receptor, and lip35, is mediated by 14-3-3 release motifs (48). At present we do not understand the mechanism(s) by which MTJ-1 is involved in the transport of GRP78 to plasma membranes. However, based upon our previous results (25–30), we hypothesize that MTJ-1/GRP78 membrane complexes involved in ER protein quality control may escape the ER and be transported to the plasma membrane.

In summary, our studies suggest that MTJ-1 is essential for cell surface targeting of GRP78. Because MTJ-1 spans the membrane, whereas GRP78 does not, we suggest that it is essential for the receptor functions of GRP78. Moreover, other proteins may be carried to the surface as part of an MTJ-1/GRP78 complex, resulting in a signaling receptor. Such a complex must include various accessory proteins as well as heterotrimeric G proteins (25–30, 36). Additional studies will be required to probe the role of MTJ-1 in the uptake of viruses such as Dengue or coxsackie (45, 46).

Acknowledgments

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References

1. Hartl, F. N. 1996. Molecular chaperones in cellular protein folding. *Nature 381:571*.
2. Bukau, B., and A. L. Horwich. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell 92:351*.
3. Corsi, A. K., and R. Schekman. 1996. Mechanism of polypeptide translocation into the endoplasmic reticulum. *J. Biol. Chem. 271:30299*.
4. Gething, M.-J. 1999. Role and regulation of the ER chaperone BiP. *Semin. Cell Dev. Biol. 10:465*.
5. Alder, N. N., and A. E. Johnson. 2004. Cotranslational membrane protein biogenesis at the endoplasmic reticulum. *J. Biol. Chem. 279:22787*.
6. McKay, D. B. 1993. Structure and mechanism of 70-kDa heat-shock-related proteins. *Adv. Protein Chem. 44:67*.
7. Lee, A. S. 2001. The glucose-regulated proteins: stress induction and clinical applications. *Trends Biochem. Sci. 26:504*.
8. Chevalier, M., H. Rhee, E. C. Elguindi, and S. Y. Blond. 2000. Interaction of murine BiP/GRP78 with the DnaJ homologue MTJ1. *J. Biol. Chem. 275:19620*.
9. Cheetham, M. E., and A. J. Caplan. 1998. Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chap. 3:28*.
10. Cyr, D. M., T. Langer, and M. G. Douglas. 1994. DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. *Trends Biochem. Sci. 19:176*.
11. Liberek, K., J. Marszalek, D. Ang, C. Georgopoulos, and M. Zylicz. 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. *Proc. Natl. Acad. Sci. USA 88:2874*.
12. Wickner, S., D. Skowyra, J. Hoskins, and K. McKenney. 1992. DnaJ, DnaK, and GrpE heat shock proteins are required in oriP1 DNA replication solely at the RepA monomerization step. *Proc. Natl. Acad. Sci. USA 89:1034*.
13. Chung, K. T., Y. Shen, and L. M. Hendershot. 2002. BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. *J. Biol. Chem. 277:47557*.
14. Dudek, J., J. Volkmer, C. Bies, S. Guth, A. Müller, M. Lerner, P. Feick, K.-H. Schäfer, E. Morgenstern, F. Hennessy, et al. 2002. A novel type of co-chaperone mediates transmembrane recruitment of DnaK-like chaperones to ribosomes. *EMBO J. 21:2958*.
15. Lu, Z., and D. M. Cyr. 1998. Protein folding activity of Hsp70 is modified differentially by the hsp40 co-chaperones Sis1 and Ydj1. *J. Biol. Chem. 273:27824*.
16. Georgopoulos, C. P., A. Lundquist-Heil, J. Yochem, and M. Feiss. 1980. Identification of the E. coli dnaJ gene product. *Mol. Gen. Genet. 178:583*.
17. Hosoda, A., Y. Kimata, A. Tsuru, and K. Kohno. 2003. JPDI, a novel endoplasmic reticulum-resident protein containing both a BiP-interacting J-domain and thioredoxin-like motifs *J. Biol. Chem. 278:2669*.
18. Brightman, S. E., G. L. Blatch, and B. R. Zetter. 1995. Isolation of a mouse cDNA encoding MTJ1, a new murine member of the DnaJ family of proteins. *Gene 153:249*.
19. Delpino, A., and M. Castelli. 2002. The 78 kDa glucose-regulated protein (GRP78/BIP) is expressed on the cell membrane, is released into cell culture medium and is also present in human peripheral circulation. *Biosci. Rep. 22:407*.
20. Xiao, G., T.-F. Chung, H. Y. Pyun, R. E. Fine, and R. J. Johnson. 1999. KDEL proteins are found on the surface of NG108–15 cells. *Mol. Brain Res. 72:121*.
21. Takemoto, H., T. Yoshimori, A. Yamamoto, Y. Miyata, I. Yahara, and K. Inoue. 1992. Heavy chain binding protein (BiP/GRP78) and endoplasmin are exported from the endoplasmic reticulum in rat exocrine pancreatic cells, similar to protein disulfide-isomerase. *Arch. Biochem. Biophys. 296:129*.
22. Altmeyer, A., R. G. Maki, A. M. Feldweg, M. Heike, V. P. Protopopov, S. K. Masur, and P. K. Srivastava. 1996. Tumor-specific cell surface expression of the-KDEL containing, endoplasmic reticular heat shock protein gp96. *Int. J. Cancer 69:340*.
23. Weist, D. I., A. Bhandoola, J. Punt, G. Kreibich, D. McKean, and A. Singer. 1997. Incomplete endoplasmic reticulum (ER) retention in immature thymocytes as revealed by surface expression of "ER-resident" molecular chaperones. *Proc. Natl. Acad. Sci. USA 94:1884*.
24. Berger, C. L., Z. Doug, D. Hanlon, E. Bisaccia, and R. L. Edelson. 1997. Incomplete endoplasmic reticulum (ER) retention in immature thymocytes as revealed by surface expression of "ER-resident" molecular chaperones. *Int. J. Cancer 71:1077*.
25. Misra, U. K., C. T. Chu, G. Gawdi, and S. V. Pizzo. 1994. Evidence for a second α_2-macroglobulin receptor. *J. Biol. Chem. 269:12541*.
26. Misra, U. K., C. T. Chu, G. Gawdi, and U. K. Misra. 1994. The relationship between low density lipoprotein-related protein/α_2-macroglobulin (α_2-M) receptors and the newly described α_2M signaling receptor. *J. Biol. Chem. 269:18303*.
27. Misra, U. K., G. Gawdi, M. Gonzalez-Gronow, and S. V. Pizzo. 1999. Coordinate regulation of the α_2-macroglobulin signaling receptor and the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor by insulin *J. Biol. Chem. 274:25785*.
28. Misra, U. K., and S. V. Pizzo. 2002. Regulation of cytosolic phospholipase A2 activity in macrophages stimulated with receptor-recognized forms of α_2-macroglobulin: role in mitogenesis and cell proliferation *J. Biol. Chem. 277:4069*.
29. Misra, U. K., M. Gonzalez-Gronow, G. Gawdi, J. P. Hart, K. E. Johnson, and S. V. Pizzo. 2002. The role of Grp 78 in α_2-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. *J. Biol. Chem. 277:42082*.
30. Misra, U. K., M. Gonzalez-Gronow, G. Gawdi, F. Wang, and S. V. Pizzo. 2004. A novel receptor function for the heat shock protein Grp78: silencing of Grp78 gene expression attenuates α_2M*-induced signalling. *Cell. Signal. 16:929*.
31. Triantafilou, M., D. Fradelizi, and K. Triantafilou. 2001. Major histocompatibility class one molecule associates with glucose regulated protein (GRP) 78 on the cell surface *Hum. Immunol. 62:764*.
32. Jindadamrongwech, S., C. Thepparit, and D. R. Smith. 2004. Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. *Arch. Virol. 149:915*.
33. Triantafilou, K., D. Fradelizi, K. Wilson, and M. Triantafilou. 2002. GRP78, a coreceptor for coxsackievirus A9, interacts with major histocompatibility complex class I molecules which mediate virus internalization. *Virology 76:633*.
34. Jorgensen, M. M., O. N. Jensen, H. K. Holst, J. J. Hansen, T. J. Corydon, P. Bross, L. Bolund, and N. Gregerson. 2000. Grp78 is involved in retention of mutant low density lipoprotein receptor protein in the endoplasmic reticulum *J. Biol. Chem. 275:33861*.
35. Xu, M., K. D. Dittmar, G. Giannoukos, W. B. Pratt, and S. S. Simons, Jr. 1998. Binding of hsp90 to the glucocorticoid receptor requires a specific 7-amino acid sequence at the amino terminus of the hormone-binding domain *J. Biol. Chem. 273:13918*.
36. Misra, U. K., and S. V. Pizzo. 1999. Upregulation of macrophage plasma membrane and nuclear phospholipase D activity on ligation of the α_2-macroglobulin signaling receptor: involvement of heterotrimeric and monomeric G proteins *Arch. Biochem. Biophys. 363:68*.
37. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem. 72:248*.
38. Stocker, J. W., and C. H. Hensser. 1979. Methods for binding cells to plastic: application to a solid-phase radioimmunoassay for cell-surface antigens *J. Immunol. Methods 26:87*.
39. Misra, U. K., C. T. Chu, D. S. Rubenstein, G. Gawdi, and S. V. Pizzo. 1993. Receptor-recognized α_2-macroglobulin-methylamine elevates intracellular calcium, inositol phosphates and cyclic AMP in murine peritoneal macrophages. *Biochem. J. 290:885*.

40. Mellman, I., and G. Warren. 2000. The road taken: past and future foundations of membrane traffic. *Cell 100:99*.
41. Ellgaard, L., M. Molinari, and A. Helenius. 1999. Setting the standards: quality control in the secretory pathway. *Science 286:1882*.
42. Munro, S., and H. R. B. Pelham. 1987. A C-terminal signal prevents secretion of luminal ER proteins. *Cell 48:899*.
43. Jackson, M. R., T. Nilsson, and P. A. Peterson. 1990. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J. 9:3153*.
44. Gray, A. J., P. W. Park, T. J. Broekelmann, G. J. Laurent, J. T. Reeves, K. R. Stenmark, and R. P. Mecham. 1995. The mitogenic effects of the Bβ chain of fibrinogen are mediated through cell surface calreticulin. *J. Biol. Chem. 270:26602*.
45. White, T. K., Q. Zhu, and M. L. Tanzer. 1995. Cell surface calreticulin is a putative mannoside lectin which triggers mouse melanoma cell spreading. *J. Biol. Chem. 270:15926*.
46. Wiest, D. L., W. H. Burgess, D. McKean, K. P. Kearse, and A. Singer. 1995. The molecular chaperone calnexin is expressed on the surface of immature thymocytes in association with clonotype-independent CD3 complexes. *EMBO J. 14:3425*.
47. Klausner, R. D., J. Lippincott-Schwartz, and J. S. Bonifacino. 1990. The T cell antigen receptor: insights into organelle biology. *Annu. Rev. Cell. Biol. 6:403*.
48. Zerangue, N., B. Schwappach, Y. N. Jan, and L. Y. Jan. 1999. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron 22:537*.
49. Standley, S., K. W. Roche, J. McCallum, N. Sans, and R. J. Wenthold. 2000. PDZ domain suppression of an ER retention signal in NMDA receptor NR1 splice variants. *Neuron 28:887*.
50. Zarei, M. M., N. Zhu, A. Alioua, M. Eghbali, E. Stefani, and L. Toro. 2001. A novel MaxiK splice variant exhibits dominant-negative properties for surface expression. *J. Biol. Chem. 276:16232*.
51. O'Kelly, I., M. H. Butler, N. Zilberberg, and S. A. N. Goldstein. 2002. Forward transport. 14-3-3 binding overcomes retention in endoplasmic reticulum by dibasic signals. *Cell 111:577*.