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LMAN1 (ERGIC-53) is a potential carrier protein for matrix metalloproteinase-9 glycoprotein secretion

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ABSTRACT

Matrix metalloproteinase-9 (MMP-9) is a secreted glycoprotein with a major role in shaping the extracellular matrix and a detailed understanding of the secretory mechanism could help identify methods to correct diseases resulting from dysregulation of secretion. MMP-9 appears to follow a canonical secretory pathway through a quality control cycle in the endoplasmic reticulum (ER) before transport of the properly folded protein to the Golgi apparatus and beyond for secretion. Through a complementation assay, we determined that LMAN1, a well-studied lectin-carrier protein, interacts with a secretion-competent N-glycosylated MMP-9 in the ER while N-glycosylation-deficient secretion-compromised MMP-9 does not. In contrast, co-immunoprecipitation demonstrated protein interaction between LMAN1 and secretion-compromised N-glycosylation-deficient MMP-9. MMP-9 secretion was reduced in the LMAN1 knockout cell line compared to control cells confirming the functional role of LMAN1. These observations support the role of LMAN1 as a lectin-carrier protein mediating efficient MMP-9 secretion.

1. Introduction

MMP-9 is a secreted glycoprotein protease with a pleotropic function playing critical roles in shaping the extracellular matrix, activating cytokines, and possibly cell signaling through proteolytic activation of intracellular substrates (reviewed in Ref. [1]). Details of the transcriptional activation of MMP-9 in cells have been reported but our understanding of the intracellular events that result in the secretion of this protease is only beginning to be explored. Like most glycoproteins, MMP-9 is thought to progress from the co-translational *en bloc* acquisition of a core oligosaccharide complex at the specified N-residues by an N-glycosidic bond with further trimming and maturation of the glycosylation tree as it traverses from the endoplasmic reticulum (ER) to the Golgi apparatus and beyond for secretion of the properly folded protein.

Secretion of glycoproteins is thought to occur by a carrier-mediated or a bulk flow mechanism. According to the carrier-mediated theory co-translationally N-glycosylated nascent glycoproteins in the ER traverse through the CANX/CALR cycle until the properly folded protein is passed on to a carrier lectin for transport to the Golgi apparatus. Alternatively, secretion can proceed via the bulk flow mechanism without involvement of a transport lectin and secretion is precluded only when misfolded proteins are retained within the ER [2–5]. Identification of a putative protein carrier interacting with the properly folded MMP-9 in an N-glycosylation-specific manner would support carrier-mediated secretion over the bulk-flow mechanism but no such carrier has been identified.

A complementation assay based on two-halves of an YFP fluorophore, one tagged to an ER resident protein bait and another tagged to a glycoprotein, reporting on an ER-specific interaction of two proteins has been described [6]. We utilized a complementation assay between the Lectin Mannose-Binding 1 (LMAN1) (also known as ERGIC-53) as the bait and MMP-9 to determine whether this lectin-carrier interacts with MMP-9 in the ER. Furthermore, a Cas9/CRISPR-mediated LMAN1-knock out cell line was created to confirm the functional role of LMAN1 in MMP-9 secretion.

2. Materials and methods

2.1. Molecular constructs

A cartoon illustration of all created molecular constructs in the eukaryotic expression vector, pCI/neo (Promega, Madison, WI) and the restriction enzyme sites used for subcloning are shown in

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Fig. S1. Table S1 and Table S2 lists all the oligonucleotide PCR primers and gene fragment sequences synthesized by a vender (GenScript, Piscataway, NJ), respectively.

2.2. YFP complementation constructs and assay

The YFP fragmentation strategy was previously described in Nyfeler et al. [6]. cDNAs encoding LMAN1 (#HG16166-G) was purchased from Sino Biological Inc (Beijing, China). LMAN1 and MMP-9 lacking the signal sequence were PCR amplified with flanking Mlu I sites and cloned into the YFP fragment pCI/neo fusion vectors (see Fig S1). Efficient complementation between the bait and the prey eYFP fusion constructs depends on the geometry of the two interacting proteins. MMP-9 is a soluble protein released into the ER-lumen and no information was available on deciding the best placement of the YFP2-fusion construct, therefore all permutations of the placement of the eYFP fragments on the N- or the C-termini were examined. α1-antitrypsin (A1AT), previously documented to secrete with LMAN1 as the lectin-carrier [7], served as the positive control for the complementation and secretion assays. For the complementation assay HEK293 cells grown overnight (~70–90% confluence) in a 12-well plate were transfected with LMAN1 tagged with nYFP1 and MMP-9 either C-terminally or N-terminally tagged with YFP2 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). 48 h post-transfection cell media was aspirated, suspended in 200 μL PBS, and transferred to a black 96-well plate. YFP fluorescence was recorded using an excitation wavelength/bandwidth of 485/20 and emission wavelength/bandwidth of 528/20 with a Synergy 2 microplate reader (BioTek Inc).

2.3. N-glycosylation-deficient mutant MMP-9 constructs

Starting with the wild type human MMP-9 complementary deoxyribonucleic acid (cDNA; Accession BC006093, Image Clone MGC: 12688) we engineered the desired nucleotide switch by overlap polymerase chain reaction (PCR) with primers listed in Table S1 and subcloned it into the wild type-MMP-9 background. We created an Asn to Ser mutation at residue 38 that contained silent mutations designed to disrupt miRNA-binding sites while conserving amino acid sequence in order to express an N38-glycosylation deficient Ser mutant that was found to be down-regulated through a single nucleotide polymorphism-specific miRNA mechanism [8]. We name this miRNA-binding disrupted N-glycosylation-deficient construct modified-S38 (mS38).

2.4. Secreted eGFP

The N-terminal signal sequence of a GPI-anchored T-cadherin was fused to the N-terminal of eGFP after removal of the endogenous start methionine [9]. The resulting T-cadherin signal sequence-eGFP chimera cDNA ($ss^{T\text{-}Cad}$.eGFP) was subcloned into the pCI/neo expression vector. When transfected into cells, the fusion eGFP protein was secreted into the cell culture media, used as a transfection control, and could be quantified by a fluorescent microplate reader [10].

2.5. Cell culture

Human embryonic kidney (HEK) 293 (#CRL-1573) cells (ATCC, Manassas, VA) were grown in Dulbecco's Modification of Eagle's Medium (Mediatech, Inc, Manassas, VA) with 4.5 g/L glucose, L-glutamine, sodium pyruvate, and supplemented with 10% heat inactivated fetal bovine serum (Mediatech Inc), 100 U/mL penicillin (Mediatech Inc), and 0.1 mg/mL streptomycin (Mediatech Inc). Castanospermine (CST) (#BML-S107) was purchased from Enzo Life Sciences (Farmingdale, NY).

2.6. CRISPR-mediated genomic Lman1 knockout

A 200 bp sequence of the N-terminal human LMAN1 cDNA (NM005570) was searched for guide RNA target sites using the online software (crispr.mit.edu/guides). Pairs of oligonucleotides encoding each of the four highest scoring guide RNA target sites were annealed and ligated into the lentiCRISPRv2 vector [11]; (Addgene #52961) after vector digestion with BsmBI following the Zhang Lab Lentiviral CRISPR Toolbox protocol (https://www.addgene.org/static/data/plasmids/52/52961/52961-attachment_B3xTwla0bkYD.pdf). The sg-blank control that had no insert was subcloned into the vector. Lentivirus rescued by co-transfection of the lentiCRISPRv2 harboring the target guide RNA site with the helper psPAX2 and pVSVG plasmids (Addgene #12260 and 12259) was used to transduce a 35 mm plate of HEK293 cells and selected with puromycin (2 μg/mL). The cells were propagated as a mixed culture and the loss of LMAN1 immunoreactive protein band confirmed by a Western blot. The most complete LMAN1 protein knock out was obtained with a guide RNA target site of TCACTCGGTCGCTTCGTCCG corresponding to nucleotides 100–119 of NM005570 on the positive strand within the coding sequence of *Lman1*.

2.7. Western blot

At various times post-transfection cell culture media and cell pellets were collected for analysis as previously described [12]. For co-immunoprecipitation, 50 μL Dynabeads Protein G (Invitrogen) were magnetically precipitated and washed with PBST (PBS with 0.2% Tween 20). A 1:150 dilution of mouse anti-HA antibody (Covance, Princeton, NJ) was incubated on a rotator at room temperature for 10 min. The beads were re-suspended in a solution of 100 μg of intracellular protein and rotationally incubated for 1 h. The beads were washed three times with 200 μL PBS, re-suspended in 20 μL of 5× PDR, boiled at 70 °C for 10 min, and analyzed by Western blot.

2.8. Chemical crosslinking

Cells were washed with cold PBS followed with crosslinking buffer (10 mM triethanolamine, pH 7.4, 250 mM sucrose, 2 mM $CaCl_2$) and then incubated for 30 min at 4 °C with 1 mM Dithiobis-(succinimidyl propionate) (DSP, #22585, Thermo Scientific, Rockford, IL). Cells were rinsed with quenching buffer (50 mM ethanolamine in crosslinking buffer) twice for 15 min each. A detailed description is available in Nyfeler et al. [13].

2.9. Immunocytochemistry and fluorescence microscopy

Fluorescently-tagged fusion proteins and overexpressed immuno-stained proteins were imaged as described previously [8] using endogenous fluorescence or anti-MMP-9 (1:250, UC-Davis) or anti-LMAN1 (1:250, #ab125006, Abcam Inc. Cambridge, MA) primary antibodies followed by Alexa 594-conjugated anti-mouse or Alexa 488-conjugated anti-rabbit secondary antibodies (Life Technologies, Carlsbad, CA).

2.10. Statistics

Comparison of the means was done by the Student t-test based on at least 3 independent biological replicates. $P < 0.05$ was considered statistically significant.

3. Results

3.1. MMP-9 secretion is reduced in LMAN1 knockout cells

We examined the role of LMAN1 in MMP-9 secretion by creating an LMAN1-knock out cell line using the Cas9/CRISPR system [11]. Sequencing of PCR products of the relevant genomic DNA from the pooled cells showed indel formations and a Western blot confirmed the complete loss of LMAN1 immunoreactivity in the LMAN1-knock out HEK293A cells (Fig. 1A, B). Comparison of the secreted wild type-MMP-9 and A1AT in the cell culture media after transfection of the respective cDNA demonstrated decreased amounts of both proteins with a trend towards increased retained intracellular proteins in the LMAN1-knock out cells (Fig. 1C, D). A1AT served as a control protein having been demonstrated previously to depend on LMAN1 for secretion [13].

3.2. Complementation assay indicates an N-glycosylation-dependent LMAN1: MMP-9 interaction

MMP-9 has two well-characterized N-glycosylation sites (Fig 2A) and we asked whether MMP-9 and LMAN1 interacted in an N-glycosylation-dependent manner in the ER as expected for a glycoprotein and its lectin-carrier. Complementation assay reports on the subcellular compartment-specific interaction between two proteins. The spatial orientation of the interacting bait and target proteins is critical since the two halves of the YPF fluorophore must be in close proximity to each other for the complementation to occur, therefore, we placed the YFP1 fluorophore on the N-terminus of LMAN1 and the YFP2 fluorophore on either the N- or C-termini of the MMP-9 protein. A complementation signal was observed for wt-MMP-9 with YFP2 tagging of the N-terminus giving a greater signal compared to the C-terminal tagging (Fig 2B). N-tagged A1AT served as a positive control yielding robust complementation signal with LMAN1. We examined whether the MMP-9 constructs with deletion of N-glycosylation at residue 38 (mS38), 120 (N120S), or both (mS38/N120S) interacted with LMAN1. The N-glycosylation-deficient MMP-9 mutants, in contrast to the wt-MMP-9, did not produce a reliable complementation signal. Fig 2C shows fluorescent images from these complementation experiments confirming the presence of fluorescence for cells transfected with LMAN1.nYFP1 and wt-MMP-9.nYPF2 or A1AT.nYPF2 but not for the N-glycosylation deletion mutants. The complementation signal was only observed when both the bait and target proteins were present supporting the specificity of the assay and immunocytochemistry confirmed the proper expression of LMAN1 and MMP-9 proteins even for the combinations that did not give a complementation signal (Fig S2).

If the interaction between the LMAN1 and the target proteins are glycosylation-dependent, treatment with castanospermine (CST), which inhibits trimming of the terminal glucose molecules essential for glycoprotein entry into the CANX/CALR folding cycle prior to potential lectin-carrier interaction, should decrease the interaction. As expected, the LMAN1 complementation signal with wt-MMP-9 and A1AT was decreased upon CST treatment (Fig 2D). Additionally the lectin-deficient N156A-LMAN1 mutant [14] serving as the bait was used to examine if the interaction was N-glycosylation-dependent. LMAN1 oligomerization using wt-LMAN1.nYFP2 was used as a negative control to verify that N156A-LMAN1 was expressed and localized in the ER since the N156A mutation doesn't effect LMAN1 oligomerization (Fig S3). A1AT.nYFP2 was used as a positive control to verify that N156A-LMAN1 was lectin-deficient [13]. The complementation signal of wt-MMP-9 was reduced to a level comparable to mS38/N120S-MMP-9 (Fig 2E) further supporting an N-glycosylation-dependent interaction between LMAN1 and MMP-9.

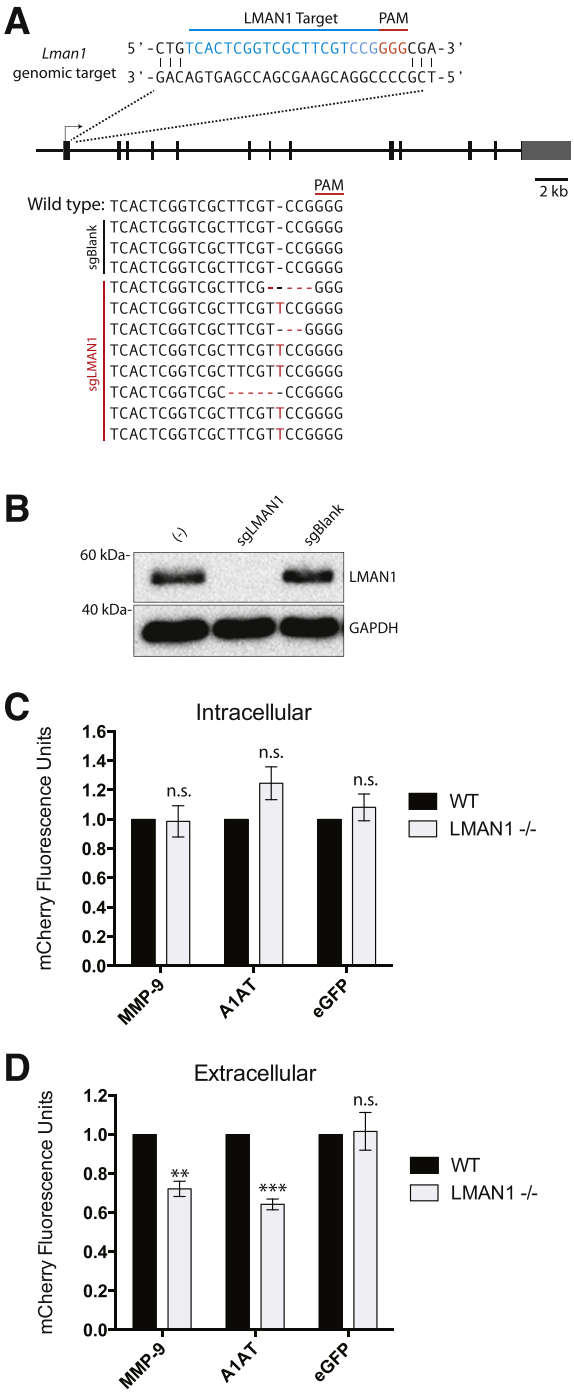

Fig. 1. Effects of LMAN1 deletion on MMP-9 and A1AT secretion. (A) Targeting scheme of the human *Lman1* locus showing SpCas9 target location. Targeted sequence indicated in blue, PAM sequence indicated in red. Indel mutation patterns detected by sequencing the HEK293 *Lman1* locus shown below with the location of the PAM site in: wild type sequence (top); sg-blank targeted sequence (middle); LMAN1 targeted sequence (bottom). Red dashes indicates deletion mutation, red bases indicates insertion mutations. PAM (protospacer adjacent motif), kb (kilo base). (B) Western blot of endogenous LMAN1 expression in HEK293 cells untreated (−), treated with LMAN1 targeting sgRNA, and treated with blank sgRNA. (C) MMP-9 or A1AT mCherry fusion levels 8 h, and $ss^{T\text{-}Cad}$ eGFP levels 24 h post-transfection in wild type or LMAN1 knockout backgrounds. Mean ± S.E.M., n.s., non-significant, n = 3. (D) MMP-9 or A1AT mCherry fusion levels averaged from 6 to 8 h, and $ss^{T\text{-}Cad}$ eGFP levels 24 h post-transfection in wild type or LMAN1 knockout backgrounds. Mean ± S.E.M., $^{**}P < 0.005$, $^{***}P < 0.0005$, n.s., non-significant, n = 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

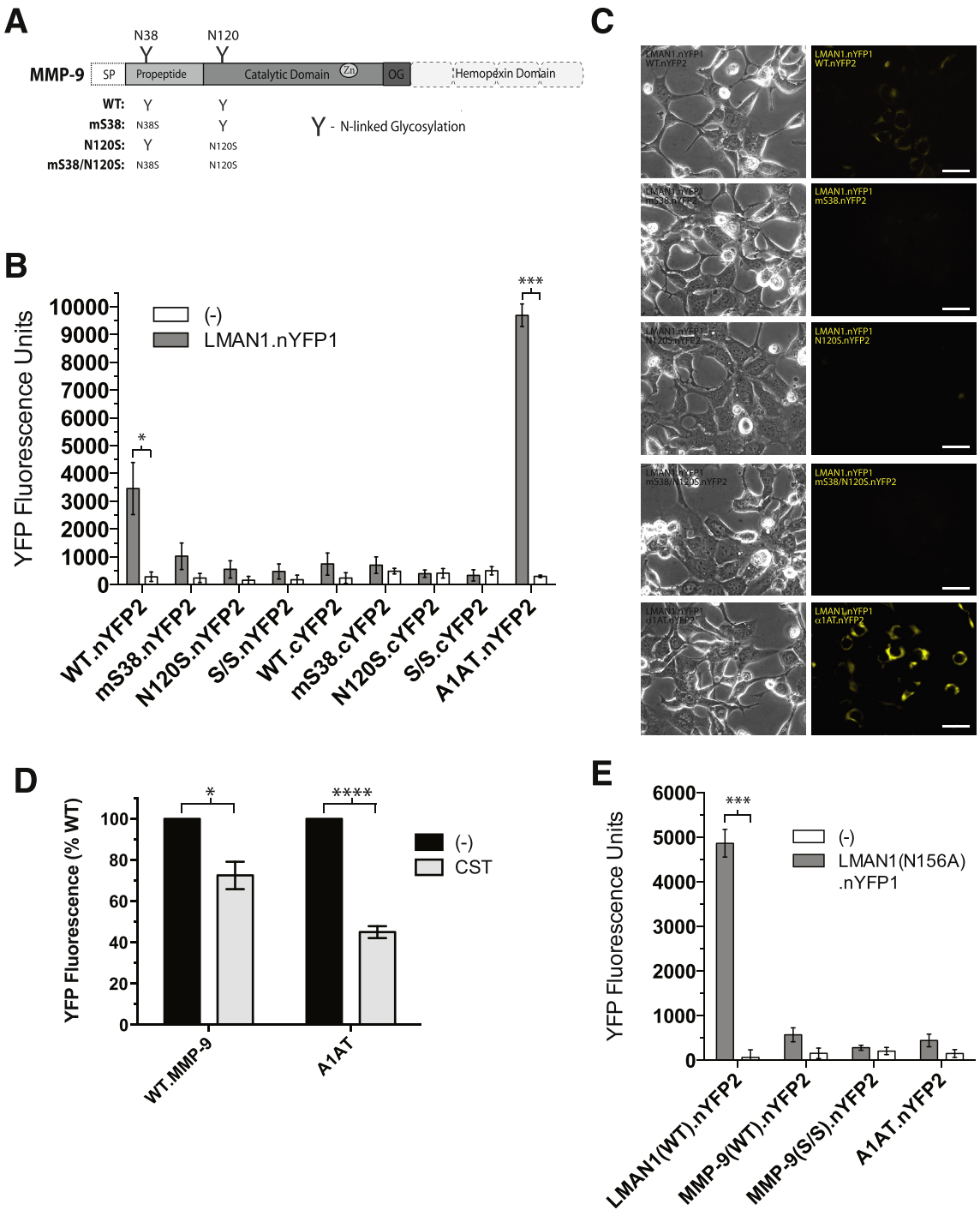

Fig. 2. Complementation assay reveal N-glycosylation-dependent interaction between LMAN1 and MMP-9. (A) Domain structure of MMP-9 including the locations of the N-linked glycosylation sites (Y). Below are the names of the molecular constructs and N-glycosylation status of the engineered N-glycosylation-deficient mutants. SP, signal peptide; OG, O-glycosylated domain; Zn, catalytic zinc molecule. (B) Complementation assay with amino-terminally tagged LMAN1 (LMAN1.nYFP1), amino-terminally tagged A1AT (A1AT.nYFP2), and amino (nYFP2)- or carboxyl (cYFP2)-terminally tagged wild type-, mS38-, N120S-, and mS38/N120S-MMP-9 (S/S). Grey bars indicated presence of LMAN1.nYFP1, open bars indicate no LMAN1.nYFP1 transfection, mean ± S.E.M., *$P < 0.05$, ***$P < 0.0005$, $n = 3$. (C) Phase contrast (left) and fluorescent (right) microscopic images of cells transfected with the noted amino-terminally (nYFP) tagged plasmids, scale bar = 40 μm. (D) Complementation assay with amino-terminally tagged wild type-MMP-9 or A1AT with YFP2 and amino-terminally tagged LMAN1 with YFP1 after 36 h treatment with (grey bars) or without (black bars) 1 mM Castanospermine (CST). Mean ± S.E.M., *$P < 0.05$, ****$P < 0.0001$, $n = 3$. (E) Complementation assay with (grey bars) or without (open bars) the amino-terminally YFP1 tagged N-glycosylation-binding-deficient LMAN1 mutant N156A with amino-terminally YFP2 tagged binding partners, mean ± S.E.M., ***$P < 0.0005$, $n = 3$.

3.3. Chloroquine inhibits MMP-9 secretion and results in intracellular accumulation

We further examined the functional role of LMAN1 in MMP-9 secretion through pharmacological inhibition of the unloading of the cargo protein from the carrier with chloroquine. Previous studies suggest that unloading of the cargo protein from LMAN1 in the ERGIC depends on acidification. Chloroquine inhibits the normal unloading of the cargo protein and secretion by inhibiting the pH change in the organelle [15]. Chloroquine inhibited

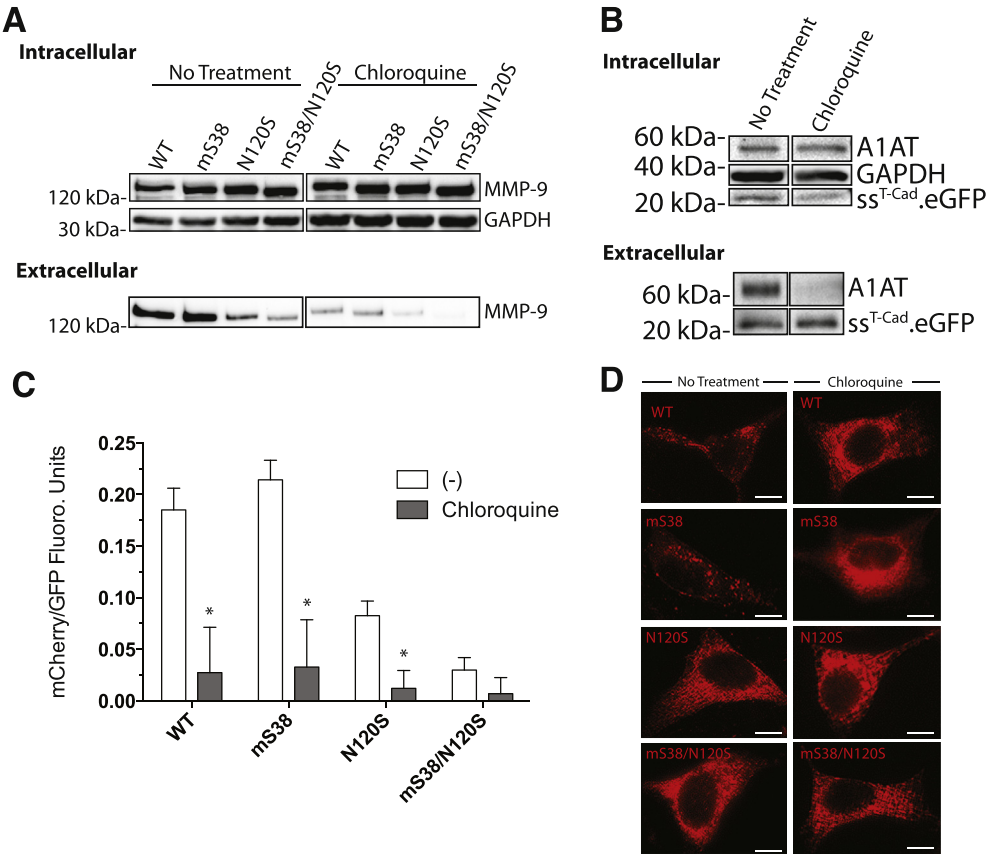

Fig. 3. Chloroquine inhibition of MMP-9 secretion. (A) Western blot of whole cell lysate (top) probed for MMP-9 and GAPDH and culture media probed for secreted MMP-9 (bottom). Panel to the left was control no treatment and panel to the right was after chloroquine treatment (50 μg/mL). (B) Western blot of whole cell lysate (top) probed for A1AT, GAPDH, and $ss^{T\text{-}Cad}$ eGFP showing equal abundance of expressed protein not affected by the chloroquine treatment (50 μg/mL). The culture media probed for secreted A1AT and $ss^{T\text{-}Cad}$ eGFP (bottom). (C) Microplate reader quantitation of secreted mCherry-tagged MMP-9 constructs after 24 h post-transfection in the absence (open bars) or presence (closed bars) of chloroquine (50 μg/mL) added at 4 h post-transfection. The mCherry signal was normalized by the co-transfected $ss^{T\text{-}Cad}$ eGFP control secretion signal, mean ± S.E.M., *$P < 0.05$, $n = 3$. (D) Fluorescent images of mCherry-tagged MMP-9 constructs show the intracellular mCherry fluorescence in non-treated cells (left column) and those treated with 50 μg/mL chloroquine (right column), scale bar = 15 μm.

secretion of wild type-, mS38-MMP-9, and the positive control A1AT, while having no effect on the intracellular expression of these proteins or on the expression and secretion of a control non-glycosylated constitutively secreted eGFP ($ss^{T\text{-}Cad}$.eGFP) (Fig 3A, B). A microplate reader-based quantitation of secreted mCherry-tagged MMP-9 confirmed that chloroquine robustly inhibited the secretion of MMP-9 with a greater inhibition of the secretion competent wild type and mS38 compared to the weakly secreted N-glycosylation mutants (Fig. 3C). Fluorescent microscopy of cells transfected with MMP-9-Cherry demonstrated the expected intracellular accumulation of the fusion protein, most likely in the ER, upon chloroquine treatment (Fig 3D).

3.4. Co-immunoprecipitation reports an N-glycosylation-independent LMAN1: MMP-9 interaction

Co-immunoprecipitation is another method of interrogating interaction between two proteins. Lysates from cells co-transfected with the MMP-9 constructs and the HA-tagged LMAN1 were subjected to immunoprecipitation with the anti-HA antibody and probed for MMP-9 co-immunoprecipitation. In contrast to the complementation assay, the secretion-compromised N120S and mS38/N120S-MMP-9 demonstrated robust co-immunoprecipitation with LMAN1 while the secretion-competent wild type showed little co-immunoprecipitation (Fig 4A). Additionally co-immunoprecipitation with N156A-LMAN1 also resulted in strong interaction with secretion-compromised MMP-9 (Fig S3). Cross-linking with DSP prior to co-immunoprecipitation previously reported to stabilize the interaction between a secreted glycoprotein and LMAN1 [7] only minimally increased the co-IP product (Fig 4B). A control immunoblot for the input proteins confirmed good intracellular expression of LMAN1 and all MMP-9 proteins including those that did not co-immunoprecipitate. This apparent discrepancy between the complementation and co-immunoprecipitation assays was not unexpected since the two assays report on different protein: protein interactions and could be detecting different types of biologically relevant interactions.

4. Discussion

Secretion of MMP-9 being N-glycosylation dependent and that this normally folded protein interacts with the ER-resident chaperone proteins such as CALR (Duellman et al., in review) suggested that the early secretory pathway of MMP-9 might involve a carrier-mediated mechanism. However, no lectin-carrier protein mediating MMP-9 secretion has been reported. Several lectin carriers mediating the transport of different secreted proteins has been described [16,17] with LMAN1 perhaps the best characterized (reviewed in Refs. [18,19]). In cells LMAN1 binds to the multiple coagulation factor deficiency protein 2 (MCFD2) forming a homo-hexameric complex [20]. MCFD2 is a small, soluble protein with 2 EF hand domains while LMAN1 contains the carbohydrate

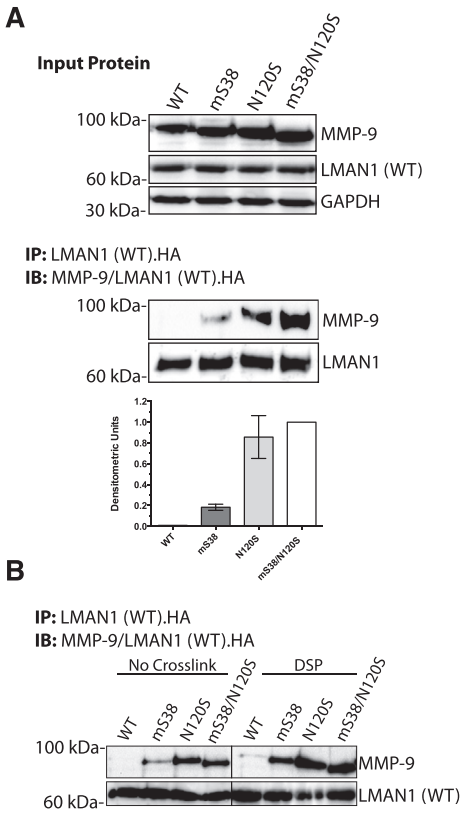

Fig. 4. Co-IP assay reveal different interaction between LMAN1 and MMP-9 N-glycosylation mutants. (A) An input whole cell lysate (top) and co-IP Western blot (bottom) showing MMP-9 N-glycosylation mutant interaction with LMAN1. Densitometric analysis presented as mean ± S.E.M., n = 3. (B) Effects of chemical crosslinking on co-IP between LMAN1.HA and MMP-9 N-glycosylation mutants. The conditions were identical to (A), with co-IP Western blot showing MMP-9 N-glycosylation mutant interaction with LMAN1 after no treatment (left) or after treatment (right) with the crosslinking agent DSP.

recognition domain, transmembrane domain, diphenylalanine ER exit motif, and dilysine ER retrieval motif which imparts the membrane localization, the lectin function, and ER trafficking, respectively, of the LMAN1-MCFD2 cargo receptor complex [19]. We examined whether LMAN1 could be the lectin carrier mediating secretion of MMP-9. The complementation assay between YFP1-LMAN1 and YFP2-MMP-9 (i.e. both N-terminal tagged) demonstrated a robust signal with a much lower fluorescence produced by the N-glycosylation-deficient mutants, indicating that secretion-competent MMP-9 can interact with LMAN1 in a likely glycosylation-dependent manner. The specificity of the complementation assay was indicated by the observation that the signal strength was dependent on the location of the YFP2-tag, with the N-terminal tag giving much greater signal than the C-terminal tag, and the expression of the YFP1-LMAN1 or YFP2-MMP-9 alone giving no signal.

LMAN1 normally shuttles between the ER and ERGIC [21]. If the secretion of wild type-MMP-9 depends on LMAN1, as suggested by the complementation assay, deletion of LMAN1 protein or inhibition of LMAN1 function by chloroquine should reduce the secretion of the glycoprotein. In a cell line with the LMAN1 protein expression deleted by genomic editing, both MMP-9 and A1AT secretion were reduced. However, it is also clear that significant secretion of both MMP-9 and A1AT remained even after complete elimination of the LMAN1 protein indicating the presence of other lectin-carriers mediating the secretion of these glycoproteins or secretion by the complementary bulk-flow pathway. Chloroquine, a drug previously demonstrated to inhibit LMAN1-dependent secretion of cathepsin Z-related protein (catZr) presumably through inhibition of cargo unloading by the carrier protein [15] also inhibited MMP-9 secretion. Together, the observations support the idea that the lectin-carrier protein LMAN1 plays a role in MMP-9 secretion while other carrier proteins and/or bulk flow can mediate the secretion as well.

The co-IP assay showed little, if any, interaction between LMAN1 and the secretion-competent MMP-9 but with robust co-IP between LMAN1 and the secretion-compromised MMP-9s. A lack of co-IP between LMAN1 and its client protein catZr where the secretion is known to be LMAN1-mediated have been reported [7] but these weak or transient interactions are not detected by co-IP. Co-IP can, however, be revealed by chemical cross-linking. Cross-linking with DSP to overcome a transient interaction between LMAN1 and wild type-MMP-9 proteins resulted in only a weak and barely distinguishable co-IP signal compared to the non-cross-linked control condition. The non-glycosylation-dependent LMAN1: MMP-9 interaction revealed by the biochemical co-IP assay clearly reports on a different interaction from that reported by the more organelle-specific complementation assay. The implication of this protein interaction revealed by co-IP is unknown but it is curious that the MMP-9 mutants with deficits in secretion efficiency (N120S-, mS38/N120S-MMP-9) appear to interact strongly with LMAN1 as reported by co-IP. MCFD2 has been reported to recruit Factors V and VII to the LMAN1-MCFD2 complex independent of the N-glycosylation status [22] and co-IP may be reporting on a similar type of interaction where MCFD2 is able to specifically interact and recruit N-glycosylation-deficient MMP-9 to the LMAN1-MCFD2 complex.

A deeper understanding of the mechanisms responsible for the secretion of MMP-9, and the role of LMAN1 in particular, has several biological and therapeutic implications. First, it appears that LMAN1, in addition to playing a traditional role as a lectin-binding carrier, interacts with MMP-9 in a non-glycosylation-dependent manner suggesting a potential role in the retention of non-glycosylated misfolded protein in the ER. It is unclear in what cellular compartment this interaction takes place. The LMAN1-MCDF2: non-glycosylated MMP-9 interaction could be a second line of quality control mediating a retrograde transport of misfolded protein escaping to the ERGIC back to the ER as has been suggested for the VIP36 lectin carrier protein [16]. Second, identification of the carrier protein may allow a more selective modulation of MMP-9 secretion. The lectin binding glycoprotein system in the ER has been suggested as a novel target for drug discovery particularly with regards to anti-viral and anti-inflammatory drugs. However, currently available small molecules such as thapsigargin, tunicamycin, CST, and others targeting the global glycosylation and protein folding mechanisms [23] are likely to result in many side effects. Targeting a specific carrier-lectin playing a role in the secretion of a particular target glycoprotein is likely to result in a specific modulation of secretion. LMAN1 has been documented to play a role in the secretion of several glycoproteins and its role as a lectin-binding carrier protein is not unique. The number of client proteins for the LMAN1 carrier is thought to be small [18,19] but nevertheless, further studies are necessary to see whether a specific interaction with LMAN1 and a given cargo protein can be selectively disrupted to achieve a focused modulation of secretion. Lastly, a clearer understanding of the determinants of MMP-9 interaction with LMAN1 could result in the secretion of misfolded proteins alleviating the cells from death due to accumulation of misfolded protein. Our preliminary experiments with the deletion of the C-terminal hemopexin domain of MMP-9 indicates that the normally non-secreted N-glycosylation-deficient m38S/N120S-MMP-9 mutant can be rendered secretion-competent upon deletion of the hemopexin domain known to mediate homo- and

hetero-protein interactions [24]. Whether this is due to a decreased interaction between the hemopexin-deleted MMP-9 and the LMAN1-MCFD2 complex is currently under investigation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.164.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.164.

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