Inhibition of MMP-9-Dependent Degradation of Gelatin, but Not Other MMP-9 Substrates, by the MMP-9 Hemopexin Domain Blades 1 and 4*

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ABSTRACT

Degradation and remodeling of the extracellular matrix by matrix metalloproteinases (MMPs) plays important roles in normal development, inflammation and cancer. MMP-9 efficiently degrades the extracellular matrix component gelatin and the hemopexin domain of MMP-9 (PEX9) inhibits this degradation. To study the molecular basis of this inhibition, we generated GST-fusion proteins containing PEX9 or truncated forms corresponding to specific structural blades (B1-B4) of PEX9. GST-PEX9 inhibited MMP-9-driven gelatin proteolysis, measured by gelatin zymography, FITC-gelatin conversion and DQ-gelatin degradation assays. However, GST-PEX9 did not prevent the degradation of other MMP-9 substrates, such as a fluorogenic peptide, αB crystallin or non-muscular-actin. Therefore, PEX9 may inhibit gelatin degradation by shielding gelatin and specifically preventing its binding to MMP-9. Accordingly, GST-PEX9 also abolished the degradation of gelatin by MMP-2, confirming that PEX9 is not an MMP-9 antagonist. Moreover, GST-B4 and, to a lesser extent, GST-B1, also inhibited gelatin degradation by MMP-9, indicating that these regions are responsible for the inhibitory activity of PEX9. Accordingly, ELISA assays demonstrated that GST-B4 and GST-B1 specifically bound to gelatin. Our results establish new functions of PEX9 attributed to blades B4 and B1 and should help in designing specific inhibitors of gelatin degradation.
domain and a carboxy-terminal hemopexin domain. Both MMP-2 and MMP-9 possess a domain with three fibronectin type II homology repeats that yield high affinity binding to gelatins. Only MMP-9 contains a serine-, threonine- and proline-rich O-glycosylated domain, which confers high flexibility to the enzyme (10).

The hemopexin domain of MMP-9 (PEX9) and other MMPs consists of four blade β-propeller structures (blades-1, -2, -3 and -4) (11). PEX9 is responsible for the interactions of MMP-9 with many molecules, including substrates, cell receptors, such as integrins and CD44, and tissue inhibitors of MMPs (12-15). PEX9 also contributes to MMP-9 trimerization (16). Because of these properties and the low homology with the hemopexin domains of other MMPs (25-30% identical residues) (12), PEX9 is revealed as an interesting target to increase the selectivity of MMP-9 inhibitors.

The main substrate of MMP-9 is gelatin, the product of denaturation or degradation of collagen by collagenases (MMP-1, MMP-8 and MMP-13). It was previously shown that the murine PEX9 binds to gelatin and a claim was made that PEX9 acts as an antagonist of MMP-9 (17). This was based on the fact that PEX9 inhibited gelatinolysis in zymography assays, as well as invasion of melanoma and colorectal cancer cells (17,18). In the present study, we have further investigated this and we demonstrate that human PEX9 inhibits gelatin degradation by shielding gelatin and specifically preventing its interaction with active MMP-9. Moreover, we have identified the specific regions of PEX9 responsible for the inhibitory effect and we show that PEX9 is not an antagonist of MMP-9 catalytic activity.

EXPERIMENTAL PROCEDURES

MEC-1 cells and transfectants – The MEC-1 cell line, established from a chronic lymphocytic leukemia (CLL) patient, was purchased from DSMZ (Braunschweig, Germany). MEC-1 cells stably expressing MMP-9 (MMP-9-MEC-1 cells) were generated by lentiviral transfection exactly as described (19). Cells were maintained in IMDM medium (Lonza, Basel, Switzerland), 10% fetal bovine serum.

Antibodies, Reagents, Proteins and Peptides
- Rabbit polyclonal antibodies to GST (GST, sc-459) and MMP-9 (sc-6841-R) were from Santa Cruz Biotechnology (Santa Cruz, CA). Recombinant full-length human pro-MMP-9 was prepared as reported (15). DQ™ gelatin was purchased from Invitrogen (Carlsbad, CA, USA), DQ™ collagen and fluorogenic peptide (DNPPro-Cha-Gly-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH2 were from Calbiochem®, (Darmstadt, Germany). MMP-2 and FITC-gelatin were from Molecular Probes (Eugene, OR). Human non-muscle-actin and subunit αB-crystallin recombinant proteins were from Cytoskeleton, Inc (Denver, CO) and Enzo Life Sciences (Farmingdale, NY), respectively. Pfu DNA polymerase was from Agilent Technologies (Waldbornn, Germany).

Construction of plasmids - The full-length human MMP-9 cDNA (cloned in pEGFP-N1) was obtained from Dr. Santos Mañes (Centro Nacional de Biotecnología, Madrid Spain) (20). The hemopexin domain cDNA was amplified by PCR using the following primers, custom-made by Sigma-Aldrich (St. Louis, MO): Forward: 5'-GAATTCCCCTTTGAGTCCGGTGGACG-3' engineering an internal EcoR1 site (underlined), Reverse 5'-CTCGAGCTAGTCCTCAGGGCACTGCA-3' containing an internal Xho-I restriction site. Amplification of DNAs was performed with the use of cloned Pfu DNA polymerase and the resultant PCR fragments were inserted into the pGEX4T3 vector (GE Healthcare Biosciences, Upsala, Sweden) to generate the GST-PEX9 DNA. To generate the PEX9 mutants we performed similar protocols, but used the primers indicated in Table 1. For GST-B1, GST-B2, GST-B3, and GST-B4 we engineered an internal EcoR1 site in the forward primers (GAATTC) and a Xho-I site (CTCGAG) in the reverse primers. For GST-ΔB1, GST-ΔB2, GST-ΔB3, and GST-ΔB4 we designed internal primers with the initial and final sequences of each removed blade and we amplified the complete construction from the GST-PEX9 plasmid. All obtained sequences were confirmed by DNA sequencing.

Expression and purification of GST and GST-fusion proteins - Recombinant proteins were prepared as previously described for GST and
GST-PEX9 (14). Briefly, protein constructs were expressed in DH5α *Escherichia coli* competent cells by induction with isopropyl-1-thio-β-D-galactopyranoside. Recombinant proteins were specifically induced in this system and produced in sufficient amount for the desired experimentation. Bacteria cultures were lysed by sonication in 1.5 M NaCl, 0.5 M Tris, 50 mM Na₂ EDTA, 10% Triton, and centrifuged. GST, GST-B1, GST-B2 and GST-B3 were soluble in this buffer and were purified using a glutathione-agarose matrix (Sigma-Aldrich). All other fusion proteins appeared in inclusion bodies and were solubilized in PBS, supplemented with 1% sarkosyl. These fusion proteins did not bind to glutathione-agarose and were purified by SDS-PAGE and electrophoresis. Purity and identity of the proteins were confirmed by SDS-PAGE and Western blotting. Purified fusion proteins were renatured by extensive dialysis against PBS.

**Cell Adhesion and Soluble Binding Assays**

Adhesion assays were performed in 96-well plates coated with 0.5% BSA or various concentrations of appropriate proteins. 1 × 10⁵ MEC-1 cells were incubated with 1.4 ng/ml 2′,7′-bis(carboxyethyl)-5(6′)-carboxyfluorescein-acetoxymethyl ester (BCECF-AM, Molecular Probes) for 20 min, suspended in RPMI 1640, 0.5% BSA (adhesion medium), and added to the coated wells. After 60 min at 37 °C, attached cells were lysed with PBS, 0.1% SDS and quantified using a fluorescence analyzer (BMG Labtech, Offenburg, Germany). For binding assays in solution, 1 × 10⁵ cells were incubated in 100 µl of adhesion medium containing the proteins under investigation and incubated for 30 min at 4 °C. After washing with ice-cold medium, cells were incubated with anti-GST pAbs (30 min, 4 °C), washed with cold PBS, and incubated (30 min, 4 °C) with Alexa488-labeled secondary Abs. Surface-bound proteins were analyzed by flow cytometry.

**Incubation of PEX9 proteins with active MMP-9**

MMP-9 in 50 mM Tris, 150 mM NaCl, 5 mM CaCl₂, 0.01% Tween 20 buffer was activated with the catalytic domain of MMP-3 (Millipore, Darmstadt, Germany), at a MMP-3:MMP-9 ratio of 1:100. GST-PEX9 (1 µg) was incubated with active MMP-9 during 100 min and aliquots of the reaction were taken every 10 min. For the analysis of non-muscular-actin and αB-crystallin cleavage, 2 µg of the recombinant proteins were incubated with active MMP-9 (enzyme:substrate ratio of 1:20) at 37°C for 2, 8 and 24 h, in the presence or absence of GST-PEX9. The incubated fractions were analyzed on 12% SDS-PAGE gels and visualized by staining with Coomassie Brilliant Blue R-250 (Sigma).

**Gelatin zymography analysis**

Samples of 20 ng of recombinant purified MMP-9 were analyzed on 7.5% polyacrylamide gels containing 0.1% gelatin (Sigma). The amount of the MMP-9 added in the gel was within the linear range of the zymogram and this was evaluated by testing a dilution series of MMP-9 by zymography analysis. After electrophoresis, gels were rinsed 3 x 30 min in 2.5% Triton X-100 and 1 x 30 min in distilled water, followed by overnight incubation in 50 mM Tris pH 7.5, 200 mM NaCl, 10 mM CaCl₂ at 37°C. To analyze the effect of GST-PEX9 recombinant proteins, mutant proteins were added to the incubation buffers overnight at a final concentration of 0.4 µM. Gels were stained with 0.2% Coomassie blue and areas of gelatinolysis were visualized as transparent lysis zones. Bands were quantitated on a densitometer (Molecular Dynamics, Sunnyvale, CA) using the Quantity-One™ program (Bio-Rad Laboratories, Hercules, CA). Control MMP-9 levels were normalized to 100 percent.

**FITC-gelatin degradation assay**

60 nM activated MMP-9 in 50 mM Tris, 150 mM NaCl, 5 mM CaCl₂, 0.01% Tween 20 was added to 96 FITC-gelatin-coated wells (100 µg/ml), in the absence or presence of 0.4 µM recombinant GST-PEX9 proteins. After 24 h at 37°C, wells were washed with PBS and fluorescence was measured using a fluorescence analyzer (BMG Labtechnologies, Offenburg Germany). The background fluorescence of the conditioned medium added to unlabeled gelatin-coated wells was subtracted from all values. To analyze the capacity of the GST-PEX9 proteins on gelatin degradation by leukemic cells, 96 well plates were coated with 100 µg/ml of FITC-labeled gelatin and the various GST-PEX9 proteins, and fixed with 0.5% glutaraldehyde. After washing with PBS, 70% ethanol/PBS and medium, 1 x 10⁵ MMP-9-MEC-1 cells were added to the wells. After 24 h.
cells were removed with several washing steps and the fluorescence was measured.

**DQ-gelatin or fluorogenic peptide degradation assay** - A DQ-gelatin degradation assay was performed as previously described (21). Briefly, to measure the degradation in fluorescence units, 0.1 nM of active MMP-9 was added to a solution of 2.5 µg/ml of DQ-gelatin in a black 96 well plate. To analyze the maximal enzyme velocity in these assays, several concentrations of the DQ-gelatin substrate (20, 10, 5, 2.5, 0.75, 0.5 and 0.25 µg/ml) were added to the 96 well plate. To test the effect of the GST-PEX9 constructs, 0.4 µM of the recombinant proteins were added to the wells. The plates were immediately placed in the fluorescence reader (FL600 Microplate fluorescence reader, Biotek, Highland Park, IL) and fluorescence was measured every 10 min for 2 h at 37°C (ex 485 nm/em 530 nm). All data were corrected by subtraction of the negative controls. In all the experiments the maximal velocity of the enzyme was the velocity observed during the first 10 minutes of the assay. Graphs and calculations were obtained with Prism 5 (GraphPad Software, Inc).

To test the degradation of a small fluorogenic peptide we used {DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH2 (ex. 365 nm/em 450 nm, MM: 1077.2/EMD/Calbiochem®, Darmstadt, Germany). This substrate is cleaved by MMP-9 into a single cleavage product, Dnm-Pro-Cha-Gly (22). Activated MMP-9 was used at a concentration of 1 nM and the fluorogenic peptide was used at 10 µg/mL. Fluorescence was measured every 10 min for 2 h in the fluorescence reader.

**ELISA** - High binding 96 wells plates were coated with 5 µg of gelatin overnight at 4°C. After washing, recombinant proteins were added at a final concentration of 0.4 µM and incubated for 1 hour at 37°C; then the plates were washed and anti-GST polyclonal antibody at 5 µg/ml was added to the wells. After incubation during 1 h at 37°C the plate was incubated with secondary anti-rabbit HRP antibody for 30 minutes, washed and developed.

**RESULTS**

The human proMMP-9 hemopexin domain inhibits gelatin degradation by MMP-9 - Roeb et al. previously showed that the murine PEX9 domain inhibits MMP-9 activity in gelatin zymography assays (17). To investigate the molecular basis of this observation, we prepared a fusion protein containing GST and human PEX9 (residues 508-707 of proMMP-9, Figure 1A). After purification, the GST-PEX9 protein ran as a single 50-kDa band in SDS gels and its identity was confirmed by Western blot analysis with an anti-MMP-9 antibody (Figure 1B). Recombinant GST alone was also prepared by bacterial expression (Figure 1B) and was used as control. We previously showed that the resulting GST-PEX9 protein, but not GST alone, mediates CLL cell adhesion and soluble cell binding via specific cell surface receptors (14, 23). GST-PEX9 also specifically inhibits chemotaxis and transendothelial migration of leukemic B cells and induced survival signals upon binding to α4β1 integrin (14, 23). Therefore, the purified GST-PEX9 protein fully retained its biological activities and was suitable for further functional analyses.

In initial experiments, we determined whether MMP-9 was able to cleave GST-PEX9. The GST-PEX9 protein (1 µg) was incubated with active MMP-9 (1 nM) and samples were taken every 10 min for a time interval of 100 min. Analyses by SDS-PAGE followed by protein staining with Coomassie blue showed that MMP-9 did not degrade the GST-PEX9 protein (Figure 1C). With the knowledge that GST-PEX9 remained intact in the presence of active MMP-9, we analyzed the effect of GST-PEX9 on gelatin degradation by MMP-9, using three different techniques: gelatin zymography, degradation of FITC-gelatin in 96-well plate format and DQ-gelatin degradation assay. In all these experiments we also tested the effect of purified GST as negative controls.

Gelatin zymography analyses demonstrated that adding GST-PEX9 (0.4 µM) reduced gelatin degradation by MMP-9 by approximately 50% (Figure 1D) while GST had no effect. To study whether GST-PEX9 inhibited the degradation of FITC-gelatin, GST-PEX9 was incubated with active MMP-9 in microtiter plate
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wells coated with FITC-gelatin and the fluorescence was measured after 24 h. GST-PEX9, but not GST, significantly decreased gelatin degradation by MMP-9 (average 50% decrease) (Figure 1E). The inhibitory function of PEX9 was also demonstrated with a dye-quenched gelatin (DQ-gelatin) degradation assay (21). As shown in Figure 1F, GST-PEX9 significantly reduced the level of fluorescence in a time-dependent manner, during the 120 min of the assay. GST-PEX9 also reduced the MMP-9 enzyme velocity in this assay, whereas no inhibition was seen after incubation with GST (Figure 1G). Altogether these results established that the human PEX9 domain inhibits the degradation of gelatin by MMP-9, in agreement with previous results with murine PEX9 (17).

The PEX9 domain does not act as an MMP-9 antagonist - To determine if PEX9 is an MMP-9 antagonist and blocks its catalytic activity or, alternatively, binds to gelatin and prevents its degradation by MMP-9, we tested different MMP-9 substrates and different gelatinases. In contrast to the inhibitory effect on the degradation of macromolecular gelatin (Figure 1D-G), GST-PEX9 was unable to block the proteolysis of a small DQ-fluorogenic peptide (FP) by MMP-9 (Figure 2A). Moreover, the enzyme velocity from the same assays was similar in the presence or absence of GST-PEX9 (Figure 2A).

We next analyzed whether GST-PEX9 affected the proteolytic activity of MMP-9 on other MMP-9 substrates, such as αβ-crystallin (24) and non-muscular-actin (6). To this end, we performed kinetic assays incubating αB-crystallin and actin with active MMP-9 in the presence or absence of GST-PEX9 and evaluated protein degradation at 2, 8 and 24 hours. As shown in Figure 2B, the presence of PEX9 did not influence the degradation of actin by MMP-9 and only minimally delayed the proteolysis of αB-crystallin by this enzyme. In contrast to these results, GST-PEX9 inhibited the degradation of DQ-collagen, another MMP-9 substrate (Figure 2C). Because DQ-gelatin can also be degraded by MMP-2 (gelatinase A), we tested whether PEX9 also inhibited DQ-gelatin proteolysis by this enzyme. Figure 2D shows that GST-PEX9 was able to inhibit DQ-gelatin degradation by MMP-2 at similar levels as observed for MMP-9. Altogether, these results corroborate the role of PEX9 as an inhibitor of gelatin/collagen degradation, and establish that PEX9 is not a direct antagonist of the catalytic activity of MMP-9.

Blades 1 and 4 of the PEX9 domain are responsible for the gelatin degradation inhibitory activity of PEX9 - The PEX9 domain contains a four blade beta-propeller structure (11), schematically shown in Figures 1A and 3A. To define the region involved in the inhibition of gelatin degradation, we prepared two GST fusion proteins containing deletions of blades 3-4 (GST-B1B2 protein, proMMP-9 residues 508-613) or blades 1-2 (GST-B3B4 protein, proMMP-9 residues 609-707) (Figure 3A). The purity of these proteins was confirmed by SDS-PAGE analyses (Figure 3B). We previously showed that purified GST-B1B2 and GST-B3B4 mediated CLL cell adhesion by binding to CD44 and α4β1 integrin, respectively (14, 23). Moreover, we also identified specific cell binding sequences in both proteins (14, 23). The soluble proteins also specifically bound to cells and inhibited CLL cell chemotaxis and transendothelial migration (14, 23), thus confirming the retention of their biological activity. GST-B1B2 and GST-B3B4 were therefore tested as inhibitors of gelatin degradation using different assays. In gelatin zymography analysis, GST-PEX9, GST-B1B2 and GST-B3B4 (all at 0.4 µM) inhibited gelatin degradation by 60%, 35% and 40%, respectively (Figure 3C). Similar results were obtained with the FITC-gelatin degradation assay. In this case, all three proteins, GST-PEX9, GST-B1B2 and GST-B3B4 reduced the gelatinolytic activity of MMP-9 to approximately 50%, compared to the control (Figure 3D). To further confirm these results, we analyzed the inhibitory role of these recombinant proteins in the DQ-gelatin degradation assay. Figure 3E shows that GST-PEX9, GST-B1B2 and GST-B3B4 similarly inhibited the velocity of the DQ-gelatin degradation by MMP-9. These data indicated that both regions, B1B2 and B3B4 appeared to contribute to the inhibitory function of PEX9.

To identify the specific sequence within PEX9 that binds to gelatin, we prepared alternative recombinant proteins, each containing only one
blade of the PEX9 domain (GST-B1, GST-B2, GST-B3 and GST-B4) (Figure 4A,B). To first confirm that these proteins retained their biological function, we analyzed their ability to mediate cell adhesion and soluble binding. Figure 4C shows that all four PEX9 blades mediated adhesion of MEC-1 cells, while GST did not. As expected, the level of cell adhesion to the individual blades was lower than adhesion to the entire PEX9 domain (Figure 4C). Additionally, these fusion proteins, but not GST, were able to bind to MEC-1 cells in suspension, as detected with an anti-GST antibody (Figure 4D).

Having confirmed that the purified GST-B1, GST-B2, GST-B3 and GST-B4 proteins were functional, we tested their ability to block gelatin degradation. Figure 4E shows for a representative experiment, how GST-B1 and GST-B4, but not GST-B2 or GST-B3 (all at 0.4 µM), inhibited gelatin degradation in a gelatin zymography assay. By comparison of the four single blade proteins, GST-B4 was found to be more effective, blocking gelatin degradation by 42%, whereas incubation with GST-B1 inhibited by 25%. In parallel experiments GST-PEX9 diminished the degradation of gelatin by 60% (average of 3 independent experiments) (Figure 4C). Additionally, these fusion proteins, but not GST, were able to bind to MEC-1 cells in suspension, as detected with an anti-GST antibody (Figure 4D).

Further characterization of the inhibitory activity of blades B1 and B4 on gelatin degradation by MMP-9 - Having identified blades 1 and 4 as the regions in PEX9 with inhibitory activity, we tested whether this activity was dose-dependent. The DQ-gelatin degradation assay was used for this purpose. Activated MMP-9 was incubated with constant concentrations of DQ-gelatin (20 µg/ml) in the absence or presence of increasing concentrations of GST-PEX9, GST-B1, or GST-B4. All three proteins inhibited gelatinolysis in a dose-dependent manner, and GST-B4 was the most effective at all concentrations tested (Figure 5D). To confirm these results, we tested whether these proteins bound to gelatin using ELISA assays. Figure 5E shows that GST-PEX9 and GST-B4 bound to gelatin while GST, GST-B2 and GST-B3 did not. These data therefore established the identity and specificity of blades 1 and 4 of PEX9 as inhibitors of gelatin degradation.

To place these biochemical findings in a biological context, we analyzed the inhibitory capacity of the PEX9 proteins on cell-induced degradation of FITC-gelatin. For these experiments, we used the leukemic cell line MEC-1, stably transfected with MMP-9 (MMP-9-EC1 cells) (19). We previously showed that these cells produce high amounts of MMP-9, most of which is in an active form (19). 1 x 10^5 MMP-9 transfected MEC-1 cells were incubated with FITC-gelatin in the presence or absence of the GST-PEX9 variants and the resulting fluorescence was determined after 24 h. Figure 5F shows that GST-PEX9 significantly inhibited FITC-gelatin degradation by 34%, compared to the control cells with no inhibitor. In agreement with the results obtained with purified MMP-9, recombinant proteins containing blades B1 and/or B4 inhibited the degradation of gelatin by MMP-9-EC1 cells, while GST-B2 and GST-B3 had no effect (Figure 5F). Altogether, these results established...
that blade 4, and to a lesser extent blade 1, are the regions responsible for the inhibitory role of PEX9 in gelatin degradation.

DISCUSSION
We have studied the effect of human PEX9 on MMP-9 proteolysis on several substrates. We report that PEX9 inhibits the degradation of gelatin but not of other MMP-9 substrates. This was due to the sequestration of gelatin by PEX9, which is therefore a binding competitor, but not an antagonist, of MMP-9.

The MMP family has been studied during many years, because of their role in many physiological and pathological processes. At first, the scientific community focused on the catalytic roles of these molecules, and most efforts were concentrated on developing inhibitors of the catalytic activity. The main problem with this was that the amino acid sequences of the catalytic domain are highly conserved among all MMPs, and even within the ADAM and ADAMTS family (25,26). Therefore, most of the inhibitors were not specific, inhibiting a range of MMPs and resulting in side-effects. In recent years it was demonstrated that most MMPs also have non-catalytic functions (27) and can act like ligands (28) or even like transcription factors (29,30). Due to the failure of first generation MMP inhibitors, the search is now open towards inhibitors that target specific functions of one or more MMPs. More detailed information about the structure and function of single MMP domains (exosites) is therefore needed (25,31).

The hemopexin domain of the MMPs is the main domain that drives the non-catalytic functions, due to its ability to interact with substrates, receptors and inhibitors (11-13). For example, in chronic lymphocytic leukemia cells, PEX9 can drive intracellular signaling and survival independently of other MMP-9 domains (14,28). Given the lower amino acid sequence homology in the hemopexin domains than in other domains among MMPs, we suggest that targeting this domain may be a useful and more specific approach to prevent MMP-9-mediated pathological functions. Indeed, strategies aimed to block the interaction of PEX with individual molecules are already in progress. For example, we recently identified the sequences in PEX9 responsible for the interaction of proMMP-9 with CD44 (FDAIAEIGNQLYLFKDGKYW, blade 1) and αβ1 integrin (FPGVPLDTHDFQYREKAYFC, blade 4) in chronic lymphocytic leukemia cells (14,23). Likewise, active sequences in blades 1 and 4 of MMP-14 were identified and shown to inhibit carcinoma cell migration, tumor metastasis and angiogenesis (32).

In the present report we have characterized a different function of PEX9, namely its ability to block gelatin and collagen degradation by MMP-9. Interestingly, this activity also involved structural blades 1 and 4, highlighting the importance of these regions in many PEX9 functions. Our data clearly show that the inhibitory function of PEX9 did not apply to the degradation of other MMP-9 substrates, such as a small fluorogenic peptide or αB-crystallin. This, together with the fact that PEX9 also abrogated gelatinolysis by MMP-2, indicates that PEX9 is not an antagonist of MMP-9 catalytic function, as previously proposed (17). Instead, PEX9 blocks gelatin degradation because it specifically binds to gelatin, and probably not to other MMP-9 substrates. Indeed, murine PEX9 was previously shown to bind to gelatin and the binding parameters have been reported (17,18). Using recombinant proteins that lack or contain specific blades of human PEX9, in the present study we have extended the binding studies and have identified blade 4 and, to a lesser extent, blade 1, as the structural modules in PEX9 that bind to gelatin and inhibit MMP-9 gelatinolysis.

Degradation and remodeling of the extracellular matrix, mostly carried out by MMPs, play crucial roles in normal development but also in the migration and metastasis of cancer cells (33,34). The extracellular matrix is therefore a dynamic structure, whose components interact with specific integrin receptors and trigger intracellular signaling and cell responses. Consequently, depending on the composition of the extracellular matrix, these ligand-receptor interactions will induce different signaling, directly affecting cell behaviour (35). To fully understand the behavior of different cell types, including metastatic cancer cells, new probes and also extracellular matrix molecules may lead to the
development of new drugs against cancer. Until now, the research focus was to target the malignant cells and the MMPs, which degrade the extracellular matrix. It might be equally important to try to block specific components of this matrix, such as gelatin. Therefore the PEX9-blades, generated in this study, may become useful probes to study gelatinolysis and/or serve to generate specific inhibitors of gelatinolysis. Since this approach specifically targets gelatin catalysis it might also serve to block the migration of metastatic cancer cells and of leukocytes in pathological inflammations.

In summary, the present study helps to understand how MMP-9 and gelatin interact with each other and points towards new directions to develop new inhibitors of specific MMP functions.

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Conflict of interest
The authors declare that they have no conflicts of interest with the contents of this article.

Author contributions:
E.U-B. designed and performed most of the experiments, analyzed the data, and wrote the paper. J.V. provided valuable help and advice with some experiments. E.B. provided valuable help and advice with some experiments and the preparation of the figures. G.O. and A.G-P. designed and supervised the experiments, discussed the results, and critically reviewed the manuscript.

REFERENCES
1. Overall, C. M., McQuibban, G. A., and Clark-Lewis, I. (2002) Discovery of chemokine substrates for matrix metalloproteinases by exosite scanning: a new tool for degradomics. J. Biol. Chem 383, 1059-1066
2. Overall, C. M., Tam, E. M., Kappelhoff, R., Connor, A., Ewart, T., Morrison, C. J., Puente, X., Lopez-Otin, C., and Seth, A. (2004) Protease degradomics: mass spectrometry discovery of protease substrates and the CLIP-CHIP, a dedicated DNA microarray of all human proteases and inhibitors. J. Biol. Chem. 385, 493-504
3. Butler, G. S., and Overall, C. M. (2009) Updated biological roles for matrix metalloproteinases and new "intracellular" substrates revealed by degradomics. Biochemistry 48, 10830-10845
4. Huesgen, P. F., and Overall, C. M. (2012) N- and C-terminal degradomics: new approaches to reveal biological roles for plant proteases from substrate identification. Physiol. plant. 145, 5-17
5. Cauwe, B., Van den Steen, P. E., and Opdenakker, G. (2007) The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases. Crit. Rev. Biochem. Mol. Biol. 42, 113-185
6. Cauwe, B., and Opdenakker, G. (2010) Intracellular substrate cleavage: a novel dimension in the biochemistry, biology and pathology of matrix metalloproteinases. Crit. Rev. Biochem. Mol. Biol. 45, 351-423
7. Nabeshima, K., Inoue, T., Shimao, Y., and Sameshima, T. (2002) Matrix metalloproteinases in tumor invasion: role for cell migration. Pathol. Int. 52, 255-264
8. Loffek, S., Schilling, O., and Franzke, C. W. (2011) Series "matrix metalloproteinases in lung health and disease": Biological role of matrix metalloproteinases: a critical balance. Eur. Respir. J. 38, 191-208
9. Coussens, L. M., Fingleton, B., and Matrisian, L. M. (2002) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295, 2387-2392
10. Vandooren, J., Van den Steen, P. E., and Opdenakker, G. (2013) Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. Crit. Rev. Biochem. Mol. Biol. 48, 222-272
11. Cha, H., Kopetzki, E., Huber, R., Lanzendorfer, M., and Brandstetter, H. (2002) Structural basis of the adaptive molecular recognition by MMP9. J. Mol. Biol. 320, 1065-1079
12. Picard, H., Van den Steen, P. E., and Opdenakker, G. (2007) Hemopexin domains as multifunctional liganding modules in matrix metalloproteinases and other proteins. J. Leukoc. Biol. 81, 870-892
13. Redondo-Munoz, J., Ugarte-Berzal, E., Garcia-Marco, J. A., del Cerro, M. H., Van den Steen, P. E., Opdenakker, G., Terol, M. J., and Garcia-Pardo, A. (2008) Alpha4beta1 integrin and 190-kDa CD44v constitute a cell surface docking complex for gelatinase B/MMP-9 in chronic leukemic but not in normal B cells. Blood 112, 169-178
14. Ugarte-Berzal, E., Bailon, E., Amigo-Jimenez, I., Vituri, C. L., del Cerro, M. H., Terol, M. J., Albar, J. P., Rivas, G., Garcia-Marco, J. A., and Garcia-Pardo, A. (2012) A 17-residue sequence from the matrix metalloproteinase-9 (MMP-9) hemopexin domain binds alpha4beta1 integrin and inhibits MMP-9-induced functions in chronic lymphocytic leukemia B cells. J. Biol. Chem. 287, 27601-27613
15. Van den Steen, P. E., Van Aelst, I., Hvidberg, V., Piccard, H., Fiten, P., Jacobsen, C., Moestrup, S. K., Fry, S., Royle, L., Wormald, M. R., Wallis, R., Rudd, P. M., Dwek, R. A., and Opdenakker, G. (2006) The hemopexin and O-glycosylated domains tune gelatinase B/MMP-9 bioavailability via inhibition and binding to cargo receptors. J. Biol. Chem. 281, 18626-18637
16. Vandooren, J., Born, B., Solomonov, I., Zajac, E., Saldoa, R., Senske, M., Ugarte-Berzal, E., Martens, E., Van den Steen, P. E., Van Damme, J., Garcia-Pardo, A., Froeyen, M., Deryugina, E. I., Quigley, J. P., Moestrup, S. K., Rudd, P. M., Sagi, I., and Opdenakker, G. (2015) Circular trimers of gelatinase B/matrix metalloproteinase-9 constitute a distinct population of functional enzyme molecules differentially regulated by tissue inhibitor of metalloproteinases-1. Biochem. J. 465, 259-270
17. Roeb, E., Schleinkofer, K., Kernebeck, T., Potsch, S., Jansen, B., Behrmann, I., Matern, S., and Grotzinger, J. (2002) The matrix metalloproteinase 9 (mmp-9) hemopexin domain is a novel gelatin binding domain and acts as an antagonist. J. Biol. Chem. 277, 50326-50332
18. Burg-Roderfeld M, Roderfeld M, Wagner S, Henkel C, Grötzinger J, Roeb E. (2007) MMP-9-hemopexin domain hampers adhesion and migration of colorectal cancer cells. Int J Oncol. 30(4):985-92.
19. Bailón, E., Ugarte-Berzal, E., Amigo-Jimenez, I., Van den Steen, P., Opdenakker, G., Garcia-Marco, J. A., and Garcia-Pardo, A. (2014) Overexpression of progelatinase B/proMMP-9 affects migration regulatory pathways and impairs chronic lymphocytic leukemia cell homing to bone marrow and spleen. J. Leukoc. Biol. 96, 185-199
20. Mira, E., Lacalle, R. A., Buesa, J. M., de Buitrago, G. G., Jimenez-Baranda, S., Gomez-Mouton, C., Martinez, A. C., and Manes, S. (2004) Secreted MMP9 promotes angiogenesis more efficiently than constitutive active MMP9 bound to the tumor cell surface. J. Cell. Sci. 117, 1847-1857
21. Vandooren, J., Geurts, N., Martens, E., Van den Steen, P. E., Jonghe, S. D., Herdevijn, P., and Opdenakker, G. (2011) Gelatin degradation assay reveals MMP-9 inhibitors and function of O-glycosylated domain. World J. Biol. Chem. 2, 14-24
22. Bickett, D. M., Green, M. D., Berman, J., Dezube, M., Howe, A. S., Brown, P. J., Roth, J. T., and McGeehan, G. M. (1993) A high throughput fluorogenic substrate for interstitial collagenase (MMP-1) and gelatinase (MMP-9). Anal. Biochem. 212, 58-64
23. Ugarte-Berzal, E., Bailon, E., Amigo-Jimenez, I., Albar, J. P., Garcia-Marco, J. A., and Garcia-Pardo, A. (2014) A novel CD44-binding peptide from the pro-matrix metalloproteinase-9 hemopexin
domain impairs adhesion and migration of chronic lymphocytic leukemia (CLL) cells. *J. Biol. Chem.* **289**, 15340-15349

24. Starckx, S., Van den Steen, P. E., Verbeek, R., van Noort, J. M., and Opdenakker, G. (2003) A novel rationale for inhibition of gelatinase B in multiple sclerosis: MMP-9 destroys alpha B-crystallin and generates a promiscuous T cell epitope. *J. Neuroimmunol.* **141**, 47-57

25. Hu, J., Van den Steen, P. E., Sang, Q. X., and Opdenakker, G. (2007) Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. *Nat. Rev. Drug. Discov.* **6**, 480-498

26. Van Wart, H. E., and Birkedal-Hansen, H. (1990) The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 5578-5582

27. Garcia-Pardo, A., and Opdenakker, G. (2015) Nonproteolytic functions of matrix metalloproteinases in pathology and insights for the development of novel therapeutic inhibitors. *Metalloproteinases Med.* **2**, 19-28

28. Redondo-Munoz, J., Ugarte-Berzal, E., Terol, M. J., Van den Steen, P. E., Hernandez del Cerro, M., Roderfeld, M., Roeb, E., Opdenakker, G., Garcia-Marco, J. A., and Garcia-Pardo, A. (2010) Matrix metalloproteinase-9 promotes chronic lymphocytic leukemia b cell survival through its hemopexin domain. *Cancer Cell* **17**, 160-172

29. Marchant, D. J., Bellac, C. L., Moraes, T. J., Wadsworth, S. J., Dufour, A., Butler, G. S., Bilawchuk, L. M., Hendry, R. G., Robertson, A. G., Cheung, C. T., Ng, J., Ang, L., Luo, Z., Heilbron, K., Norris, M. J., Duan, W., Bucyk, T., Karpov, A., Devel, L., Georgiadis, D., Hegele, R. G., Luo, H., Granville, D. J., Dive, V., McManus, B. M., and Overall, C. M. (2014) A new transcriptional role for matrix metalloproteinase-12 in antiviral immunity. *Nat. Med.* **20**, 493-502

30. Eguchi, T., Kubota, S., Kawata, K., Mukudai, Y., Uehara, J., Ohgawara, T., Ibaragi, S., Sasaki, A., Kuboki, T., and Takigawa, M. (2008) Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene. *Mol. Cell. Biol.* **28**, 2391-2413

31. Sela-Passwell, N., Rosenblum, G., Shoham, T., and Sagi, I. (2010) Structural and functional bases for allosteric control of MMP activities: can it pave the path for selective inhibition? *Biochim. Biophys. Acta.* **1803**, 29-38

32. Zarrabi, K., Dufour, A., Li, J., Kuscu, C., Pulkoski-Gross, A., Zhi, J., Hu, Y., Sampson, N. S., Zucker, S., and Cao, J. (2011) Inhibition of matrix metalloproteinase 14 (MMP-14)-mediated cancer cell migration. *J. Biol. Chem.* **286**, 33167-33177

33. Egeblad, M., and Werb, Z. (2002) New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer.* **2**, 161-174

34. Ethell, I. M., and Ethell, D. W. (2007) Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. *J. Neurosci. Res.* **85**, 2813-2823

35. Hynes, R. O. (2009) The extracellular matrix: not just pretty fibrils. *Science.* **326**, 1216-1219
Inhibition of gelatin degradation by PEX9 blades 1 and 4

Footnotes
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FIGURE LEGENDS

FIGURE 1. Effect of human GST-PEX9 on the degradation of gelatin by MMP-9. A, Schematic drawing of the structural domains of proMMP-9. The four structural blades of PEX9 (B1–B4) are indicated. The GST-PEX9 fusion protein prepared in this study contains amino acid residues 508-707 of human proMMP-9. B, SDS-PAGE (10% acrylamide) and Western blot analyses of the purified GST and GST-PEX9 proteins. C, 1 µg of GST-PEX9 was incubated with 1 nM active MMP-9 for the indicated times. The samples were analyzed by 10% SDS-PAGE and stained with Coomassie Blue. D, Gelatin zymography analysis of 20 ng of MMP-9 (within the linearity of the assay) in the presence or absence of 0.4 µM GST-PEX9. A representative zymography is shown. E, 60 nM of MMP-9 was added to FITC-gelatin-coated plates, in the absence or presence of 0.4 µM GST or GST-PEX9. After 24 h at 37°C, fluorescence was determined and control values were normalized to 100. F, Effect of GST and GST-PEX9 (0.4 µM) on the conversion of DQ-gelatin into fluorogenic gelatin. G, Maximal enzyme velocity as a function of the amount of substrate. The maximal velocity of each point is the first read time point at 10 minutes and these measurements were within the linearity of the assay. Bars represent standard deviation. AU: Arbitrary units. **, p < 0.01; ***, p < 0.001.

FIGURE 2. Role of GST-PEX9 on the degradation of MMP-9 and MMP-2 substrates. A, 1 nM MMP-9 was incubated with 2.5 µg/ml DQ-peptide in the absence or presence of 0.4 µM GST-PEX9. The conversion of DQ-peptides into fluorogenic peptides was monitored on a fluorescence reader. The fluorescence and the maximal velocity of the enzyme (initial velocity at 10 minutes read point) from the same experiment are shown in the two graphs. B, Preparations of 2 µg actin or αB-crystallin were incubated with active MMP-9 (actMMP-9) in the absence or presence of 0.4 µM GST-PEX9. After the indicated times at 37°C, cleaved proteins were analyzed by SDS-PAGE and Coomassie blue staining. C, D, Velocity of the degradation of DQ-collagen by MMP-9 (C) and DQ-gelatin by MMP-2 (D). Maximum velocity (initial velocity time point at 10 minutes) was normalized to 100 and enzymes were used at 0.1 nM. AU: Arbitrary units.

FIGURE 3. Effect of the B1B2 and B3B4 regions of PEX9 on the inhibition of gelatin degradation by MMP-9. A, Schematic drawing of the truncated GST-B1B2 and GST-B3B4 fusion proteins prepared in this study. B, SDS-PAGE analysis of the purified GST-fusion proteins, visualized by Coomassie-blue staining. C, Representative gelatin zymography analysis using 20 ng of proMMP-9 and with or without the indicated proteins (0.4 µM each). D, 60 nM of MMP-9 was added to FITC-gelatin-coated plates in the absence or presence of 0.4 µM of the indicated proteins. After 24 h at 37°C, fluorescence was determined and control values were normalized to 100. E, Effect of the indicated proteins on the conversion of DQ-gelatin into fluorogenic gelatin. The lines show the maximal enzyme velocity evolution as a function of the amount of substrate. Bars represent standard deviation. *, p < 0.05; **, p < 0.01. AU: Arbitrary units.
FIGURE 4. **Blades B1 and B4 of PEX9 inhibit gelatin degradation by MMP-9.** A. Schematic drawing of the truncated recombinant GST-fusion proteins. B, SDS-PAGE analysis of the purified proteins shown in (A). C, BCECF-AM-labeled MEC-1 cells were added to wells coated with the indicated proteins (0.4 μM) and adhesion was quantitated as explained. D, MEC-1 cells were incubated for 30 min with or without the indicated proteins (0.8 μM) and analyzed by flow cytometry using anti-GST Abs. E, Representative gelatin zymography analysis of 20 ng of purified recombinant proMMP-9 in the presence or absence of the indicated proteins. Values (arbitrary units) represent the average of 3 different experiments. F, 60 nM of MMP-9 was added to FITC-gelatin-coated plates in the absence or presence of the indicated proteins. After 24 h, the fluorescence was determined. G, Effect of the indicated proteins on the conversion of DQ-gelatin into fluorogenic gelatin. The graph represents the maximal enzyme velocity evolution as a function of the amount of substrate. *, p < 0.05; **, p < 0.01. AU: Arbitrary units.

FIGURE 5. **Further characterization of the effect of PEX9 blades B1 or B4 on gelatin degradation by MMP-9.** A, Schematic drawing of the truncated GST-fusion proteins lacking specific blades of PEX9 and SDS-PAGE analysis of the purified proteins. B, Cell adhesion of BCECF-AM-labeled MEC-1 cells to the indicated proteins (all at 0.4 μM). C, Effect of the indicated mutant proteins on the conversion of DQ-gelatin into fluorogenic gelatin. The maximal enzyme velocity (initial velocity t=10min) evolution as a function of the amount of substrate is shown. D, Dose-dependent inhibition of MMP-9-driven DQ-gelatin degradation by the indicated recombinant proteins. The graph shows the inhibition of the maximal velocity in each experiment, after normalizing control signals to 100. E, Binding of the indicated proteins to 5 μg immobilized gelatin determined by ELISA. F, Effect of PEX9-derived proteins on the degradation of FITC-gelatin by MMP-9-MEC-1 cells. 1 x 10^5 MMP-9-MEC-1 cells were added to 96-well plates coated with FITC-gelatin, in the absence or presence of the indicated proteins. After 24 h at 37ºC, wells were washed and the remaining fluorescence was determined. The values from control wells (FITC coated without cells) were normalized to 100. Bars represent standard deviation. AU: Arbitrary units. *, p < 0.05; **, p < 0.01.
TABLE 1: Primers designed to generate the various GST-PEX9 recombinant proteins

| Protein | Primers 5'-3' |
|---------|--------------|
| PEX9 F  | GAATTCCTTTTGGAGTCCGGGACG |
| PEX9 R  | CTCGAGCTAGTCCTCAGGGCAGTCA |
| B1B2 F  | GAATTCCTTTTGGAGTCCGGGACG |
| B1B2 R  | CTCGAGTCACCTGGCCACGTC |
| B3B4 F  | GAATTCAGCCGCAGTGGCCAG |
| B3B4 R  | CTCGAGCTAGTCCTCAGGGCAGTCA |
| B1 F    | GAATTCCTTTTGGAGTCCGGGACG |
| B1 R    | CTCGAGTCACCTGGCCACGTC |
| B2 F    | GAATTCACCGCTGGAGTGCCTTGT |
| B2 R    | CTCGAGTCACCTGGCCACGTC |
| B3 F    | GAATTCAGCCGCAGTGGCCAG |
| B3 R    | CTCGAGTCACCTGGCCACGTC |
| B4 F    | GAATTCCTTTTGGACACGCAG |
| B4 R    | CTCGAGTCACCTGGCCACGTC |
| ΔB1 F   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB1 R   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB2 F   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB2 R   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB3 F   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB3 R   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB4 F   | TCTAGAGCTGGACTCGGTCTTTGA |
| ΔB4 R   | TCTAGAGCTGGACTCGGTCTTTGA |
A. Diagram showing the structure of proMMP-9, including catalytic domain, Hemopexin domain (PEX9), and SH domain.

B. Western blots showing GST-PEX9 and GST bands.

C. Graph showing % FITC Gelatin degradation over time (min) with and without act MMP-9.

D. Gelatin degradation assays comparing control, GST, and GST-PEX9 with proMMP-9 trimer and monomer.

E. % FITC Gelatin degradation graphs comparing control, GST, and GST-PEX9.

F. Graph showing MMP-9 velocity (AU) with and without GST-PEX9.

G. Graph showing MMP-9 velocity (AU) with varying concentrations of DQ™-Gelatin.

Figure 1
PEX9-derived recombinant proteins

GST-PEX9  GST B1 B2 B3 B4
GST-B1B2  GST B1 B2
GST-B3B4  GST B3 B4

---

**Figure 3**

(A) PEX9-derived recombinant proteins

(B) Mw (kDa) of GST-PEX9, GST-B1B2, and GST-B3B4

(C) % Gelatin degradation of Control, GST-PEX9, GST-B1B2, and GST-B3B4

(D) % FITC Gelatin degradation of Control, GST-PEX9, GST-B1B2, and GST-B3B4

(E) MMP-9 velocity (AU) for Control, GST-PEX9, GST-B1B2, and GST-B3B4 with DQ™-Gelatin µg/ml

---

n=4

* p < 0.05

** p < 0.01
Figure 4

A

PEX9-derived recombinant proteins

GST-PEX9  GST B2 B3 B4
GST-B1  GST B1
GST-B2  GST B2
GST-B3  GST B3
GST-B4  GST B4

B

Mw (kDa)

50  37  25

C

n=3

% Cell adhesion

GST-PEX9  GST-B1  GST-B2  GST-B3  GST-B4

D

Mean Fluorescence Intensity

3%  95%  44%

GST-B2  GST-B3  GST-B4

n=3

% Gelatin degradation

E

proMMP-9

Control  GST-PEX9  GST-B1  GST-B2  GST-B3  GST-B4

F

n=3

% FITC Gelatin degradation

Control  GST-PEX9  GST-B1  GST-B2  GST-B3  GST-B4

G

MMP-9 velocity (AU)

n=3

DQ™-Gelatin µg/ml
A) PEX9-derived recombinant proteins

B) % Cell adhesion

C) MMP-9 velocity (AU)

D) % Inhibition of MMP-9 velocity

E) Absorbance (492nm)

F) % Gelatin degradation by CLL cells

Figure 5

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Inhibition of MMP-9-Dependent Degradation of Gelatin, but Not Other MMP-9 Substrates, by the MMP-9 Hemopexin Domain Blades 1 and 4
Estefania Ugarte-Berzal, Jennifer Vandooren, Elvira Bailon, Ghislain Opdenakker and Angeles Garcia-Pardo

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