Matrix metalloproteinases (MMPs) including membrane type 1 MMP (MT1-MMP) can degrade extracellular matrix and cell surface receptor molecules and have an essential function in malignancy. Recently, we established a functional link between MT1-MMP and the receptor of complement component 1q (gC1qR). The gC1qR is known as a compartment-specific regulator of diverse cellular and viral proteins. A fragment encoded by proliferating cells, soluble gC1qR may inhibit complement component 1q hemolytic activity and play important roles in vivo in assisting tumor cells to evade destruction by complement. Here, we report that gC1qR is susceptible to MT1-MMP proteolysis in vitro and in cell cultures. The major MT1-MMP cleavage site (Gly79-Gln80) is localized within the structurally disordered loop connecting the β2 and the β4 strands of gC1qR. The recombinant MT1-MMP construct that included the catalytic domain but lacked the hemopexin-like domain lost the proteolytic capacity; however, it retained the ability to bind gC1qR. Inhibition of MT1-MMP activity by a hydroxamate inhibitor converted the protease into a cell surface receptor of gC1qR and promoted co-precipitation MT1-MMP with the soluble gC1qR protein. It is tempting to hypothesize that these novel mechanisms may play important roles in vivo and have to be taken into account in designing hydroxamate-based cancer therapy.

Human gC1qR (also known as p33 or p32), originally cloned as a splicing factor 2-associated protein (1), has been reported to interact with a variety of molecules including, but not limited to, cell surface adrenergic and GABA receptors, protein kinase C, and certain viral and bacterial proteins (2–11). In addition to these proteins, gC1qR represents a receptor of complement component 1q (C1q1; the subcomponent of the C1 complex of the classical pathway of complement activation) in tumor cells and can interact with globular heads of C1q (12–15). In cells, gC1qR is present in the cytosol, the plasma membrane, and mitochondria, and the soluble forms of gC1qR are released into the surrounding milieu by proliferating cells (3, 16). The soluble gC1qR protein inhibits C1q hemolytic activity (3, 16). Once released by cells, soluble gC1qR may play important roles in vivo as regulator of complement activation. In this manner, proliferating cells may evade destruction or elimination by complement.

Recently, a membrane-tethered membrane type 1 matrix metalloproteinase (MT1-MMP) (17), an enzyme shown to be essential to tumor invasion and metastasis (18–20), has been found to associate with gC1qR via the enzyme’s cytoplasmic tail. MT1-MMP is largely localized to the invasive front of highly motile cancer cells (21–23). This protease is thought to be directly involved in matrix breakdown (24) and proteolysis of cell surface receptors (25–27). Although a direct functional link between gC1qR and MT1-MMP is still uncertain, our observations suggested that there might be reciprocal interactions involving these proteins in vivo. This prompted us to evaluate whether gC1qR is susceptible to MT1-MMP proteolysis.

Here, we report that breast carcinoma cells release soluble gC1qR in the medium. MT1-MMP forms an enzyme-substrate complex with released gC1qR and cleaves this protein in vitro and in cell culture conditions. Our findings imply that the proteolytically inactive full-length MT1-MMP species may represent cell surface receptors of gC1qR, while MT1-MMP activity expressed at the surface of aggressive tumor cells is likely to reduce the levels of soluble gC1qR in the tumor vicinity.

MATERIALS AND METHODS

Antibodies and Reagents—A murine mAb 60.11, directed against the N-terminal portion of gC1qR, and rabbit polyclonal antibody pAb-p33 were described earlier (12, 28). Where indicated, mAb 60.11 was labeled with sulfo-N-hydroxyssuccinimide-LC-biotin (Pierce) and separated from excess biotin according to the manufacturer’s recommendations. Rabbit antibody ABs15 against a 285–318 hinge region of MT1-MMP was from Chemicon International (Temecula, CA). Goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP); donkey anti-mouse IgG conjugated with HRP; and soluble Ser24–Val524 pro-MT1-MMP consisting of the prodomain, the catalytic (CAT) domain and the hemopexin-like (PEX) domain (pro-MT1-MMP-PEX) were from Chemicon. The recombinant individual Tyr112–Gly289 CAT domain of the wild type MT1-MMP (MT1-MMP-CAT) was isolated from Escherichia coli, purified, and have to be taken into account in designing hydroxamate-based cancer therapy.

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§ The abbreviations used are: C1q, complement component 1q; CAT, catalytic domain; HRP, horseradish peroxidase; MMP, matrix metalloproteinase; MMP-2, matrix metalloproteinase-2; MT1-MMP, membrane type 1 MMP; TIMP, tissue inhibitor of MMPs; PEX, hemopexin domain; PMA, phorbol 12-myristate 13-acetate; WT, wild type; mAb, monoclonal antibody.

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Proteolysis of gC1qR by MT1-MMP

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ELISA—ELISA was used to quantify biotin-labeled gC1qR. The gC1qR protein was labeled with sulfo-N-hydroxysuccinimide-LC-biotin according to the manufacturer's recommendations (Pierce). Wells of a 96-well plate (Costar, Cambridge, MA) were coated with anti-gC1qR mAb 60.11 (1 μg/ml) in PBS at 4 °C overnight. After removing unbound gC1qR, the plates were blocked with 1% casein in PBS for 1 h at 37 °C. Medium samples (100 μl) were allowed to bind immobilized mAb 60.11 for 1 h at 37 °C. After washing, bound biotin-labeled material was identified by ExtrAvidin-HRP and the TMB/E substrate. The A450 of the samples was measured in a microplate reader. The concentrations of gC1qR in the medium samples were calculated by using a calibration curve that ranged from 0.1 to 10 ng/ml. Washes between reactions were done in PBS containing 0.1% Tween 20.

Binding of gC1qR with MT1-MMP-CAT and MT1-MMP-PEX—Wells of a 96-well plate (Costar) were coated overnight at 4 °C with 100 μl of PBS containing MT1-MMP-CAT (200 ng/ml) or MT1-MMP-PEX (500 ng/ml; an equimolar amount relative to that of MT1-MMP-CAT). After binding with 1% casein in PBS, MT1-MMP-CAT or MT1-MMP-PEX was incubated for 1 h at 37 °C in 100 μl of PBS supplemented with AGN3340 (1 μM), GM6001 (1 μM), TIMP-2 (2 μg/ml), or TIMP-1 (2 μg/ml) or left untreated. Afterward, gC1qR solution (10 μg/ml) in 1% casein-PBS was allowed to bind to the MT1-MMP samples for 1 h at 37 °C. Bound gC1qR was detected by incubating the plate with mAb 60.11 (1 μg/ml in PBS-1% casein) followed by incubation with donkey anti-mouse IgG conjugated with HRP and the TMB/E substrate. The plate was washed with PBS containing 0.1% Tween 20 between each change of reagents. The reading of casein control (background) was subtracted from the A450 values of the experimental samples.

Determination of Protein Concentration—Protein concentration was determined by dye methods with a Bio-Rad assay kit with bovine serum albumin as the standard.

RESULTS

gC1qR Cleavage by MT1-MMP in Vitro—Two soluble truncated forms of MT1-MMP, specifically pro-MT1-MMP-PEX, comprising both the CAT domain and the PEX domain, and the MT1-MMP-CAT construct that represents the individual catalytic domain of the enzyme, were used in our studies. The MT1-MMP-CAT construct isolated from E. coli exhibits high proteolytic activity after refolding (29). Activation is required to convert the pro-MT1-MMP-PEX latentzymogen into the catalytically active MT1-MMP-PEX enzyme. The pro part of MT1-MMP-PEX was cleaved by MT1-MMP-CAT to generate catalytically active MT1-MMP-PEX. MT1-MMP-PEX was immediately used in the gC1qR cleavage reactions. At the 1:30 enzyme-substrate molar ratio, proteolytically active MT1-MMP-PEX (2 pmol) cleaved gC1qR (60 pmol), generating the predominant proteolytic fragment with a molecular mass of about 17 kDa and two minor fragments, 12 and 11 kDa (Fig. 1A).

Since glucose oxidase has an acidic pI value similar to that of gC1qR, this control protein was used in our studies. In a 5-fold molar excess relative to gC1qR, glucose oxidase failed to affect the gC1qR proteolysis, thus supporting the unique sensitivity of gC1qR to MT1-MMP-PEX (Fig. 1B). Both GM6001 (1 μM), a wide range hydroxamine inhibitor of MMPs, and TIMP-2 (200 ng, 10 pmol) fully inhibited the cleavage of gC1qR by MT1-MMP-PEX. In contrast, TIMP-1 (270 ng, 9 pmol), known as a poor inhibitor of MT1-MMP (36), had no effect (Fig. 1B).

Earlier, we found that the cytoplasmic tail sequence of MT1-MMP associates with gC1qR (2). Our present observations show that, apparently, there is a second gC1qR binding site in the MT1-MMP molecule. We suggest that this site comprises the enzyme’s CAT domain involved in the enzyme-substrate MT1-MMP-gC1qR complex.

Intriguingly, the individual MT1-MMP-CAT construct that lacks the PEX domain in contrast to MT1-MMP-PEX was incapable of cleaving gC1qR (Fig. 1C). Evidently, the presence of the PEX domain is critical to accomplishing the cleavage of gC1qR by the protease.

Further, we examined whether MMP-2 is capable of cleaving...
gC1qR (Fig. 2). For these purposes, pro-MMP-2 essentially free of TIMP-2 was isolated from medium conditioned by p2AHT2A72 cells (a derivative of the HT1080 fibrosarcoma cell line doubly transfected with E1A and pro-MMP-2) (30, 31). Purified MMP-2 was activated by MT1-MMP-CAT. The conversion of the 68-kDa latent zymogen into the 62-kDa enzyme of MMP-2 was confirmed by gelatin zymography (Fig. 2A). The activated MMP-2 enzyme was immediately employed in our cleavage studies. However, as can be seen in Fig. 2B, activated MMP-2 failed to induce any significant hydrolysis of gC1qR.

The Cleavage Site Sequence—To identify relative positions of the cleavage fragments within the gC1qR molecule, we used Western blotting with mAb 60.11 directed to the N-terminal portion of gC1qR. This antibody recognized the 12- and 11-kDa cleavage fragments but failed to interact with the 17-kDa fragment of gC1qR (Fig. 3). These data indicated that the MT1-MMP cleavage sites were located within the N-terminal part of the gC1qR molecule. The N-terminal sequencing of the 17-kDa...
fragment indicated that MT1-MMP cleaved the mature gC1qR protein at the PSQG\(^20\) \(\rightarrow\) QVE site, providing the 17-kDa fragment with the N-terminal QKVEEQE sequence. In the known crystal structure of the doughnut-shaped gC1qR (5), the PSQG\(^20\)QVKE segment represents a highly disordered \(\beta\)-strand loop connecting the \(\beta\)-strands to the \(\beta\)-strands (Fig. 3B). The surface location of this structurally disordered loop explains its sensitivity to proteolysis. Evidently, the binding of the loop sequence to the PEX domain is necessary to achieve the proper orientation of the loop relative to the active site of MT1-MMP-PEX. This may explain the inability of MT1-MMP-CAT that lacks the PEX sequence to accomplish the cleavage of gC1qR. The subsequent computer modeling supports this hypothesis (see “Discussion”).

Cleavage of gC1qR in Cell Cultures—The \(\textit{in vitro}\) results prompted us to examine whether gC1qR is sensitive to MT1-MMP expressed in MCF7 breast carcinoma cells. We specifically selected this cell line for our studies, since it is deficient in pro-MMP-2 and MT1-MMP (34, 37). This provided us with the opportunity to specifically identify the effects of MT1-MMP expressed in stably transfected cells.

First, we evaluated by Western blotting the levels of soluble gC1qR in medium conditioned by mock cells and by cells expressing the MT1-MMP-E240A, MT1-MMP-WT, and MT1-MMP-\(\Delta\)CT constructs. All cell lines exhibited soluble gC1qR in medium. We found that there was no significant difference in the levels of soluble natural gC1qR (about 10–20 ng/ml) in these cell lines (Fig. 4A).

Second, we evaluated the ability of cellular MT1-MMP to degrade soluble gC1qR. To eliminate the contribution of the biosynthesis of gC1qR and to specifically target the degradation of this protein, we used biotin-labeled gC1qR. For these purposes, 30 ng/ml biotin-labeled gC1qR was added to medium conditioned by mock cells and by cells expressing the MT1-MMP-E240A, MT1-MMP-WT, and MT1-MMP-\(\Delta\)CT constructs. After incubation of biotin-gC1qR with cells for 16 h, the residual concentrations of biotin-gC1qR in medium were measured by ELISA and calculated by using a calibration curve (Fig. 4B), which ranged from 10 to 100 ng/ml biotin-labeled gC1qR (Fig. 4B, inset). The levels of biotin-gC1qR remained largely unchanged in medium conditioned by the cell lines expressing no MT1-MMP activity such as mock cells and MT1-MMP-E240A-transfected cells. In turn, cell lines expressing MT1-MMP activity such as MT1-MMP-WT and MT1-MMP-\(\Delta\)CT cells were both competent in depleting biotin-gC1qR (Fig. 4C). Thus, there was a correlation between expression of functionally active MT1-MMP in the cells and the levels of soluble gC1qR in the medium, thereby suggesting the degradation of gC1qR by this protease. Importantly, biotinylation did not significantly alter sensitivity of gC1qR to the cleavage by MT1-MMP-PEX (data not shown).

The contribution of MT1-MMP to proteolysis of gC1qR was further confirmed by employing HT1080 fibrosarcoma cells in our studies. This cell line is known to express endogenous MT1-MMP, which upon stimulation for example by phorbol esters is recruited to the plasma membrane (31, 38, 39). At the cell surface, MT1-MMP participates in pro-MMP-2 activation (30, 40). This inducible expression allowed us to examine how cell surface MT1-MMP contributes to the proteolysis of gC1qR (Fig. 4C, inset). For these purposes, biotin-labeled gC1qR was added to medium conditioned by PMA-stimulated or untreated HT1080 cells. Pretreatment of HT1080 cells with PMA resulted in efficient activation of pro-MMP-2, while less significant activation of pro-MMP-2 was observed in untreated cells (Fig. 4C, inset). Concomitantly, PMA pretreatment enhanced degradation of biotin-labeled gC1qR by HT1080 cells and, accordingly, decreased the levels of the labeled protein in the medium. The addition of the hydroxamate GM6001 inhibitor alone or its co-addition with PMA strongly inhibited both the proteolysis of gC1qR and activation of MMP-2 in HT1080 cells (Fig. 4C). Since purified activated MMP-2 failed to cleave gC1qR (Fig. 2), our data suggest that MT1-MMP naturally expressed in HT1080 cells is directly involved in proteolysis of gC1qR.

FIG. 4. MT1-MMP expressed on cell surfaces cleaves soluble gC1qR released by the cells in the extracellular milieu. A, immunoblotting of extracellular gC1qR. One-mL aliquots of medium conditioned by mock cells and cells expressing MT1-MMP-WT (WT), MT1-MMP-E240A (E240A), or MT1-MMP-\(\Delta\)CT (\(\Delta\)CT) were 50-fold concentrated by a SpeedVac Concentrator® (Savant, Farmingdale, NY) and analyzed by Western blotting employing mAb 60.11, HRP-conjugated donkey anti-mouse IgG, and the TMB/M substrate. Left lane, the purified gC1qR protein (100 ng). B, gC1qR ELISA. The purified gC1qR protein was labeled with biotin. The inset shows Western blot of biotin-labeled gC1qR developed with ExtrAvidin-HRP. Increasing concentrations of biotin-labeled gC1qR were allowed to bind plastic coated with anti-gC1qR mAb 60.11. Bound biotin-labeled material was detected with ExtrAvidin-HRP and the TMB/E substrate. The A\(_{405}\) was measured on a microplate reader. C, functionally active MT1-MMP but not MMP-2 degrades soluble biotin-labeled gC1qR in medium. HT1080 cells naturally expressing MT1-MMP and pro-MMP-2, MCF7 mock cells and MCF7 cells expressing the MT1-MMP-WT, MT1-MMP-E240A, and MT1-MMP-\(\Delta\)CT constructs were incubated for 18 h in medium supplemented with biotin-labeled gC1qR. Where indicated, cells were pre-treated with PMA (50 ng/ml) for 18 h, GM6001 (50 \(\mu\)M), or both. The inset shows gelatin zymography of MMP-2 from medium conditioned by HT1080 cells to confirm efficient activation of MMP-2 induced by PMA. The residual concentrations of gC1qR in medium were measured by ELISA and quantified using the calibration curve shown in B. Data bars represent the levels of the gC1qR cleavage from one representative experiment.
gC1qR in Vitro Binds the Catalytic Domain of MT1-MMP—
Next, we examined the ability of MT1-MMP-CAT and MT1-MMP-PEX to directly bind gC1qR. For these purposes, the purified and refolded MT1-MMP-CAT or MT1-MMP-PEX were each coated on plastic and then allowed to bind gC1qR in the presence and absence of MT1-MMP inhibitors including hydroxamate class inhibitors AGN3340 and GM6001 and TIMPs such as TIMP-1 (a poor inhibitor of MT1-MMP activity) and TIMP-2 (a potent inhibitor of MT1-MMP activity). After washings, the bound gC1qR was identified by mAb 60.11 followed by incubation of immune complexes with an HRP-conjugated secondary antibody. Our data indicated that MT1-MMP-CAT was capable of directly associating with gC1qR. Inhibition of MT1-MMP activity reduced but not abolished the ability of the recombinant construct to bind gC1qR. Similarly to MT1-MMP-CAT, MT1-MMP-PEX was capable of binding to gC1qR, and the binding was sensitive to inhibition by a hydroxamate (Fig. 5A).

The cleavage of α1-antitrypsin by MT1-MMP was used to confirm the proteolytic activity of the MT1-MMP construct and the effects caused by the inhibitors. Consistent with the observations of other authors (41, 42), α1-antitrypsin was sensitive to MT1-MMP. Thus, at the 1:15 enzyme-substrate ratio, MT1-MMP-CAT (20 ng) efficiently cleaved α1-antitrypsin (1 μg) (Fig. 5B). All three potent inhibitors of MT-MMP activity (AGN3340, GM6001, and TIMP-2) fully blocked the gC1qR proteolysis by MT1-MMP-CAT, while TIMP-1 failed to significantly affect MT1-MMP activity. These findings presumably indicate that there is a partial but not complete overlap of the gC1qR- and inhibitor-binding sites within the active site of the catalytic domain of MT1-MMP. Particularly, these data suggest that once inhibited by hydroxamates or TIMP-2, MT1-MMP expressed at the plasma membrane may represent a cellular receptor of gC1qR.

gC1qR Binds with Hydroxamate-inactivated MT1-MMP in the Cell System—To confirm our in vitro observations, we employed cells expressing MT1-MMP-WT in biotinylation and immunoprecipitation studies. A sensitive procedure for surface biotinylation and immunoprecipitation provided data about cell surface expression of MT1-MMP. Several major forms of MT1-MMP have been described in cells, including a 63-kDazymogen, an active species at 55–60 kDa, and the catalytically inactive truncated fragments of 38–45 kDa (34, 43). In agreement, anti-MT1-MMP antibody precipitated the full-length MT1-MMP and its degradation fragments from lysates of surface-biotinylated cells preincubated with or without GM6001, respectively (Fig. 6). No specific bands were observed in mock cells.

Anti-gC1qR pAB-p33 precipitated a 33-kDa biotin-labeled protein from the lysates of MT1-MMP-WT cells pretreated with GM6001. This band was highly similar in its mobility to the purified biotin-labeled gC1qR control. No similar bands were observed in mock cells and untreated MT1-MMP-WT cells. Remarkably, the material precipitated with anti-MT1-MMP antibody demonstrated the presence of the same 33-kDa protein in cells pretreated with GM6001, while no co-precipitation of this protein with MT1-MMP was observed in untreated cells (Fig. 6). Conversely, a band that resembled the catalytically inactive truncated fragment of MT1-MMP was co-immunoprecipitated with gC1qR. Similar results were obtained with another potent hydroxamate inhibitor AGN3340 (data not shown).

Computer Modeling of the MT1-MMP-gC1qR Complex—To specifically address the role of the PEX domain peptide sequence in the MT1-MMP-gC1qR complex, we studied the possible binding modes of a gC1qR monomer to both MT1-MMP-CAT and MT1-MMP-PEX by computer modeling. The Insight software package (available on the World Wide Web at www.msi.com) and the GRAMM computer program (44–46) were used in our modeling studies. The crystal structures of MT1-MMP-CAT (47) and gC1qR (Protein Data Bank code 1P32) (5) are known, but the structure of MT1-MMP’s PEX domain is unavailable so far. To build the model of MT1-MMP-PEX, we used the CAT domain of MT1-MMP (Protein Data Bank code 1CK7; residues 461–660) (48). The sequence of the PEX domain of MT1-MMP is about 40% identical to that of the PEX domain of MMP-2. The structures of MT1-MMP-CAT and the
MMP-2's PEX domain were merged to build the three-dimensional model of the CAT(MT1-MMP)-PEX(MMP-2) chimera. To refine our model and to build a mutant CAT(MT1-MMP)-PEX(MMP-2) chimera, the amino acid residues that distinguish the PEX domain of MMP-2 (Leu461, Gly462, Val464, Thr465, Ile468, Cys469, Gln471, Thr496, Val497, Thr498, Pro499, Arg500, Asp501, Lys502, Pro503, Met504, and Gly505) were replaced by the residues of the PEX domain of MT1-MMP (Ser461, Arg462, Ser464, Val465, Asp467, Lys468, Pro490, Arg497, Asn498, Asn499, Gln501, Val501, Met502, Asp503, Gly504, and Thr505) in those sequence regions that, according to our modeling (Fig. 8), are likely to be involved in the binding with gC1qR. The mutations did not affect the docking results. The overall orientation of the domains and the general shape of the molecule were highly similar in MMP-2 and the mutant CAT(MT1-MMP), PEX(MMP-2) chimera (Fig. 7).

Then we docked these two molecules, the CAT domain of MT1-MMP and the CAT(MT1-MMP), PEX(MMP-2) mutant chimera, to the monomer of gC1qR. This was accomplished by using the GRAMM program. In each case, the 10 most probable models of the respective complexes were generated. The models with the highest probability scores are shown in Fig. 8, C and D. Evidently, the binding mode of the individual MT1-MMP-CAT to gC1qR is totally distinct from the one found for the CAT(MT1-MMP), PEX(MMP-2)-gC1qR chimeric complex.

**DISCUSSION**

The sustained presence of MMPs in the tumor environment produced both by the activated host cells and the cancer cells leads to the destruction of normal extracellular matrix and remodeling of tissue. Among known human MMPs, membrane-tethered MT1-MMP has been shown to be most important in promoting tumor cell invasion (17–19, 24, 26, 27, 49–53). Evidence emerges that, in addition to its ability to directly degrade matrix components, MT1-MMP is involved in cleavage of cell surface receptors including CD44 (27), tissue transglutaminase (25), and precursor of integrin α-subunit (62). This allows the cancer cells to adjust their receptor profile to continually changing matrix environment. Recently, we identified a functional link between MT1-MMP and the gC1qR intracellular protein, which is presumably associated with the mechanisms of trafficking and internalization of the protease (62).

gC1qR exhibits many puzzling features, and its functional role is not understood in detail (3). gC1qR exists in two molecular forms, the 1–282 precursor and the 74–282 mature protein. The mature gC1qR is generated by post-transcriptional proteolytic processing, whose mechanisms are unknown. Since the N-terminal region of premature gC1qR has a structure typical to mitochondrial signal peptide, the gC1qR precursor is directed to the mitochondrial matrix, where it is thought to be important for the maintenance of mitochondrial oxidative phosphorylation (54, 55). The mature multiligand-binding gC1qR protein participates in many interactions outside mitochondria and may be found at the cell membrane, in the cytosol, in the nucleus, and in the extracellular milieu (3, 16). The gC1qR protein was shown to directly regulate the functional activity of the splicing factor SF2/ASF (1), protein kinase C (11), adrenergic receptor (10), GABA receptor β-subunit (9), the plasma complement component C1q (12, 15), high molecular weight kininogen (56), factor XII (6), and some other plasma and extracellular matrix proteins. In addition, mature gC1qR is believed to interact with certain viral proteins (57, 58) and to be involved in their trafficking to the nucleus. Once released by cells into the extracellular milieu, the soluble gC1qR protein may inhibit C1q hemolytic activity (16). This function of soluble...
strongly affect the mechanisms involved in the binding and proteolysis of gC1rR by MT1-MMP.

Our data suggest that both MT1-MMP in the cells naturally expressing the enzyme and MT1-MMP exhibited by the transfected cells hydrolyze soluble gC1rR existing in medium. This may modulate the levels of gC1rR in the tumor cell microenvironment. To our knowledge, this is the first evidence that cellular MT1-MMP is capable of cleaving a soluble, nonmatrix protein. Remarkably, inhibition of cell surface MT1-MMP activity by hydroxamate inhibitors promoted co-precipitation of the protease with the soluble gC1rR protein from the extracellular milieu. These observations suggest that inhibition of MT1-MMP activity is likely to convert the protease into a cell surface receptor of gC1rR and, subsequently, C1q. The calculations based on our immunoprecipitation studies suggest that MT1-MMP-WT cells preincubated with hydroxamate inhibitor GM6001 are able to manifest hundreds of the gC1rR molecules per cell. It is tempting to hypothesize that inhibition of MT1-MMP by hydroxamates and the subsequent complex formation of the inhibited MT1-MMP molecules with gC1rR may promote an immune-mediated attack on those target cells in vivo (60, 61). This may superficially contribute to invasion of tumor cells, which have escaped inhibitor and still expressed functionally active MT1-MMP. Experiments are currently under way to determine whether the MT1-MMP-gC1rR complex represented by the hydroxamate-treated cells can be a designated receptor for C1q and to facilitate selective elimination of tumor cells by the immune system. Our data support the emerging concept that an integration of matrix degradation and limited proteolysis of both soluble and cellular key regulatory proteins influences the fate of tumor cells and their abilities to invade and metastasize.

REFERENCES


FIG. 8. Modeling of the MT1-MMP-gC1qR complexes. A surface representation of the individual MT1-MMP-CAT (magenta; A) and the CAT-MT1-MMP–PEX chimera (red; B) and their complexes with the gC1qR monomer, chain A (Protein Data Bank code 1P32) (C and D, respectively) is shown. The gC1qR monomer is depicted as a stick model in light blue. Note the difference of the binding mode of gC1qR to the individual MT1-MMP-CAT and to the CAT-MT1-MMP–PEX chimera. The arrow points to the catalytic Zn2+ ion (yellow) of the active site. The MT1-MMP cleavage site (PSQQ80QKVE) is localized in the disordered loop that connects the β3 strand to the β4 strand of the gC1qR molecule. Since its structure is unknown (5), the loop is not depicted in our model.
Proteolysis of gC1qR by MT1-MMP

The Hemopexin-like C-terminal Domain of Membrane Type 1 Matrix Metalloproteinase Regulates Proteolysis of a Multifunctional Protein, gC1qR
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