J Neurooncol (2011) 104:33–43
DOI 10.1007/s11060-010-0468-2

LABORATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

A role for MT1-MMP as a cell death sensor/effector through the regulation of endoplasmic reticulum stress in U87 glioblastoma cells

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Received: 23 August 2010 / Accepted: 8 November 2010 / Published online: 19 November 2010

Abstract Recent findings in cell death signalling show that membrane type 1 matrix metalloproteinase (MT1-MMP), an MMP known for its involvement in cancer cell invasion and metastasis, can act as a "bioswitch" in the invasion versus cell death decision in brain tumour cells. Given that the endoplasmic reticulum (ER) is a subcellular compartment involved in metabolic control and cell death signalling and that cytoskeleton disruption, as encountered during cancer cell invasion, can lead to ER stress, we questioned whether MT1-MMP contributes to ER stress. We found that MT1-MMP gene silencing or pharmacological inhibition of vesicular trafficking with Brefeldin-A abrogated MT1-MMP cell surface-mediated proMMP-2 activation by the lectin Concanavalin-A (ConA) in U87 glioblastoma cells. ConA, also known to trigger the expression of pro-inflammatory cyclooxygenase (COX)-2 through MT1-MMP signalling from the plasma membrane, failed to do so when MT1-MMP was prevented from reaching the cell surface by Brefeldin-A. Gene silencing of MT1-MMP antagonized the expression of ConA-induced COX-2 and of the ER stress marker glucose-related protein 78 (GRP78), further suggesting that plasma membrane localization of MT1-MMP contributes to signalling ER stress. MT1-MMP maturation, which partially occurs during its trafficking from the ER to the plasma membrane, showed correlation of the 60 kDa MT1-MMP with GRP78 expression. Finally, Brefeldin-A treatment of glioblastoma cells led to Akt dephosphorylation; this effect was reversed when MT1-MMP was silenced. Collectively, our results provide a molecular rationale for a new role for MT1-MMP in the regulation of cancer cell death processes through ER stress signalling.

Keywords Endoplasmic reticulum stress · MT1-MMP · Concanavalin-A

Abbreviations

ConA	Concanavalin-A
ECM	Extracellular matrix
ER	Endoplasmic reticulum
GRP78	Glucose-related protein 78
MT1-MMP	Membrane type 1 matrix metalloproteinase

Introduction

The endoplasmic reticulum (ER) is a membrane-bound organelle present in all eukaryotic cells. Recently, ER stress signalling has been linked to disease states involving insulin resistance and disordered lipid metabolism, and to hypoxia tolerance in tumour progression [1, 2]. Accordingly, tumour cells often show evidence of constitutive ER stress, possibly due to hypoxia and glucose depletion [3]. Moreover, the ATP depleting agents and ER stress inducers 2-deoxyglucose and 5-thioglucose have been shown to inhibit MMP-2 secretion from U87 glioblastoma-derived cells [4], a process known to contribute to tumour development [5]. In addition to its role as a multifunctional metabolic compartment that controls entry and release of calcium, sterol biosynthesis, apoptosis and the release of arachidonic acid [6, 7], the ER is primarily known as the

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site of synthesis and folding of secreted, membrane-bound, and some organelle-targeted proteins [8]. Despite its complex organization, the ER is a continuous membrane compartment whose architecture depends upon microtubule dynamics [9]. In accordance with this, the microtubulin cytoskeleton and the centrosomes (the microtubulin cytoskeleton-organizing centers) were found to be essential for the trafficking and internalization of the membrane-bound matrix metalloproteinase MT1-MMP [10], involved in brain tumour cell invasion, extracellular matrix (ECM) degradation and cell–ECM interaction [11].

The expression levels of several members of the MMP family have been shown to correlate with the grade level of gliomas, including that of MT1-MMP, the best-characterized MT-MMP. While most MMPs are secreted, the MT-MMPs are membrane-associated and a number of them have cytoplasmic domains which were recently shown to bear important functions in cellular signalling [12–14]. Aside from its well-known roles in the activation of proMMP-2 and in intrinsic proteolytic activity towards ECM molecules, many new functions of MT1-MMP have recently been associated with PGE_2-induced angiogenesis [15], platelet-mediated calcium mobilization [16], regulation of proinflammatory signalling [17, 18] and radioresistance in both glioma cells [19, 20] and endothelial cells [21]. More importantly, a role for MT1-MMP as a cell death/survival bioswitch has been inferred [14, 22] although the mechanisms and biological significance are poorly understood.

Interactions between carbohydrate-binding proteins (lectins) and the oligosaccharide moieties of glycoproteins at the cell surface are involved in numerous cellular recognition processes including development, differentiation, morphogenesis and cell migration. We used the lectin from *Canavalia ensiformis* (Concanavalin-A, ConA), one of the most abundant lectins known [23], to mimic the biological lectin/carbohydrate interactions that regulate ECM protein recognition. As such, ConA is routinely used to trigger MT1-MMP-mediated activation of latent proMMP-2 [24–26], and was found to increase the sub-G1 cell cycle phase and to trigger cell death. All these events are indicative of a potential role for MT1-MMP in cell surface clustering that affects cell survival [27]. Whether any MT1-MMP-mediated signalling that leads to ER stress is a factor in ConA-induced cell death remains unknown.

In the present study, we provide evidence for MT1-MMP as an actor in the early signalling cascade that leads to ER stress, as reflected by the increase in the ER stress marker glucose-related protein 78 (GRP78). We show that ConA treatment also regulated the pro-inflammatory cyclooxygenase (COX)-2 expression concomitantly with ER stress. Vesicular trafficking evidence, with the use of Brefeldin-A, further enabled us to assess the requirement of MT1-MMP at the cell surface in order to trigger ConA-mediated ER stress. The use of the lectin ConA, as an MT1-MMP-inducing agent, further linked MT1-MMP-mediated intracellular signalling to proinflammatory COX-2 expression that could account for the cell survival and inflammatory balance responsible for the therapy resistance phenotype of glioblastoma cells.

Materials and methods

Materials

Sodium dodecylsulfate (SDS) and bovine serum albumin (BSA) were purchased from Sigma (Oakville, ON). Cell culture media was obtained from Invitrogen (Burlington, ON). Electrophoresis reagents were purchased from Bio-Rad (Mississauga, ON). The enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Baie d'Urfé, QC). Micro bicinchoninic acid protein assay reagents were from Pierce (Rockford, IL). The polyclonal antibody against COX-2 was from Cayman Chemical (Ann Arbor, MI). The polyclonal antibody against MT1-MMP was from Chemicon (Temecula, CA). The polyclonal antibodies against Akt and phosphorylated-Akt were purchased from Cell Signalling (Danvers, MA). The monoclonal antibody against GRP78 was from New England Biolabs (Pickering, ON). The GAPDH antibody was from Advanced Immunochemicals (Long Beach, CA). Horseradish peroxidase-conjugated donkey anti-rabbit and anti-mouse IgG secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). All other reagents were from Sigma-Aldrich Canada.

Cell culture

The human U87 glioblastoma cell line was purchased from American Type Culture Collection (Manassas, VA) and maintained in Eagle's Minimum Essential Medium containing 10% (v/v) calf serum (HyClone Laboratories, Logan, UT), 1 mM sodium pyruvate, 2 mM glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin. Cells were incubated at 37°C with 95% air and 5% CO_2.

Total RNA isolation, cDNA synthesis and real-time quantitative RT-PCR

Total RNA was extracted from cell monolayers using TriZol reagent (Life Technologies, Gaithersburg, MD). For cDNA synthesis, 2 μg of total RNA were reverse-transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). cDNA was stored at −80°C prior to PCR. Gene expression was quantified by real-time

quantitative PCR using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). DNA amplification was carried out using an Icycler iQ5 (Bio-Rad, Hercules, CA) and product detection was performed by measuring binding of the fluorescent dye SYBR Green I to double-stranded DNA. The QuantiTect primer sets were provided by Qiagen (Valencia, CA): MT1-MMP (Hs_Mmp14_1_SG QT00001533) and β-actin (Hs_Actb_2_SG QT01680476). GAPDH primer sets were synthesized by Biocorp (Dollard-des-Ormeaux, QC) with the following sequences: forward CCATCAC CATCTTCCAGGAG and reverse CCTGCTTCACCAC CTTCTTG. The relative quantities of target gene mRNA compared against two internal controls, GAPDH and β-actin RNA, were measured by following a ΔC_T method employing an amplification plot (fluorescence signal versus cycle number). The difference (ΔC_T) between the mean values in the triplicate samples of target gene and those of GAPDH and β-actin mRNAs were calculated by iQ5 Optical System Software version 2.0 (Bio-Rad, Hercules, CA) and the relative quantified value (RQV) was expressed as $2^{-\Delta C_T}$.

Transfection method and RNA interference

Cells were transiently transfected with 20 nM siRNA against MT1-MMP (Hs_MMP14_6 HP validated siRNA, SI03648841) or scrambled sequences (AllStar Negative Control siRNA, 1027281) using Lipofectamine 2000 (Invitrogen, ON). MT1-MMP-specific gene knockdown was evaluated by qRT-PCR as described above. Small interfering RNA and mismatch siRNA were synthesized by Qiagen and annealed to form duplexes.

Gelatin zymography

Gelatin zymography was used to assess the extracellular levels of proMMP-2 and MMP-2 activities. Briefly, an aliquot (20 µl) of the culture medium was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a gel containing 0.1 mg/ml gelatin. The gels were then incubated in 2.5% Triton X-100 and rinsed in nanopure distilled water. Gels were further incubated at 37°C for 20 h in 20 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35, 50 mM Tris–HCl buffer, pH 7.6 and then stained with 0.1% Coomassie Brilliant blue R-250 and destained in 10% acetic acid, 30% methanol in water. Gelatinolytic activity was detected as unstained bands on a blue background.

Immunoblotting procedures

Proteins from control and treated cells were separated by SDS-PAGE. After electrophoresis, proteins were electrotransferred to polyvinylidene difluoride membranes which were then blocked for 1 h at room temperature with 5% non-fat dry milk in Tris-buffered saline (150 mM NaCl, 20 mM Tris–HCl, pH 7.5) containing 0.3% Tween-20 (TBST). Membranes were further washed in TBST and incubated with the primary antibodies (1/1,000 dilution) in TBST containing 3% BSA and 0.1% sodium azide, followed by a 1 h incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (1/2,500 dilution) in TBST containing 5% non-fat dry milk. Immunoreactive material was visualized by ECL (Amersham Biosciences, Baie d'Urfée, QC).

Immunofluorescent microscopy

U87 cells were serum-starved in media containing vehicle or 100 ng/ml Brefeldin-A for 24 h. Brefeldin effect was compared to that of siMT1-MMP-transfected cells as described above. Media was then removed and cells fixed in formalin phosphate buffer (Fisher Scientific, Ottawa, ON) for 20 min, then blocked 1 h in 1% BSA/PBS. Immunostaining was performed for 1 h with the anti-MT1-MMP catalytic domain antibody 1:200 in 1% BSA/PBS (Millipore, Laval, QC), followed by 1:200 anti-mouse-alexa fluor 488 (Invitrogen, ON), and fluorescence examined by microscopy.

Statistical data analysis

Data are representative of three or more independent experiments. Statistical significance was assessed using Student's unpaired *t*-test. Probability values of less than 0.05 were considered significant and an asterisk identifies such significance in the figures.

Results

Brefeldin-A inhibits cell surface localization of MT1-MMP

The efficacy of Brefeldin-A as an inhibitor of vesicular traficking was first assessed. U87 cells were treated with vehicle or Brefeldin-A and cell surface localization of MT1-MMP evaluated by immunofluorescence as described in the "Methods" section. We demonstrate, in fixed non-permeabilized cells, that untreated cells had cell surface fluorescent staining with anti-MT1-MMP antibody directed against its extracellular catalytic domain (Fig. 1, control). In contrast, significantly diminished staining was found in either Brefeldin-A-treated cells (Fig. 1, Brefeldin), or in cells where MT1-MMP expression was silenced (Fig. 1, siMT1-MMP). These data support the cell surface localization of

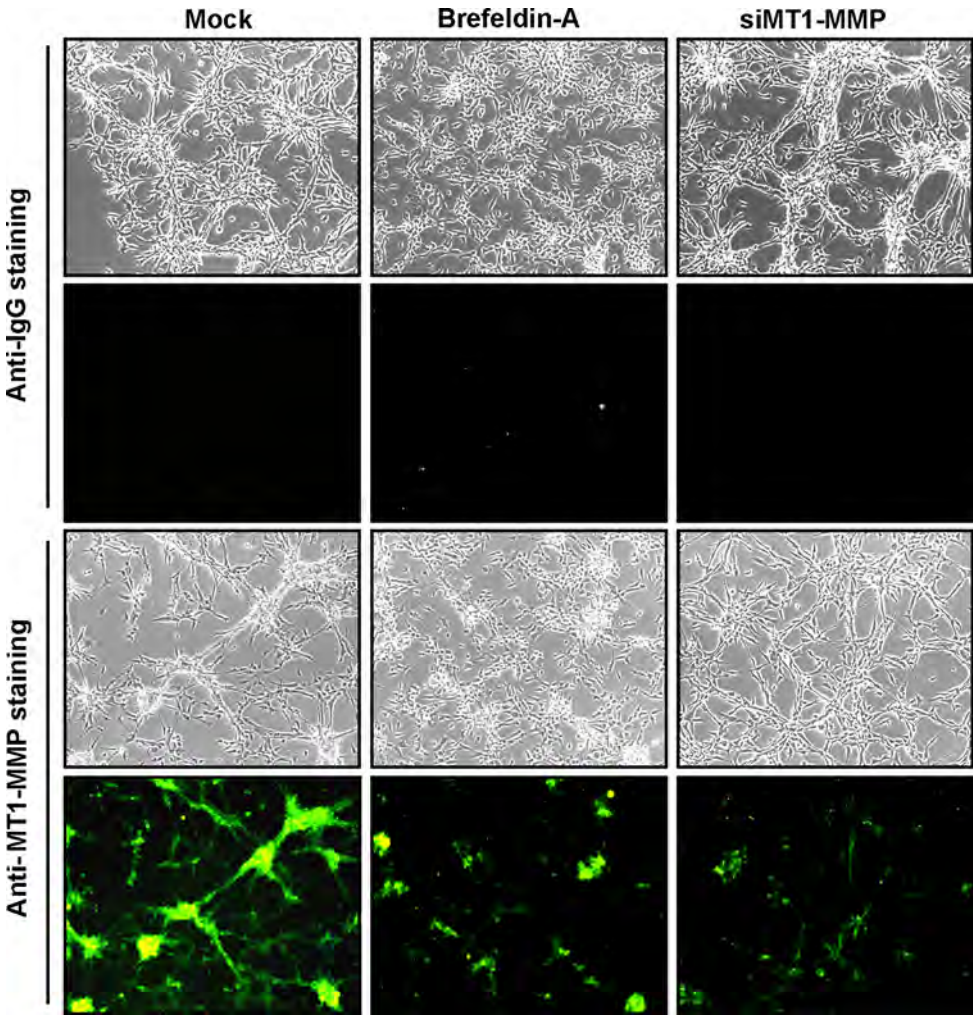

Fig. 1 Brefeldin-A inhibits cell surface localization of MT1-MMP. U87 glioblastoma-derived cells were serum starved and treated with vehicle or 100 ng/ml Brefeldin-A for 18 h. U87 cells were also transfected with siMT1-MMP. Anti-IgG or anti-MT1-MMP catalytic antibody staining was performed as described in the "Methods" section, and phase contrast visible cells or MT1-MMP-associated cell surface fluorescence acquired by microscopy

MT1-MMP and that Brefeldin-A prevented efficient targeting of MT1-MMP to the plasma membrane.

Con-A triggers MT1-MMP gene expression

ConA is a well-documented lectin which, through its binding to carbohydrate moieties on cell surface proteins, elicits very efficient in vitro induction of MT1-MMP expression [14, 28]. Serum-starved U87 glioblastoma cells were therefore treated with ConA, Brefeldin-A, or a combination of both. Total RNA was then isolated and MT1-MMP gene expression assessed using qRT-PCR. We found that ConA effectively induced MT1-MMP gene expression, while Brefeldin was ineffective at doing so (Fig. 2a, Mock). When MT1-MMP gene expression was silenced, the MT1-MMP transcript levels were accordingly decreased under all conditions and ConA's ability to induce MT1-MMP was abrogated (Fig. 2a, siMT1-MMP). Brefeldin's effect on MT1-MMP trafficking to the cell surface was also evaluated. Cell lysates were generated and MT1-MMP protein expression was assessed using immunodetection as described in the "Methods" section. In agreement with the gene expression data, we found that ConA-treated cells exhibited increased MT1-MMP protein expression as reflected by the mature 55 kDa form (Fig. 2b, upper panel). Brefeldin treatment trapped the unprocessed 60 kDa form of MT1-MMP, which intracellular trapped form is in agreement with previous reports highlighting the required molecular maturation and subsequent trafficking to the plasma membrane [29, 30]. In order to evaluate the impact of cellular compartment targeting of MT1-MMP, the levels of proMMP-2 activation were assessed in the conditioned media by gelatin zymography as described in the "Methods" section. We observed that ConA treatment resulted in an increase in proMMP-2 activation, while the combined Brefeldin/ConA treatment resulted in lower activation of proMMP-2 in agreement with the fact that MT1-MMP remained trapped within the cell (Fig. 2b, lower panel). Brefeldin-A treatment alone did not affect cell surface localized MT1-MMP

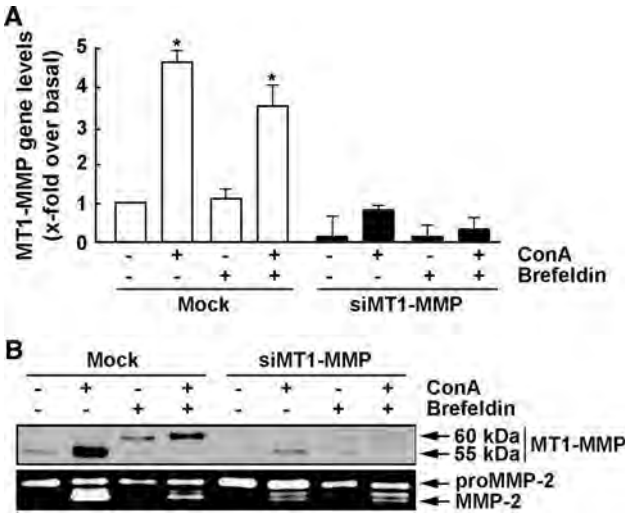

Fig. 2 Concanavalin-A triggers MT1-MMP gene expression. U87 glioblastoma-derived cells were serum starved and treated with 30 μg/ml Concanavalin-A (ConA), 100 ng/ml Brefeldin-A, or a combination of both for 18 h. **a** Total RNA isolation and qRT-PCR were performed as described in the "Methods" section to assess MT1-MMP gene expression in Mock- (*white bars*) or siMT1-MMP-transfected cells (*black bars*). **b** Cell lysates as well as conditioned media were isolated as described in the "Methods" section in order to perform MT1-MMP imunodetection (*top*) and gelatin zymography (*bottom*) to assess proMMP-2 activation states

functions in proMMP-2 activation (data not shown). Gene silencing of MT1-MMP significantly abrogated any ConA-mediated proMMP-2 activation (Fig. 2b).

Brefeldin-A inhibits MT1-MMP trafficking to the plasma membrane

Most of the MT1-MMP functions are performed at the cell surface and include ECM degradation and activation of latent proMMPs as well as hydrolysis of cell surface receptors [12–14]. MT1-MMP's intracellular domain enables additional functions in signal transduction including activation of signalling pathways such as ERK [12], NK-κB [17, 18], and RhoA [31]. We assessed the relevance of MT1-MMP localization at the plasma membrane on the expression of COX-2 and GRP78. ConA and Brefeldin-A treatment of U87 cells all led to increased GRP78 expression (Fig. 3a, upper panel). Only ConA treatment was able to also trigger COX-2 expression (Fig. 3a, middle panel). This suggests that both ConA-induced MT1-MMP synthesis or Brefeldin-A ER trapping of MT1-MMP lead to ER stress while only cell surface localization of MT1-MMP enables ConA to trigger signalling that leads to COX-2 expression in agreement with previous reports [17, 18]. The importance of MT1-MMP expression was further confirmed through gene silencing strategies, where cells transfected with MT1-MMP siRNA not only inhibited

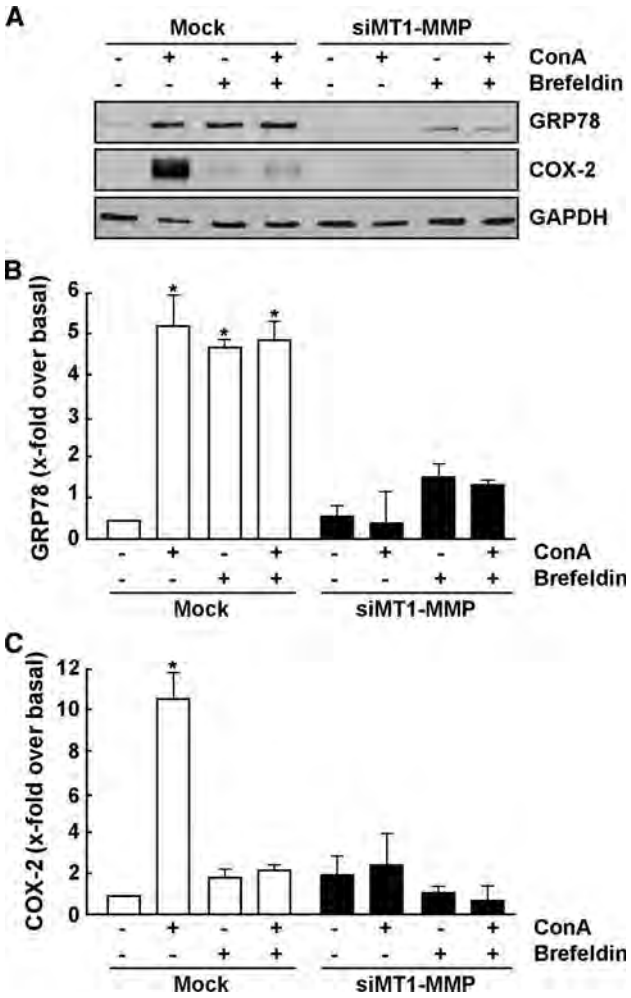

Fig. 3 Brefeldin inhibits MT1-MMP trafficking to the plasma membrane. U87 glioblastoma-derived cells were serum starved and treated with 30 μg/ml Concanavalin-A (ConA), 100 ng/ml Brefeldin-A or a combination of both for 18 h. **a** Cell lysates were isolated as described in the "Methods" section to assess GRP78, COX-2 and GAPDH expression in Mock- (*white bars*) or siMT1-MMP-transfected cells (*black bars*) by western blot. Scanning densitometry was used to assess the extent of **b** GRP78 or **c** COX-2 protein expression

ConA and Brefeldin-A from inducing GRP78 (Fig. 3b, black bars) but also inhibited ConA-induced COX-2 expression (Fig. 3c, black bars).

MT1-MMP proteolytic processing does not contribute to the induction of ER stress by Con-A

The importance of MT1-MMP maturation through proteolytic processing was next assessed using ConA and Brefeldin-A treatment. MT1-MMP proteolytic processing was first triggered by ConA, which is known to trigger proteolytic processing of the 60 kDa immature MT1-MMP form to a 55 kDa processed form [18, 22, 24, 28]. Serum-starved cells were treated for 18 h, and ConA (Fig. 4a, filled

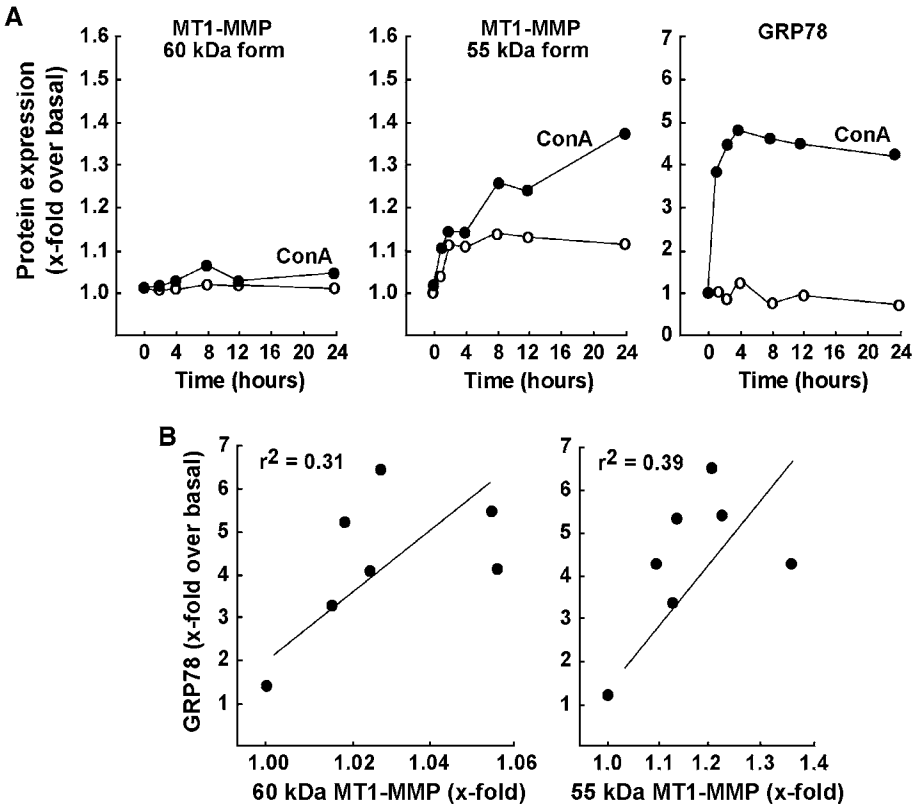

Fig. 4 MT1-MMP proteolytic processing does not contribute to the induction of endoplasmic reticulum stress by Concanavalin-A. U87 glioblastoma-derived cells were serum starved for 18 h in the absence (*open circles*) or presence (*closed circles*) of 30 μg/ml ConA. Cell lysates were then isolated at different time points as described in the "Methods" section. **a** The 60 and 55 kDa forms of MT1-MMP, and GRP78 were immunodetected and scanning densitometry data performed. **b** Correlation plots were made between expression levels of GRP78 and either the 60 kDa MT1-MMP or the 55 kDa MT1-MMP

circles) was found to rapidly induce the expression of the processed 55 kDa form of MT1-MMP, while expression of the 60 kDa form remained largely unchanged through that same time period as compared to non-treated cells (open circles) (Fig. 4a, left and middle panels). GRP78 expression was also found to be rapidly (<2 h) induced and to reach a plateau at 4 h (Fig. 4a, right panel). When correlations were performed between the levels of GRP78 and of the 60 kDa MT1-MMP (Fig. 4b, left panel) or between the levels of GRP78 and 55 kDa MT1-MMP (Fig. 4b, right panel), no significant correlations were found, with respective correlation factors of $r^2 = 0.39$ and $r^2 = 0.31$, respectively. This suggests that cell surface localization of MT1-MMP, rather than proteolytic processing, may contribute to the ER stress induction processes.

Inhibition of MT1-MMP cell surface localization by Brefeldin-A triggers ER stress

MT1-MMP maturation occurs along its trafficking from the ER to the cell surface [25]. Furthermore, we showed above that MT1-MMP gene silencing abrogated the ability of Brefeldin-A to trigger ER stress (Fig. 3). We next investigated which MT1-MMP form affects ER stress induction upon Brefeldin-A treatment. Cells were treated with Brefeldin-A for 18 h as described in the "Methods" section. Intracellular trapping through the inhibition of vesicular trafficking of MT1-MMP led to increased expression of the 60 kDa form of MT1-MMP, while the level of the 55 kDa form decreased with time (Fig. 5a, left and middle panels). Brefeldin-A also time-dependently led to GRP78 expression (Fig. 5a, right panel). When correlations were assessed, we found that GRP78 expression was directly correlated with that of the 60 kDa MT1-MMP form trapped within the ER (Fig. 5b, left panel), while an inverse, non-significant correlation was observed between GRP78 and the 55 kDa MT1-MMP form (Fig. 5b, right panel). Collectively, this lends further support to a role for MT1-MMP in the ER stress processes and highlights the 60 kDa MT1-MMP form among the major contributors.

ER trapping of MT1-MMP triggers cell death signalling

The effect on cell survival of the MT1-MMP-mediated signalling that leads to ER stress was next assessed. The phosphorylation status of Akt is a well established marker that enables assessment of the cell signalling regulating survival. Cells were treated with either ConA, Brefeldin-A, or a combination of both and phosphorylated Akt levels were then measured in cell lysates. As expected, high phosphorylated Akt levels were observed in the control conditions of Mock or siMT1-MMP-transfected cells (Fig. 6a). When cells were treated with ConA, Akt phosphorylation decreased (Fig. 6b) in agreement with the

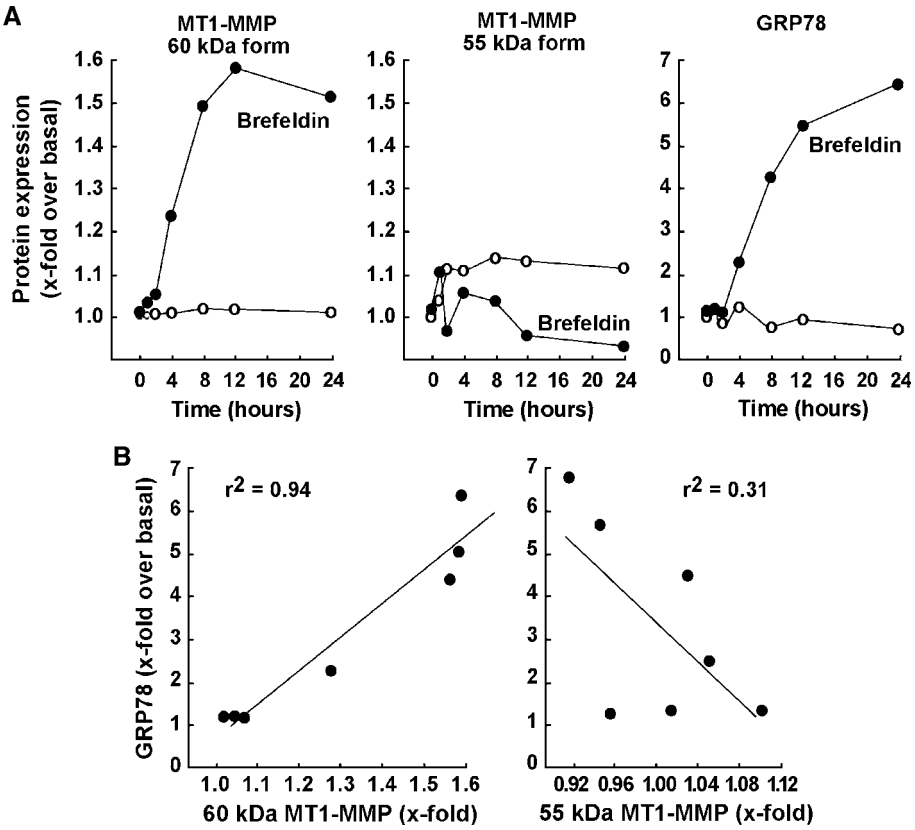

Fig. 5 Inhibition of MT1-MMP vesicular trafficking triggers endoplasmic reticulum stress. U87 glioblastoma-derived cells were serum starved for 24 h in the absence (*open circles*) or presence (*closed circles*) of 100 ng/ml Brefeldin-A. Cell lysates were then isolated at different time points as described in the "Methods" section. **a** The 60 and 55 kDa forms of MT1-MMP, and GRP78 were immunodetected and scanning densitometry data was performed. **b** Correlation plots were made between between expression levels of GRP78 and either the 60 kDa MT1-MMP or the 55 kDa MT1-MMP

pro-apoptotic actions of ConA [22, 27]. Brefeldin-A treatment of the cells also led to decreased Akt phosphorylation levels in Mock cells, but this effect was abrogated when MT1-MMP was silenced (Fig. 6b). Altogether, this suggests that MT1-MMP trapping within the ER by Brefeldin-A is in part responsible for the cell death signalling triggered by Brefeldin-A.

Discussion

Gliomas remain a great challenge in oncology today as they account for more than 50% of all brain tumours and are by far the most common primary brain tumours in adults [32]. Moreover, the mechanisms involved in the resistance of migrating glioblastoma cells to chemotherapy or to radiation-induced cell death have long been recognized [33], and still receive much attention [34]. While diffuse infiltration of glioma cells into normal brain tissue is considered to be a main reason for the unfavorable outcomes for patients with malignant gliomas, it was recently reported that MT1-MMP was up-regulated in glioma-associated microglia rather than in glioma cells

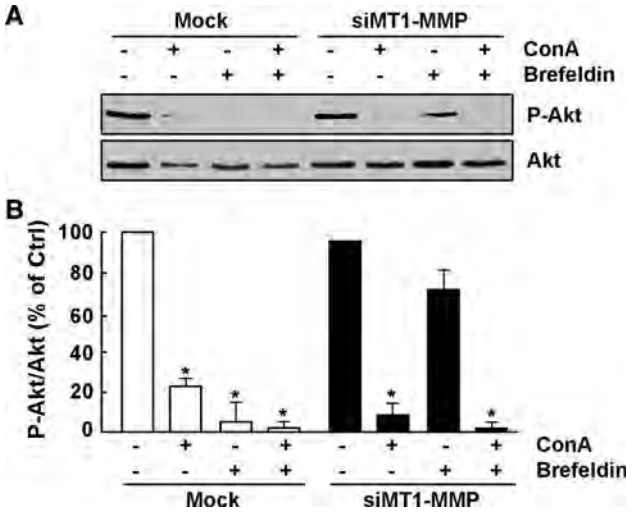

Fig. 6 Endoplasmic reticulum trapping of MT1-MMP triggers cell death signalling. U87 glioblastoma-derived cells were serum starved and treated with 30 μg/ml Concanavalin-A (ConA), 100 ng/ml Brefeldin-A, or a combination of both for 18 h. **a** Cell lysates were isolated as described in the "Methods" section and used to detect Akt and phosphorylated Akt from Mock- or siMT1-MMP-transfected cells by western blot. **b** Scanning densitometry was used to assess the extent of Akt phosphorylation in Mock- (*white bars*) or siMT1-MMP-transfected cells (*black bars*)

themselves, the pathological consequences of which suggest that gliomas induce and exploit microglial MT1-MMP expression for tumour expansion [35]. Overexpression of MT1-MMP was, in fact, found to be lethal for glioma cells in agreement with our current study but the specific subcellular requirements in the MT1-MMP-mediated cell death signalling still remain undefined.

Our data provide vesicular trafficking evidence linking MT1-MMP's role in cell death signalling to protein maturation from the ER to the plasma membrane (Fig. 7). We conclude that MT1-MMP is an absolute requirement for lectin-induced ER stress and COX-2 expression, and that failure of MT1-MMP to localize at the plasma membrane abrogated signalling leading to COX-2 expression. Induction of ER stress was also abrogated when either MT1-MMP expression was silenced or when the mature 60 kDa form of MT1-MMP was trapped within the ER (Figs. 3, 4b). Accordingly, the processing of the prodomain leading to MT1-MMP activation was demonstrated to be accomplished while the de novo synthesized MT1-MMP proenzyme is on its way to the plasma membrane, possibly through the known association of furin with the Golgi compartment and the secretory vesicles [36]. As a consequence in tumourigenic processes, it is hypothesized that MT1-MMP may become active inside cancer cells prior to its presentation at the cell surface. The implications of such observations are that, in addition to regulating pericellular proteolysis, MT1-MMP could cleave crucial co-compartmentalized substrate proteins. Concomitantly, MT1-MMP could also trigger cell death signalling directly in the course of protein processing in cancer cells.

The intracellular metabolic compartments regulating cell survival and invasiveness are of particular interest since tumour cells must adapt to hypoxic stress by upregulating a variety of genes involved in glucose uptake, glycolysis and angiogenesis, all essential to maintaining nutrient availability and intracellular ATP levels [37]. Aside from mitochondria and lysosomes, the ER is also very important in this respect as it is now recognized as an important sensor of cellular stress and it plays a key role in the release and activation of death factors such as cathepsins, calpains, and other proteases through intracellular calcium flux [38]. When experiencing cellular stresses, maintenance of cytoarchitecture is required for cell survival since its perturbation by Cytochalasin-D- or ConA-mediated MT1-MMP mechanisms diminished cell survival but were correlated to MT1-MMP's ability to activate proMMP-2 [14, 39, 40]. In fact, MT1-MMP's intracellular domain has been demonstrated to be an absolute requirement for transducing the intracellular signalling that leads to cell death [14, 27]. Finally, a caspase-dependent mechanism has recently been associated with MT1-MMP function in endothelial cell morphogenic differentiation

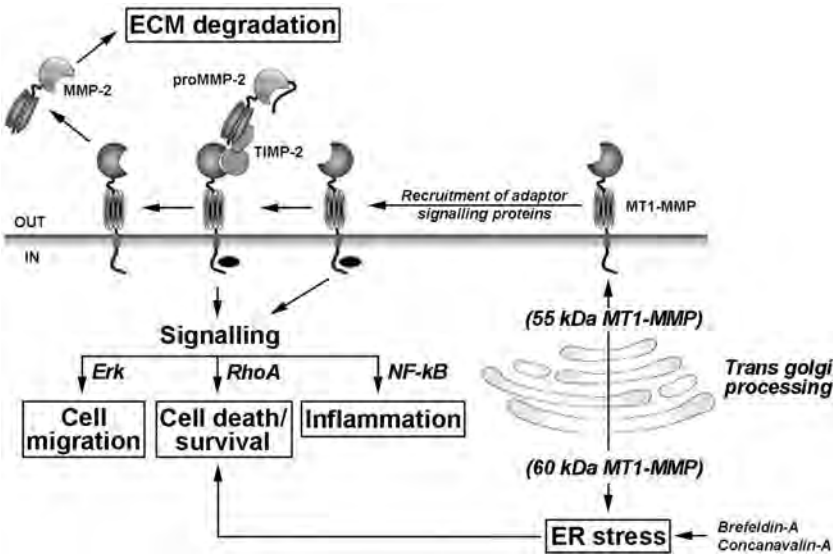

Fig. 7 Schematic representation of the possible mechanisms involved in MT1-MMP-mediated ER stress and cell death signalling in U87 glioblastoma cells. Cellular stress induction such as hypoxia, or pharmacological stress inducers such as Concanavalin-A of Brefeldin-A can trigger ER stress that can consequently lead to cell death. Our study provides evidence for the involvement of ER-trapped 60 kDa MT1-MMP in the induction of ER stress. MT1-MMP furin-mediated trans golgi proteolytic processing [36] can lead to the generation of a 55 kDa MT1-MMP at the cell surface. Recruitement of adaptor signalling proteins [49, 50] is thought to regulate subsequent MT1-MMP-mediated functions in ECM degradation through TIMP-2/proMMP-2 activation, or signalling that leads to the regulation of cell migration (Erk, [12, 13]) cell death/survival (RhoA, [51]), or inflammation (NF-κB, [17, 18])

[41], and confirms that MT1-MMP acts as a cell death sensor/effector that signals ECM degradation processes.

Along with vesicular trafficking evidence for a role for MT1-MMP in ER stress induction, we also showed that COX-2 expression was regulated, in part, through MT1-MMP. In human glioblastoma, COX-2 performs important functions in tumourigenesis [42] and inhibitors of eicosanoid biosynthesis have been shown to suppress cell proliferation and to promote astrocytic differentiation [43]. Since COX-2 protein is overexpressed in the majority of gliomas, it is therefore considered an attractive therapeutic target [44, 45]. Paradoxically, the effectiveness of direct COX-2 inhibitors on glioma cell proliferation and radioresponse enhancement has been shown to be independent of COX-2 protein expression [46]. This evidence suggests that alternate initiator molecules, possibly involving cell surface transducing mechanisms, are associated with therapy resistance and involved in the regulation of COX-2 expression. Whether MT1-MMP (our data), or any cell surface carbohydrate structures, are involved in such regulation remains to be confirmed. Nevertheless, it becomes tempting to suggest that targeting MT1-MMP pleiotropic intracellular transducing functions that, among other targets, lead to COX-2 expression may help design or optimize current therapeutic strategies.

Poor chemosensitivity and the development of chemoresistance remain major obstacles to successful chemotherapy of malignant gliomas. GRP78, which maintains ER homeostasis and suppresses stress-induced apoptosis, is expressed at low levels in normal adult brain, but is significantly elevated in malignant glioma specimens and human malignant glioma cell lines, correlating with their rate of proliferation. Down-regulation of GRP78 by small interfering RNA lead to a slowdown in glioma cell growth, and knockdown of GRP78 in glioblastoma cell lines induces CHOP and activates caspase-7 in temozolomide-treated cells [47]. Here, we show a correlation between MT1-MMP intracellular signaling that leads to GRP78 expression and that may act as a cell death sensor/effector. Our results further show that combination of drugs capable of suppressing MT1-MMP and/or induction of GRP78 might represent a novel approach to eliminate residual tumor cells after surgery and increase the effectiveness of malignant glioma chemotherapy. As an example, aggravated ER stress has proved to enhance glioblastoma cell killing by the proteasome inhibitor bortezomib in combination with COX-2 inhibitor celecoxib [48].

In summary, we highlight new functions of MT1-MMP in cell death signalling, as previously reported [14, 22, 27], which may potentially be linked to those of the ER-mediated stress signalling. We believe that this signalling axis may not be exclusive to one cell type, and that it may regulate cell mobilization processes through metabolic and/or cell survival control such as have been demonstrated for endothelial cells [41] and for bone marrow-derived stromal cells [27]. Our study further indicates a molecular axis linking the invasive phenotype of brain tumour cells to their potential metabolic control through the ER. By revealing tumour-specific metabolic shifts in tumour cells, metabolic profiling studies will further enable drug developers to identify the metabolic steps that control cell proliferation, thus aiding the identification of new anti-cancer targets and screening of lead compounds for anti-proliferative metabolic effects.

Acknowlegments BA holds a Canada Research Chair in Molecular Oncology from the Canadian Institutes of Health Research (CIHR). SPB is a Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT) awardee. JP is a Natural Sciences and Engineering Research Council of Canada (NSERC) awardee. This study was funded by a grant from the NSERC to BA.

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