RESEARCH ARTICLE

The lectin concanavalin-A signals MT1-MMP catalytic independent induction of COX-2 through an IKKγ/NF-κB-dependent pathway

Asmaa Sina · Sébastien Proulx-Bonneau · Alain Roy ·
Laurent Poliquin · Jian Cao · Borhane Annabi

Abstract The lectin from *Canavalia ensiformis* (Concanavalin-A, ConA), one of the most abundant lectins known, enables one to mimic biological lectin/carbohydrate interactions that regulate extracellular matrix protein recognition. As such, ConA is known to induce membrane type-1 matrix metalloproteinase (MT1-MMP) which expression is increased in brain cancer. Given that MT1-MMP correlated to high expression of cyclooxygenase (COX)-2 in gliomas with increasing histological grade, we specifically assessed the early proinflammatory cellular signaling processes triggered by ConA in the regulation of COX-2. We found that treatment with ConA or direct overexpression of a recombinant MT1-MMP resulted in the induction of COX-2 expression. This increase in COX-2 was correlated with a concomitant decrease in phosphorylated AKT suggestive of cell death induction, and was independent of MT1-MMP's catalytic function. ConA- and MT1-MMP-mediated intracellular signaling of COX-2 was also confirmed in wild-type and in Nuclear Factor-kappaB (NF-κB) $p65^{-/-}$ mutant mouse embryonic fibroblasts (MEF), but was abrogated in NF-κB1 $(p50)^{-/-}$ and in I kappaB kinase (IKK) $\gamma^{-/-}$ mutant MEF cells. Collectively, our results highlight an IKK/NF-κB-dependent pathway linking MT1-MMP-mediated intracellular signaling to the induction of COX-2. That signaling pathway could account for the inflammatory balance responsible for the therapy resistance phenotype of glioblastoma cells, and prompts for the design of new therapeutic strategies that target cell surface carbohydrate structures and MT1-MMP-mediated signaling. *Concise summary* Concanavalin-A (ConA) mimics biological lectin/carbohydrate interactions that regulate the proinflammatory phenotype of cancer cells through yet undefined signaling. Here we highlight an IKK/NF-κB-dependent pathway linking MT1-MMP-mediated intracellular signaling to the induction of cyclooxygenase-2, and that could be responsible for the therapy resistance phenotype of glioblastoma cells.

Keywords Concanavalin-A · Inflammation · Cyclooxygenase · MT1-MMP · Cancer

Abbreviations

ConA	concanavalin-A
COX	cyclooxygenase
ECM	extracellular matrix
IKK	I kappaB kinase
MEF	mouse embryonic fibroblasts
MT1-MMP	membrane type-1 matrix metalloproteinase
NF-κB	nuclear factor kappaB

A. Sina · S. Proulx-Bonneau · A. Roy · L. Poliquin · B. Annabi
Centre de Recherche BioMED, Université du Québec à Montréal, Montréal, Québec, Canada

J. Cao
Department of Medicine, State University of New York, Stony Brook, NY 11794, USA

B. Annabi (✉)
Laboratoire d'Oncologie Moléculaire, Département de Chimie, Université du Québec à Montréal,
C.P. 8888, Succ. Centre-ville,
Montréal, Québec, Canada H3C 3P8
e-mail: annabi.borhane@uqam.ca

Introduction

Membrane-type matrix metalloproteinases (MT-MMP) constitute a growing subclass of MMP (Fillmore et al. 2001). The expression levels of several members of the MMP family have been shown to correlate with the graded level of gliomas, including that of MT1-MMP, the best-characterized

MT-MMP. While most of the MMPs are secreted, the MT-MMPs are membrane-associated and a number of these have cytoplasmic domains which were recently shown to bear important functions in cellular signaling (Gingras et al. 2001; Annabi et al. 2004; Belkaid et al. 2007). Aside from its well-known roles in the activation of proMMP-2 and in intrinsic proteolytic activity towards extracellular matrix (ECM) molecules, many new functions of MT1-MMP have recently been associated with PGE$_2$-induced angiogenesis (Alfranca et al. 2008), platelet-mediated calcium mobilization (Fortier et al. 2008a), regulation of cell death/survival bioswitch (Belkaid et al. 2007; Fortier et al. 2008b), and radioresistance in both glioma cells (Wild-Bode et al. 2001; Wick et al. 2002) and endothelial cells (Annabi et al. 2003a). The recent demonstration that MT1-MMP also plays a role in medulloblastoma CD133(+) neurosphere-like formation (Annabi et al. 2008) and in CD133(+) glioblastoma invasiveness (Annabi et al. 2009) reinforces the need to design new therapeutic strategies that either directly target MT1-MMP expression/functions or its associated downstream signaling.

Tetra- and hexavalent mannosides were recently demonstrated to specifically target MT1-MMP pleiotropic functions in cell survival, proliferation, and ECM degradation (Fortier et al. 2008b). These glycocluster constructions may therefore serve in carbohydrate-based anticancer strategies since transformed cells express selective carbohydrate motifs in the form of glycoproteins or glycolipids (Danishefsky and Allen 2000; Roy 2004; Verez-Bencomo et al. 2004). Interactions between carbohydrate-binding proteins (lectins) and the oligosaccharide moieties of glycoprotein at the cell surfaces are, in fact, involved in extensive cellular recognition processes including development, differentiation, morphogenesis and cell migration. The lectin from *Canavalia ensiformis* (Concanavalin-A, ConA), one of the most abundant lectins known (Lin and Levitan 1991), enables one to mimic biological lectin/carbohydrate interactions that regulate extracellular matrix (ECM) protein recognition and, as such, is routinely used to trigger MT1-MMP-mediated activation of latent proMMP-2 (Yu et al. 1997; Zucker et al. 2002; Lafleur et al. 2006). ConA was also found to increase the sub-G1 cell cycle phase as well as cell death; indicative of a potential role in cell surface clustering that affects cell survival (Currie et al. 2007; Fortier et al. 2008b). Furthermore, MT1-MMP gene silencing significantly abrogated chemotaxis in response to the tumorigenic growth factor sphingosine 1-phosphate in mesenchymal stromal cells, again suggesting a crucial role for that MMP in transducing intracellular signaling processes (Currie et al. 2007; Annabi et al. 2003b).

In human glioblastoma, COX-2 performs important functions in tumorigenesis (Deininger et al. 1999) and inhibitors of eicosanoid biosynthesis have been shown to suppress cell proliferation and to promote astrocytic differentiation (Wilson et al. 1990). Since the COX-2 protein is overexpressed in the majority of gliomas, it is therefore considered an attractive therapeutic target (New 2004; Sminia et al. 2005). Paradoxically, the effectiveness of direct COX-2 inhibitors on glioma cell proliferation and radioresponse enhancement was also found to be independent of COX-2 protein expression (Kuipers et al. 2007). This evidence suggests that alternate initiator molecules, possibly involving some cell surface transducing mechanisms, are associated with therapy resistance and are involved in the regulation of COX-2 expression. Whether any cell surface carbohydrate structures are involved in such regulation remains unclear.

In the present study, we provide evidence of cell surface carbohydrate involvement and identified MT1-MMP as a candidate in the early signaling cascade regulating COX-2 expression. As such, the use of the lectin ConA, as a MT1-MMP-inducing agent, further documents an IKK/NF-κB-dependent pathway linking MT1-MMP-mediated intracellular signaling to proinflammatory COX-2 expression that could mimic for the cell survival and inflammatory balance responsible of 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 Advanced Immunochemical Inc. (Long Beach, CA). The polyclonal antibody against MT1-MMP (AB815) was from Chemicon (Temecula, CA). The polyclonal antibodies against AKT and phosphorylated-AKT were purchased from Cell Signaling (Danvers, MA). 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 was maintained in Eagle's Minimum Essential Medium (EMEM) containing 10% (v/v) calf serum (CS) (HyClone Laboratories,

Logan, UT), 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. Murine embryo fibroblasts (MEFs) were maintained in Dulbecco's modified Eagle medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml of penicillin, 100 μg/ml streptomycin, and 25 ng/ml of amphotericin B (Invitrogen, Carlsbad, CA). MEFs deficient in IKKα (Hu et al. 1999), IKKβ (Li et al. 1999), and IKKγ/Nemo (Makris et al. 2000) were described previously and were kindly provided by Dr Terence Dermody (Vanderbilt University, USA).

Cell transfection method

Sub-confluent U87 cell monolayers were transiently transfected with 10 μg of a vector containing cDNA encoding full length (Wt) MT1-MMP fused to GFP (Belkaid et al. 2007) using Lipofectamine 2000 (Invitrogen, Burlington, ON). Mock transfections of U87 cultures with the empty vector, pcDNA (3.1+), were used as controls. Transfected cells were left to recuperate and were used 48 h post-transfection. MT1-MMP specific gene expression and function was evaluated by immunoblotting procedures, and was validated by assessing MT1-MMP-mediated proMMP-2 activation using gelatin zymography.

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 H_2O. 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 H_2O. 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 electro-transferred to polyvinylidene difluoride membranes which were then blocked for 1 hr 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% bovine serum albumin, followed by a 1 hr 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 enhanced chemiluminescence (Amersham Biosciences, Baie d'Urfée, QC).

Results

Concanavalin-A triggers proMMP-2 activation, MT1-MMP and COX-2 expression

Concanavalin-A (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 (Belkaid et al. 2007; Fortier et al. 2008b; Sina et al. 2009). Serum-starved U87 glioblastoma cells were therefore treated with increasing concentrations of ConA and the levels of proMMP-2 activation were assessed in the conditioned media by gelatin zymography as described in the "Materials and methods" section. ProMMP-2 activation into MMP-2 was observed (Fig. 1a), and this was accompanied by MT1-MMP and COX-2 protein expression, as assessed in the cell lysates by Western Blotting (Fig. 1a). When MT1-MMP expression was plotted against that of COX-2, positive linear correlation ($r^2 = 0.92$) was observed following treatment of the cells with the different ConA concentrations (Fig. 1b). Immunodetection of total and phosphorylated AKT was also performed on those same ConA-treated cells. ConA dose-dependently decreased the basal levels of phosphorylated AKT, though not the total AKT protein expression (Fig. 1a). AnnexinV-PI staining and flow cytometry analysis following treatment with ConA confirmed cell death induction through necrosis (data not shown) in agreement with previous reports (Belkaid et al. 2007; Currie et al. 2007). An inverse correlation ($r^2 = 0.91$) was also observed between the extent of AKT phosphorylation status and COX-2 expression (Fig. 1c).

Concanavalin-A-induced COX-2 expression is independent of MT1-MMP's catalytic function

In order to address which MT1-MMP protein domain is responsible for the ConA-induced COX-2 expression, U87 cells were treated with or without Ilomastat, a broad range MMP inhibitor known to target the MT1-MMP extracellular domain catalytic functions (Currie et al. 2007). While Ilomastat, as expected, inhibited the MT1-MMP-mediated proMMP-2 activation into MMP-2 (Fig. 2a), it was unable to significantly reverse ConA-induced MT1-MMP and COX-2 expression (Fig. 2b). These observations suggest that MT1-MMP's extracellular catalytic domain is not involved in the COX-2 induction process. Cell-based
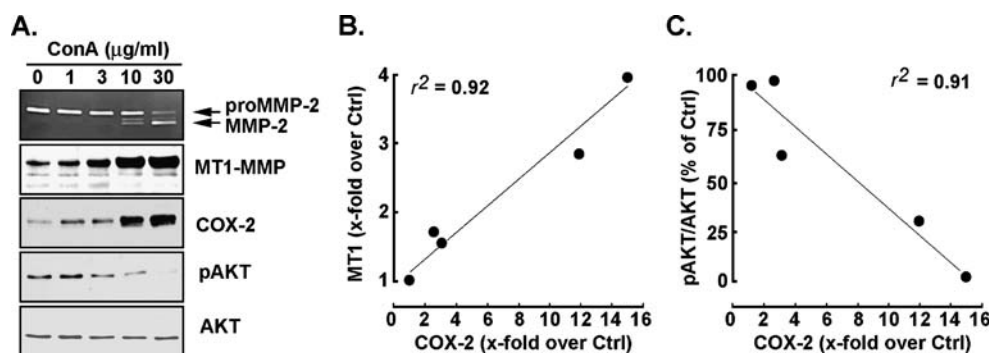
Fig. 1 Concanavalin-A triggers proMMP-2 activation, MT1-MMP and COX-2 expression. **a** U87 glioblastoma cells were cultured as described in the "Materials and methods" section until they reached ~75–90% confluence. They were then serum-starved for 24 h prior to the addition of increasing concentrations of concanavalin-A (ConA). Gelatin zymography (upper panel) was performed to assess the extent of proMMP-2 activation levels, as described in the "Materials and methods" section, using conditioned media isolated from each of the serum-starved cells conditions. Cell lysates were isolated and SDS-PAGE performed (20 μg protein/well). MT1-MMP, COX-2, phosphorylated-AKT, and total AKT expression were assessed by Western blotting and immunodetection was performed as described in the "Materials and methods" section. Scanning densitometry data of the (**b**) MT1-MMP vs COX-2 and (**c**) pAKT/AKT vs COX-2 autoradiograms were plotted and are from a representative experiment

evidence for the aminopeptidase N/CD13 inhibitor actinonin's targeting of MT1-MMP-mediated proMMP-2 activation was also recently demonstrated (Sina et al. 2009). Similar to Ilomastat, actinonin was found to dose-dependently inhibit ConA-induced proMMP-2 activation (Fig. 3a), while it had no effects on ConA-induced MT1-MMP and COX-2 expression (Fig. 3b).

Constitutive recombinant MT1-MMP expression triggers COX-2 expression

In order to rule out any alternative signaling driven by ConA, we transiently transfected U87 cells with a cDNA plasmid encoding recombinant MT1-MMP/GFP (Belkaid et al. 2007). Efficient overexpression of the MT1-MMP recombinant form yielded a higher molecular weight band (Fig. 4, top panel) and led to COX-2 expression (Fig. 4,

middle panel). Again, the MMP catalytic function inhibitors were unable to reverse MT1-MMP-mediated COX-2 expression although they were still efficient in inhibiting MT1-MMP-mediated proMMP-2 activation (Fig. 4). This demonstrates an alternate role, i.e. in intracellular signaling inducing COX-2, as a new function for the cell surface MT1-MMP.

Concanavalin-A- and recombinant MT1-MMP-mediated COX-2 induction requires an intact NF-κB p50 and IKKγ signaling axis

COX-2 transcriptional expression is thought to be regulated, in part, through NF-κB-mediated signaling involving nuclear translocation of the NF-κB heterodimer p50:p65 (Tsatsanis et al. 2006). In an attempt to elucidate the NF-κB signaling axis needed to trigger COX-2 expression, wild-type
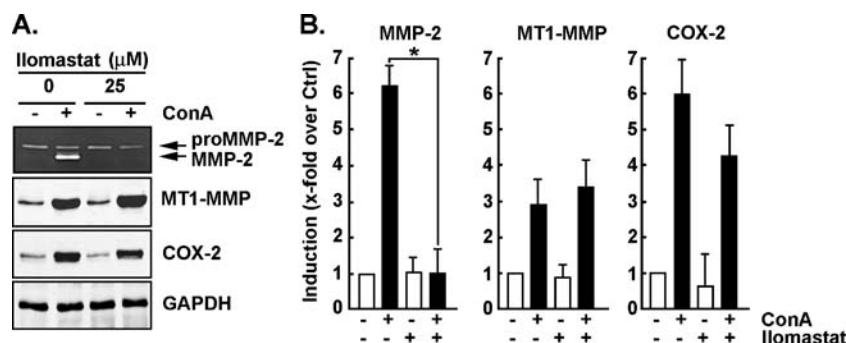
Fig. 2 Concanavalin-A-induced COX-2 expression is independent of MT1-MMP's catalytic function. **a** Cell lysates were isolated from U87 glioma cells that had been treated with or without ConA, 10 μM Ilomastat, or a combination of both. Gelatin zymography (upper panel) was carried out to assess the extent of proMMP-2 activation levels, as described in the "Materials and methods" section, using conditioned media isolated from each of the serum-starved cell conditions. The extent of MT1-MMP and COX-2 expression were assessed as described in the legend to Fig. 1. **b** Scanning densitometry data of MMP-2, MT1-MMP, COX-2 and GAPDH expression were plotted and are from 3 independent experiments
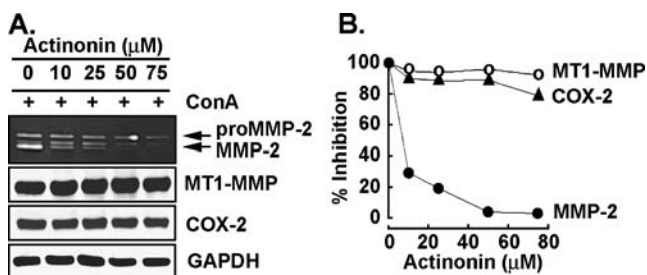
Fig. 3 Actinonin is unable to antagonize concanavalin-A-induced COX-2 expression. **a** Gelatin zymography (upper panel) was carried out to assess the extent of proMMP-2 activation levels, as described in the "Materials and methods" section, using conditioned media isolated from serum-starved U87 glioblastoma cells that had been treated with increasing actinonin concentrations in the presence of ConA. The extent of MT1-MMP and COX-2 expression was assessed in cell lysates as described in the legend to Fig. 1. **b** Scanning densitometry data of the respective MMP-2, MT1-MMP, COX-2 and GAPDH expression were plotted and are from a representative experiment

mouse embryonic fibroblasts (MEF), $p50^{-/-}$ and $p65^{-/-}$ NF-κB MEF mutants were used, as were $IKK\alpha^{-/-}$, $IKK\beta^{-/-}$, and $IKK\gamma^{-/-}$. Cells were either treated with ConA or transfected with a cDNA plasmid encoding MT1-MMP. Cell lysates as well as conditioned media were isolated following these treatments. ConA triggered a strong activation of proMMP-2 in Wt, $p50^{-/-}$, $p65^{-/-}$, and $IKK\gamma^{-/-}$ cells, while it was lower in $IKK\alpha^{-/-}$ and $IKK\beta^{-/-}$ cells (Fig. 5a). ConA treatment concomitantly induced COX-2 expression in all cell lines except the $p50^{-/-}$ and $IKK\alpha^{-/-}$ MEF mutants (Fig. 5a). Expression and cell surface activity of the recombinant MT1-MMP were also confirmed in transfected cells as Wt, $p50^{-/-}$, $p65^{-/-}$, and $IKK\gamma^{-/-}$ cells all exhibited significantly increased proMMP-2 activation into active MMP-2 form, as judged by gelatin zymography (Fig. 5b). Low but detectable proMMP-2 activation was achieved in $IKK\alpha^{-/-}$ and $IKK\beta^{-/-}$ cells (Fig. 5b). When COX-2 protein expression was measured, we observed the induction of COX-2 by recombinant MT1-MMP in Wt-MEF (Fig. 5b), confirming the results observed in U87 glioma cells (Fig. 4a). Similar MT1-MMP-mediated COX-2 induction was also observed in $p65^{-/-}$, $IKK\alpha^{-/-}$, and $IKK\beta^{-/-}$ mutant MEF, while COX-2 expression was completely abrogated in $p50^{-/-}$ and in $IKK\gamma^{-/-}$ mutant MEF (Fig. 5b). This cell-based evidence directly demonstrates the specific involvement of p50 and of IKKγ in NF-κB-mediated MT1-MMP regulation of COX-2 expression.

Discussion

Dysregulated NF-κB activity occurs in a number of chronic inflammatory diseases and certain types of cancers, making NF-κB signaling an attractive target for the development of anti-inflammatory and anti-cancer drugs. A pivotal regula-

tor of all inducible NF-κB signaling pathways is the IκB kinase (IKK) complex that consists of two kinases (IKKα and IKKβ) and a regulatory subunit named NF-κB essential modulator (NEMO, or IKKγ). In the present study, we have identified an IKKγ/NF-κB-dependent pathway that links ConA-induced MT1-MMP intracellular signaling to COX-2 expression in two cellular models, the U87 glioblastoma cells and the Wt mouse embryonic fibroblasts. An NF-κB-mediated induction of MT1-MMP had already been demonstrated in human dermal fibroblasts, and this was promoted by TNF-α (Han et al. 2001). More recently, IKKγ was shown to regulate an early NF-κB-independent cell-death checkpoint during TNF signaling (Legarda-Addison et al. 2009), supporting molecular signaling interplay between MT1-MMP and COX-2 and which likely regulates cell survival status.

Aside from glioblastoma cells, such a link has also been observed in cells derived from malignant fibrous histiocytoma, one of the highest-grade sarcomas arising in bone and soft tissue, where concomitantly increased levels of expression of COX-2 and MT1-MMP were described (Maeda et al. 2008). Co-distribution of MT1-MMP, MMP-2 and COX-2 was also demonstrated in grade IV atheroma, again indicating a possible link between these enzymes in the destabilization of atherosclerotic plaques (Kuge et al. 2007). Altogether, these published data support a molecular signaling convergence linking MT1-MMP to COX-2 expression through an NF-κB-mediated pathway which may constitute the point of convergence of many oncogenic pathways by virtue of its ability to regulate the expression of genes involved in cell apoptosis, differentiation,
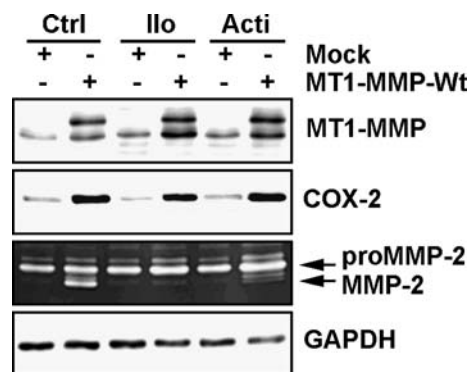
Fig. 4 Constitutive recombinant MT1-MMP expression triggers COX-2 expression. U87 glioblastoma cells were either Mock-transfected or transfected with a cDNA plasmid encoding recombinant MT1-MMP/GFP (MT1-MMP-Wt). Cells were then incubated in serum-free media in the presence or absence of Ilomastat (Ilo) or Actinonin (Acti). Gelatin zymography was performed to monitor the extent of latent proMMP-2 and active MMP-2 expression from the conditioned media of the serum-starved cells (lower panel). Cell lysates were isolated and SDS-PAGE performed (20 μg protein/well), followed by Western blotting and MT1-MMP, COX-2 and GAPDH immunodetection
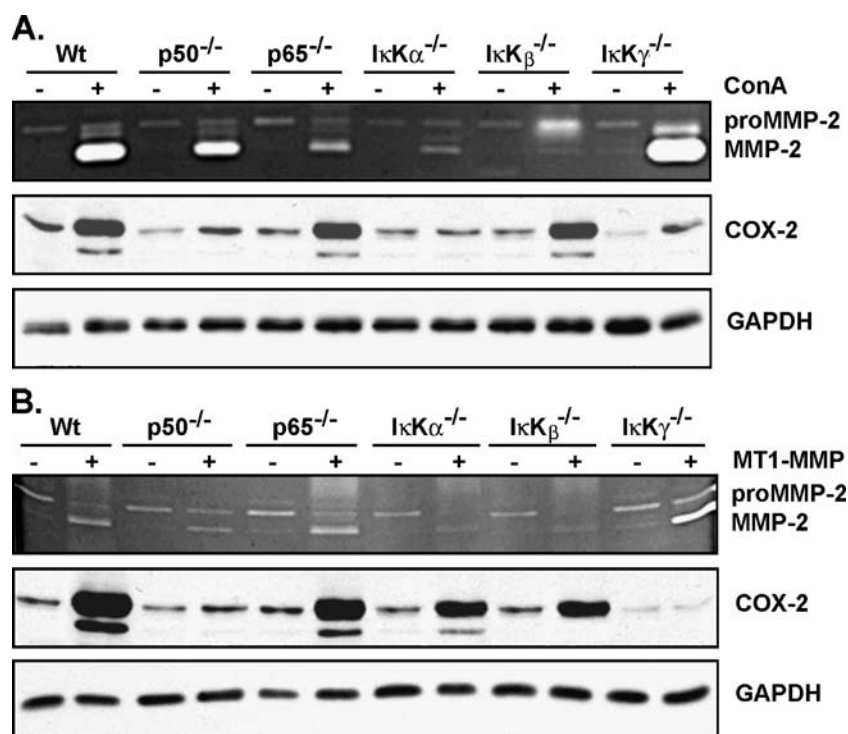
Fig. 5 Concanavalin-A- and recombinant MT1-MMP-mediated COX-2 induction necessitates an intact NF-κB1 p50 and IKKγ⁻ signaling axis. Wild-type (Wt), p65⁻/⁻, p50⁻/⁻, IKKα⁻/⁻, IKKβ⁻/⁻, and IKKγ⁻/⁻ mouse embryonic fibroblasts (MEF) were either treated with ConA (**a**), Mock-transfected or transfected with a cDNA plasmid encoding MT1-MMP (**b**). Gelatin zymography was used to monitor the extent of latent proMMP-2 and active MMP-2 expression from the conditioned media of the serum-starved cells (upper panels of **a** and **b**). Cell lysates were isolated and SDS-PAGE performed (20 μg protein/well), followed by Western blotting and immunodetection of COX-2 and GAPDH

adhesion, and survival (Dolcet et al. 2005). Aside from its critical role in the development of human cancer, NF-κB has also been implicated at the molecular level in the promotion of angiogenesis, which is of particular interest since malignant astrocytomas are highly vascular tumors (Korkolopoulou et al. 2008). NF-κB is also a transcriptional regulator in the inducible expression of numerous genes including COX-2 (Lim et al. 2001). Interestingly, a consensus binding site for NF-κB p65 (TGGAGCTTCC) was found in the 5′-flanking region of the human MT1-MMP gene (Han et al. 2001) and NF-κB-mediated induction of MT1-MMP was confirmed in murine melanoma cells (Philip et al. 2001) and in human fibrosarcoma cells (Park et al. 2007). Further studies also implicated NF-κB as a potentially critical factor in astrocytic tumorigenesis and astrocytoma progression through analysis of cell lines and preclinical models (Nagai et al. 2002; Hayashi et al. 2001; Yamamoto et al. 2000). In the current study, we provide evidence for an MT1-MMP-mediated signaling cascade that leads to activation of COX-2 expression and that is independent of MT1-MMP's catalytic function. We also demonstrate that this new MT1-MMP/COX-2 signaling axis absolutely requires IKKγ/NF-κB p50. In support of our results, an increase in NF-κB p50 was recently found to rapidly induce MT1-MMP expression in trabecular meshwork cells (Miller et al. 2007).

Gliomas remain a great challenge in oncology as they account for more than 50% of all brain tumors and are by far the most common primary brain tumors in adults (Stewart 2002). More importantly, the mechanisms responsible for the resistance of migrating glioblastoma cells to chemotherapy or to radiation-induced cell death have long been recognized (Berens and Giese 1999) and still receive much attention in order to optimize future cellular targets for the treatment of glioblastomas (Lefranc et al. 2005). In addition, tissue necrosis is a characteristic feature of malignant gliomas, in particular glioblastoma, and is most likely the consequence of rapidly increasing tumor mass that is inadequately oxygenated by the pre-existing vasculature (Raza et al. 2002). At the cellular level, maintenance of cytoarchitecture is required for cell survival, since its perturbation by Cytochalasin-D- or ConA-mediated MT1-MMP mechanisms diminishes cell survival and has been correlated with proMMP-2 activation (Belkaid et al. 2007; Hinoue et al. 2005; Preaux et al. 2002; this study). Accordingly, MT1-MMP's intracellular domain was shown to be an absolute requirement for transducing the intracel-

lular signaling that leads to cell death since overexpression of a membrane-bound catalytically active but cytoplasmic domain-deleted MT1-MMP was unable to trigger necrosis (Belkaid et al. 2007). Although the exact identity of the amino acid residues from the MT1-MMP intracellular domain remain to be identified, speculation about Tyr-573, Cys-574, and Val-582 have been put forward as important for MT1-MMP signaling (Labrecque et al. 2004; Anilkumar et al. 2005). Similarly, a caspase-dependent mechanism has recently been associated with MT1-MMP function in endothelial cell morphogenic differentiation (Langlois et al. 2005). This suggests that MT1-MMP acts as a potential cell death sensor/effector that signals ECM degradation processes to be activated.

In summary, the use of ConA proves that cell surface carbohydrate structures account for MT1-MMP intracellular signaling that regulates COX-2 expression through an IKKγ/NF-κB-dependent pathway in U87 glioblastoma cells. By revealing such a new signaling axis in tumor cells, profiling studies will be needed in order to enable drug developers to more efficiently target those pathways, thus aiding the identification of new anti-cancer lead compounds.

References

Alfranca A, López-Oliva JM, Genís L, López-Maderuelo D, Mirones I, Salvado D, Quesada AJ, Arroyo AG, Redondo JM (2008) PGE2 induces angiogenesis via MT1-MMP-mediated activation of the TGFbeta/Alk5 signaling pathway. Blood 112:1120–1128

Anilkumar N, Uekita T, Couchman JR, Nagase H, Seiki M, Itoh Y (2005) Palmitoylation at Cys574 is essential for MT1-MMP to promote cell migration. FASEB J 19:1326–1328

Annabi B, Lee YT, Martel C, Pilorget A, Bahary JP, Béliveau R (2003a) Radiation induced-tubulogenesis in endothelial cells is antagonized by the antiangiogenic properties of green tea polyphenol (−) epigallocatechin-3-gallate. Cancer Biol Ther 2:642–649

Annabi B, Thibeault S, Lee YT, Bousquet-Gagnon N, Eliopoulos N, Barrette S, Galipeau J, Beliveau R (2003b) Matrix metalloproteinase regulation of sphingosine-1-phosphate-induced angiogenic properties of bone marrow stromal cells. Exp Hematol 31:640–649

Annabi B, Thibeault S, Moumdjian R, Béliveau R (2004) Hyaluronan cell surface binding is induced by type I collagen and regulated by caveolae in glioma cells. J Biol Chem 279:21888–21896

Annabi B, Rojas-Sutterlin S, Laflamme C, Lachambre MP, Rolland Y, Sartelet H, Béliveau R (2008) Tumor environment dictates medulloblastoma cancer stem cell expression and invasive phenotype. Mol Cancer Res 6:907–916

Annabi B, Lachambre MP, Plouffe K, Sartelet H, Béliveau R (2009) Modulation of invasive properties of CD133+ glioblastoma stem cells: a role for MT1-MMP in bioactive lysophospholipid signaling. Mol Carcinog 48:910–919

Belkaid A, Fortier S, Cao J, Annabi B (2007) Necrosis induction in glioblastoma cells reveals a new "bioswitch" function for the MT1-MMP/G6PT signaling axis in proMMP-2 activation versus cell death decision. Neoplasia 9:332–430

Berens ME, Giese A (1999) "...those left behind". Biology and oncology of invasive glioma cells. Neoplasia 1:208–219

Currie JC, Fortier S, Sina A, Galipeau J, Cao J, Annabi B (2007) MT1-MMP down-regulates the glucose 6-phosphate transporter expression in marrow stromal cells: a molecular link between pro-MMP-2 activation, chemotaxis, and cell survival. J Biol Chem 282:8142–8149

Danishefsky SJ, Allen JR (2000) From the laboratory to the clinic: a retrospective on fully synthetic carbohydrate-based anticancer vaccines frequently used abbreviations are listed in the appendix. Angew Chem Int Ed 39:836–863

Deininger MH, Weller M, Streffer J, Mittelbronn M, Meyermann R (1999) Patterns of cyclooxygenase-1 and -2 expression in human gliomas in vivo. Acta Neuropathol 98:240–244

Dolcet X, Llobet D, Pallares J, Matias-Guiu X (2005) NF-kB in development and progression of human cancer. Virchows Arch 446:475–482

Fillmore HL, VanMeter TE, Broaddus WC (2001) Membrane-type matrix metalloproteinases (MT-MMPs): expression and function during glioma invasion. J Neurooncol 53:187–202

Fortier S, Labelle D, Sina A, Moreau R, Annabi B (2008a) Silencing of the MT1-MMP/ G6PT axis suppresses calcium mobilization by sphingosine-1-phosphate in glioblastoma cells. FEBS Lett 582:799–804

Fortier S, Touaibia M, Lord-Dufour S, Galipeau J, Roy R, Annabi B (2008b) Tetra- and hexavalent mannosides inhibit the pro-apoptotic, antiproliferative and cell surface clustering effects of concanavalin-A: impact on MT1-MMP functions in marrow-derived mesenchymal stromal cells. Glycobiology 18:195–204

Gingras D, Bousquet-Gagnon N, Langlois S, Lachambre MP, Annabi B, Béliveau R (2001) Activation of the extracellular signal-regulated protein kinase (ERK) cascade by membrane-type-1 matrix metalloproteinase (MT1-MMP). FEBS Lett 507:231–236

Han YP, Tuan TL, Wu H, Hughes M, Garner WL (2001) TNF-alpha stimulates activation of pro-MMP2 in human skin through NF-(kappa)B mediated induction of MT1-MMP. J Cell Sci 114:131–139

Hayashi S, Yamamoto M, Ueno Y, Ikeda K, Ohshima K, Soma G, Fukushima T (2001) Expression of nuclear factor-kappa B, tumor necrosis factor receptor type 1, and c-Myc in human astrocytomas. Neurol Med Chir (Tokyo) 41:187–195

Hinoue A, Takigawa T, Miura T, Nishimura Y, Suzuki S, Shiota K (2005) Disruption of actin cytoskeleton and anchorage-dependent cell spreading induces apoptotic death of mouse neural crest cells cultured in vitro. Anat Rec A Discov Mol Cell Evol Biol 282:130–137

Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, Johnson R, Karin M (1999) Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase. Science 284:316–320

Korkolopoulou P, Levidou G, Saetta AA, El-Habr E, Eftichiadis C, Demenagas P, Thymara I, Xiromeritis K, Boviatsis E, Thomas-Tsagli E, Panayotidis I, Patsouris E (2008) Expression of nuclear factor-kappaB in human astrocytomas: relation to pI kappa Ba,

vascular endothelial growth factor, Cox-2, microvascular characteristics, and survival. Hum Pathol 39:1143–1152

Kuge Y, Takai N, Ishino S, Temma T, Shiomi M, Saji H (2007) Distribution profiles of membrane Type-1 matrix metalloproteinase (MT1-MMP), matrix metalloproteinase-2 (MMP-2) and cyclooxygenase-2 (COX-2) in rabbit atherosclerosis: comparison with plaque instability analysis. Biol Pharm Bull 30:1634–1640

Kuipers GK, Slotman BJ, Wedekind LE, Stoter TR, Berg J, Sminia P, Lafleur MV (2007) Radiosensitization of human glioma cells by cyclooxygenase-2 (COX-2) inhibition: independent on COX-2 expression and dependent on the COX-2 inhibitor and sequence of administration. Int J Radiat Biol 83:677–685

Labrecque L, Nyalendo C, Langlois S, Durocher Y, Roghi C, Murphy G, Gingras D, Béliveau R (2004) Src-mediated tyrosine phosphorylation of caveolin-1 induces its association with membrane type 1 matrix metalloproteinase. J Biol Chem 279:52132–52140

Lafleur MA, Mercuri FA, Ruangpanit N, Seiki M, Sato H, Thompson EW (2006) Type I collagen abrogates the clathrin-mediated internalization of membrane type 1 matrix metalloproteinase (MT1-MMP) via the MT1-MMP hemopexin domain. J Biol Chem 281:6826–6840

Langlois S, Di Tomasso G, Boivin D, Roghi C, Murphy G, Gingras D, Béliveau R (2005) Membrane type 1-matrix metalloproteinase induces endothelial cell morphogenic differentiation by a caspase-dependent mechanism. Exp Cell Res 307:452–464

Lefranc F, Brotchi J, Kiss R (2005) Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. J Clin Oncol 23:2411–2422

Legarda-Addison D, Hase H, O'Donnell MA, Ting AT (2009) NEMO/IKKgamma regulates an early NF-kappaB-independent cell-death checkpoint during TNF signaling. Cell Death Differ 16:1279–1288

Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, Karin M (1999) The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. J Exp Med 189:1839–1845

Lim JW, Kim H, Kim KH (2001) NF-kappaB, inducible nitric oxide synthase and apoptosis by Helicobacter pylori infection. Free Radic Biol Med 31:355–366

Lin SS, Levitan IB (1991) Concanavalin A: a tool to investigate neuronal plasticity. Trends Neurosci 14:273–277

Maeda T, Hashitani S, Zushi Y, Segawa E, Tanaka N, Sakurai K, Urade M (2008) Establishment of a nude mouse transplantable model of a human malignant fibrous histiocytoma of the mandible with high metastatic potential to the lung. J Cancer Res Clin Oncol 134:1005–1011

Makris C, Godfrey VL, Krahn-Senftleben G, Takahashi T, Roberts JL, Schwarz T, Feng L, Johnson RS, Karin M (2000) Female mice heterozygous for IKK gamma/NEMO deficiencies develop a dermatopathy similar to the human X-linked disorder incontinentia pigmenti. Mol Cell 5:969–979

Miller AM, Nolan MJ, Choi J, Koga T, Shen X, Yue BY, Knepper PA (2007) Lactate treatment causes NF-kappaB activation and CD44 shedding in cultured trabecular meshwork cells. Invest Ophthalmol Vis Sci 48:1615–1621

Nagai S, Washiyama K, Kurimoto M, Takaku A, Endo S, Kumanishi T (2002) Aberrant nuclear factor-kappaB activity and its participation in the growth of human malignant astrocytoma. J Neurosurg 96:909–917

New P (2004) Cyclooxygenase in the treatment of glioma: its complex role in signal transduction. Cancer Control 11:152–164

Park JM, Kim A, Oh JH, Chung AS (2007) Methylseleninic acid inhibits PMA-stimulated pro-MMP-2 activation mediated by MT1-MMP

expression and further tumor invasion through suppression of NF-kappaB activation. Carcinogenesis 28:837–847

Philip S, Bulbule A, Kundu GC (2001) Osteopontin stimulates tumor growth and activation of promatrix metalloproteinase-2 through nuclear factor-kappa B-mediated induction of membrane type 1 matrix metalloproteinase in murine melanoma cells. J Biol Chem 276:44926–44935

Preaux AM, D'ortho MP, Bralet MP, Laperche Y, Mavier P (2002) Apoptosis of human hepatic myofibroblasts promotes activation of matrix metalloproteinase-2. Hepatology 36:615–622

Raza SM, Lang FF, Aggarwal BB, Fuller GN, Wildrick DM, Sawaya R (2002) Necrosis and glioblastoma: a friend or a foe? A review and a hypothesis. Neurosurgery 51:2–12

Roy R (2004) New trends in carbohydrate-based vaccines. Drug Discov Today: Technologies 1:327–336

Sina A, Lord-Dufour S, Annabi B (2009) Cell-based evidence for aminopeptidase N/CD13 inhibitor actinonin targeting of MT1-MMP-mediated proMMP-2 activation. Cancer Lett 279:171–176

Sminia P, Stoter TR, van der Valk P, Elkhuizen PH, Tadema TM, Kuipers GK, Vandertop WP, Lafleur MV, Slotman BJ (2005) Expression of cyclooxygenase-2 and epidermal growth factor receptor in primary and recurrent glioblastoma multiforme. J Cancer Res Clin Oncol 131:653–661

Stewart LA (2002) Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials. Lancet 359:1011–1018

Tsatsanis C, Androulidaki A, Venihaki M, Margioris AN (2006) Signalling networks regulating cyclooxygenase-2. Int J Biochem Cell Biol 38:1654–1661

Verez-Bencomo V, Fernandez-Santana V, Hardy E, Toledo ME, Rodriguez MC, Heynngnezz L, Rodriguez A, Baly A, Herrera L, Izquierdo M, Villar A, Valdes Y, Cosme K, Deler ML, Montane M, Garcia E, Ramos A, Aguilar A, Medina E, Torano G, Sosa I, Hernandez I, Martinez R, Muzachio A, Carmenates A, Costa L, Cardoso F, Campa C, Diaz M, Roy R (2004) A synthetic conjugate polysaccharide vaccine against Haemophilus influenzae type b. Science 305:522–525

Wick W, Wick A, Schulz JB, Dichgans J, Rodemann HP, Weller M (2002) Prevention of irradiation-induced glioma cell invasion by temozolomide involves caspase 3 activity and cleavage of focal adhesion kinase. Cancer Res 62:1915–1919

Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W (2001) Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 61:2744–2750

Wilson DE, Anderson KM, Seed TM (1990) Ultrastructural evidence for differentiation in a human glioblastoma cell line treated with inhibitors of eicosanoid metabolism. Neurosurgery 27:523–531

Yamamoto M, Fukushima T, Hayashi S, Ikeda K, Tsugu H, Kimura H, Soma G, Tomonaga M (2000) Correlation of the expression of nuclear factor-kappa B, tumor necrosis factor receptor type 1 (TNFR 1) and c-Myc with the clinical course in the treatment of malignant astrocytomas with recombinant mutant human tumor necrosis factor-alpha (TNF-SAM2). Anticancer Res 20:611–618

Yu M, Bowden ET, Sitlani J, Sato H, Seiki M, Mueller SC, Thompson EW (1997) Tyrosine phosphorylation mediates ConA-induced membrane type 1-matrix metalloproteinase expression and matrix metalloproteinase-2 activation in MDA-MB-231 human breast carcinoma cells. Cancer Res 57:5028–5032

Zucker S, Hymowitz M, Conner CE, DiYanni EA, Cao J (2002) Rapid trafficking of membrane type 1-matrix metalloproteinase to the cell surface regulates progelatinase a activation. Lab Invest 82:1673–1684