Cancer Letters 279 (2009) 171–176

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Cell-based evidence for aminopeptidase N/CD13 inhibitor actinonin targeting of MT1-MMP-mediated proMMP-2 activation

Asmaa Sina, Simon Lord-Dufour, Borhane Annabi *

Laboratoire d'Oncologie Moléculaire, Département de Chimie, Centre de Recherche BioMED, Université du Québec à Montréal, C.P. 8888, Succ. Centre-ville Montréal, Québec, Canada H3C 3P8

ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 19 December 2008
Accepted 22 January 2009

Keywords:
MT1-MMP
Aminopeptidase N/CD13 inhibitor
Glioblastoma
Concanavalin
RECK

ABSTRACT

Recent profiling has identified the aminopeptidase N/CD13 inhibitor actinonin as a selective soluble secreted matrix metalloproteinase (MMP) inhibitor. Given that actinonin's effects against membrane-bound MMPs remain unknown and that MT1-MMP has been linked to chemo- and radio-therapy resistance in brain tumor development, we therefore assessed MT1-MMP functional inhibition by actinonin in U87 glioblastoma cells. We show that actinonin inhibits concanavalin-A (ConA)-induced proMMP-2 activation, while it does not inhibit ConA-induced MT1-MMP gene expression suggesting post-transcriptional effects of the drug possibly mediated through the membrane-anchored protease regulator RECK. Specific gene silencing of MT1-MMP with siRNA abrogated the ability of ConA to activate proMMP-2. Functional recombinant MT1-MMP whose constitutive expression led to proMMP-2 activation was also efficiently antagonized by actinonin. We provide evidence for actinonin's new therapeutic application in the direct targeting of MT1-MMP-mediated proMMP-2 activation, an essential step in both brain tumor infiltration and in brain tumor-associated angiogenesis.

1. Introduction

Actinonin has been ascribed several inhibitory activities, amongst which inhibition of transmembrane protease aminopeptidase N (APN)/CD13 [1] and peptide deformylase [2,3], as well as antiproliferative effects against human tumor cells [4] were reported. Tumor-infiltrating T cells in renal and lung cancers [5,6] as well as in tumoral, but not normal, vasculature [7], are all characterized by high APN/CD13 levels, and also represent potential targets for actinonin. While enhanced expression of membrane and/or soluble forms of APN/CD13 is observed in many cancers [8–11], it must be pointed out that actinonin, intriguingly, also inhibited the growth of both CD13-positive myeloid and CD13-negative lymphoma cell lines, suggesting that the effects induced by actinonin are unlikely to be solely mediated by CD13 inhibition [12]. Furthermore, gene silencing of APN/CD13 in endothelial cells resulted in the inhibition of *in vitro* tubulogenesis, suggesting that APN/CD13 inhibition may affect important processes such as in vascular endothelial morphogenesis and during angiogenesis [13].

Apart from all these observations, it has emerged that potential alternate transmembrane proteases which, similar to APN/CD13, may impact those same critical processes involved in angiogenesis and in tumor development and thus be targeted by actinonin. Among these, membrane-type-1 matrix metalloproteinase (MT1-MMP), an important protein related to tumor growth and angiogenesis, is expressed on malignant tumor cells and in activated endothelial cells [14]. Furthermore, MT1-MMP is part of the specific transmembrane proteases family that regulates

* Corresponding author. Tel.: +1 514 987 3000x7610; fax: +1 514 987 0246.
E-mail address: annabi.borhane@uqam.ca (B. Annabi).

Abbreviations: APN, aminopeptidase N; ConA, concanavalin-A; ECM, extracellular matrix; HUVEC, human umbilical vein endothelial cells; MMP, matrix metalloproteinase; MT1-MMP, membrane-type-1 MMP; RECK, reversion-inducing cysteine-rich protein with Kazal motifs.

0304-3835/$ - see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2009.01.032

cell growth and invasion and that may also contribute to angiogenesis [15]. MT1-MMP is involved in cell migration, extracellular matrix (ECM) degradation and endothelial cell tubulogenesis [16]. Detailed studies by RNA interference recently revealed that MT1-MMP also controls mesenchymal stromal cell mobilization and homing processes, which require invasion through ECM barriers [17,18] and which may possibly contribute to brain tumor development [19]. In addition, previous demonstration that MT1-MMP can function as an activator of latent proMMP-2 [20] was later strengthened by demonstrations that reduced activation of proMMP-2 was observed in various tissues of MT1-MMP null mice [21] and that inhibition of MT1-MMP by cancer drugs interfered with the homing of diabetogenic T cells into the pancreas [22].

Because of MT1-MMP's ability to promote directed cell migration across reconstituted basement membranes both in metastasis and in tumor angiogenesis processes, newly developed oncogenic strategies, including specific immunoliposomal anticancer MT1-MMP targeting [23] and interference RNA technology [24] are currently under consideration. The design, use, and evaluation of these approaches have, however, been more complex than expected. More recently, we identified glycocluster constructions that may serve in carbohydrate-based anticancer strategies through their ability to specifically target MT1-MMP pleiotropic functions in cell survival, proliferation and ECM degradation [25]. In the present study, we extend our efforts to identify new molecules with anti-MT1-MMP inhibitory functions and to provide the first cell-based evidence that the APN/CD13 inhibitor actinonin directly abrogates MT1-MMP's ability to activate latent proMMP-2 in highly invasive U87 glioma cells.

2. Materials and methods

2.1. Materials

Sodium dodecylsulfate (SDS) and bovine serum albumin (BSA) were purchased from Sigma (Oakville, ON). Cell culture media were obtained from Life Technologies (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 MT1-MMP was from Chemicon (Temecula, CA).

2.2. Cell culture, transfection method, and survival assay

The U87 glioblastoma cell line was purchased from American Type Culture Collection (Manassas, VA) and cultured in Eagle's Minimum essential medium (MEM) containing 10% (v/v) fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT) and 2 mM glutamine, at 37 °C under a humidified atmosphere containing 5% CO_2. U87 glioblastoma cells were either transiently transfected with a cDNA construct encoding full length MT1-MMP fused to GFP [26], or with 20 nM siRNA (see below) using Lipofectamine 2000 (Invitrogen, Burlington, ON). The occurrence of MT1-MMP specific gene knockdown was evaluated by assessing ConA's ability to trigger MT1-MMP-mediated proMMP-2 activation. Mock transfections of U87 cultures with cDNA encoding GFP expression vectors alone were used as controls. Cell death was quantified by lactate dehydrogenase (LDH) release assay according to the manufacturer's protocol (Fisher Scientific, Nepean, ON).

2.3. RNA interference

RNA interference experiments were performed using Lipofectamine 2000. A small interfering RNA against MT1-MMP (siMT1-MMP) and mismatch siRNA were synthesized by EZBiolab Inc. (Westfield, IN) and annealed to form duplexes. The sequence of the siMT1-MMP used in this study is as follows: 5′-CCAGAAGCUGAAGGUAGAAdTdT-3′ (sense) and 5′-UUCUACCUUCAGCUUCUGGdTdT-3′ (antisense) [27]. The diminution of MT1-MMP expression, as assessed by RT-PCR, was routinely over ~85% (not shown). U87 cells were also transiently transfected with 20 nM siRNA against RECK (Hs_RECK_5 HP siRNA, SI04146072; Hs_RECK_6 HP siRNA, SI04174282; Hs_RECK_7 HP siRNA, SI04195464; Hs_RECK_8 HP siRNA, SI04350318, QIAGEN, CA). RECK-specific gene knockdown was evaluated by qRT-PCR as described below.

2.4. Gelatin zymography

Gelatin zymography was used to assess the extent of latent and proMMP-2 and active MMP-2 activity. Briefly, an aliquot (20 μl) of the culture medium was subjected to 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, 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.

2.5. cDNA synthesis and real-time quantitative RT-PCR

Total RNA was extracted from cultured U87 cells using TRIzol reagent (Invitrogen). For cDNA synthesis, ~1 μg total RNA was reverse-transcribed into cDNA using a high capacity cDNA reverse Transcription Kit (Applied Biosystems, Foster City, CA). cDNA was stored at −80 °C for 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 machine (BIO-RAD, Hercules, CA) and product detection was performed by measuring the binding of the fluorescent dye SYBR Green I to double-stranded DNA. MT1-MMP and RECK primer sets were derived from human sequences and provided by QIAGEN (Valencia, CA). The relative quantities of target gene mRNA against an internal control, 18S ribosomal RNA, was measured by following a ΔC_T method. An amplification plot of fluorescence signal *vs*. cycle number was drawn. The difference (ΔC_T) between the mean values in the triplicate samples

of target gene and those of 18S ribosomal RNA were calculated by iQ5 Optical System Software version 2.0 (BIO-RAD, Hercules, CA) and the relative quantified value (RQV) was expressed as $2_T^{-\Delta C}$.

2.6. Immunoblotting procedures

Lysates or plasma membrane preparations [28] from control and treated cells were separated by SDS-polyacrylamide gel electrophoresis (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 MT1-MMP primary antibody (1/1000 dilution) in TBST containing 3% bovine serum albumin and 0.02% NaN_3, followed by a 1 h incubation with horseradish peroxidase-conjugated anti-rabbit IgG (1/2500 dilution) in TBST containing 5% non-fat dry milk. Immunoreactive material was visualized by enhanced chemiluminescence (Amersham Biosciences, Baie d'Urfée, QC).

3. Results

3.1. Actinonin inhibits concanavalin-A-induced proMMP-2 activation and MT1-MMP proteolytic processing

Concanavalin-A (ConA) is thought to trigger latent proMMP-2 activation into its active MMP-2 form [25,29]. Therefore, U87 glioma cells were serum-starved and treated with increasing concentrations of ConA, and then conditioned media were harvested in order to assess the extent of proMMP-2 activation by gelatin zymography. We observed that ConA dose-dependently induced proMMP-2 activation and that this was correlated with both induction of the mature MT1-MMP form expression and with MT1-MMP proteolytic processing, as shown by the appearance of its proteolytic 43 kDa fragment (Fig. 1A). When actinonin was added in conjunction with ConA, we observed a dose-dependent decrease in proMMP-2 activation by ConA, which was also correlated with a decrease in MT1-MMP proteolytic processing (Fig. 1B). Actinonin's inhibitory effect on MMP-2 was found complete at 50 μM (Fig. 1C), while it did not affect cell survival or proMMP-2 expression (Fig. 1C). In order to compare actinonin's efficacy at inhibiting ConA-induced proMMP-2 activation, we also showed that ilomastat, a broad-spectrum MMP inhibitor [30], abrogated proMMP-2 activation similarly to actinonin (Fig. 1D). These observations suggest that actinonin antagonizes proMMP-2 activation processes possibly involving MT1-MMP functions.

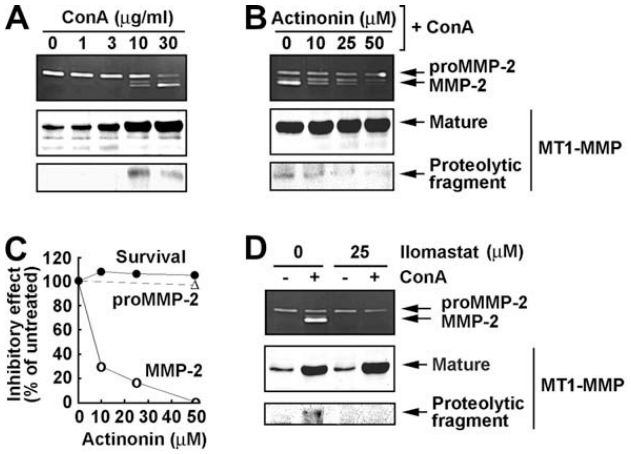

Fig. 1. Actinonin inhibits concanavalin-A-induced proMMP-2 activation and MT1-MMP proteolytic processing. Serum-starved U87 glioma cells were cultured as described in Section 2 in the presence of: (A) increasing concentrations of Concanavalin-A (ConA); (B) increasing concentrations of actinonin in the presence of 30 μg/ml ConA; or (D) 25 μM Ilomastat in the presence of 30 μg/ml ConA. Conditioned media were harvested in order to assess the extent of proMMP-2 activation by gelatin zymography (upper panels in A, B, and D). MT1-MMP expression (middle panels in A, B, and D) and MT1-MMP proteolytic processing (lower panels in A, B, and D) were assessed by western blotting and immunodetection performed as described in Section 2. (C) ProMMP-2 (open triangle) and MMP-2 (open circles) levels were quantified by densitometry of the zymograms from (B). Lactate dehydrogenase (closed circles) release into the conditioned media was assessed to monitor cell survival.

3.2. Actinonin does not inhibit concanavalin-A-induced MT1-MMP gene expression

In order to further delineate the molecular impact of actinonin on MT1-MMP-mediated processes, we next explored whether gene expression was affected. U87 glioma cells were treated (or not) with ConA, total RNA was isolated as described in Section 2, and MT1-MMP gene expression was assessed using qRT-PCR. ConA-induced MT1-MMP gene expression (Fig. 2, black bars) in agreement with the increase in protein levels we observed in Fig. 1A and with previous work [17,29,31]. Interestingly, while ilomastat and actinonin were efficient in inhibiting ConA-induced proMMP-2 activation (Fig. 1), neither was able to downregulate MT1-MMP gene expression. This suggests that actinonin affects MT1-MMP functions at the cell surface rather than at the level of gene expression.

3.3. Actinonin inhibits recombinant MT1-MMP-induced proMMP-2 activation

While most previous assays of actinonin's effects on MMPs were performed against secreted soluble MMP (MMP-1, -2, -3, -7, -8, -9, -10, -12, and -13), actinonin's direct impact on MT1-MMP functions remained to be demonstrated [32]. In order to assess the contribution of MT1-MMP to the ConA-mediated activation of proMMP-2, we first had to confirm that MT1-MMP was involved in ConA-mediated proMMP-2 activation. We used siRNA gene silencing strategies to specifically downregulate MT1-MMP gene expression in U87 glioma cells. Mock and siMT1-MMP cells were then treated (or not) with ConA and MT1-MMP silencing effectively antagonized ConA's ability to trigger proMMP-2 activation (Fig. 3A). We next transiently transfected U87 cells with a cDNA plasmid encoding MT1-MMP as previously validated by us [17,29]. Constitutive expression of recombinant MT1-MMP protein in transfected cells was confirmed by western blotting and was not inhibited by either actinonin or ilomastat (Fig. 3B, upper panel). The recombinant MT1-MMP protein was further shown to be fully active as its cell surface expression led to increased proMMP-2 activation as assessed by zymography from the isolated conditioned media (Fig. 3B, lower panel). Finally, the recombinant MT1-MMP-mediated proMMP-2 activation was completely abrogated by both

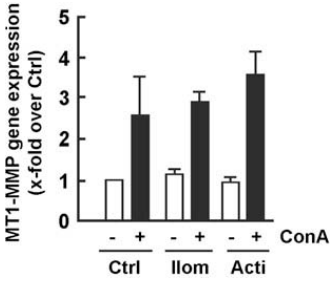

Fig. 2. Actinonin does not inhibit concanavalin-A-induced MT1-MMP gene expression. Serum-starved U87 glioma cells were treated (black bars) or not (white bars) with 30 μg/ml ConA for 18 h and total RNA was isolated as described in Section 2. MT1-MMP gene expression was assessed using qRT-PCR.

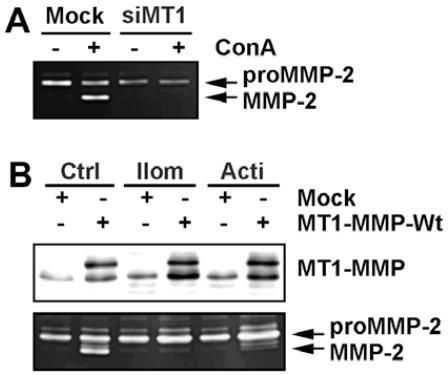

Fig. 3. Actinonin inhibits recombinant MT1-MMP-induced proMMP-2 activation. Silencing of MT1-MMP gene expression in U87 glioma cells was performed as described in Section 2: (A) serum-starved mock-transfected and siMT1-MMP-transfected (siMT1) cells were treated (or not) with 30 μg/ml ConA and the extent of proMMP-2 activation was assessed by gelatin zymography and (B) transient transfection of U87 glioma cells with a cDNA plasmid encoding MT1-MMP was performed as described in Section 2. Serum-starved cells were then treated with either 25 μM ilomastat (Ilo) or 25 μM actinonin (Acti) for 18 h. Constitutive expression of recombinant MT1-MMP protein in transfected cells was confirmed by western blotting (upper panel), while recombinant MT1-MMP-mediated activation of proMMP-2 was assessed by gelatin zymography from the isolated conditioned media (lower panel).

ilomastat and actinonin (Fig. 3B). Altogether, this is the first cell-based demonstration of a novel and direct effect of actinonin on MT1-MMP function.

3.4. Gene silencing of RECK prevents concanavalin-A-induced MT1-MMP proteolytic activation

Recently, the reversion-inducing cysteine-rich protein with Kazal motifs (RECK) was demonstrated to interact with MT1-MMP and CD13/aminopeptidase N, and to modulate their endocytic pathways [33]. This modulation correlated with the accelerated internalisation and decay of MT1-MMP and CD13. Based on the relevance of those data to the current study, we used gene silencing (siRNA) strategies to address whether such mechanism is in fact involved upon U87 treatment with actinonin. The rationale being that if RECK was involved in any MT1-MMP-internalisation process upon actinonin treatment, then this would result in the differential depletion of MT1-MMP from plasma membrane preparations. We therefore tested four different siRNA sequences to knockdown RECK gene expression and used qRT-PCR to demonstrate that efficient knockdown of RECK by more than ~72% in U87 cells was achieved with siRNA construct #4 (Fig. 4a). Furthermore, we now also demonstrate that actinonin's effects (i.e. inhibition of MT1-MMP-mediated proMMP-2 activation) are in fact possibly dependent of RECK's-mediated MT1-MMP-internalisation processes. Gene silencing of RECK with construct #4 partially prevented concanavalin-A-induced MT1-MMP proteolytic activation (Fig. 4b, upper panel). Actinonin was further unable to antagonize MT1-MMP proteolytic activation in siRECK-transfected cells, in contrast to Mock-transfected cells (Fig. 4b). Intriguingly, actinonin was able to reverse proMMP-2 activation in both Mock- and siRECK-transfected cells as shown by gelatin zymography (Fig. 4b, lower panel). This is suggesting that a pool of cell surface MT1-MMP remains insensitive to RECK's interaction Altogether, these results confirm a potential molecular link between MT1-MMP and CD13/aminopeptidase N [33], which could partly explain actinonin's inhibitory effects on MT1-MMP-mediated proMMP-2 activation.

4. Discussion

One of the most exciting findings over recent years was the discovery of a cell-associated membrane-bound MMP, MT1-MMP/MMP-14, and the subsequent demonstration that this molecule can function as an activator of latent

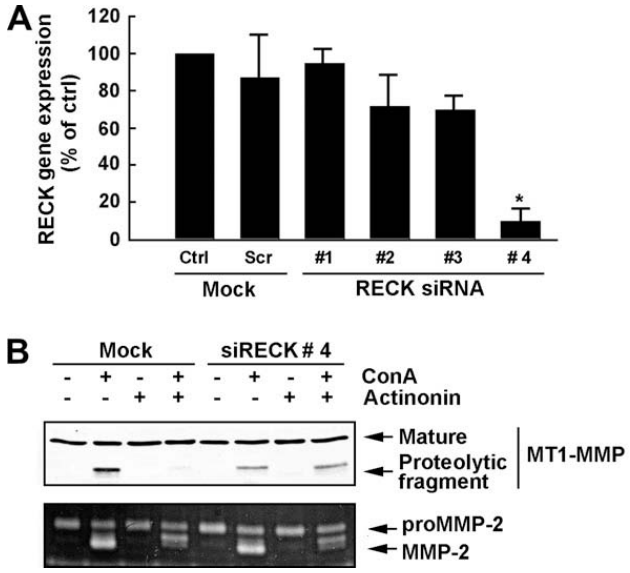

Fig. 4. Gene silencing of RECK prevents concanavalin-A-induced MT1-MMP proteolytic activation: (A) silencing of RECK gene expression in U87 glioma cells was performed as described in Section 2 and RECK gene expression assessed by qRT-PCR (Scr, scrambled sequence) and (B) serum-starved mock-transfected and siRECK-transfected (siRECK) cells were treated (or not) with 30 μg/ml ConA or with 25 μM actinonin (Acti) for 18 h. Plasma membrane preparation was performed and the extent of MT1-MMP proteolytic processing assessed by western blotting (upper panel), while proMMP-2 activation was assessed by gelatin zymography (lower panel).

proMMP-2 [20]. While disruption of MMP-2 in mice had only a minor effect on development and growth [34], disruption of MT1-MMP caused craniofacial dysmorphism, arthritis, osteopenia, dwarfism, and fibrosis of soft tissues due, in part, to ablation of collagenolytic activity [35]. Moreover, MT1-MMP gene silencing also abrogated angiogenesis-related disease [24] as well as cell invasive properties [27]. These findings implicate pivotal functions for MT1-MMP not only in physiological connective tissue metabolism, but also in pathological tumor-associated invasive processes [16,36].

Gliomas remain a great challenge in oncology today as they account for more than 50% of all brain tumors and are by far the most common primary brain tumors in adults [37]. Many of the mechanisms involved in brain tumor resistance to chemotherapy or to radiation-induced cell death have been recognized [38], and high expression of MT1-MMP was clearly identified as a potential molecular contributor to therapy resistance in glioblastomas [39,40]. As ECM proteolysis is a necessary part of the invasive and infiltrative phenotype of brain tumors, and MT1-MMP plays a central role in proteolysis [41,42], the screening of new molecules such as actinonin combined with the development of current pharmaceutical approaches that directly affect the expression and/or function interplay between MT1-MMP, APN/CD13 as well as of RECK involvement in the cell surface recycling of these two molecules, may prove beneficial in targeting invading glioma cells.

Our data, most importantly, suggests reconsidering the molecular targets and mechanisms underlying the anti-angiogenic effects of actinonin, which were initially

thought to involve APN/CD13 metalloprotease. APN/CD13 has been shown to be expressed on the human umbilical vein endothelial cells (HUVEC) of angiogenic, but not normal, vasculature [43]. Functional antagonists of APN (bestatin, amastatin, CD13 antibody) abrogated the ability of HUVEC, cultured on Matrigel, to organize a capillary network [10,44]. Still, the direct involvement of APN/CD13 in the anti-angiogenic molecular mechanism of action of actinonin remained, however, unclear. One main argument being that pathological angiogenesis is often accompanied by the formation of a fibrinous matrix, which consists of fibrin, collagen fibers and plasma proteins, all features characteristic of alternate cell surface proteases such as MT1-MMP [45,46]. Accordingly, endothelial cell assembly into capillary-like structures within collagen/fibrin gels is also thought to require the activity of MT1-MMP [47–49]. Inhibitors such as actinonin may therefore find a dual function in inhibiting specific membrane-bound proteases such as APN/CD13 and MT1-MMP, with a combined impact in both ECM degradation and in tubulogenesis processes.

In conclusion, we report the first direct, cell-based biological evaluation of actinonin against MT1-MMP. The specific action of actinonin revolves around its capacity to directly antagonize MT1-MMP-mediated activation of latent proMMP-2 in U87 glioma cells. While novel approaches to the inhibition of MT1-MMP activity are currently being explored [23–25], our observations may in fact find broader molecular and therapeutic implications which are not limited to MT1-MMP's ability to activate proMMP-2 or to contribute to therapy resistance. Based on our results, it is tempting to suggest that inhibition of MT1-MMP functions may also abrogate cell migration through MT1-MMP-mediated epidermal growth factor (EGF) receptor transactivation [50] or signaling through the EGF receptor [51]. Other avenues to explore involve actinonin's direct effect on MT1-MMP functions that may also impact on the new and appealing MT1-MMP's ability to promote CD133-positive cancer stem cells invasive properties and that could possibly be targeted by actinonin [52].

Conflicts of interest statement

None declared.

Acknowledgements

B.A. holds a Canada Research Chair in Molecular Oncology from the Canadian Institutes of Health Research (CIHR). A.S. is a recipient of a Fonds de la Recherche en Santé du Québec (FRSQ) Fellowship. This study was funded by a Grant of the Natural Sciences and Engineering Research Council of Canada (NSERC) to B.A.

References

[1] B. Bauvois, D. Dauzonne, Aminopeptidase-N/CD13 (EC 3.4.11.2) inhibitors: chemistry, biological evaluations, and therapeutic prospects, Med. Res. Rev. 26 (2006) 88–130.
[2] C. Giglione, M. Pierre, T. Meinnel, Peptide deformylase as a target for new generation broad spectrum antimicrobial agents, Mol. Microbiol. 36 (2000) 1197–1205.
[3] G.S. Van Aller, R. Nandigama, C.M. Petit, W.E. DeWolf, C.J. Quinn, K.M. Aubart, M. Zalacain, S.B. Christensen, R.A. Copeland, Z. Lai, Mechanism of time-dependent inhibition of polypeptide deformylase by actinonin, Biochemistry 44 (2005) 253–260.
[4] M.D. Lee, Y. She, M.J. Soskis, C.P. Borella, J.R. Gardner, P.A. Hayes, B.M. Dy, M.L. Heaney, M.R. Philips, W.G. Bornmann, F.M. Sirotnak, D.A. Scheinberg, Human mitochondrial peptide deformylase, a new anticancer target of actinonin-based antibiotics, J. Clin. Invest. 114 (2004) 1107–1116.
[5] D. Riemann, A. Kehlen, K. Thiele, M. Löhn, J. Langner, Induction of aminopeptidase N/CD13 on human lymphocytes after adhesion to fibroblast-like synoviocytes, endothelial cells, epithelial cells, and monocytes/macrophages, J. Immunol. 158 (1997) 3425–3432.
[6] A. Kehlen, J. Olsen, J. Langner, D. Riemann, Increased lymphocytic aminopeptidase N/CD13 promoter activity after cell–cell contact, J. Cell. Biochem. 80 (2000) 115–123.
[7] R. Rangel, Y. Sun, L. Guzman-Rojas, M.G. Ozawa, J. Sun, R.J. Giordano, C.S. Van Pelt, P.T. Tinkey, R.R. Behringer, R.L. Sidman, W. Arap, R. Pasqualini, Impaired angiogenesis in aminopeptidase N-null mice, Proc. Natl. Acad. Sci. USA 104 (2007) 4588–4593.
[8] H. Fujii, M. Nakajima, I. Saiki, J. Yoneda, I. Azuma, T. Tsuruo, Human melanoma invasion and metastasis enhancement by high expression of aminopeptidase N/CD13, Clin. Exp. Metast. 13 (1995) 337–344.
[9] N. Ikeda, Y. Nakajima, T. Tokuhara, N. Hattori, M. Sho, H. Kanehiro, M. Miyake, Clinical significance of aminopeptidase N/CD13 expression in human pancreatic carcinoma, Clin. Cancer Res. 9 (2003) 1503–1508.
[10] H. Hashida, A. Takabayashi, M. Kanai, M. Adachi, K. Kondo, N. Kohno, Y. Yamaoka, M. Miyake, Aminopeptidase N is involved in cell motility and angiogenesis: its clinical significance in human colon cancer, Gastroenterology 122 (2002) 376–386.
[11] A. Kehlen, U. Lendeckel, H. Dralle, J. Langner, C. Hoang-Vu, Biological significance of aminopeptidase N/CD13 in thyroid carcinomas, Cancer Res. 63 (2003) 8500–8506.
[12] Y. Xu, L.T. Lai, J.L. Gabrilove, D.A. Scheinberg, Antitumor activity of actinonin in vitro and in vivo, Clin. Cancer Res. 4 (1998) 171–176.
[13] K. Fukasawa, H. Fujii, Y. Saitoh, K. Koizumi, Y. Aozuka, K. Sekine, M. Yamada, I. Saiki, K. Nishikawa, Aminopeptidase N (APN/CD13) is selectively expressed in vascular endothelial cells and plays multiple roles in angiogenesis, Cancer Lett. 243 (2006) 135–143.
[14] H. Sato, T. Takino, H. Miyamori, Roles of membrane-type matrix metalloproteinase-1 in tumor invasion and metastasis, Cancer Sci. 96 (2005) 212–217.
[15] B. Bauvois, Transmembrane proteases in cell growth and invasion: new contributors to angiogenesis?, Oncogene 23 (2004) 317–329
[16] L. Genís, B.G. Gálvez, P. Gonzalo, A.G. Arroyo, MT1-MMP: universal or particular player in angiogenesis?, Cancer Metast Rev. 25 (2006) 77–86.
[17] J.C. Currie, S. Fortier, A. Sina, J. Galipeau, J. Cao, B. Annabi, 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 (2007) 8142–8149.
[18] C. Ries, V. Egea, M. Karow, H. Kolb, M. Jochum, P. Neth, MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines, Blood 109 (2007) 4055–4063.
[19] A. Nakamizo, F. Marini, T. Amano, A. Khan, M. Studeny, J. Gumin, J. Chen, S. Hentschel, G. Vecil, J. Dembinski, M. Andreeff, F.F. Lang, Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas, Cancer Res. 65 (2005) 3307–3318. Erratum in: Cancer Res. 66 (2006) 5975.
[20] A.Y. Strongin, I. Collier, G. Bannikov, B.L. Marmer, G.A. Grant, G.I. Goldberg, Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease, J. Biol. Chem. 270 (1995) 5331–5338.
[21] Z. Zhou, S.S. Apte, R. Soininen, R. Cao, G.Y. Baaklini, R.W. Rauser, J. Wang, Y. Cao, K. Tryggvason, Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I, Proc. Natl. Acad. Sci. USA 97 (2000) 4052–4057.
[22] A.Y. Savinov, D.V. Rozanov, V.S. Golubkov, F.S. Wong, A.Y. Strongin, Inhibition of membrane type-1 matrix metalloproteinase by cancer drugs interferes with the homing of diabetogenic T cells into the pancreas, J. Biol. Chem. 280 (2005) 27755–27758.
[23] K. Atobe, T. Ishida, E. Ishida, K. Hashimoto, H. Kobayashi, J. Yasuda, T. Aoki, K. Obata, H. Kikuchi, H. Akita, T. Asai, H. Harashima, N. Oku, H. Kiwada, In vitro efficacy of a sterically stabilized immunoliposomes

targeted to membrane type 1 matrix metalloproteinase (MT1-MMP), Biol. Pharm. Bull. 30 (2007) 972–978.
[24] A.G. Arroyo, L. Genís, P. Gonzalo, S. Matías-Román, A. Pollán, B.G. Gálvez, Matrix metalloproteinases: new routes to the use of MT1-MMP as a therapeutic target in angiogenesis-related disease, Curr. Pharm. Des. 13 (2007) 1787–1802.
[25] S. Fortier, M. Touaibia, S. Lord-Dufour, J. Galipeau, R. Roy, B. Annabi, Tetra- and hexa-valent 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 (2008) 195–204.
[26] J. Cao, C. Chiarelli, P. Kozarekar, H.L. Adler, Membrane type 1-matrix metalloproteinase promotes human prostate cancer invasion and metastasis, Thromb. Haemostasis 93 (2005) 770–778.
[27] P. Neth, M. Ciccarella, V. Egea, J. Hoelters, M. Jochum, C. Ries, Wnt signaling regulates the invasion capacity of human mesenchymal stem cells, Stem Cells 24 (2006) 1892–1903.
[28] B. Annabi, M. Lachambre, N. Bousquet-Gagnon, M. Pagé, D. Gingras, R. Béliveau, Localization of membrane-type 1 matrix metalloproteinase in caveolae membrane domains, Biochem. J. 353 (2001) 547–553.
[29] A. Belkaid, S. Fortier, J. Cao, B. Annabi, 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 (2007) 332–340.
[30] A. Saghatelian, N. Jessani, A. Joseph, M. Humphrey, B.F. Cravatt, Activity-based probes for the proteomic profiling of metalloproteases, Proc. Natl. Acad. Sci. USA 101 (2004) 10000–10005.
[31] B. Annabi, S. Thibeault, Y.T. Lee, N. Bousquet-Gagnon, N. Eliopoulos, S. Barrette, J. Galipeau, R. Béliveau, Matrix metalloproteinase regulation of sphingosine-1-phosphate-induced angiogenic properties of bone marrow stromal cells, Exp. Hematol. 31 (2003) 640–649.
[32] C. Antczak, C. Radu, H. Djaballah, A profiling platform for the identification of selective metalloprotease inhibitors, J. Biomol. Screen. 13 (2008) 285–294.
[33] T. Miki, Y. Takegami, K. Okawa, T. Muraguchi, M. Noda, C. Takahashi, The reversion-inducing cysteine-rich protein with Kazal motifs (RECK) interacts with membrane type 1 matrix metalloproteinase and CD13/aminopeptidase N and modulates their endocytic pathways, J. Biol. Chem. 282 (2007) 12341–12352.
[34] T. Itoh, M. Tanioka, H. Yoshida, T. Yoshioka, H. Nishimoto, S. Itohara, Reduced angiogenesis and tumor progression in gelatinase A-deficient mice, Cancer Res. 58 (1998) 1048–1051.
[35] K. Holmbeck, P. Bianco, J. Caterina, S. Yamada, M. Kromer, S.A. Kuznetsov, M. Mankani, P.G. Robey, A.R. Poole, I. Pidoux, J.M. Ward, H. Birkedal-Hansen, MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover, Cell 99 (1999) 81–92.
[36] Y. Itoh, M. Seiki, MT1-MMP: a potent modifier of pericellular microenvironment, J. Cell. Physiol. 206 (2006) 1–8.
[37] Glioma Meta-analysis Trialists (GMT) Group, Lancet 359 (2002) 1011–1018.
[38] M.E. Berens, A. Giese, Those left behind biology and oncology of invasive glioma cells, Neoplasia 1 (1999) 208–219.
[39] B. Annabi, M. Bouzeghrane, R. Moumdjian, A. Moghrabi, R. Béliveau, Probing the infiltrating character of brain tumors: inhibition of RhoA/ROK-mediated CD44 cell surface shedding from glioma cells by the green tea catechin EGCg, J. Neurochem. 94 (2005) 906–916.
[40] N. McLaughlin, B. Annabi, M. Bouzeghrane, A. Temme, J.P. Bahary, R. Moumdjian, R. Béliveau, The Survivin-mediated radioresistant phenotype of glioblastomas is regulated by RhoA and inhibited by the green tea polyphenol (−)-epigallocatechin-3-gallate, Brain Res. 1071 (2006) 1–9.
[41] H.L. Fillmore, T.E. VanMeter, W.C. Broaddus, Membrane-type matrix metalloproteinases (MT-MMPs): expression and function during glioma invasion, J. Neurooncol. 53 (2001) 187–202.
[42] M. Nakada, D. Kita, K. Futami, J. Yamashita, N. Fujimoto, H. Sato, Y. Okada, Roles of membrane type 1 matrix metalloproteinase and tissue inhibitor of metalloproteinases 2 in invasion and dissemination of human malignant glioma, J. Neurosurg. 94 (2001) 464–473.
[43] R. Pasqualini, E. Koivunen, R. Kain, J. Lahdenranta, M. Sakamoto, A. Stryhn, R.A. Ashmun, L.H. Shapiro, W. Arap, E. Ruoslahti, Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis, Cancer Res. 60 (2000) 722–727.
[44] S.V. Bhagwat, N. Petrovic, Y. Okamoto, L.H. Shapiro, The angiogenic regulator CD13/APN is a transcriptional target of Ras signaling pathways in endothelial morphogenesis, Blood 101 (2003) 1818–1826.
[45] N. Hiraoka, E. Allen, I.J. Apel, M.R. Gyetko, S.J. Weiss, Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins, Cell 95 (1998) 365–377.
[46] A. Collen, R. Hanemaaijer, F. Lupu, P.H. Quax, N. van Lent, J. Grimbergen, E. Peters, P. Koolwijk, V.W. van Hinsbergh, Membrane-type matrix metalloproteinase-mediated angiogenesis in a fibrin–collagen matrix, Blood 101 (2003) 1810–1817.
[47] M.P. d'Ortho, H. Stanton, M. Butler, S.J. Atkinson, G. Murphy, R.M. Hembry, MT1-MMP on the cell surface causes focal degradation of gelatin films, FEBS Lett. 421 (1998) 159–164.
[48] M.A. Lafleur, M.M. Handsley, V. Knäuper, G. Murphy, D.R. Edwards, Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs), J. Cell. Sci. 115 (2002) 3427–3438.
[49] T. Koike, R.B. Vernon, M.A. Hamner, E. Sadoun, M.J. Reed, MT1-MMP, but not secreted MMPs, influences the migration of human microvascular endothelial cells in 3-dimensional collagen gels, J. Cell. Biochem. 86 (2002) 748–758.
[50] S. Langlois, C. Nyalendo, G. Di Tomasso, L. Labrecque, C. Roghi, G. Murphy, D. Gingras, R. Béliveau, Membrane-type 1 matrix metalloproteinase stimulates cell migration through epidermal growth factor receptor transactivation, Mol. Cancer Res. 5 (2007) 569–583.
[51] F. Kheradmand, K. Rishi, Z. Werb, Signaling through the EGF receptor controls lung morphogenesis in part by regulating MT1-MMP-mediated activation of gelatinase A/MMP2, J. Cell. Sci. 115 (2002) 839–848.
[52] B. Annabi, S. Rojas-Sutterlin, C. Laflamme, M.P. Lachambre, Y. Rolland, H. Sartelet, R. Béliveau, Tumor environment dictates medulloblastoma cancer stem cell expression and invasive phenotype, Mol. Cancer Res. 6 (2008) 907–916.