Matrix Biology 31 (2012) 229–233

Contents lists available at SciVerse ScienceDirect

Matrix Biology

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

Brief report

# Microvesicles shed by oligodendroglioma cells and rheumatoid synovial fibroblasts contain aggrecanase activity

Alessandra Lo Cicero [a,b], Iwona Majkowska [c], Hideaki Nagase [b], Italia Di Liegro [a], Linda Troeberg [b,*]

[a] Dipartimento di Biomedicina sperimentale e Neuroscienze cliniche, via del Vespro 129, 90127 Palermo, Italy
[b] Kennedy Institute of Rheumatology, University of Oxford, 65 Aspenlea Road, London, W6 8LH, UK
[c] Imperial College London, 65 Aspenlea Road, London, W6 8LH, UK

ARTICLE INFO

*Article history:*
Received 3 January 2012
Received in revised form 22 February 2012
Accepted 22 February 2012

*Keywords:*
Membrane vesicles
Aggrecan
Metalloproteinase
ADAMTS

ABSTRACT

Membrane microvesicle shedding is an active process and occurs in viable cells with no signs of apoptosis or necrosis. We report here that microvesicles shed by oligodendroglioma cells contain an 'aggrecanase' activity, cleaving aggrecan at sites previously identified as targets for adamalysin metalloproteinases with disintegrin and thrombospondin domains (ADAMTSs). Degradation was inhibited by EDTA, the metalloproteinase inhibitor GM6001 and by tissue inhibitor of metalloproteinases (TIMP)-3, but not by TIMP-1 or TIMP-2. This inhibitor profile indicates that the shed microvesicles contain aggrecanolytic ADAMTS(s) or related TIMP-3-sensitive metalloproteinase(s). The oligodendroglioma cells were shown to express the three most active aggrecanases, namely *Adamts1*, *Adamts4* and *Adamts5*, suggesting that one or more of these enzymes may be responsible for the microvesicle activity. Microvesicles shed by rheumatoid synovial fibroblasts similarly degraded aggrecan in a TIMP-3-sensitive manner. Our findings raise the novel possibility that microvesicles may assist oligodendroglioma and rheumatoid synovial fibroblasts to invade through aggrecan-rich extracellular matrices.



## 1. Introduction

An increasing number of cell types have been shown to shed microvesicles (MVs) under both physiological and pathological conditions. MVs have been demonstrated to contain a wide array of biological effector molecules, including proteins, lipids, DNA, microRNA and mRNA, and since MVs can interact with other cells, they can serve as a means of cell-to-cell communication (Mathivanan et al., 2010). MVs can interact with other cells either through direct membrane fusion, or by utilising adhesion molecules such as L1 (Gutwein et al., 2005) and CD44 (Stoeck et al., 2006), or signalling molecules such as integrins (Dolo et al., 1998; Taraboletti et al., 2002) located on the MV surface. MVs can initiate various biological events in target cells, ranging from apoptosis (D'Agostino et al., 2006; Lo Cicero et al., 2011) to cell survival and proliferation (Deregibus et al., 2007; Skog et al., 2008). They have also been shown to mediate horizontal mRNA transfer (Deregibus et al., 2007; Skog et al., 2008).

Fibroblasts (Lee et al., 1993), reticulocytes (Johnstone et al., 1987), endothelial cells (Taraboletti et al., 2002), lymphocytes (Raposo et al., 1996), dendritic cells (Zitvogel et al., 1998) and platelets (Heijnen et al., 1999), as well as by numerous cancer cell types (Ginestra et al., 1997; Dolo et al., 1998) have been shown to shed MVs. The MV population can be subdivided into exosomes and shedding vesicles based on their size and origin. Exosomes are 40–100 nm in diameter and are derived from endosomal multivesicular bodies (Théry et al., 2002), while shedding vesicles are 100–1000 nm in diameter and are generated by outward budding of the plasma membrane (Cocucci et al., 2009). Cells commonly release both exosomes and shedding vesicles into the extracellular space, with this mixed population referred to as 'microvesicles' (MVs).

MVs shed by tumour cells contain tumour antigens and can thus contribute to immune system evasion (Andre et al., 2002). Tumour MVs may also contribute to tumour invasion, as they contain proteinases that degrade the ECM. For example, MVs have been demonstrated to contain cathepsin B (Giusti et al., 2008), matrix metalloproteinase (MMP)-2 (Ginestra et al., 1997), MMP-9 (Ginestra et al., 1997; Dolo et al., 1998), MT1-MMP (Taraboletti et al., 2002), and urokinase-type plasminogen activator (Ginestra et al., 1997), as well as the adamalysins ADAM10 (Gutwein et al., 2005; Stoeck et al., 2006) and ADAM17 (Stoeck et al., 2006).

We have previously shown that G26/24 oligodendroglioma cells shed MVs, which contain Fas ligand and tumour necrosis factor α-related apoptosis-inducing ligand and induce apoptosis

*Abbreviations:* ADAM, adamalysin; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; ECM, extracellular matrix; MEF, mouse embryonic fibroblasts; MMP, matrix metalloproteinase; MVs, microvesicles; RA, rheumatoid arthritis; TIMP, tissue inhibitor of metalloproteinase.

* Corresponding author. Tel.: +44 20 8383 4444; fax: +44 20 8383 4999.
*E-mail address:* linda.troeberg@kennedy.ox.ac.uk (L. Troeberg).

0945-053X © 2012 Elsevier B.V. Open access under CC BY-NC-ND license.
doi:10.1016/j.matbio.2012.02.005

in neurons and astrocytes (D'Agostino et al., 2006; Lo Cicero et al., 2011). In addition to their effects on healthy cells within the brain, oligodendrogliomas remodel the brain extracellular matrix (ECM) and invade into surrounding tissues (Bellail et al., 2004). Along with other lecticans, aggrecan is a constituent of the brain ECM (Yamaguchi, 2000; Bellail et al., 2004). In particular, aggrecan is thought to be an important component of perineuronal nets (Matthews et al., 2002), with its expression altered in pathological conditions such as Alzheimer's disease (Morawski et al., 2010) and early-life seizure (McRae et al., 2010).

Aggrecan is also a major constituent of the ECM of cartilage, and aggrecan degradation is associated with progression of various types of arthritis (Lohmander et al., 1993). During the development of rheumatoid arthritis (RA), the cartilage matrix is invaded by fibroblasts originating from the synovial lining (Ospelt et al., 2004). The invading synovial cells have been shown to express a number of ECM-degrading MMPs (Murphy and Nagase, 2008) as well as metalloproteinases with disintegrin and thrombospondin motifs (ADAMTSs) (Yamanishi et al., 2002). ADAMTS-4 and ADAMTS-5 are thought to be the primary 'aggrecanases' responsible for aggrecan degradation in osteoarthritis (Murphy and Nagase, 2008).

Our results demonstrate for the first time that MVs shed by both oligodendroglioma cells and rheumatoid synovial fibroblasts contain a TIMP-3-sensitive aggrecanase activity. This suggests that MVs may contribute to cellular invasion of aggrecan-rich extracellular matrices.

## 2. Results

### 2.1. G26/26 MVs cleave aggrecan at several sites

Using a panel of antibodies that recognise aggrecan degradation products, we found that MVs shed by G26/24 oligodendroglioma cells are able to degrade aggrecan at several sites.

Following incubation of 500 nM aggrecan with MVs (4 μg protein) for 24 h at 37 °C, aggrecan degradation was visible using the 2-B-6 antibody (Caterson et al., 1985), which recognises chondroitinase-treated aggrecan fragments (Fig. 1A). The pattern of the fragments generated by 4 μg of G26/24 MVs mirrored that obtained after incubation of aggrecan with 0.01 nM of recombinant ADAMTS-5.

Aggrecan degradation by G26/24 MVs was further investigated using a panel of neoepitope antibodies that recognise aggrecan cleavage at sites previously shown to be hydrolysed by ADAMTS-4 and ADAMTS-5 (Kashiwagi et al., 2004; Gendron et al., 2007; Troeberg et al., 2008). Following 24 h of incubation at 37 °C with 4 μg of MVs, aggrecan degradation was visible using anti-$^{1772}$AGEG and anti-GELE$^{1480}$ antibodies, indicating that MVs cleaved aggrecan at the TAQE$^{1771}$–$^{1772}$AGEG and GELE$^{1480}$–$^{1481}$GRGT sites in the chondroitin sulfate-rich region (Fig. 1A). The aggrecanase activity of MVs was the same whether 0.05% Brij 35 was included in the assay buffer or not. The degradation products were comparable with those generated by incubation of aggrecan with 0.01 nM recombinant ADAMTS-5 (Fig. 1A).

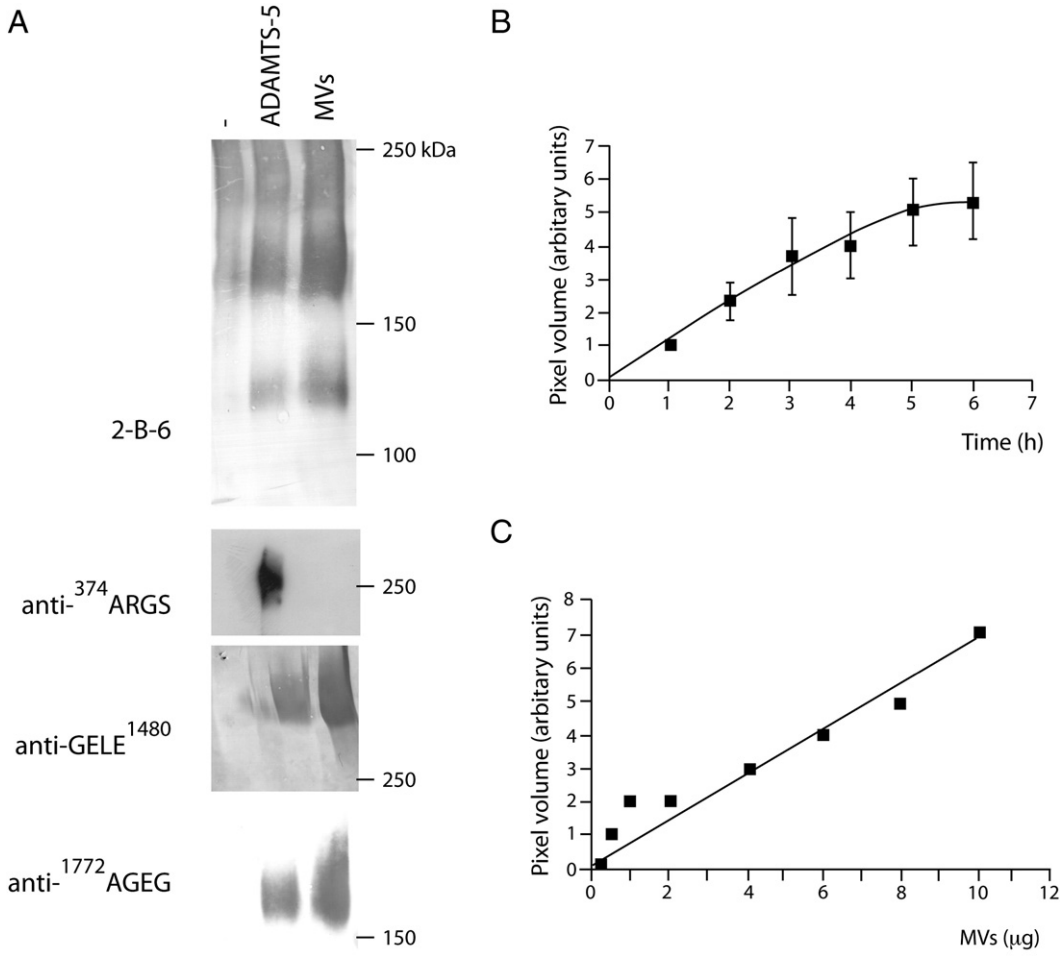


**Fig. 1.** G26/24 MVs degrade aggrecan. (A) Aggrecan was incubated alone (−), or with recombinant ADAMTS-5 (5 nM for detection with anti-$^{374}$ARGSV, 0.01 nM for all other antibodies), or G26/24 MVs (4 μg) for 24 h at 37 °C and degradation products analysed by immunoblotting with the indicated antibodies. (B) Aggrecan was incubated with G26/24 MVs (2 μg) at 37 °C for various times and formation of the $^{1772}$AGEG neo-epitope visualised by immunoblotting. (C) Aggrecan was incubated with G26/24 MVs (0.1–10 μg, 3 h, 37 °C) and formation of the $^{1772}$AGEG neo-epitope visualised by immunoblotting.

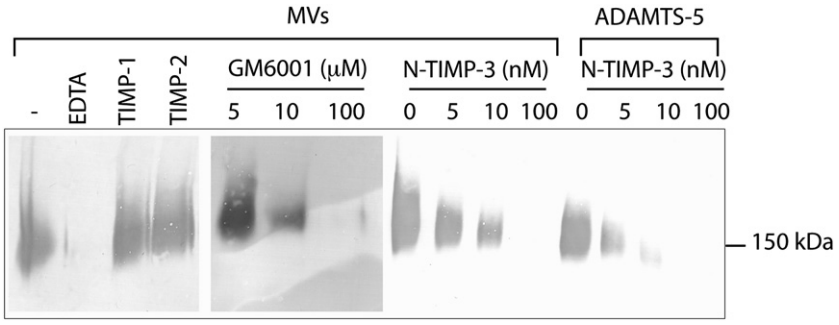


**Fig. 2.** Aggrecan degradation by G26/24 MVs is due to a TIMP-3-sensitive metalloproteinase. G26/24 MVs (4 μg) or ADAMTS-5 (0.001 nM) were incubated alone (−) or with EDTA (10 mM), TIMP-1 (100 nM), TIMP-2 (100 nM), GM6001 (5–100 μM) or N-TIMP-3 (5–100 nM) (1 h, 37 °C). Residual activity against aggrecan (3 h, 37 °C) was visualised using the anti-$^{1772}$AGEG neo-epitope antibody.

MV cleavage of aggrecan at the NITEGE$^{373}$–$^{374}$ARGSV site in the interglobular domain was not detectable with an anti-$^{374}$ARGSV neoepitope antibody (Hughes et al., 1995) following incubation of 500 nM aggrecan with 4 μg of MVs for 24 h at 37 °C (Fig. 1A). Incubation of aggrecan with recombinant ADAMTS-5 (5 nM) generated the expected cleavage product.

Aggrecan degradation by G26/24 MVs was shown to be time- and dose-dependent. Using the anti-$^{1772}$AGEG antibody, degradation of aggrecan by 2 μg of MVs was linear for up to 4 h (Fig. 1B). Aggrecan degradation was also dose-dependent, with formation of the $^{1772}$AGEG neoepitope increasing linearly over 3 h for 0.1 to 10 μg of MVs (Fig. 1C).

### 2.2. G26/26 MV aggrecan-degrading activity is inhibited by TIMP-3

We investigated the inhibitor sensitivity of the aggrecan-degrading activity of G26/24 MVs by incubating MVs with various proteinase inhibitors and measuring residual activity against the TAQE$^{1771}$–$^{1772}$AGEG site.

The metal chelator EDTA (10 mM) blocked the formation of the $^{1772}$AGEG neoepitope, indicating that a metalloproteinase is responsible for degradation (Fig. 2). GM6001, a hydroxamate metalloproteinase inhibitor, also inhibited aggrecan cleavage by G26/24 MV, with GM6001 inhibiting activity completely at 100 μM and partially at 10 μM (Fig. 2).

TIMP-1 and TIMP-2 (100 nM) had no effect on aggrecan degradation by G26/24 MV, while N-TIMP-inhibited activity strongly and similarly to N-TIMP-3 inhibition of ADAMTS-5. This inhibitory profile indicates that an ADAMTS or related TIMP-3-sensitive metalloproteinase is responsible for the observed aggrecanase activity.

### 2.3. G26/26 cells express aggrecanolytic ADAMTSs

RT-PCR confirmed the expression of *Adamts1*, *Adamts4* and *Adamts5* by G26/24 cells (Fig. 3).

### 2.4. Rheumatoid synovial fibroblast MVs cleave aggrecan in a TIMP-3-sensitive manner

To investigate whether other cell types also shed MVs containing aggrecan-degrading activity, we isolated MVs shed by primary rheumatoid synovial fibroblasts, normal skin fibroblasts and HTB94 chondrosarcoma. The RA synovial fibroblast MVs (4 μg of protein) cleaved aggrecan at the TAQE$^{1771}$–$^{1772}$AGEG bond, while no activity was detected with normal skin fibroblast or HTB94 MVs (4 μg) (Fig. 4A). Aggrecan degradation by RA fibroblast MVs was inhibited by 100 nM TIMP-3, indicating that this activity is also likely to be due to an ADAMTS or related metalloproteinase.

## 3. Discussion

To our knowledge, this is the first description of aggrecanase activity being present in microvesicles shed from two different cell types: oligodendroglioma and rheumatoid synovial fibroblasts. Based on our inhibitor studies, the aggrecanase activity in the shed MVs is likely to be due to ADAMTS metalloproteinase(s). ADAMTS-1, -4, -5, -8, -9, 15, -16 and -18 have all been reported to degrade aggrecan (Murphy and Nagase, 2008). Among these, ADAMTS-1 (Kuno et al., 2000), ADAMTS-4 (Kashiwagi et al., 2004) and ADAMTS-5 (Gendron et al., 2007) are the best characterised to date and show the strongest aggrecanase activity. By RT-PCR, we confirmed that G26/24 oligodendroglioma cells express all three of these enzymes, as previously

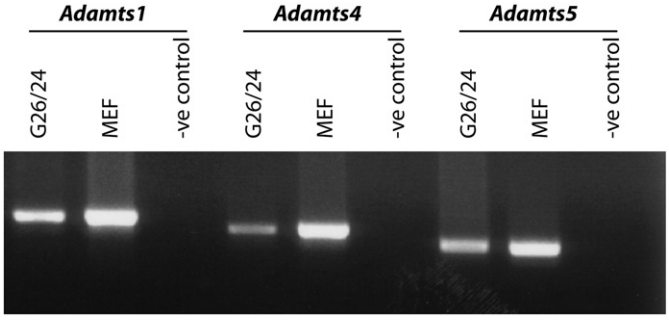


**Fig. 3.** G26/24 cells express ADAMTS-1, ADAMTS-4 and ADAMTS-5. RT-PCR for *Adamts1*, *Adamt4* and *Adamts5* was performed on total RNA isolated from G26/24 cells and MEF cells. Negative controls were performed without template.

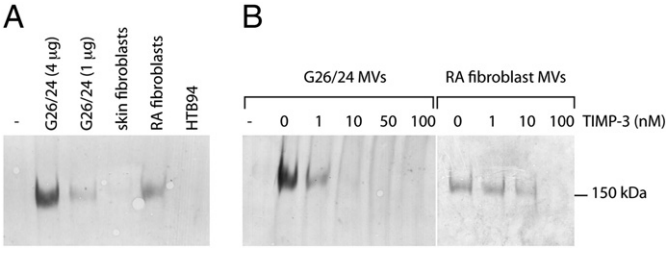


**Fig. 4.** RA synovial fibroblast MVs degrade aggrecan. (A) Aggrecan was incubated alone (−) or with MVs from G26/24 (4 or 1 μg), normal skin fibroblasts (4 μg), RA synovial fibroblasts (4 μg) or HTB94 chondrosarcoma (4 μg) (24 h, 37 °C). Degradation products were analysed by immunoblotting using an anti-$^{1772}$AGEG neo-epitope antibody. (B) MVs from G26/24 (4 μg) or RA synovial fibroblasts (4 μg) were pre-incubated with TIMP-3 (0–100 nM, 1 h, 37 °C) and residual activity against aggrecan (24 h, 37 °C) visualised using an anti-$^{1772}$AGEG neo-epitope antibody.

shown for glioblastomas (Held-Feindt et al., 2006). RA synovial fibroblasts are known to express ADAMTS-4 and ADAMTS-5 (Yamanishi et al., 2002). These enzymes are thus candidates for the aggrecanase activity observed in the MVs. The MVs may contain a number of ADAMTSs, including those whose expression has been confirmed as well as others that remain to be characterised. We were unable to identify ADAMTSs present in the MVs by immunohistochemistry due to the insensitivity of currently available antibodies. This limitation, coupled with the low level at which the enzymes are expressed in tissues, has hampered direct detection of aggrecanases in various tissue samples.

G26/24 MVs cleaved aggrecan at two sites in the chondroitin sulfate-rich region, namely at the TAQE$^{1771}$–$^{1772}$AGEG and GELE$^{1480}$–$^{1481}$GRGT bonds. However, no cleavage of aggrecan at the characteristic NITEGE$^{373}$–$^{374}$ARGSV aggrecanase site in the interglobular region was detected. This may be because higher concentrations of enzyme are required to detect cleavage at the NITEGE$^{373}$–$^{374}$ARGSV site than at the TAQE$^{1771}$–$^{1772}$AGEG and GELE$^{1480}$–$^{1481}$GRGT sites. For example, 5 nM recombinant ADAMTS-5 was required to detect aggrecan cleavage using the anti-$^{374}$ARGSV antibody, while 0.01 nM ADAMTS-5 was sufficient to detect cleavage using the anti-$^{1772}$AGEG and anti-GELE$^{1480}$ antibodies, in line with previous findings (Gendron et al., 2007). We found that 4 μg of MVs from G26/26 cells cleaved aggrecan comparably to 0.01 nM ADAMTS-5 at the TAQE$^{1771}$–$^{1772}$AGEG and GELE$^{1480}$–$^{1481}$GRGT sites, suggesting that 2000 μg of MVs would be required to detect activity at NITEGE$^{373}$–$^{374}$ARGSV. We were not able to isolate sufficient amounts of MVs to test this hypothesis.

Furthermore, we have found that heparin inhibits ADAMTS-4 hydrolysis of aggrecan, and that hydrolysis of the NITEGE$^{373}$–$^{374}$ARGSV site is more sensitive to inhibition by heparin than the GELE$^{1480}$–$^{1481}$GRGT site (Fushimi et al., 2008). Our data may thus indicate that the aggrecan-degrading activity in G26/24 MVs has similar properties and is associated with a heparan sulfate proteoglycan on the surface of MVs. Membrane-associated aggrecanase activity was first described by Billington et al. in preparations of bovine nasal chondrocyte membranes (Billington et al., 1998). ADAMTS-4 is thought to associate with syndecan 1 on the surface of chondrosarcoma cells (Gao et al., 2004), and ADAMTS-5 to associate with syndecan 4 on chondrocytes (Echtermeyer et al., 2009). We thus postulate that the aggrecanase activity is associated with the surface of the MVs through interaction with heparan sulfate proteoglycans.

Both oligodendroglioma and rheumatoid synovial fibroblasts are capable of invading into brain and cartilage extracellular matrices, which are rich in lectican chondroitin sulfate proteoglycans such as aggrecan, versican, brevican and neurocan. Our findings suggest that shed MVs may contribute to the ECM-degrading and invasive capacity of the cells. Degradation of brevican by ADAMTSs has been shown to generate a cleaved fragment that increases the invasiveness of glioma cells (Nakada et al., 2005), suggesting that aggrecanase activity in shed MVs may also indirectly increase invasive capacity. The presence of ADAMTSs in shed MVs may represent a novel mechanism by which cells target these enzymes to lectican substrates in diverse extracellular matrices.

## 4. Experimental procedures

### 4.1. Materials

Human ADAMTS-5 lacking the C-terminal thrombospondin domain (Gendron et al., 2007), bovine aggrecan (Hascall and Sajdera, 1969), TIMP-1 (Troeberg et al., 2002), TIMP-2 (Troeberg et al., 2002), TIMP-3 (Troeberg et al., 2009) and N-TIMP-3 (Kashiwagi et al., 2001) were prepared as previously described. GM6001 was from Elastin Products (Owensville, USA).

### 4.2. Cell culture

Murine G26/24 oligodendroglioma and human HTB94 chondrosarcoma cells were maintained in DMEM supplemented with 10% foetal calf serum, and 1% penicillin/streptomycin. Human synovial fibroblasts were obtained with ethics approval and informed consent from synovial tissue of RA patients undergoing joint replacement surgery as previously described (Miller et al., 2009). Normal human skin fibroblasts were obtained with ethics approval and informed consent from patients undergoing surgery for Dupuytren's disease as previously described (Verjee et al., 2009).

### 4.3. Preparation of MVs from conditioned medium

MVs were prepared from media conditioned for 24 h on subconfluent cells. Media were centrifuged at 2000 *g* (15 min, 4 °C) and 4000 *g* (15 min, 4 °C) to remove debris. MVs were collected from the supernatant by centrifugation at 105 000 *g* (90 min, 4 °C) and re-suspended in phosphate-buffered saline, pH 7.5. MVs were quantified based on protein content using the Bradford assay (Protein Assay Reagent, Bio-Rad, UK) with BSA as a standard protein.

### 4.4. Aggrecanase assay

Aggrecan (500 nM, 50 μg in 50 μl assay volume) was incubated with MVs or ADAMTS-5 in 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$, and 0.05% Brij 35 at 37 °C. For inhibitor analysis, ADAMTSs or MVs were pre-incubated with inhibitors (1 h, 37 °C) before addition of aggrecan. Samples were prepared for immunoblotting by treatment with chondroitinase ABC and keratanase (Seikagaku, Japan) (0.01 units each, 16 h, 37 °C) and precipitation with acetone (5 volumes, −20 °C, 18 h). Degradation was visualised using antibodies recognising degraded aggrecan fragments as before (Troeberg et al., 2008). The 2-B-6 antibody recognises chondroitinase-treated aggrecan (Caterson et al., 1985); anti-$^{374}$ARGSV recognises aggrecan cleaved at NITEGE$^{373}$–$^{374}$ARGSV (Hughes et al., 1995), anti-GELE$^{1480}$ recognises aggrecan cleaved at GELE$^{1480}$–$^{1481}$GRGT (Kashiwagi et al., 2004) and anti-$^{1772}$AGEG recognises aggrecan cleaved at TAQE$^{1771}$–$^{1772}$AGEG (Troeberg et al., 2008). Blots were quantified using ImageJ software.

### 4.5. RT-PCR

*Adamts* expression was evaluated by RT-PCR of the total RNA extracted from G26/24 and mouse embryonic fibroblasts (MEF) using a QIAamp RNA Blood Mini Kit (Qiagen Ltd, UK). Primers for *Adamts1* amplification were: 5′-CAGGAAGCATAAGGAAGAAG-3′ (forward) and 5′-GCACAGTGCTTAGCATCATCA-3′ (reverse), for *Adamts4*: 5′-ATGTGGGCACAGTGTGTGAT-3′ (forward) and 5′-CAAGGTGAGTGCTTCGTCTG-3′ (reverse), and for *Adamts5*: 5′-GGCATCATTCATGTGACACC-3′ (forward) and 5′-CGAGTACTCAGGCCCAAATG-3′ (reverse) (Stanton et al., 2005).

## Acknowledgements

We thank Salvatore Santamaria (Kennedy Institute of Rheumatology) for preparing ADAMTS-5, and Prof. B. Caterson and Dr C. Hughes (University of Cardiff, UK) for providing 2-B-6 and anti-$^{374}$ARGSV antibodies.

Alessandra Lo Cicero was supported by a PhD studentship from the University of Palermo (Università degli Studi di Palermo), Palermo, Italy. Linda Troeberg is supported by an Arthritis Research UK Career Development Fellowship (grant 19466). Hideaki Nagase is supported by Arthritis Research UK Core grant to the Kennedy Institute of Rheumatology, and National Institute of Arthritis and Musculoskeletal and Skin Diseases (grant AR40994). The content is

solely the responsibility of the authors and does not necessarily represent the official views of NIAMS or NIH.

## References


Andre, F., Schartz, N.E., Movassagh, M., Flament, C., Pautier, P., Morice, P., Pomel, C., Lhomme, C., Escudier, B., Le Chevalier, T., Tursz, T., Amigorena, S., Raposo, G., Angevin, E., Zitvogel, L., 2002. Malignant effusions and immunogenic tumour-derived exosomes. Lancet 360, 295–305.

Bellail, A.C., Hunter, S.B., Brat, D.J., Tan, C., Van Meir, E.G., 2004. Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. Int. J. Biochem. Cell Biol. 36, 1046–1069.

Billington, C.J., Clark, I.M., Cawston, T.E., 1998. An aggrecan-degrading activity associated with chondrocyte membranes. Biochem. J. 336, 207–212.

Caterson, B., Christner, J.E., Baker, J.R., Couchman, J.R., 1985. Production and characterization of monoclonal antibodies directed against connective tissue proteoglycans. Fed. Proc. 44, 386–393.

Cocucci, E., Racchetti, G., Meldolesi, J., 2009. Shedding microvesicles: artefacts no more. Trends Cell Biol. 19, 43–51.

D'Agostino, S., Salamone, M., Di Liegro, I., Vittorelli, M.L., 2006. Membrane vesicles shed by oligodendroglioma cells induce neuronal apoptosis. Int. J. Oncol. 29, 1075–1085.

Deregibus, M.C., Cantaluppi, V., Calogero, R., Lo Iacono, M., Tetta, C., Biancone, L., Bruno, S., Bussolati, B., Camussi, G., 2007. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 110, 2440–2448.

Dolo, V., Ginestra, A., Cassarà, D., Violini, S., Lucania, G., Torrisi, M.R., Nagase, H., Canevari, S., Pavan, A., Vittorelli, M.L., 1998. Selective localization of matrix metalloproteinase 9, beta1 integrins, and human lymphocyte antigen class I molecules on membrane vesicles shed by 8701-BC breast carcinoma cells. Cancer Res. 58, 4468–4474.

Echtermeyer, F., Bertrand, J., Dreier, R., Meinecke, I., Neugebauer, K., Fuerst, M., Lee, Y.J., Song, Y.W., Herzog, C., Theilmeier, G., Pap, T., 2009. Syndecan-4 regulates ADAMTS-5 activation and cartilage breakdown in osteoarthritis. Nat. Med. 15, 1072–1076.

Fushimi, K., Troeberg, L., Nakamura, H., Lim, N.H., Nagase, H., 2008. Functional differences of the catalytic and non-catalytic domains in human ADAMTS-4 and ADAMTS-5 in aggrecanolytic activity. J. Biol. Chem. 283, 6706–6716.

Gao, G., Plaas, A., Thompson, V.P., Jin, S., Zuo, F., Sandy, J.D., 2004. ADAMTS4 (aggrecanase-1) activation on the cell surface involves C-terminal cleavage by glycosylphosphatidyl inositol-anchored membrane type 4-matrix metalloproteinase and binding of the activated proteinase to chondroitin sulfate and heparan sulfate on syndecan-1. J. Biol. Chem. 279, 10042–10051.

Gendron, C., Kashiwagi, M., Lim, N.H., Enghild, J.J., Thøgersen, I.B., Hughes, C., Caterson, B., Nagase, H., 2007. Proteolytic activities of human ADAMTS-5: comparative studies with ADAMTS-4. J. Biol. Chem. 282, 18294–18306.

Ginestra, A., Monea, S., Seghezzi, G., Dolo, V., Nagase, H., Mignatti, P., Vittorelli, M.L., 1997. Urokinase plasminogen activator and gelatinases are associated with membrane vesicles shed by human HT1080 fibrosarcoma cells. J. Biol. Chem. 272, 17216–17222.

Giusti, I., D'Ascenzo, S., Millimaggi, D., Taraboletti, G., Carta, G., Franceschini, N., Pavan, A., Dolo, V., 2008. Cathepsin B mediates the pH-dependent proinvasive activity of tumor-shed microvesicles. Neoplasia 10, 481–488.

Gutwein, P., Stoeck, A., Riedle, S., Gast, D., Runz, S., Condon, T.P., Marmé, A., Phong, M.C., Linderkamp, O., Skorokhod, A., Altevogt, P., 2005. Cleavage of L1 in exosomes and apoptotic membrane vesicles released from ovarian carcinoma cells. Clin. Cancer Res. 11, 2492–2501.

Hascall, V.C., Sajdera, S.W., 1969. Proteinpolysaccharide complex from bovine nasal cartilage. The function of glycoprotein in the formation of aggregates. J. Biol. Chem. 244, 2384–2396.

Heijnen, H.F., Schiel, A.E., Fijnheer, R., Geuze, H.J., Sixma, J.J., 1999. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood 94, 3791–3799.

Held-Feindt, J., Paredes, E.B., Blömer, U., Seidenbecher, C., Stark, A.M., Mehdorn, H.M., Mentlein, R., 2006. Matrix-degrading proteases ADAMTS4 and ADAMTS5 (disintegrins and metalloproteinases with thrombospondin motifs 4 and 5) are expressed in human glioblastomas. Int. J. Cancer 118, 55–61.

Hughes, C.E., Caterson, B., Fosang, A.J., Roughley, P.J., Mort, J.S., 1995. Monoclonal antibodies that specifically recognize neoepitope sequences generated by 'aggrecanase' and matrix metalloproteinase cleavage of aggrecan: application to catabolism in situ and in vitro. Biochem. J. 305, 799–804.

Johnstone, R.M., Adam, M., Hammond, J.R., Orr, L., Turbide, C., 1987. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J. Biol. Chem. 262, 9412–9420.

Kashiwagi, M., Tortorella, M., Nagase, H., Brew, K., 2001. TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5). J. Biol. Chem. 276, 12501–12504.

Kashiwagi, M., Enghild, J.J., Gendron, C., Hughes, C., Caterson, B., Itoh, Y., Nagase, H., 2004. Altered proteolytic activities of ADAMTS-4 expressed by C-terminal processing. J. Biol. Chem. 279, 10109–10119.

Kuno, K., Okada, Y., Kawashima, H., Nakamura, H., Miyasaka, M., Ohno, H., Matsushima, K., 2000. ADAMTS-1 cleaves a cartilage proteoglycan, aggrecan. FEBS Lett. 478, 241–245.

Lee, T.L., Lin, Y.C., Mochitate, K., Grinnell, F., 1993. Stress-relaxation of fibroblasts in collagen matrices triggers ectocytosis of plasma membrane vesicles containing actin, annexins II and VI, and beta 1 integrin receptors. J. Cell Sci. 105, 167–177.

Lo Cicero, A., Schiera, G., Proia, P., Saladino, P., Savettieri, G., Di Liegro, C.M., Di Liegro, I., 2011. Oligodendroglioma cells shed microvesicles which contain TRAIL as well as molecular chaperones and induce cell death in astrocytes. Int. J. Oncol. doi:10.3892/ijo.2011.1160.

Lohmander, L.S., Neame, P.J., Sandy, J.D., 1993. The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis. Arthritis Rheum. 36, 1214–1222.

Mathivanan, S., Ji, H., Simpson, R.J., 2010. Exosomes: extracellular organelles important in intercellular communication. J. Proteomics 73, 1907–1920.

Matthews, R.T., Kelly, G.M., Zerillo, C.A., Gray, G., Tiemeyer, M., Hockfield, S., 2002. Aggrecan glycoforms contribute to the molecular heterogeneity of perineuronal nets. J. Neurosci. 22, 7536–7547.

McRae, P.A., Baranov, E., Sarode, S., Brooks-Kayal, A.R., Porter, B.E., 2010. Aggrecan expression, a component of the inhibitory interneuron perineuronal net, is altered following an early-life seizure. Neurobiol. Dis. 39, 439–448.

Miller, M.C., Manning, H.B., Jain, A., Troeberg, L., Dudhia, J., Essex, D., Sandison, A., Seiki, M., Nanchahal, J., Nagase, H., Itoh, Y., 2009. Membrane type 1 matrix metalloproteinase is a crucial promoter of synovial invasion in human rheumatoid arthritis. Arthritis Rheum. 60, 686–697.

Morawski, M., Brückner, G., Jäger, C., Seeger, G., Arendt, T., 2010. Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease. Neuroscience 169, 1347–1363.

Murphy, G., Nagase, H., 2008. Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? Nat. Clin. Pract. Rheumatol. 4, 128–135.

Nakada, M., Miyamori, H., Kita, D., Takahashi, T., Yamashita, J., Sato, H., Miura, R., Yamaguchi, Y., Okada, Y., 2005. Human glioblastomas overexpress ADAMTS-5 that degrades brevican. Acta Neuropathol. 110, 239–246.

Ospelt, C., Neidhart, M., Gay, R.E., Gay, S., 2004. Synovial activation in rheumatoid arthritis. Front. Biosci. 9, 2323–2334.

Raposo, G., Nijman, H.W., Stoorvogel, W., Liejendekker, R., Harding, C.V., Melief, C.J., Geuze, H.J., 1996. B lymphocytes secrete antigen-presenting vesicles. J. Exp. Med. 183, 1161–1172.

Skog, J., Würdinger, T., van Rijn, S., Meijer, D.H., Gainche, L., Sena-Esteves, M., Curry, W.T.J., Carter, B.S., Krichevsky, A.M., Breakefield, X.O., 2008. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat. Cell Biol. 10, 1470–1476.

Stanton, H., Rogerson, F.M., East, C.J., Golub, S.B., Lawlor, K.E., Meeker, C.T., Little, C.B., Last, K., Farmer, P.J., Campbell, I.K., Fourie, A.M., Fosang, A.J., 2005. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature 437, 648–652.

Stoeck, A., Keller, S., Riedle, S., Sanderson, M.P., Runz, S., Le Naour, F., Gutwein, P., Ludwig, A., Rubinstein, E., Altevogt, P., 2006. A role for exosomes in the constitutive and stimulus-induced ectodomain cleavage of L1 and CD44. Biochem. J. 393, 609–618.

Taraboletti, G., D'Ascenzo, S., Borsotti, P., Giavazzi, R., Pavan, A., Dolo, V., 2002. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. Am. J. Pathol. 160, 673–680.

Théry, C., Zitvogel, L., Amigorena, S., 2002. Exosomes: composition, biogenesis and function. Nat. Rev. Immunol. 2, 569–579.

Troeberg, L., Tanaka, M., Wait, R., Shi, Y.E., Brew, K., Nagase, H., 2002. E. coli expression of TIMP-4 and comparative kinetic studies with TIMP-1 and TIMP-2: insights into the interactions of TIMPs and matrix metalloproteinase 2 (gelatinase A). Biochemistry 41, 15025–15035.

Troeberg, L., Fushimi, K., Khokha, R., Emonard, H., Ghosh, P., Nagase, H., 2008. Calcium pentosan polysulfate is a multifaceted exosite inhibitor of aggrecanases. FASEB J. 22, 3515–3524.

Troeberg, L., Fushimi, K., Scilabra, S.D., Nakamura, H., Dive, V., Thøgersen, I.B., Enghild, J.J., Nagase, H., 2009. The C-terminal domains of ADAMTS-4 and ADAMTS-5 promote association with N-TIMP-3. Matrix Biol. 28, 463–469.

Verjee, L.S., Midwood, K., Davidson, D., Essex, D., Sandison, A., Nanchahal, J., 2009. Myofibroblast distribution in Dupuytren's cords: correlation with digital contracture. J. Hand Surg. Am. 34, 1785–1794.

Yamaguchi, Y., 2000. Lecticans: organizers of the brain extracellular matrix. Cell. Mol. Life Sci. 57, 276–289.

Yamanishi, Y., Boyle, D.L., Clark, M., Maki, R.A., Tortorella, M.D., Arner, E.C., Firestein, G.S., 2002. Expression and regulation of aggrecanase in arthritis: the role of TGF-beta. J. Immunol. 168, 1405–1412.

Zitvogel, L., Regnault, A., Lozier, A., Wolfers, J., Flament, C., Tenza, D., Ricciardi-Castagnoli, P., Raposo, G., Amigorena, S., 1998. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat. Med. 4, 594–600.