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MMPs and ADAMTSs: functional studies

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1. ABSTRACT

Members of the MMP (matrix metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin type I motifs) families of enzymes are capable of cleaving a diverse array of cellular, extracellular and extracellular matrix substrates, including collagens and procollagens, proteoglycans, cytokines and cytokine ligands, chemokines, elastin and von Willebrand factor, thereby modulating tissue structure and function during both health and disease. Physiologically relevant roles attributable to various members of these metalloproteinase families have been discerned from functional studies correlating *in vitro* substrate processing events with catabolic cleavages occurring *in vivo/in situ*, and the consequences thereof. Mechanisms regulating the post-translational activities of MMPs and ADAMTSs can clearly also have an influential impact on cell metabolism and tissue structure/function, and a number of functional studies have addressed the contributions of ancillary (non-catalytic) domains and endogenous inhibitors in this regard. Further revelations and affirmations of proteinase function, in an *in vivo* context, have emanated with the characterization of genetically manipulated animals misexpressing specific MMPs or ADAMTSs (or their substrates). An increased understanding thereby attained for the physiological functions of MMPs and ADAMTSs, and the means by which their activities are controlled, may lead to the realization of rational therapeutic strategies to counteract pathologies associated with aberrant proteolysis of homeostatic tissue macromolecules.

Table 1. Human MMP nomenclature and database identifiers.

Gene Symbol[1]	Other Name(s)	MEROPS ID[2]	Entrez GeneID[3]	GenBank Locus[3]	OMIM[3]
MMP1	interstitial/fibroblast collagenase	M10.001	4312	NM_002421	120353
MMP2	gelatinase A, 72 kDa gelatinase	M10.003	4313	NM_004530	120360
MMP3	stromelysin-1	M10.005	4314	NM_002422	185250
MMP7	matrilysin, PUMP-1	M10.008	4316	NM_002423	178990
MMP8	neutrophil collagenase	M10.002	4317	NM_002424	120355
MMP9	gelatinase B, 92 kDa gelatinase	M10.004	4318	NM_004994	120361
MMP10	stromelysin-2	M10.006	4319	NM_002425	185260
MMP11	stromelysin-3	M10.007	4320	NM_005940	185261
MMP12	macrophage metalloelastase	M10.009	4321	NM_002426	601046
MMP13	collagenase-3	M10.013	4322	NM_002427	600108
MMP14	MT1-MMP	M10.014	4323	NM_004995	600754
MMP15	MT2-MMP	M10.015	4324	NM_002428	602261
MMP16	MT3-MMP	M10.016	4325	NM_005941, NM_022564	602262
MMP17	MT4-MMP	M10.017	4326	NM_016155	602285
MMP19	n.a.	M10.021	4327	NM_002429, NM_022791, NM_022792	601807
MMP20	enamelysin	M10.019	9313	NM_004771	604629
MMP21	n.a.	M10.026	118856	NM_147191	608416
MMP23A, *MMP23B*	CA-MMP	M10.037, M10.022	8511, 8510	NM_004659, NM_006983	603320, 603221
MMP24	MT5-MMP	M10.023	10893	NM_006690	604871
MMP25	MT6-MMP	M10.024	64386	NM_022468, NM_022718	608482
MMP26	matrilysin-2, endometase	M10.029	56547	NM_021801	605470
MMP27	n.a.	M10.027	64066	NM_022122	n.a.
MMP28	epilysin	M10.030	79148	NM_024302, NM_032950	608417

[1](http://www.gene.ucl.ac.uk/nomenclature/); [2](http://merops.sanger.ac.uk/); [3](http://www.ncbi.nlm.nih.gov/); n.a., not assigned.

2. INTRODUCTION

Proteolytic processing of cellular, extracellular and extracellular matrix (ECM) substrates by enzymes from the MMP (matrix metalloproteinase) (1,2) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin type I motifs) (3,4) families of clan MA peptidases (5) impacts the structural and functional properties of a variety of tissues during development, growth, homeostasis and pathology. Molecular analyses and genome sequencing efforts have revealed the identities of 23 separate human MMPs and 19 different human ADAMTSs, notwithstanding variants occurring due to alternative mRNA splicing and/or post-translational processing events (see Tables 1 and 2). Attentive research efforts are thus engaged with the ongoing challenge of continuing to unveil the physiological functions of these proteinases, and determining the mechanisms whereby their activities are regulated. Results from some of the functional studies undertaken to address these issues are reviewed herein, including data from experiments providing validation for the ability of particular MMPs or ADAMTSs to cleave substrate(s) *in vivo/in situ* with coinciding specificity to that discerned *in vitro*, findings from studies designed to elucidate the roles of ancillary (non-catalytic) domains and endogenous inhibitors in the post-translational control of MMP or ADAMTS function, and information on the consequences of modifying MMP or ADAMTS expression and/or activity *in vivo/in situ*. By providing insights into the pertinent ramifications of MMP and ADAMTS actions, and the regulation thereof, functional studies on MMPs and ADAMTSs may help facilitate rational approaches toward moderating proteinase activity for therapeutic benefit.

3. PHYSIOLOGICAL SUBSTRATES IDENTIFIED FOR MMPs

3.1. Collagens

Observations from some of the first functional studies on MMPs were reported in the early 1960s by Gross and colleagues, who described an enzymatic activity in metamorphosing tadpoles which was capable of degrading helical collagen fibrils (6,7). A collagenase cleavage site was subsequently identified for collagen types I, II and III at a specific Gly-Leu/Gly-Ile bond (see Table 3) located three-quarters distant from the N-terminus of the polypeptide chain, which matches the point of initial collagen proteolysis *in vivo* (8,9). In humans, this activity is principally attributed to the actions of the collagenases MMP-1 (“interstitial” or “fibroblast” collagenase) (10), MMP-8 (“neutrophil” collagenase) (11,12) and MMP-13 (collagenase-3) (13). Moreover, it has been reported that *Xenopus* MMP-18 (collagenase-4) can function in this capacity (14), and that MMP-2 (gelatinase A) and MMP-14 (MT1-MMP) are also able to exhibit collagenase

Table 2. Human ADAMTS nomenclature and database identifiers.

Gene Symbol[1]	Other Name(s)	MEROPS ID[2]	Entrez GeneID[3]	GenBank Locus[3]	OMIM[3]
ADAMTS1	METH-1	M12.222	9510	NM_006988	605174
ADAMTS2	procollagen I N-proteinase (pNPI)	M12.301	9509	NM_014244, NM_021599	604539
ADAMTS3	n.a.	M12.220	9508	NM_014243	605011
ADAMTS4	aggrecanase-1	M12.221	9507	NM_005099	603876
ADAMTS5	aggrecanase-2	M12.225	11096	NM_007038	605007
ADAMTS6	n.a.	M12.230	11174	NM_014273	605008
ADAMTS7	n.a.	M12.231	11173	NM_014272	605009
ADAMTS8	METH-2	M12.226	11095	NM_007037	605175
ADAMTS9	n.a.	M12.021	56999	NM_182920, NM_182921, NM_020249	605421
ADAMTS10	n.a.	M12.2235	81794	NM_030957	608990
ADAMTS12	n.a.	M12.237	81792	NM_030955	606184
ADAMTS13	von Willebrand factor-cleaving protease (vWF-cp)	M12.241	11093	NM_139025, NM_139026, NM_139027, NM_139028	604134
ADAMTS14	n.a.	M12.024	140766	NM_139155, NM_080722	607506
ADAMTS15	n.a.	M12.025	170689	NM_139055	607509
ADAMTS16	n.a.	M12.026	170690	NM_139056	607510
ADAMTS17	n.a.	M12.027	170691	NM_139057	607511
ADAMTS18	n.a.	M12.028	170692	NM_199355, NM_139054	607512
ADAMTS19	n.a.	M12.029	171019	NM_133638	607513
ADAMTS20	n.a.	M12.246	80070	NM_025003, NM_175851	n.a.

[1](http://www.gene.ucl.ac.uk/nomenclature/); [2](http://merops.sanger.ac.uk/); [3](http://www.ncbi.nlm.nih.gov/); n.a., not assigned.

Table 3. Physiological cleavage sites (human sequence enumeration, *except as indicated*) identified for MMP substrates

Enzyme(s)	Substrate[1] (Cleavage Site)	Reference(s)
MMP-1,-2,-8,-13,-14	Collagen I alpha1 (Gly^{775}-Ile^{776}); Collagen I alpha2 (Gly^{775}-Leu^{776}); Collagen II alpha1 (Gly^{775}-Leu^{776}); Collagen III alpha1 (Gly^{784}-Ile^{785})	8-13,15-17,20-23
MMP-1,-2,-3,-7,-8,-9,-10,-13,-14,-19,-20	Aggrecan (Asn^{341}-Phe^{342})	32-55
MMP-8,-14	Aggrecan (Glu^{373}-Ala^{374})	36,39
MMP-1,-2,-3,-9,-10	Link Protein (His^{16}-Ile^{17})	60-63
MMP-13	Fibromodulin (Tyr^{63}-Ala^{64}: *bovine*)	71
MMP-1,-2,-3,-7,-9,-12,-14,-17	ProTNF-alpha (Ala^{76}-Val^{77})	75-79
MMP-2,-3,-7,-9,-13	BM-40/SPARC (Glu^{196}-Leu^{197}: *mouse*)	81,82
MMP-2	MCP-3 (Gly^{4}-Ile^{5})	83

[1]Abbreviations used: TNF, tumor necrosis factor; BM-40, basement membrane-40; SPARC, secreted protein, acidic and rich in cysteine; MCP-3, monocyte chemoattractant protein-3.

activity *in vitro* (15-17). Conventionally, MMP-2 (and MMP-9, or gelatinase B) are considered as gelatinases, enzymes which degrade denatured collagens, although both MMP-2 and MMP-9 can degrade native collagens such as types IV and V (18,19). The determination that MMP-2 (although not MMP-9) is able to degrade helical collagen types I and II (15,17) emphasizes the proteolytic potentiality (and capability) of extracellular metalloproteinases. Antithetically, collagenases may also exhibit substrate specificity. Thus, MMP-13 has been shown to preferentially degrade collagen II (20-23), the principal structural collagen of articular cartilage. Expression of MMP-13 is upregulated in cartilage and synovial tissue in association with degenerative joint diseases such as osteoarthritis and rheumatoid arthritis (21,24-29), and collagenase-generated collagen II cleavage products colocalize with MMP-13 expression patterns in osteoarthritic human cartilage (27,30), indicating a major functional role for MMP-13 in cartilage collagenolysis.

3.2. Aggrecan

In situ catabolism of aggrecan, the major proteoglycan component of articular cartilage which is vital

for biomechanical competence of the tissue, results in the separation of diffusible, hydrophilic sulfated glycosaminoglycan (sGAG)-bearing regions from the N-terminal hyaluronan-binding G1 domain (reviewed in (31)). During this process, pernicious cleavage occurs within the "interglobular domain" (IGD) between G1 and G2, a region which is susceptible to digestion by a variety of proteolytic enzymes, including MMPs and ADAMTSs. Numerous MMPs are capable of endoproteolytic cleavage at Asn^{341}-Phe^{342} within the IGD (32-41) (see Table 3), and proteolytic processing at this site also occurs *in vivo*. Thus, aggrecan fragments generated by cleavage at Asn^{341}-Phe^{342} have been purified from, and/or detected in, articular cartilage (33,42-48), growth plate (49), synovial fluid samples (46,50,51), intervertebral disc (44,52) and spinal cord (53). However, while MMP activities appear to contribute significantly to processing of aggrecan *in situ* at sites C-terminal to the IGD (46,54), the involvement of MMPs in aggrecan IGD catabolism occurs as a late/secondary event in cartilage degeneration (45,46,54-56), whereas primary physiological cleavage in the IGD at Glu^{373}-Ala^{374} is mediated by the "aggrecanase" activity of ADAMTS proteinase(s) (see section 4.2.1).

3.3. Link protein

In articular cartilage, link protein (LP) engages in the formation of ternary complexes with aggrecan and hyaluronan to stabilize proteoglycan aggregates in the tissue (57). A truncated isoform of LP (LP3) which is generated *in vivo* in human articular cartilage (58,59) has an N-terminus initiating at Ile^{17}, consistent with scission of the His^{16}-Ile^{17} bond which is cleaved *in vitro* by MMP-1, -2, -3, -9 and -10 (60-63). MMP-mediated cleavage at His^{16}-Ile^{17} does not appear to compromise the ability of LP3 to bind to hyaluronan, however the further fragmentation of LP which occurs in cartilage with aging (59,61) indicates that the molecule can be detrimentally catabolized.

3.4. Elastin

Macrophage infiltration and elastolysis are principal pathological features of chronic obstructive pulmonary disease (COPD) and attendant pulmonary emphysema (64). MMP-12 is a potent elastolytic enzyme which is expressed by alveolar macrophages (65), and while several other MMPs and serine proteases are capable of degrading elastin, MMP-12 knockout mice are dramatically less susceptible to emphysema induced by smoke exposure, and macrophages and lung extracts from these mice display no elastolytic activity (66,67). The utility of transgenic animals to evaluate MMP and ADAMTS functionality *in vivo* is further discussed in section 9.

3.5. Fibromodulin

Fibromodulin (FM) is a small keratan sulfate-substituted proteoglycan with a leucine-rich repeat protein (LRRP) core which binds to collagen types I and II and is believed to be influential in organizing collagen fiber orientation (68-70). *In situ* catabolism of FM in bovine cartilage explant cultures results in removal of the N-terminal tyrosine sulfate-rich region *via* cleavage of the Tyr^{63}-Ala^{64} peptide bond (Tyr^{63}-Thr^{64} in human FM), a site which is cleaved *in vitro* by MMP-13 (but not MMP-2, -8 or -9) (71). Moreover, as assessed by neoepitope-Western immunoblotting, FM fragments generated by cleavage at this site are detectable in extracts of MMP-13-treated bovine articular cartilage (71). Incubation of FM with recombinant (C-terminally truncated) ADAMTS-4 generates a 29 kDa fragment which also results from cleavage at Tyr^{63}-Ala^{64} (72). Fragmentation of FM increases with age in human intervertebral discs (73), although it remains to be determined whether such processing involves cleavage of the human FM Tyr^{63}-Thr^{64} bond by MMP-13 (or ADAMTS-4).

3.6. Cytokines, cytokine ligands and chemokines: proTNF-alpha, BM-40/SPARC and MCP-3

In addition to their actions on ECM substrates, MMPs are also capable of cleaving physiologically relevant peptide bonds in bioregulatory substrates such as tumor necrosis factor-alpha (TNF-alpha), BM-40 ("basement membrane"-40; also termed SPARC and osteonectin) and monocyte chemoattractant protein-3 (MCP-3).

The membrane bound precursor of TNF-alpha (proTNF-alpha) is cleaved specifically at Ala^{76}-Val^{77} to yield the soluble cytokine. Whilst the principal activity responsible for such TNF-alpha 'shedding' is attributed to ADAM-17 ("TNF-alpha converting enzyme", or TACE) (74), nonetheless MMP-1, -2, -3, -7, -9, -12, -14 and -17 all cleave proTNF-alpha substrates at Ala^{76}-Val^{77} *in vitro*, although inhibitor studies suggest that their role in this capacity *in vivo* is likely to be minor (75-79).

BM-40/SPARC, an extracellular calcium- and cytokine-binding protein (80), is cleaved by MMP-2, -3, -7, -9 and -13, resulting in enhanced collagen-binding affinity and potential localization of bound ligands in the ECM (81). BM-40/SPARC fragments generated by MMP-mediated cleavage at Glu^{196}-Leu^{197} have been immunodetected in mouse tissues, although processing at an adjacent site (Leu^{197}-Leu^{198}), by an unidentified proteinase, appears to be more predominant *in vivo* (82).

Using an "exosite scanning"-yeast two-hybrid strategy, McQuibban *et al.* have demonstrated that MMP-2 binds (*via* its C-terminal hemopexin-like domain) and cleaves MCP-3 at Gly^{4}-Ile^{5}, and that MCP-3-MMP-2 complexes and MCP-3 fragments initiating at Ile^{5} are present in human synovial fluid (83). MMP-2-cleaved MCP-3 retains receptor binding ability, and can act as a non-signaling antagonist to dampen inflammation (83). In addition to providing a novel tool for identifying MMP substrates (see also section 5.6), these studies also highlight the potential involvement of proteinase exosites (i.e. non-catalytic substrate binding sites) (84,85), and/or other ancillary (non-catalytic) domains (see section 6) in catalytic specificity/efficiency.

4. PHYSIOLOGICAL SUBSTRATES IDENTIFIED FOR ADAMTSs

4.1. Procollagens

Proteolytic removal of the N- and C-propeptides from fibrillar collagens (i.e. collagens I, II, III, V and XI) is essential for efficient collagen fibril assembly *in vivo*. The

Table 4. Physiological cleavage sites (human sequence enumeration, *except as indicated*) identified for ADAMTS substrates

Enzyme(s)	Substrate (Cleavage Site(s))	Reference(s)
ADAMTS-1	Aggrecan (Glu^{373}-Ala^{374}, Glu^{1545}-Gly^{1546}, Glu^{1919}-Leu^{1920}, Asn^{341}-Phe^{342})	112,113
ADAMTS-1,-4	V1 Versican (Glu^{441}-Ala^{442}); V0 Versican (Glu^{1428}-Ala^{1429})	125
ADAMTS-2	Procollagen I alpha1 (Pro^{161}-Gln^{162}); Procollagen I alpha2 (Ala^{79}-Gln^{80}); Procollagen II alpha1 (Ala^{181}-Gln^{182}); Procollagen III alpha1 (Pro^{148}-Gln^{149})	86-91
ADAMTS-3	Procollagen II alpha1 (Ala^{181}-Gln^{182})	92
ADAMTS-4,-5	Aggrecan (Glu^{373}-Ala^{374}, Glu^{1545}-Gly^{1546}, Glu^{1714}-Gly^{1715}, Glu^{1819}-Ala^{1820}, Glu^{1919}-Leu^{1920})	43-48,51-56,103-111
ADAMTS-4	Aggrecan (Asn^{341}-Phe^{342}); Brevican (Glu^{395}-Ser^{396}: *rat*); V2 Versican (Glu^{405}-Gln^{406})	119-123,128
ADAMTS-8,-15	Aggrecan (Glu^{373}-Ala^{374})	115,116
ADAMTS-9	Aggrecan (Glu^{1819}-Ala^{1820}); V1 Versican (Glu^{441}-Ala^{442})	114
ADAMTS-13	von Willebrand factor (Tyr^{842}-Met^{843})	130-137
ADAMTS-14	Procollagen I alpha1 (Pro^{161}-Gln^{162}); Procollagen I alpha2 (Ala^{79}-Gln^{80})	93

first procollagen N-proteinase identified, ADAMTS-2 (also referred to as pNPI), cleaves the appropriate Pro-Gln/Ala-Gln bond in procollagen type I, II or III chains (see Table 4) which is hydrolyzed during conversion of these procollagens to mature collagens (86-91). More recently, ADAMTS-3 has been identified as a procollagen II N-proteinase which is preferentially expressed, relative to ADAMTS-2, in articular cartilage (92). Additionally, ADAMTS-14, which is highly homologous to ADAMTS-2 and ADAMTS-3, can cleave procollagen I with the appropriate N-proteinase specificity (93).

Substrate conformation is evidently critical for procollagen N-proteinase activity, since cleavage is not observed for denatured or unfolded procollagen type I or II (94,95). The N-propeptide of native procollagen I folds back in a hair-pin configuration and binds to the major triple helical region of the collagen monomer; following N-proteinase cleavage, retention of the N-telopeptide in a hair-pin conformation is essential for appropriate crosslink formation and collagen fibril assembly (96-99). Thus, functional regulation of collagen N-proteinase activity by its substrate can act as part of a 'quality control' process, ensuring optimal collagen fibrillogenesis and organization (100).

It is also worth noting that MMP-3, MMP-9 and MMP-14 are able to hydrolyse the procollagen N-proteinase cleavage site on a recombinant type IIA collagen N-propeptide fusion protein (101), suggesting that these enzymes could perform this function *in vivo*. However, MMP-3, MMP-9 and MMP-14 appear to have lower stringency in this regard, since they (as well as MMP-7 and MMP-13) cleave at several other sites in the collagen II N-propeptide and N-telopeptide regions (101,102).

4.2 Hyaluronan-binding proteoglycans

4.2.1. Aggrecan

As discussed in section 3.2, catabolism of aggrecan in articular cartilage results in the release of sGAG-bearing fragments from the tissue, and involves cleavage within the IGD to separate the hyaluronan-binding G1 domain from the remainder of the molecule. In 1991, Sandy *et al.* reported that prominent cleavage of the IGD Glu^{373}-Ala^{374} bond occurs during catabolism of aggrecan *in situ* in cartilage explants (103), and similar findings were further described by other investigators, including the identification of homologous cleavage sites (see Table 4) processed in the aggrecan chondroitin sulfate-substituted region (104,105). Ensuing investigations demonstrated that aggrecan fragments present in human arthritic synovial fluids manifest a pre-eminent N-terminal sequence initiating at Ala^{374}, resulting from cleavage at Glu^{373}-Ala^{374} by a proteolytic activity referred to as "aggrecanase" (106,107). Subsequent development of monoclonal antibody BC-3, which specifically recognizes the aggrecanase-generated catabolic neoepitope ^{374}ARG*XX*… (37), was influential in facilitating the ultimate identification of two proteinases exhibiting this novel activity, revealed in 1999 by researchers from the DuPont Pharmaceutical Company following the purification (from cartilage) and sequence elucidation of ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) (108,109). To date, these two enzymes appear to be the most potent aggrecanases, although ADAMTS-1, -8, -9 and -15 can also exhibit such activity *in vitro* (110-116) (see Table 4), as can C-terminally truncated constructs of ADAMTS-10, -16 and -18 (117).

In addition to their presence in articular cartilage and synovial fluids (43-48,51,106,107), aggrecanase-generated aggrecan fragments have also been detected in intervertebral disc (44,52), tendon (118) and spinal cord (53), indicating a role for ADAMTS-mediated aggrecanolysis in a variety of extracellular matrices. Furthermore, both ADAMTS-1 and ADAMTS-4 are also capable of (secondarily) cleaving at the aggrecan Asn^{341}-Phe^{342} bond, a site which is typically associated with MMP activity (see section 3.2), suggesting that ADAMTS(s)/aggrecanase(s) *per se* may also be responsible for this cleavage specificity in *vivo/in situ* (113,119).

4.2.2. Brevican and versican

In 1995, Yamada *et al.* (120) described the isolation of a C-terminal fragment of brevican from rat brain generated by cleavage at an "aggrecanase-like" site at Glu^{395}-Ser^{396} (corresponding to Glu^{378}-Ser^{379} in human brevican). The N-terminal fragment generated by this

cleavage, referred to as "BEHAB" (brain-enriched hyaluronan-binding protein), increases the invasiveness of tumor cells growing *in vitro* and *in vivo* (121), suggesting inhibition of 'brevicanase' activity as a potential therapeutic consideration for the treatment of brain tumors. ADAMTS-4 is able to process brevican at the relevant 'brevicanase' cleavage site *in vitro* (122,123), and ADAMTS-4 mRNA expression colocalizes with BEHAB in specific regions of the brain, and in association with loss of synaptic density (124).

Proteolysis of versican can also be elicited by ADAMTS family members. ADAMTS-1 and ADAMTS-4 cleave versican isoforms V1/V0 at Glu^{441}-Ala^{442}/Glu^{1428}-Ala^{1429}, and the N-terminal versican fragments generated by this cleavage (70 kDa and 220 kDa products from V1 and V0 versican, respectively) are present in aorta (125). Generation of the V1 versican N-terminal 70 kDa fragment is increased in a graft repair model exposed to high blood flow, indicating that ADAMTS activity may be regulated by shear stresses (126) (see also section 4.3). Elucidation of the specific V1 versican N-terminal 70 kDa fragment is also increased during cumulus matrix expansion and ovulation (127), indicating a role for ADAMTS proteinase(s) in fertility. ADAMTS-4 can also effect cleavage of brain versican isoform V2 at Glu^{405}-Gln^{406} (128), a site which is processed *in vivo* to generate "GHAP" (glial hyaluronate binding protein), a hylauronan-binding proteoglycan fragment structurally analogous to brevican-derived BEHAB.

4.3. von Willebrand factor

Deficiencies in von Willebrand factor (vWF) cleavage are associated with thrombotic thrombocytopenic purpura (TTP), a disorder wherein unusually large vWF multimers in the plasma agglutinate circulating platelets and lead to microangiopathic hemolytic anemia, thrombocytopenia, neurological and renal dysfunction, cardiac arrhythmias and fever (129). Under normal conditions, vWF multimers undergo limited proteolysis, involving specific cleavage at Tyr^{842}-Met^{843} within the vWF A2 domain by a "vWF-cleaving protease" (vWF-cp) (130,131). In 2001, several independent reports converged to identify ADAMTS-13 as a physiological vWF-cp, and thereby ascertain that inapt ADAMTS-13 activity is a cardinal contributing factor in TTP. Thus, Levy *et al.* (132) utilized a positional cloning approach to link mutations in the ADAMTS-13 gene with congenital TTP, while other independent laboratories purified and sequenced ADAMTS-13 protein from human plasma and described the cDNA sequence and structural features of the enzyme (133-136). Recombinant ADAMTS-13 was further demonstrated to exhibit appropriate vWF-cp activity *in vitro* (137), and it is interesting to note that the efficiency of cleavage by vWF-cp/ADAMTS-13 is markedly enhanced by exposure of vWF to chaotropic agents such as urea or guanidine HCl, or by exposure to shear stress (as may be encountered in the lumen of blood vessels), illustrating the importance of substrate conformation in this process (138-140).

5. EXAMPLES OF OTHER POTENTIAL SUBSTRATES FOR MMPs AND ADAMTSs

Numerous other putative substrates for MMPs and ADAMTSs have been identified based on evidence of proteolytic fragmentation and/or determination of cleavage sites following incubations performed *in vitro* in the presence of purified/recombinant enzymes. Several representative examples of these types of studies are presented in this section to emphasize the value and importance of 'fingerprinting' the specificity (or diversity) of MMP/ADAMTS activities in this way, thereby establishing a foundation for increased understanding of MMP/ADAMTS functionality and for correlating *in vitro* activity with physiological processes. For further reference, comprehensive listings of ECM and bioactive/non-ECM substrates cleaved by MMPs are highlighted in recent reviews, i.e. (2,141-143).

5.1. ECM structural/organizing proteins: COMP, decorin, fibrillin and laminin

Various additional ECM proteins have been identified as potential substrates for MMPs and/or ADAMTSs. For example, COMP (cartilage oligomeric matrix protein), a pentameric glycoprotein resident in cartilage, tendon and ligament, is cleaved *in vitro* by MMPs (MMP-1, -3, -9, -13, -19 and -20) and by ADAMTS-4 (but not ADAMTS-1 or ADAMTS-5), suggesting that these enzymes may contribute to COMP fragmentation, as has been observed in human arthritic synovial fluid samples (41,144,145). COMP can bind to fibronectin and collagen types I, II and IX, and is likely to play a role in ECM organization and structure *in vivo* (146-148), such that its degradation could lead to architectural disintegration in a number of different tissues.

The LRRP proteoglycan decorin (DCN) can be cleaved *in vitro* by several MMPs as well as by ADAMTS-4. Multiple fragments of DCN are observed following incubation with MMP-1, -2, -3, -7 or -9, and a number of the cleavage sites have been mapped (149), whereas DCN cleavage by ADAMTS-4 seems to be less extensive (72). DCN appears to perform a regulatory role in collagen fibrillogenesis, but is also able to bind and potentially sequester transforming growth factor-beta (TGF-beta) in the ECM (150). Incubation with MMP-2, MMP-3 and MMP-7 resulted in the release of TGF-beta from DCN-TGF-beta1 complexes (149), suggesting a mechanism for MMP-mediated growth factor 'mobilization'/ 'activation'.

MMP cleavage sites have also been mapped for the ECM proteins fibrillin-1 and fibrillin-2, and the basement membrane component laminin-5. Digestion of fibrillins by MMP-2, -3, -9, -12, -13 or -14 results in the disruption of fibrillin-rich microfibrils which impart elasticity to connective tissues (151). Fibrillin-1 plays an additional role in regulating TGF-beta activation, through sequestration of latent TGF-beta complexes in the ECM (152), such that its proteolysis (i.e. by MMPs) might also contribute to modulation of growth factor accessibility. MMP-2 (but not MMP-9) is capable of cleaving the laminin-5 gamma2 subunit at Ala^{586}-Leu^{587} (rat sequence

enumeration), and this (and similar proteolysis of laminin-5 by MMP-14) induces migration of breast epithelial cells (153,154), thus implicating a further defined role for specific MMPs in cancer cell invasiveness, and suggesting possible targets for oncology therapies (143,155).

5.2. Pericellular and intracellular substrates: CD44, PAR1, PARP and pericentrin

Closer to the cell surface, MMP-14 can cleave and 'shed' membrane-bound CD44H (156), an isoform of CD44 which is frequently expressed, along with MMP-14 itself, by many migratory cells and invasive cancer cells (157,158). CD44 also acts as a hyaluronan receptor on chondrocytes and other cell types, and can function both in the assembly and organization of pericellular matrices, as well as in the uptake and degradation of hyaluronan (159,160).

The protease-activated receptor (PAR) PAR1 has also recently been identified as a MMP substrate. PARs are tethered-ligand G-protein coupled receptors (GPCRs) which are typically activated by serine proteinases such as thrombin and trypsin (161). MMP-1, however, also cleaves the serine proteinase-susceptible Arg^{41}-Ser^{42} peptide bond located within the extracellular N-terminal region of PAR1, thereby inducing signaling *via* exposure of the cryptic receptor-activating sequence ^{42}SFLLRN and promoting cancer cell invasion and tumorigenesis (162). Of additional interest is the observation that PAR1-deficient mice develop less arthritis severity in an antigen-induced arthritis model than their wild-type counterparts (163), indicating abrogation of PAR1 signalling (by preventing, for example, MMP-mediated cleavage) as a potential therapeutic strategy for treating inflammatory as well as oncological disorders.

Another novel and emergent theme for MMP function is proteolytic competency at intracellular sites. For example, MMP-2 has been localized to the nucleus of cardiac myocytes, and is capable of *in vitro* cleavage of poly (ADP-ribose) polymerase (PARP), an ATP-dependent DNA repair enzyme (164,165). More recently, it has been reported that MMP-14 can accumulate in the centrosomal compartment, and is capable of cleaving the centrosomal protein pericentrin (see also section 5.6), potentially leading to chromosomal instability, malignant transformation and cancer (166).

5.3. Cytokines, growth factors and bioactive peptides: IL-1, CTGF and ET-1

The pro-inflammatory cytokine interleukin-1 (IL-1) is a potent cytoregulatory inducer of MMP expression/activity (167,168). In a countereffective manner, MMP-2 is capable of cleaving the Glu^{25}-Leu^{26} bond of IL-1beta, and degradation of IL-1beta by MMP-2, as well as by MMP-1, MMP-3 and MMP-9, results in inactivation of the cytokine (169). Thus, MMP-mediated 'inhibition' of cytokine activity could act as a control mechanism during, for example, inflammation and tissue injury, which might in turn modulate a 'feedback loop' of cytokine-stimulated MMP expression.

Stimulation of angiogenesis by vascular endothelial growth factor (VEGF) is a key contributing factor in a number of physiological and pathological conditions (170). MMP-1, MMP-3, MMP-7 and MMP-13 (but not MMP-2, MMP-9 or ADAMTS-4) can process VEGF-bound connective tissue growth factor (CTGF) at specific cleavage sites, prevalently Met^{194}-Ile^{195}, to "reactivate" the *in vitro* angiogenic activity of the $VEGF_{165}$ isoform, thereby potentially contributing to the induction/progression of inflammatory diseases, diabetic retinopathy and tumor growth (171).

MMP-2, which is a dominant gelatinase on arterial smooth muscle cells, also actuates the vasoconstrictor activity of endothelin-1 (ET-1) by cleavage of big ET-1 at Gly^{32}-Leu^{33}. The bioactive peptide generated, ET-1[1-32], is more potent than endothelin-converting enzyme (ECE)-generated ET-1[1-21], suggesting alternative therapeutic strategies (i.e. MMP inhibition) for regulating vascular reactivity in pathological conditions such as allergic inflammation (172).

5.4. Proteinase substrates for MMPs: proMMPs and ADAMTSs

A number of reports have documented the ability of active MMPs to cleave proMMPs, thereby contributing to zymogen activation (see section 6.1). For example, MMP-3 cleaves at specific sites within the inhibitory propeptides of proMMP-1, proMMP-8 and proMMP-9, (173-175), and proMMP-2 can be activated by MMP-14 in a TIMP-2-coordinated trimeric complex (176,177). Several other MT-MMPs can also activate proMMP-2, although cellular activation of proMMP-2 by MMP-15 (MT2-MMP) is not TIMP-2-dependent (178). Furthermore, other TIMPs, including TIMP-4, do not support activation of proMMP-2 (179). MMP-14 can also activate proMMP-13 independent of TIMP-2, in a process which requires interaction(s) with the MMP-13 C-terminal domain (180,181).

It has also been shown that MMP-2, MMP-8 and MMP-15 (but not MMP-1, -3, -9, -14, -16 or -17) can cleave ADAMTS-1 *in vitro* to generate fragments similar to those produced *in situ* during recombinant protein expression (which are generated predominantly *via* cleavage at Ala^{717}-Lys^{718} within the spacer domain) (182). Similar C-terminal processing of ADAMTS-4 by MMP-17 (MT4-MMP) has also been observed (183), wherein cleavage occurs at specific sites (Thr^{581}-Phe^{582} and Lys^{694}-Phe^{695}, located in the cysteine-rich and spacer domains, respectively) which are also susceptible to autocatalytic cleavage (184). Such C-terminal truncation can yield ADAMTS isoforms with altered sGAG/ECM-binding properties and modified enzymatic activities (see section 6.2), demonstrating the potential combined involvement of MMPs and ADAMTSs in degradative 'cascades', and suggesting multiple target points in therapeutic strategies therefor.

5.5. Proteinase inhibitor substrates for ADAMTSs: alpha 2-macroglobulin and TIMP-4

Alpha 2-macroglobulin, a general endoproteinase inhibitor, is a substrate for both ADAMTS-4 and ADAMTS-5, and cleavage of the alpha 2-macroglobulin "bait region" by these enzymes causes entrapment and

inactivation of the proteinases (185). Cleavage of alpha 2-macroglobulin by ADAMTS-4 or ADAMTS-5 occurs at Met^{690}-Gly^{691}, and while both intact and fragmented forms of the inhibitor are present in synovial joint fluids (185-187), neoepitope immunoblotting studies did not detect ADAMTS-4- or ADAMTS-5-generated alpha 2-macroglobulin fragments in samples of synovial fluid from osteoarthritic patients (185), although this may reflect rapid clearance of degradation products *via* the vascular/lymphatic systems.

Tissue inhibitors of metalloproteinases (TIMPs) are physiological regulators of metalloproteinase activities (see section 7), binding tightly to MMPs, for example, in a 1:1 stoichiometric fashion (2,188). It is interesting to speculate, therefore, that aggrecanase (ADAMTS) activity in cartilage, which precedes MMP-engendered catabolism (i.e. collagenolysis), might contribute to disruption of MMP inhibition *via* cleavage and inactivation of TIMP(s). Following incubation with either ADAMTS-4 or ADAMTS-5, no significant digestion of TIMP-1, TIMP-2 or TIMP-3 is observed. However, TIMP-4 can be cleaved by ADAMTS-4 (but not ADAMTS-5) in a dose-dependent manner, generating a 20 kDa TIMP-4 degradation product resulting from cleavage at Ala^{192}-Gln^{193} within the C-terminal "loop 6" domain (189). Pre-incubation of TIMP-4 with MMP-2 blocks cleavage by ADAMTS-4, suggesting that cleavage within the TIMP-4 C-terminus could disrupt critical binding interactions with target MMPs.

5.6. Additional strategies for MMP and ADAMTS substrate identification

Finally in this section, it is worth noting several other strategies which can be employed to identify and/or optimize MMP and ADAMTS substrates. For example, synthetic peptide libraries designed around known cleavage sites may be generated, and a positional-scanning approach may be adopted to assess iterative amino acid substitutions, as has been described for MMP-1, -3, -8, -9 and -26 (190-195).

Proteolytic cleavage sites have also been identified for MMP-3, -7, -13 and -14 using substrate phage display (196-198), which capitalizes on the expression of random recombinant peptide libraries and bacteriophage-amplification strategies for extensive (high-throughput) screening. In a recent applied example of this technology, the identification of pericentrin as a potential MMP-14 substrate (see section 5.2) was facilitated *via* determination of probabalistic cleavage motifs based on substrate phage display, and hydrolysis of pericentrin sequence-derived synthetic peptides by MMP-14 at predicted Gly-Leu bonds (166). Cleavage site motifs for MMP-1, -2, -3, -7, -9 and -14 have additionally been determined using mixture-based oriented peptide libraries, which involves pooled sequencing of digested peptide mixtures to identify optimal P' motifs (i.e. amino acids C-terminal to the cleavage site), followed by optimization of P residues (i.e. amino acids N-terminal to the cleavage site) (199). Cleavage of putative substrates may then be assessed after mapping such motifs to authentic protein sequences, as was performed to identify neurocan as a novel substrate processed by MMP-2 (199).

In a more physiological setting, isotope-coded affinity tag labeling has been utilized to identify cleavage products of cell- or ECM-associated proteins generated during culture of cells transfected with MMP-14 (79). In this approach, tryptic peptides from differentially tagged proteins (i.e. derived from cells transfected with a catalytically active *versus* an inactive proteinase contruct) are identified by mass spectrometry to determine the identity of the parent substrate. Using this procedure, Tam *et al.* identified several previously unreported substrates for MMP-14, including interleukin-8 (IL-8), proTNF-alpha, secretory leukocyte protease inhibitor (SLPI), CTGF and death receptor-6 (DR-6) (79). One significant potential advantage of this methodology relative to peptide library-based schemes is the presentation of native substrates to the proteinases under investigation, since substrate structure and/or the presence of proteinase-interacting sequences remote from the actual cleavage site may be critical for hydrolytic susceptibility.

6. REGULATION OF MMP and ADAMTS ACTIVITIES BY ANCILLARY (NON-CATALYTIC) DOMAINS

6.1. MMPs

The post-translational activity of many MMPs can be regulated by retention of the prodomain *via* a "cysteine-switch" mechanism, wherein an unpaired cysteine residue located within the consensus propeptide sequence PRCG*X*PD is coordinated to the active site zinc (200). Stepwise activation of proMMPs involves sequential processing and ultimate removal of the prodomain (*via* intra- and inter-molecular proteolysis), and can be initiated by serine proteinases, MMPs (see section 5.4), chemical agents such as 4-aminophenylmercuric acetate (APMA), N-ethylmaleimide and SDS, and by alterations in temperature or pH (201,202). However, the prodomains of MMP-11 (stromelysin-3), MMP-21, MMP-23 (CA-MMP), MMP-28 (epilysin) and all of the membrane-type MMPs (MMP-14, -15, -16, -17, -24 and -25) end with a furin/proprotein convertase (PC) recognition/cleavage site (Figure 1). Consequently, these MMPs are likely to be secreted as active enzymes (2,203), such that interaction with endogenous inhibitors (i.e. TIMPs; see section 7) could be imperative in regulating their activities.

At their C-terminus, most MMPs have a hemopexin-like domain, structurally configured as a 4-bladed beta-propeller fold (Figure 1), which plays an important role in substrate specificity, binding and subsequent cleavage (2,85). Notably, the hemopexin-like domain of collagenases is particularly required to degrade native helical collagens. Thus, C-terminally depleted or chimeric mutants (generated by hemopexin domain 'swapping' with non-collagenolytic MMPs) of MMP-1, MMP-8 and MMP-13 fail to cleave native collagen; furthermore, a chimera comprising the catalytic domain of MMP-3 and the MMP-1 hemopexin domain is collagenolytically inactive (22,204-208). A major function associated with collagenase hemopexin domains is the unwinding of triple-helical collagen to allow hydrolysis of individual chains. In fact, incubation of type I collagen with

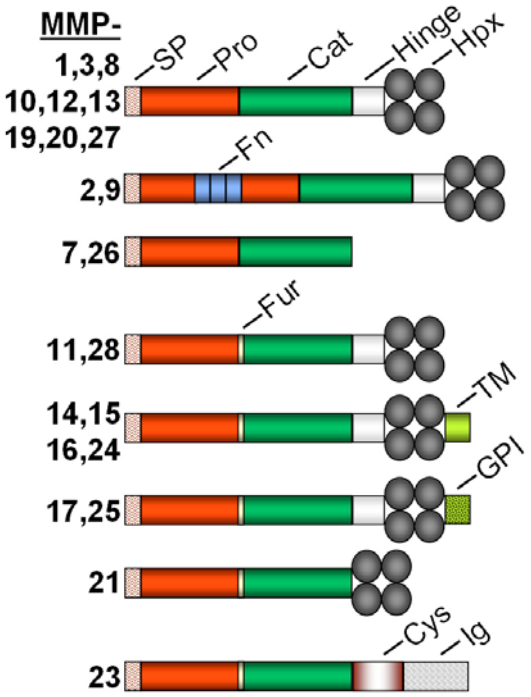

Figure 1. Domain structure and organization of MMPs. SP, signal peptide; Pro, propeptide; Cat, catalytic domain; Hinge, hinge region; Hpx, hemopexin-like domain (4-bladed beta-propeller fold); Fn, fibronectin-like domain (fibronectin type II-like repeats); Fur, furin/PC recognition/cleavage site (consensus sequence R*X*(K/R)R); TM, transmembrane domain; GPI, glycosyl phosphatidylinositol anchor; Cys, cysteine-rich domain; Ig, immunoblobulin-like domain.

a full-length MMP-1 active site mutant can allow non-collagenolytic enzymes to digest the molecule, albeit relatively inefficently, into typical 3/4 and 1/4 fragments (209). Distinctively, however, the collagen binding and triple helicase (unwinding) activity of MMP-2 appears to involve both the hemopexin-like and fibronectin-like domains, with the latter domain also playing a critical role in gelatin-binding and gelatinolysis (17,210).

Of further relevant interest, MMPs can undergo autoproteolytic degradation to separate their catalytic and hemopexin-like domains, thereby modulating their own enzymatic capability (65,211-213). Such processing of MMP-1 and MMP-8, for example, would clearly impact their collagenolytic potential (211,212), and autocatalytic 'shedding' of MMP-14 (involving consecutive cleavages at Gly^{284}-Gly^{285} and Ala^{255}-Ile^{256}) effectively inactivates the enzyme (213), such that autocatalysis may therefore serve as an additional mechanism regulating physiological MMP activity.

6.2. ADAMTSs

Akin to MMPs, ADAMTSs are synthesized with a prodomain that contains an unpaired cysteine, although there is no evidence to date indicating that ADAMTS activities are regulated by a cysteine-switch mechanism. However, ADAMTSs also have a furin/PC recognition sequence located at the C-terminus of their prodomains (see Figure 2), cleavage of which (i.e. in the Golgi) would result in the secretion of mature, potentially active enzymes lacking the propeptide region. Such prodomain processing has been confirmed for ADAMTS-1, -2, -4, -5, -7, -8, -9, -10, -12 and -13 (91,108,109,114,115,182,184,214-221); furthermore, ADAMTS-4 colocalizes with furin in the trans-Golgi, and synthetic furin inhibitors block both ADAMTS-4 prodomain removal and aggrecanase activity (215,218). Furin/PC-processed ADAMTS-4 has also been immunolocalized in growth plate cartilage to areas of active resorption (220). Interestingly, prodomain retention does not appear to affect ADAMTS-13 activity (221), although the ADAMTS-13 prodomain is significantly shorter than that of other family members (41 amino acids *versus* approximately 200 amino acids, respectively). Similarly, however, a C-terminally truncated ADAMTS-7 construct retaining most of the prodomain is catalytically active against alpha 2-macroglobulin (216).

In the extracellular matrix, ADAMTS C-terminal ancillary domains (see Figure 2) perform functions of growth factor sequestration and ECM- and substrate-binding, and can influence enzymatic activity and substrate specificity. Thus, the C-terminus of ADAMTS-1 binds VEGF, preventing interaction of the growth factor with its receptor VEGFR2, thereby defining a mechanism whereby ADAMTS-1 (as well as ADAMTS-8) exerts anti-angiogenic effects (222,223). Deletion of ADAMTS-1, ADAMTS-4, ADAMTS-7 and ADAMTS-9 C-terminal domains can attenuate association of the proteins with ECM or soluble sGAGs (72,114,182-184,215,216,224), and C-terminal truncation also tempers binding and inhibition of ADAMTS-4 by fibronectin (225). In addition, truncated isoforms of ADAMTS-4 lacking portions of the C-terminal cysteine-rich and/or spacer domains exhibit altered substrate specificity and proteolytic efficiency, including enhanced cleavage of aggrecan and V1 versican at Glu^{373}-Ala^{374} and Glu^{441}-Ala^{442}, respectively (72,215), implicating C-terminal truncation as a mechanism for ADAMTS-4 'activation'. Retention of the thrombospondin type I repeat (TSR)-1 domain of ADAMTS-4, however, appears to be requisite for efficient substrate (aggrecan) binding and proteolysis (226). Nevertheless, while aggrecanase activity may be potentiated *via* such enzyme interactions with aggrecan sGAGs (226-228), it is worth noting that sGAG-free recombinant aggrecan substrates are also hydrolyzed (229,230), and that ADAMTS-4 can cleave a short, sGAG-free synthetic peptide substrate *in vitro* (231), although at a substantially higher enzyme:substrate ratio than that sufficient for effective aggrecanolysis of native (glycosylated) substrate (115). For ADAMTS-13, the TSR-1, cysteine-rich and spacer domains are required for efficient binding and cleavage of vWF under static conditions *in vitro* (232-235), however an ADAMTS-13 mutant terminating immediately after TSR-1 (i.e. lacking the cysteine-rich, spacer and C-terminal TSR domains) is hyperactive under flow conditions, indicating that C-terminal domain(s) may negatively regulate physiological ADAMTS-13 activity (234).

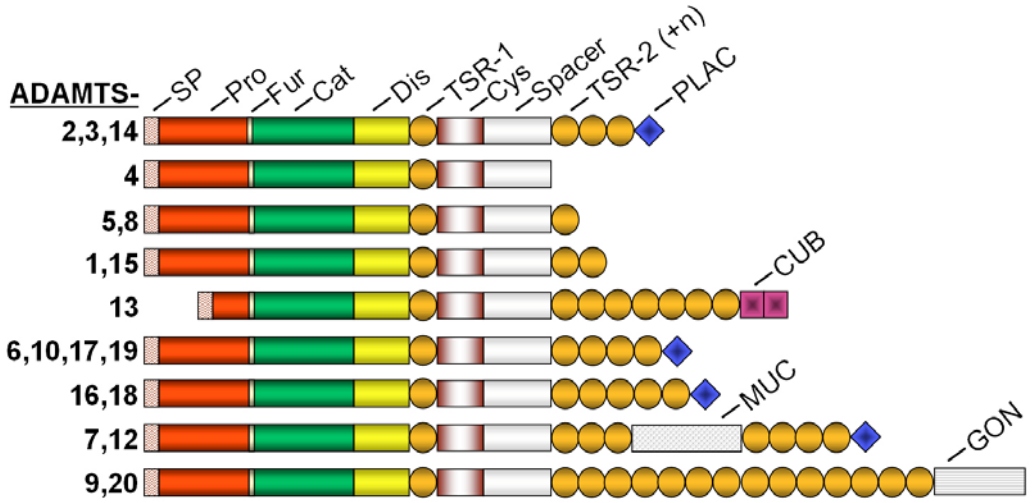

Figure 2. Domain structure and organization of ADAMTSs. SP, signal peptide; Pro, propeptide; Fur, furin/PC recognition/cleavage site (consensus sequence R*X*(K/R)R); Cat, catalytic domain; Cys, cysteine-rich domain; Dis, disintegrin-like domain; TSR, thrombospondin type I repeat; Spacer, spacer domain; PLAC, protease and lacunin domain; CUB, cubulin domain; MUC, mucin-like domain; GON, *Caenorhabditis elegans* GON-1-like domain.

7. REGULATION OF MMP AND ADAMTS ACTIVITIES BY ENDOGENOUS INHIBITORS (TIMPs)

TIMPs (tissue inhibitors of metalloproteinases) are specific inhibitors of MMPs, and readers are directed to recent reviews for comprehensive information on this topic (2,188). All four TIMPs which have been identified (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) form inhibitory complexes with most MMPs, wherein the TIMP N-terminal domain binds the MMP catalytic domain, and the TIMP C-terminal region interacts with the MMP hemopexin-like domain. TIMP-3 exhibits additional (selective) inhibitory activity toward members of the ADAM and ADAMTS families, including ADAM-10, ADAM-12, ADAM-17, ADAMTS-4 and ADAMTS-5 (236-240). In fact, the N-terminal domain of TIMP-3 is a more potent inhibitor of ADAMTS-4 and ADAMTS-5 than of MMP-1, MMP-2 or MMP-3 (239), and is able to inhibit aggrecanase-mediated aggrecan catabolism when added exogenously to cultured cartilage explants (241). Genetic deletion of TIMP-3, which is expressed normally in cartilage (242,243), temporally exacerbates aggrecan (and collagen) degradation in the joint tissues (articular cartilage, menisci) of knockout mice (244), further suggesting that ADAMTSs/aggrecanases may be principal physiological target enzymes of this inhibitor.

8. EFFECTS ELICITED BY SELECTIVELY AUGMENTING MMP OR ADAMTS ACTIVITIES *IN VIVO/IN SITU*

8.1. Cartilage-specific transgenic overexpression of MMP-13

As discussed in section 3.1, MMP-13 appears to be a principal collagenase involved in collagen II proteolysis in cartilage. In order to further evaluate the actions of MMP-13 in the joint, mice expressing a tetracycline-regulated transgene comprising a constitutively active human MMP-13 construct, controlled by a cartilage-specific (collagen type II) promoter, have been generated and characterized (245). The enhanced postnatal MMP-13 activity in the cartilage of these mice results in elevated collagen II and aggrecan degradation, and cartilage erosion similar in pathology to that observed in osteoarthritis. In addition, overexpression of the MMP-13 transgene results in synovial hyperplasia and proliferation, as may also occur in osteoarthritis. Pathology more typical of rheumatoid arthritis (i.e. cellular infiltration, pannus formation), however, was not observed in the MMP-13 overexpressors.

8.2. Macrophage-specific transgenic overexpression of MMP-12

In studies conducted to investigate macrophage involvement in rheumatoid arthritis, Liu *et al.* observed elevated MMP-12 expression levels in synovial tissues and fluids from patients with rheumatoid arthritis relative to those with osteoarthritis (246). In order to further examine the hypothesis that MMP-12 intensifies the progression of rheumatoid arthritis, rabbits expressing a human MMP-12 transgene under the control of a macrophage-specfic promoter were generated and challenged in an inflammatory arthritis model. Disease progression was potentiated in the transgenic rabbits relative to control animals, leading to proteoglycan depletion and articular cartilage destruction, suggesting that inhibition of MMP-12 activity could be therapeutically beneficial in treating inflammatory joint diseases (247,248).

8.3. Exposure of cartilage to exogenous ADAMTS-4 or ADAMTS-5

In addition to promoting the loss of sGAG-bearing aggrecan fragments, stimulation of live cartilage explant cultures with IL-1 or retinoic acid also causes the release of hyaluronan and hyaladherins (aggrecan G1 domain and link proteins) (249-257), and incubation of

either live or freeze-killed articular cartilage with exogenously added recombinant ADAMTS-4 or ADAMTS-5 elicits similar effects (256). Thus, ADAMTS-4 and ADAMTS-5 generate G1-TEGE^{373} (*via* cleavage at Glu^{373}-Ala^{374} in the aggrecan IGD), and the concomitant release of sGAG-bearing aggrecan fragments, together with hyaluronan and functionally competent hyaluronan-binding G1 domains and link proteins, emphasizes a crucial role for aggrecan in maintenance of the physical integrity of the non-collagenous cartilage infrastructure. ADAMTS (and perhaps MMP) activities in cartilage may therefore perturb not only aggrecan content, but also the abundance of aggrecan-tethering molecules (i.e. hyaluronan), thereby potentially hindering replacement of degraded proteoglycan aggregate components by newly synthesized molecules. Aggrecan can also protect the cartilage collagen network from MMP-mediated damage (258), thus advocating further the inhibition of aggrecanase activity as a rational strategy for therapeutic intervention in joint diseases such as osteoarthritis (see also section 9.2).

9. EFFECTS ELICITED BY SELECTIVELY ATTENUATING MMP OR ADAMTS ACTIVITIES *IN VIVO/IN SITU*

9.1. MMP knockout mice

A number of informative studies on various MMP-null/knockout (KO) mice have been described, and these are accentuated in several recent reviews (143,259,260). As discussed in section 3.4, MMP-12 KO mice demonstrate reduced susceptibility in a model of smoke-induced emphysema, and macrophages and lung extracts from these mice display no elastolytic activity (66,67). Based on recent results reported for rabbits overexpressing MMP-12 *in vivo* (247,248), MMP-12 KO animals may also be anticipated to demonstrate refractivity in inflammatory arthritis model(s) (see section 8.2). Notably, MMP-3 KO mice are not protected in models of inflammatory arthritis or osteoarthritis, despite the correlative expression of MMP-3 with arthritis severity which is observed in joint tissues from patients and animals (261,262). Interestingly, however, the same MMP-3 KOs displayed alterations at their neuromuscular junctions, with increased levels of acetylcholine receptors, potentially due to lack of MMP-3-mediated agrin degradation (263).

MMP-14 KO mice exhibit impaired collagen turnover and are characterized by dwarfism, arthritis, soft tissue fibrosis, osteopenia and reduced osteocytogenesis (264,265), while MMP-13 KO mice have recently been shown to exhibit altered endochondral bone and growth plate cartilage development (including expansion of the growth plate hypertrophic zone), as well as increases in trabecular bone mass (266,267). Comparative analyses described by Stickens *et al.* (utilizing MMP-13-null and MMP-9-null mice) revealed MMP-13 as the dominant collagenase in cartilage, but substantiated that other collagenases (i.e. MMP-14) are involved in collagen turnover in tissues such as bone (266). However, trabecular bone formation in MMP-13/MMP-9 double KOs was severely impaired, suggesting that cooperative processing of collagen by MMP-13 and MMP-9 also contributes to long bone development (266) .

In relation to tumorigenesis, metastasis and cancer, KOs for MMP-2, -7, -9, -11 and -19 exhibit a protective phenotype, while MMP-8 KO mice display increased skin tumor frequencies relative to wild-type animals, signifying a paradoxical protective role for this enzyme in cancer pathology (143,155,268,269).

9.2. ADAMTS knockout mice

To date, data from four ADAMTS KO mice have been reported. The phenotypic consequences observed for ADAMTS-1 KO mice are complex, and indicate a role for this protein during growth, in female fertility, and during the development and organization of renal, adipose and adrenal tissues/organs (270-272). Moreover, the articular cartilage of ADAMTS-1 KOs does not exhibit reduced susceptibility to aggrecan catabolism, either in an *in vivo* inflammatory model or under *in vitro* culture conditions ((273), and see below). Mutations in the gene for ADAMTS-2 result in ineffective procollagen processing to elicit Ehlers Danlos syndrome type VII C in humans and dermatosparaxis in cattle (90), and ADAMTS-2 KO mice recapitulate pertinent phenotypic features of these disorders, (i.e. severe skin fragility), as well as exhibiting decreased spermatogenesis and male sterility (274). Cartilage defects were not observed in the ADAMTS-2 KOs, presumably due to efficient procollagen II processing by other procollagen N-proteinase(s) such as ADAMTS-3 (see section 4.1).

More recently, mice with a targeted in-frame genetic depletion of the catalytic domain of either ADAMTS-4 (aggrecanase-1) or ADAMTS-5 (aggrecanase-2) have been generated, and utilized to examine the relative contributions of these enzymes to articular cartilage degradation *in vivo*/*in situ* (275-277). ADAMTS-4 and ADAMTS-5 KOs are viable and fertile, with no observable gross or histologic abnormalities. When challenged in a surgical model of osteoarthritis, no difference in progression or severity of disease was observed for ADAMTS-4 KOs relative to wild-type mice (275), however there was significant diminution of cartilage degradation in ADAMTS-5 KOs (276). Similarly, the articular cartilage of ADAMTS-5 KOs (but not ADAMTS-4 KOs) is protected in a model of inflammatory arthritis (277). Furthermore, when cultured *ex vivo*, articular cartilage from wild-type and ADAMTS-4 KO mice responds to catabolic stimuli by releasing aggrecanse-generated aggrecan fragments, whereas the aggrecan in equivalent ADAMTS-5 KO mouse cartilage cultures is spared (275-277). Collectively, these data identify ADAMTS-5 as a primary candidate for aggrecan degradation leading to cartilage erosion in degenerative joint diseases, and suggest that attenuation of ADAMTS-5 activity (i.e. by pharmacologic inhibition) could be clinically efficacious in the treatment of osteoarthritis/inflammatory arthritis.

9.3. Transgenic mice expressing mutated MMP or ADAMTS substrates

In other approaches designed to further assess the functions of MMPs and ADAMTSs *in vivo*, transgenic mice expressing substrates with mutated, proteinase-

resistant cleavage sites have been studied. Amino acid substitutions at or near the collagenase cleavage site in the collagen I alpha1 chain prevent collagenase-mediated digestion (278), and mice harboring one such mutation in the endogenous ColI alpha1 gene exhibit deficiencies in tissue remodeling, resulting in dermal fibrosis and collagen accumulation in postpartum uteri (279). Such phenotypic changes are associated with aging or collagen-dependent alterations in tissue architecture, although the development of collagenase-resistant mutant mice to adulthood is normal, probably due to sufficient collagen turnover *via* novel cleavage at a Gly-Val bond identified in the collagen I alpha1 N-telopeptide (279).

Mutations at the MMP- and aggrecanase-susceptible sites within the interglobular domain of aggrecan also affect substrate processing *in vivo*/*in situ*. Thus, mutation of the aggrecan sequences ^{342}FFGVG to ^{342}GTRVG, and ^{374}AGRSV to ^{374}NVYSV, block cleavage following Asn^{341} or Glu^{373} by MMPs or aggrecanases, respectively (280). In transgenic mice, such mutations also prevent MMP or aggrecanase cleavage at these sites *in vivo*, but skeletal growth and development of the animals is unimpaired (281-283). Interestingly, however, aggrecan release from the cartilage of aggrecanase-cleavage site mutants, stimulated *in vitro* by IL-1 and retinoic acid, is significantly lower than that observed for wild-type cartilage explants (282), further corroborating the contribution of aggrecanase activity to pathological aggrecan turnover.

10. SUMMATION AND THERAPEUTIC PERSPECTIVE

MMPs and ADAMTSs are manifestly capable of processing and degrading a host of disparate physiological substrates, and the multifaceted versatility evidenced by members of these metalloproteinase families emphasizes their critical roles in governing tissue structure and metabolism. An increased understanding of the mechanisms regulating MMP and ADAMTS functionality also provides important cues to aid in conceptualizing rational strategies for controlling aberrant proteolysis associated with multiple pathologies. Potential targets for therapeutic intervention therefore clearly include not only the primary MMPs or ADAMTSs themselves, but also agents contributing to post-translational activation/modulation of the causative enzymes (i.e. see section 6). To date, safe and effective small molecule-based approaches designed to pharmaceutically inhibit deleterious metalloproteinase activities have not achieved anticipated success, either through lack of clinical efficacy or due to side-effects likely associated with broad-spectrum metalloproteinase inhibition; it may be anticipated, therefore, that by enhancing potency and selectivity to improve targeted inhibition of germane MMPs or ADAMTSs (or their 'activators'), new inhibitors can be developed to deliver on the promise of novel treatments for diseases such as arthritis, cancer and cardiovascular disease (143,155,284-292). Additional strategic insights may also evolve with further cogent analyses of native MMP/ADAMTS inhibitors, such as efforts to selectively target specific enzymes with 'designer' TIMPs (293-296). While considerable progress has been made in increasing our understanding of the functions of MMPs and ADAMTSs, the myriad of physiological processes which may be impacted by the members of these proteinase families remains to be fully disclosed. There is still much to be learned from future functional studies on these influential and intriguing biological effectors and disassemblers.

11. ACKNOWLEDGMENTS

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