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## Profiling of soluble and membrane-bound metalloproteinases

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## **Chapter 1**

### **Metzincin proteinases in health and disease**

#### **General introduction**

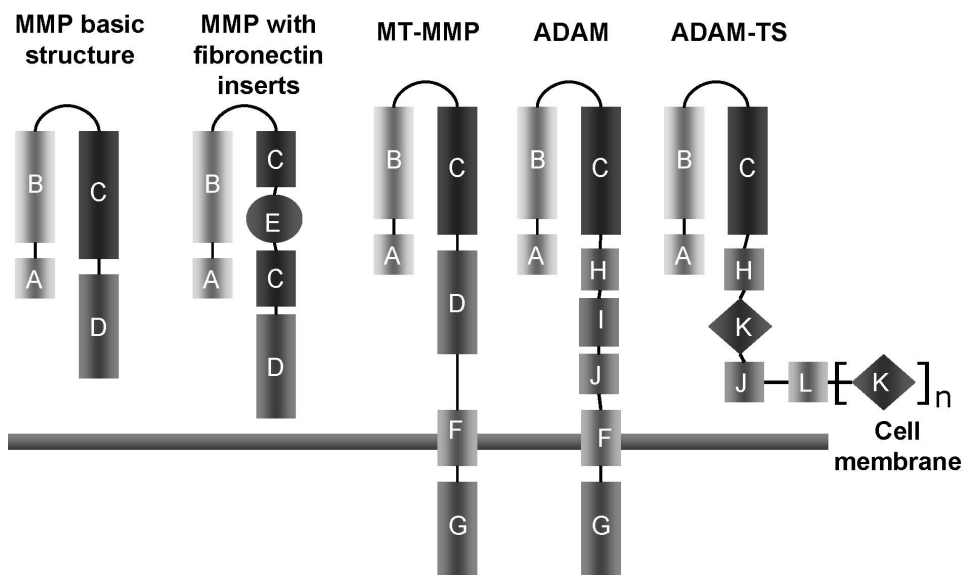
## 1.1 Introduction

Metzincins are a ubiquitously expressed family of multi-domain zinc (II)-dependent endopeptidases<sup>1</sup> whose members include well-known metalloproteases such as the Matrix Metalloproteases (MMPs)<sup>2</sup>, the A Disintegrin And Metalloproteases (ADAMs)<sup>3</sup>, the ADAMs with a thrombospondin motif (ADAMTS)<sup>4</sup>, the bacterial serralysins<sup>5</sup> and proteases such as the astacins (including the meprins)<sup>6,7</sup>. This superfamily of proteases is defined by the presence of a Zn<sup>2+</sup> ion at the catalytic centre which is coordinated by three histidine residues in the zinc binding consensus sequence HExxHxxGxxH that is present in all proteolytically active metzincins, and a characteristic, strictly conserved methionine containing tight 1,4 beta turn forming a hydrophobic cleft for the catalytic zinc ion<sup>8</sup>. Catalysis of protein substrates is (most probably) carried out via a general base mechanism involving activation of a zinc-bound water molecule by the carboxylate group of the conserved glutamate residue in the catalytic pocket followed by attack of water on the polarized carbonyl group in the substrate's scissile bond<sup>9</sup>.

The main physiological function of these proteases lies in the modulation and regulation of extracellular matrix (ECM) turnover by either direct proteolytic degradation of the ECM proteins (e.g. collagen, proteoglycans and fibronectin)<sup>10</sup> or by liberation of biologically active proteins such as cytokines, growth factors and chemokines from their membrane-anchored proforms (so-called shedding).

## 1.2 Matrix metalloproteinases

The largest human subfamily of the metzincins is the matrix metalloproteases or matrixins (see table 1) which consists of 23 distinct proteases in human (24 in mouse). The first MMP identified in 1962 as the protease responsible for the degradation of fibrillar collagen in tadpole tails during metamorphosis was dubbed interstitial collagenase<sup>11</sup>. After identification of a similar collagenase in human skin, this protease was renamed MMP-1. MMPs have since been identified as the major enzymes responsible for turnover of extracellular matrix by proteolytic degradation of virtually all proteinaceous components of the ECM<sup>10</sup>.



**Figure 1**

*Schematic representation of the domain structure of metzincin proteases*

*A: signal peptide; B: prodomain; C: catalytic domain; D: hemopexin-like domain; E: fibronectin type II insert; F: transmembrane domain; G: cytoplasmic tail; H: disintegrin domain; I: cysteine-rich domain; J: EGF-like domain, K: thrombospondin type I-like repeat, L: spacer region.*

MMPs are largely excreted proteins with several conserved domains (see figure 1). All MMPs contain the catalytic domain, which is shielded off in the inactive form of the enzyme by the prodomain. This propeptide interacts with the catalytic region through a conserved cysteine residue and the  $Zn^{2+}$  ion in the catalytic pocket (the so-called cysteine switch)<sup>12,13</sup>. Except for MMP-7, MMP-23 and MMP-26 all MMPs contain a C-terminal hemopexin-like domain which functions primarily as a recognition sequence for the substrate<sup>14</sup>. Although MMPs retain catalytic activity towards a wide range of substrates when missing this domain, the hemopexin domain, which is structured like a four-bladed propeller structure with each blade consisting of 4 antiparallel  $\beta$ -sheets and 1  $\alpha$ -helix, is an absolute necessity for the degradation of triple helical collagens<sup>15</sup>. The gelatinases (MMP-2 and -9) further contain a series of three fibronectin type II inserts in the catalytic domain which facilitate binding of gelatine and collagen<sup>16</sup>.

**Table 1:** Overview of the 23 identified human matrix metalloproteinases and their common names.

MMP	Alternative name	MMP	Alternative name
1	Collagenase-1 Interstitial collagenase	16	Membrane type-3 MMP
2	Gelatinase A 72 kDa type IV collagenase	17	Membrane type-4 MMP
3	Stromelysin-1 Transin-1	19	Human orthologue of <i>Xenopus</i> MMP-18
7	Matrilysin Pump-1	20	Enamelysin
8	Collagenase-2 Neutrophil collagenase	21	Human orthologue of <i>Xenopus</i> xMMP
9	Gelatinase B 92 kDa type IV collagenase	23	Cysteine array MMP Femalysin MMP-22
10	Stromelysin-2	24	Membrane type-5 MMP
11	Stromelysin-3	25	Membrane type-6 MMP Leukolysin
12	Macrophage metallo-elastase	26	Matrilysin-2 / endometase
13	Collagenase-3	27	None
14	Membrane type-1 MMP	28	Epilysin
15	Membrane type-2 MMP		

MMP function is regulated at several levels. Firstly induction of gene expression is controlled by a number growth factors and cytokines, and may be suppressed by transforming growth factor  $\beta$  and glucocorticoids<sup>2,17</sup>. Besides soluble factors, MMP expression may also be regulated by cell-cell contact or interaction of cells with ECM components such as EMMPRIN (extracellular matrix metalloproteinase inducer or CD147)<sup>18</sup>. The expressed MMPs are largely excreted as inactive zymogens with the propeptide effectively limiting entrance into and catalysis of a substrate in the catalytic pocket by blocking the catalytic zinc (II) ion via the cysteine-switch mechanism. Activation of proMMPs can occur through several mechanisms (reviewed in <sup>19</sup>) that all lead to disruption of the cysteine switch. Perhaps the most important mechanism is proteolytic

removal of the prodomain by other endopeptidases such as furin<sup>20</sup>. Removal of the prodomain of MMPs, which contains a furin-like proprotein convertase recognition site (RRKR or RxKR), has been described for 9 MMPs including all membrane-type MMPs. Alternatively the prodomain can be proteolytically removed by plasmin and other serine proteases, or even other MMPs. This mechanism is well described for MMP-2 where the proMMP2 binds the endogenous MMP inhibitor TIMP-2 (tissue inhibitor of metalloproteinases 2). This complex in turn functions as a ligand for the membrane-bound MMP-14 (or membrane-type 1 MMP) leading to activation of MMP-2<sup>21</sup>.

The cysteine switch may also be broken by chemical reactions, either physiologically by oxidation of the cysteine by reactive oxygen species, or artificially by mercury-containing compounds such as 4-aminophenylmercuric acetate (APMA) or denaturing surfactants such as sodium dodecyl sulphate (SDS). This disruption of the thiol-zinc interaction leads to allosteric relocation of the prodomain leading to active forms of the enzyme with the propeptide still attached or to autoproteolytic removal of the relocated prodomain.

MMPs are inhibited by the general protease inhibitor  $\alpha_2$ -macroglobulin and a small family of natural inhibitors specifically geared towards inhibiting metalloprotease activity. These TIMPs are a group of 4 proteins (21-30 kDa in size) that as a group effectively inhibit all MMPs *in vivo*<sup>22</sup>.

Excreted MMPs are generally classified according to their substrate specificity, leading to four classes: the collagenases (MMP-1, -8 and -13), the gelatinases (MMP-2 and -9), the stromelysins (MMP-3, -10 and -11) and a heterogeneous rest group containing matrilysin (MMP-7), metallo-elastase (MMP-12), enamelysin (MMP-20), endometase (MMP-26) and epilysin (MMP-28). In this nomenclature the membrane anchored MMPs (MMP-14, -15, -16, -17, -24 and -25) are considered a separate class. An alternate classification arranges the MMPs according to their domain structure<sup>23</sup>.

### 1.2.1 Collagenases

#### MMP-1

MMP-1 or collagenase-1 was the first described matrix metalloprotease and has sparked a large volume of research on the physiological and pathological role of MMPs. MMP-1 is a secreted enzyme and contains the hemopexin-like domain necessary for degradation of triple helical collagens. ProMMP-1 is probably activated via a two-step proteolytic process involving either mast cell tryptase<sup>24</sup> or urokinase<sup>25</sup> and MMP-3. Mature collagenase-1 can be found as two distinct forms: a major 57 kDa species and a minor, glycosylated 61 kDa form<sup>26</sup>. The active enzyme is inhibited by TIMP-1.

MMP-1 has a wide substrate specificity and is capable of degradation of aggrecan, versican, perlecan, casein, nidogen, serpins and tenascin-C<sup>27</sup>. Since MMP-1 does contain the hemopexin domain, it is one of the MMPs capable of proteolytic cleavage of fibrillar collagen leading to unwinding of the triple-helical structure leaving the collagen (or gelatine) highly susceptible to degradation by other proteases. Proteolysis by MMP-1 has been implicated in release of membrane-anchored proforms of insulin growth-factor binding proteins (IGFBP-3 and -5), IL-1 $\beta$  and L-selectin, amongst others<sup>27</sup>.

Since mice lack a clear orthologue of human MMP-1 definition of the physiological role by creating knock-out mice has not been possible, but transgenic mice which express human MMP-1 are known to develop lung emphysema, indicating a possible role for MMP-1 in development of this disease<sup>28</sup>. Other experiments with transgenic mouse strains have revealed development of skin disorders such as hyperkeratosis, and bone growth retardation<sup>29,30</sup>. Since MMP-1 appears to play a key role in turnover of ECM, deregulation of MMP-1 activity has been hypothesized to be involved in various diseases where excessive or insufficient ECM turnover is involved, such as arthritis<sup>31,32</sup>, cancer<sup>33,34</sup>, wound healing disorders<sup>35</sup>, and fibrotic diseases<sup>36</sup>. Although many studies have found a positive correlation between MMP-1 expression and disease it has proven difficult to clearly identify MMP-1 as a systemic marker of the investigated disease. Experiments with local sampling have proven more accurate, for instance in analysis of MMP-1 in synovial fluid of rheumatoid arthritis patients where MMP-1 level correlated with inflammatory activity<sup>37,38</sup>.

### **MMP-8**

MMP-8 (neutrophil collagenase or collagenase-2) is very similar to MMP-1 in structure and physiological function, although subtle differences in substrate selectivity exist. MMP-8 has a stronger affinity towards type I collagen than MMP-1, while MMP-1 preferentially cleaves type III collagen<sup>39</sup>. Neutrophil collagenase was first described in 1990 when it was cloned from neutrophils obtained from a patient with granulocytic leukaemia<sup>40</sup>. Contrary to MMP-1, MMP-8 is not released immediately after synthesis, but rather stored in specific granules that release the active enzyme upon stimulation<sup>41</sup>. As with MMP-1, MMP-8 can be activated by proteolytic removal of the propeptide by stromelysin-1 (MMP-3), but also by matrilysin (MMP-7)<sup>42</sup>. The mature enzyme is 64 kDa in size, with glycosylation increasing the size to 75 kDa. Autoproteolytic degradation has been described, yielding a 40 kDa fragment, which retains catalytic activity, but does not cleave fibrillar collagen. MMP-8 activity can be inhibited by both endogenous inhibitors TIMP-1 and TIMP-2<sup>43</sup>.

MMP-8 seems to have an important function during embryogenesis and postpartum remodelling of uterine tissue, as demonstrated by expression studies in mice<sup>39</sup>. Collagenase-2 has also been implicated as one of the major contributors to connective tissue turnover in inflammation due to oxidative auto-activation of the enzyme<sup>44</sup>. Experimental evidence has shown that purified MMP-8 is capable of degrading  $\alpha_1$ -proteinase inhibitor which could imply increased MMP-8 activity in the pathology associated with pulmonary emphysema<sup>45</sup>. The role of MMP-8 in cancer is interesting, since recent findings have suggested this MMP to have a protective effect by suppressing tumour metastasis<sup>46,47</sup>, and by aiding therapy by increasing the susceptibility to oncolytic viruses<sup>48</sup>. On the other hand studies have identified MMP-8 as a promoter of ovarian tumour proliferation<sup>49</sup> and high MMP-8 expression seems to be an indicator for poor prognosis<sup>50</sup>. This contradicting evidence of the role of MMP-8 in cancer is an indication of the complex, and so far still poorly understood biological roles of MMPs. MMP-8 is further involved in various inflammatory processes, such as atherosclerosis, where both the expression as well as the protease activity is higher in vulnerable plaques than in stable plaques<sup>51,52</sup>, indicating that MMP-8 may be useful as a marker for plaque instability in atherosclerosis.

### **MMP-13**

MMP-13 (collagenase-3) is the latest human collagenase described in the literature. This enzyme exhibits preference towards cleavage of type II collagen, effectively completing the substrate spectrum of the collagenases. Collagenase-3 was first cloned from breast cancer tissue in 1994<sup>53</sup>. MMP-13 expression can be influenced by a wide range of hormones and cytokines, such as parathyroid hormone (indicative of the important role of MMP-13 in bone development), insulin-like growth factors I and II, platelet derived growth factor, basic fibroblast growth factor, transforming growth factor  $\beta$ 1 (interestingly both up- and downregulates MMP-13 expression depending on the tissue), interleukin-1 and -6, tumour necrosis factor  $\alpha$  and many more<sup>54</sup>. ProMMP-13 can be activated by auto-proteolysis or propeptide removal by various other MMPs like stromelysin-1 (MMP-3), yielding a mature enzyme of 48 kDa which in turn can be inhibited by TIMP-1, -2 and -3<sup>55</sup>. Active MMP-13 is a key factor in the activation pathway of several MMPs. Besides the TIMP route of inactivation, MMP-13 can bind to a specific receptor on the surface of osteoblasts and fibroblasts resulting in internalisation and degradation of the protease<sup>56</sup>.

MMP-13 plays an important role in bone development and remodelling, as may be anticipated from its capability to cleave type II collagen (a major component of cartilage). This rather specific function is reflected in a limited expression profile of MMP-13 during development and adulthood, which is restricted to developing skeletal tissue.

Contrary to the other collagenases, MMP-13 has a relatively high specific activity towards gelatine, indicating that the proteolytic role of MMP-13 expands past the first-step of cleavage of triple-helical collagens. Further identified substrates of MMP-13 include aggrecan and perlecan, TGF $\beta$ , biglycan, the large isoform of tenascin-C, fibrillin-1 and -2, fibrinogen and two serpins ( $\alpha$ 2-antichymotrypsin and plasminogen activator inhibitor-2)<sup>54</sup>.

MMP-13 expression has been regularly described in literature as indicative of various cancerous processes including chondrosarcoma, breast cancer, head and neck tumours and melanoma<sup>57</sup>. In all cases high expression of MMP-13 seems to be related to aggressiveness of the tumour.

Regarding the important role of MMP-13 in bone turnover it is not surprising that this enzyme has been linked to various bone-related diseases. Since MMP-13 degrades both type II collagen and aggrecan, it has been linked to cartilage destruction in rheumatoid and osteoarthritis<sup>58</sup>. The specialized role of MMP-13 in bone development and disease have made it an interesting target for selective MMP-13 inhibitors as therapeutic compounds<sup>59,60</sup>.

### **1.2.2 Gelatinases**

#### **MMP-2**

MMP-2 (gelatinase A, 72 kDa type IV collagenase) is one of the two described human gelatinases in the MMP family, named for their ability to proteolytically degrade gelatine (denatured collagen). MMP-2 is ubiquitously expressed as a 72 kDa zymogen and subject to extensive glycosylation. Expression of MMP-2 is constitutive and most pro-inflammatory stimuli fail to increase the expression level since the gene, in contrast to that



of MMP-9, lacks binding sites for pro-inflammatory transcription factors such as activator protein-1<sup>61</sup>.

The MMP-2 zymogen is activated by the MMP-activation cascade depicted in figure 2. Under favourable stoichiometric conditions TIMP-2 complexes with proMMP-2 and this complex forms a ligand for the membrane-bound membrane type1-MMP which subsequently removes the prodomain of proMMP2 by proteolytic cleavage (aided by free active MMP-2), yielding the truncated 64 kDa active enzyme<sup>62</sup>. If the concentration of TIMP-2 is too high, both MT1-MMP and active MMP-2 will be inhibited, and no further activation will ensue. Besides this activation pathway, proMMP2 can also be activated by thrombin and activated protein C<sup>63</sup>.

MMP-2 differs from other MMPs in the fact that the catalytic domain contains cysteine-rich inserts that resemble the collagen binding regions of the type II repeats in fibronectin. These inserts are required for binding and cleavage of collagen and elastin<sup>64</sup>.

The main function of MMP-2, as with other MMPs, lies in the degradation of extracellular matrix proteins. MMP-2 is capable of cleaving gelatine, type I, IV and V collagens, elastin and vitronectin<sup>65</sup>. Through their ability to degrade collagen in the vascular basal membranes the gelatinases are involved in neovascularization<sup>66</sup> both under physiological conditions and in pathologies such as tumour metastasis. MMP-2 can also facilitate migration of cells by direct degradation of the basement membrane thus allowing infiltration of for instance neutrophils and lymphocytes, or liberation of chemo-attractants<sup>67</sup>. This latter process, named 'ectodomain shedding' is one of the important physiological functions of the membrane-bound ADAM proteases, but has also been described for many other members of the metzincin superfamily. MMP-2 has been known to be involved in both promoting and inhibiting inflammation by liberation of pro-inflammatory mediators (for instance the active form of interleukin-1 $\beta$ <sup>68</sup>), proteolytic degradation of chemoattractants (for instance transforming monocyte chemoattractant protein-3 into the truncated form with antagonistic properties on the CC chemokine receptor<sup>69</sup>) and a profound role in the chemotactic gradient that is necessary in clearance of recruited inflammatory cells from tissue<sup>70</sup>. Recent evidence from proteomics studies show that the role of MMP-2 in processing of signalling proteins may be much greater than originally anticipated which, when confirmed for other MMPs could lead to a paradigm shift in the physiological role of these proteases<sup>71,72</sup>.

Interestingly, MMP-2 knockout mice exhibit a normal phenotype under physiological conditions, although the animals do show different response patterns in allergen challenge which may be attributed to disturbance of the important role of clearing immune cells<sup>70</sup>. These findings indicate that MMP-2 function may be interchangeable with other metalloproteases, a hypothesis that is supported by the observation that expression of the second gelatinase, MMP-9 is greatly increased in MMP-2 null mice<sup>73</sup>.

### **MMP-9**

MMP-9 (gelatinase B, 92 kDa type IV collagenase) was first discovered in neutrophils in 1974<sup>74</sup>. MMP-9 is expressed as a 92 kDa zymogen which can be activated to the 83 kDa mature enzyme. The larger size of MMP-9 relative to MMP-2 can be contributed to a heavily O-glycosylated collagen V-like insert that links the metalloprotease domain to the

hemopexin-like domain<sup>75</sup>. MMP-9 activation may be mediated by removal of the prodomain by serine proteases or other MMPs<sup>76</sup>, or may be a direct response to oxidative stress which disrupts the cysteine switch<sup>77</sup>. As expected, a considerable overlap exists in the substrates degraded by MMP-2 and -9, but MMP-9 is incapable of direct proteolysis of collagen I<sup>65</sup>.

MMP-9 has been described to release the biologically active form of vascular endothelial growth factor (VEGF) which plays an important role in angiogenesis. This process is complemented by the direct proteolytic degradation of the vascular basement membrane proteins, indicating that MMP-9 (even more than MMP-2) may play a crucial role in the formation of new blood vessels<sup>78</sup>. MMP-9 further has an important role in migration of immune cells as demonstrated by the reduced presence of neutrophils, lymphocytes and dendritic cells in bronchoalveolar lavage fluid (BALF) of MMP-9 knockout mice after antigen challenge<sup>79</sup>. MMP-9 null mice show decreased fertility since MMP-9 is crucial in several stages of the female reproductive cycle (implantation of the embryo and the remodelling of endometrial tissue that occurs during the menstrual cycle)<sup>80</sup>. Absence of MMP-9 also leads to disorders in bone development, specifically delayed bone ossification due to insufficient angiogenesis in growth plates<sup>81</sup> and reduced osteoclast recruitment<sup>82</sup>. The biological role of MMP-9 is extensively reviewed in<sup>83</sup>.

As MMP-2, MMP-9 is capable of processing cytokines and chemokines. MMP-9 cleaves interleukin-8 to its more potent truncated form, activates IL-1 $\beta$  and transforming growth factor  $\beta$ <sup>65</sup>. Where MMP-2 is naturally inhibited by TIMP-2, MMP-9 is mostly inhibited by TIMP-1<sup>84</sup>. Contrary to MMP-2 which is expressed ubiquitously under physiological conditions, MMP-9 is only present constitutively in neutrophils<sup>85</sup> where it is stored in granules to be rapidly released after stimulation. Expression in many other cell types is inducible by (inflammatory) stimuli<sup>86</sup>, is increased in malignant cell lines and correlates with the metastatic potential<sup>87</sup>. Neutrophil-derived MMP-9 is distinguishable from other sources since it forms a covalent complex with neutrophil gelatinase B-associated lipocalin (NGAL)<sup>88</sup>.

The role of gelatinases in pathology has been studied extensively, especially in lung diseases (reviewed in<sup>65</sup>) and cancer (reviewed in e.g. <sup>89</sup> and <sup>90</sup>). The amount of both gelatinases in BALF and sputum of patients suffering from chronic asthma is higher than in healthy individuals and this increase is hypothesized to be mainly due to gelatinases originating from eosinophils and epithelial cells<sup>91,92</sup>. This increase may be responsible for the characteristic tissue remodelling events observed in chronic asthma such as thickening of the basement membrane, smooth muscle tissue hypertrophy and reduced epithelial thickness. MMP-2 does not seem to play an important role in the pathophysiology of acute asthma, but MMP-9 and MMP-9/TIMP-1 ratio are increased in exacerbations of acute asthma<sup>79</sup>. This phenomenon may be explained by the presence of a pool of MMP-9 inside the neutrophils that is released during the asthmatic attack. Since neutrophils do not produce TIMP-1, degranulation leads to a strong increase in the local concentration of proteolytically active MMP-9 which may cause several of the symptoms observed in acute asthma such as airway obstruction due to desquamation of epithelial cells and increased mucus production by goblet cells<sup>65</sup>.

The relevance of gelatinases in the pathophysiology of chronic obstructive pulmonary disease (COPD) is not clear, but excess protease activity certainly plays an important role in development and progression of the disease. Sputum and BALF of patients contain high concentration of both MMP-2 and -9, and especially MMP-9 may be an important factor since MMP-9 activity not only causes ECM destruction itself, but has also been described to degrade  $\alpha$ 1-antitrypsin leading to increased activity of neutrophil elastase and cathepsin G<sup>65,93</sup>. MMP-9 is further capable of promoting infiltration of neutrophils (loaded with MMP-9 containing granules) by production of the biologically more active truncated form of IL-8, causing a vicious circle of MMP-9 activity in the diseased lung. Gelatinase presence and activity has been described as elevated in many other pulmonary diseases, such as cystic fibrosis, bronchiectasis, acute respiratory distress syndrome (ARDS) and infectious diseases (reviewed in<sup>65</sup> and<sup>83</sup>).

The obvious relation of gelatinases to tumour metastasis and angiogenesis has led to a plethora of research papers on the role of MMP-2 and -9 in diverse malignant processes. This hypothesis was first affirmed by the observation that MMP-2 knockout mice show decreased tumour angiogenesis and progression<sup>94</sup> and since then MMPs have been identified as important players in angiogenesis, growth and metastasis of tumours.

The development of a new vascular system is necessary for development a tumour, since without new blood vessels the size of a tumour will be restricted. Gelatinases are primarily involved in this process by enabling proteolytic degradation of the vascular basal membrane, opening the way for endothelial migration to the formation of a new vessel<sup>95</sup>. MMP-2 is further capable of cleavage of laminin-5, which after degradation yields a cryptic site that increases endothelial cell migration<sup>95</sup>, and the release of VEGF by MMP-9 stimulates angiogenesis not only under physiological conditions but also in cancer. Tumour growth can be stimulated by gelatinase activity since MMP-2 and -9 have been known to release growth factors<sup>96</sup>. Tumour metastasis is a process that involves release of single tumour cells, migration of these single cells to a vessel and penetration into the blood stream or lymph system and finally adhesion to vessel endothelium and extravasation into the tissue at the metastatic location. The ECM degrading properties of gelatinases are crucial in both exit of the metastatic cells from the bulk tumour as well as entrance into the new seeding site.

Increased gelatinase expression and activity has been described in hundreds of publications describing malignant diseases ranging from breast cancer<sup>97</sup>, urogenital cancers<sup>98-100</sup>, brain tumours<sup>101</sup>, lung cancer<sup>102</sup>, skin cancer<sup>103</sup> and many more. Interestingly many authors have found a positive correlation between gelatinase expression or activity and invasive potential of the tumour involved, again stressing the crucial role MMP-2 and -9 play in metastasis. A comprehensive review of the literature on gelatinase involvement in individual cancers is beyond the scope of this thesis, but excellent reviews are available(e.g.<sup>83</sup>).

Besides pulmonology and oncology gelatinase activity is under investigation in several other research fields. MMP-2 has been identified as a possible target in cardiovascular disease since it was identified as the protease responsible for degradation of the vasodilator peptide adrenomedullin, with one of the resulting fragment peptides having vasoconstrictive properties, possibly leading to hypertension<sup>104</sup>.

### 1.2.3 Stromelysins

#### MMP-3

MMP-3 or stromelysin-1 was the first described in 1985 as a 51 kDa protein secreted by rabbit fibroblasts<sup>105</sup> that was able to degrade casein, and could be distinguished from collagenase by the inability to degrade type I collagen. More or less simultaneously a protease named transin was described in transformed rat cells, which later was identified to correspond to stromelysin<sup>106,107</sup>. Stromelysins have a basic MMP structure, with a hemopexin-like domain. The 51 kDa latent zymogen can be activated by proteolytic removal of the prodomain by for instance the serine proteases trypsin-2<sup>108</sup> and matriptase<sup>109</sup>, yielding a 43 kDa active enzyme in humans. MMP-3 is upregulated by exposure to interleukin 1 $\beta$ , and downregulated by retinoic acid and dexamethasone<sup>110</sup>.

The substrate specificity is broad and MMP-3 has been described to degrade many ECM proteins such as fibronectin, denatured collagens (gelatin), laminin and proteoglycans. MMP-3 is incapable of degrading triple helical collagens, but can cleave the globular portion of type IV collagen<sup>111</sup>. Besides degradation of ECM components MMP-3 is also involved in the activation cascade of the gelatinases and MMP-13 (see figure 2). The physiological function of MMP-3 is, surprisingly, not well described in literature, but is assumed to be mainly in turnover of extracellular matrix. MMP-3 is highly upregulated in mammary tissue during involution after the lactation period and seems to have a proapoptotic effect<sup>112</sup>.

In vitro experiments with cultured cells have identified some membrane-bound signalling proteins that may be released by MMP-3 (e.g. E-cadherin<sup>113</sup> and Fas ligand<sup>114</sup>), but the physiological relevance of these finding is unclear. One well-described substrate of MMP-3 is plasminogen activator inhibitor-1 (PAI-1)<sup>115</sup>, and proMMP-3 can form a complex with tissue-type plasminogen activator which increases the activity of t-PA<sup>116</sup>. These findings may be indicative of an important regulatory function of MMP-3 in the fibrinolytic pathway.

MMP-3 has been described as a factor of importance in development of arthritis<sup>117</sup>, asthma<sup>118</sup>, aneurism<sup>119</sup>, impaired wound healing<sup>120</sup>, Alzheimer's disease<sup>121</sup> and various cancers<sup>122,123</sup>. Recent insights and studies demonstrate that association of MMP-3 with disease states (especially cancer) is difficult, as many newer studies do not find a positive correlation between MMP-3 and the disease. This observation may be (partially) explained by the function of MMP-3 as an activator of other MMPs which makes identification of the protease 'culprit' difficult.

#### MMP-10

The cloning of rat transin/MMP-3 in 1985 quickly lead to the identification of a second stromelysin. This protease, named transin-2<sup>124</sup>, and later identified in humans as stromelysin-2<sup>125</sup> or MMP-10 has 82% sequence homology with MMP-3<sup>126</sup>. MMP-10 is secreted as a 53 kDa zymogen, and is activated to a 47 kDa mature protease. Originally, MMP-10 production was thought to be to be relatively unaffected by stimuli such as

cytokines and hormones<sup>127</sup>, but recent studies have shown results that indicate the contrary<sup>128</sup>.

The physiological function of MMP-10 is poorly understood, with only a handful of publications dealing with characterization of this protease. The in-vitro substrate specificity seems similar to that of MMP-3, but catalytic activity towards type III, IV and V collagens is weaker<sup>129</sup>. Contrary to MMP-3, MMP-10 is not produced by fibroblasts, but is expressed in keratinocytes which in turn do not produce MMP-3<sup>130</sup>. MMP-10 seems to have an important role in skin wound healing and cellular migration, since it is primarily found at the front of the migrating epithelial 'tongue'<sup>131</sup> and has been observed in migrating enterocytes in inflammatory bowel disease<sup>132,133</sup>. MMP-10 is, in vitro, capable of processing laminin-5 which may be an additional mechanism in which the enzyme enables cellular migration<sup>134</sup>.

Stromelysin-2 presence and activity at sites of resorption in developing bone has been demonstrated by histochemistry and casein in-situ zymography, and may play a role in the remodelling events taking place during ossification<sup>135</sup>.

### **MMP-11**

The third human stromelysin is MMP-11, which was first described in 1990 in a breast carcinoma cDNA library<sup>136</sup>. Although MMP-11 is often categorized as a stromelysin, it is very different from the other two proteases in this group. Production of MMP-11 is highest in fibroblasts, and is particularly observed in remodelling tissues at later stages of the process. MMP-11 has been associated with many physiological processes where ECM remodelling occurs, such as during embryonic development, female reproductive cycle and wound healing. The 56 kDa MMP-11 zymogen is activated intracellularly by furin<sup>20</sup> or paired basic amino acid cleaving enzyme-4 (PACE-4)<sup>137</sup> and is secreted as a 47 kDa active protease. The physiological role of MMP-11 is unclear, but differs significantly from other MMPs. No major ECM proteins such as collagens, gelatin and fibronectin can be degraded by MMP-11, and contrary to many other MMPs, exhibits an anti-apoptotic effect. The biological mechanism underlying this effect is not known, but probably involves proteolytic cleavage of yet uncharacterized protein substrates that promote cell survival<sup>138</sup>. Several possible substrates of MMP-11 have been identified, mainly being protease inhibitors such as  $\alpha 1$  proteinase inhibitor and  $\alpha 2$  macroglobulin. MMP-11 further has a weak caseinolytic activity and has been shown to cleave insulin-like growth factor binding protein-1 (IGF-BP-1) in a carcinoma cell line<sup>139</sup>. Recent research has demonstrated that although MMP-11 does not cleave many ECM proteins, degradation of type VI collagen is one of the physiological functions and is related to inhibition of adipogenesis by stromelysin-3<sup>140</sup>.

The relatively narrow and aberrant substrate specificity of MMP-11 probably stems from a mutation that occurred in the highly conserved methionine-turn. Whereas all other MMPs contain an MxP sequence in the met-turn, in MMP-11 the proline is replaced by an alanine. This substitution has a profound effect on the structure of the S'1 selectivity pocket leading to greatly changed substrate specificity<sup>141</sup>.

Although the physiological role of MMP-11 is still poorly understood, the involvement of this enzyme in especially the early stages of the process of tumour formation and metastasis

has been thoroughly investigated (reviewed in e.g. <sup>142</sup>). MMP-11 is rarely present in sarcoma tumours, but almost always expressed in carcinomas. An interesting observation is that the MMP-11 is produced not by the malignant cells themselves, but by the surrounding mesenchymal cells. High levels of MMP-11 have predictive value for tumour aggressiveness and low survival rate. MMP-11 plays a role in early invasion of the surrounding tissue by the tumour cells, a process which is dependent on the catalytic activity of the protease<sup>143</sup>. This is surprising since MMP-11 is not capable of degradation of the major ECM constituents. Cancer cells are able to stimulate nearby fibroblasts to produce MMP-11, a process which is associated with modification of the invaded ECM to a stroma phenotype by desmoplasia<sup>144</sup>. The anti-apoptotic effect of MMP-11 may play a role in establishment of the tumour, and early survival. MMP-11 deficient tumours exhibit higher levels of apoptosis, and implantation of experimental tumours is lower in MMP-11 null mice<sup>145</sup>. Although development of primary tumours is favourably affected by high MMP-11 levels, the metastatic potential of MMP-11 expressing tumours seems to be lower. In experiments with MMP-11 null mice, the number and size of secondary tumours was greater than in wild type mice implanted with similar sized tumours, indicating a protective effect of MMP-11<sup>142</sup>.

#### 1.2.4 Membrane-type MMPs

In addition to the soluble matrix metalloproteinases, a small group of membrane anchored MMPs has been described. The first member of this subfamily, MMP-14 or membrane type-1 MMP (MT1-MMP) was discovered only in 1994<sup>146</sup> and cloning experiments have since revealed the existence of five additional MT-MMPs. The domain structure of MT1-MMP is very similar to that of soluble MMPs (see figure 1) with the characteristic zinc binding catalytic domain, the prodomain in the inactive zymogen form of the protease, and a linked hemopexin-like domain, but most MT-MMPs are membrane-anchored by a single-pass transmembrane domain, and contain an intracellular cytoplasmic tail that contains three putative phosphorylation sites and is presumed to have significance in localization of the enzyme on the cell surface<sup>147</sup>. All MT-MMPs described contain the furin-like recognition site in their prodomain, allowing activation of the zymogen by proteolytic removal of this domain by furin and other proprotein convertases<sup>148</sup>.

##### **MMP-14**

MT1-MMP is present at the cell surface as a 55-60 kDa active protease but may be processed by autocatalysis into a smaller species of 45 kDa by proteolytic removal of the catalytic domain. This truncated form, which still contains the hemopexin-like domain is assumed to play a role in autoregulation of MT1-MMP catalytic activity<sup>149</sup>. The substrate specificity of MT1-MMP is well described in literature (reviewed in <sup>150</sup>). The enzyme is capable of proteolytic degradation of type I, II and III collagens following the characteristic cleavage pathway used by collagenases<sup>151</sup>, a finding that was corroborated by knockout experiments that confirmed the role of MT1-MMP as an important interstitial collagenase. MT1-MMP null mice die within 3 weeks, showing severe developmental abnormalities

related to deficiencies in ECM processing, such as dwarfism, skeletal dysplasia and defective vascularization<sup>152,153</sup>. MT1-MMP may be actually regulated by availability of type I collagen as a substrate, a hypothesis stating that migrating cells are triggered by clustering of cell surface integrins upon encountering a 3-dimensional collagen matrix leading to transcriptional activation of the MT1-MMP promoter<sup>154</sup>. Besides type I collagen, MT1-MMP is capable of degradation of many other ECM components, such as fibronectin, vitronectin, tenascin, nidogen, aggrecan, fibrin, fibrinogen and laminin-5 (leading to a possible stimulation of cellular migration as described earlier)<sup>150</sup>. Although the major proteolytic function of MT1-MMP lies in cleavage of extracellular substrates, some studies indicate a role in intracellular proteolysis after incorporation and accumulation of active MT1-MMP in the centrosomal compartment where it could contribute to development of mitotic spindle changes by degradation of pericentrin<sup>155,156</sup>. This mechanism could give rise to a role for MT1-MMP in malignant transformation of cells. MT1-MMP has further been identified as a cell-surface sheddase, and is capable of cleavage of many membrane-anchored proteins such as E- and N cadherin, integrins, hyaluronan receptor CD44, receptor activator of NF- $\kappa$ B ligand (RANKL) and several cell-surface proteoglycans and their receptors<sup>150</sup>.

MT1-MMP was originally identified as the extracellular protease responsible for activation of proMMP-2, and this process remains the best described proteolytic function of the enzyme. In this process one of the subunits of an MT1-MMP dimer forms a trimeric complex with proMMP-2 and TIMP-2 at the cell surface leading to proteolytic removal of the propeptide of the MMP-2 zymogen by the 'free' MT1-MMP unit. Besides proMMP-2, also proMMP-13<sup>157</sup> and proMMP-8<sup>158</sup> have been identified as possible targets for activation by this mechanism, which could be complementary to the intrinsic collagenolytic activity of MT1-MMP.

The role of MT1-MMP in cancer is manifold, but the involvement in angiogenesis is well described and demonstrated by the insufficient vascularization in knockout mice. Firstly MT1-MMP is capable of degradation of the deposited fibrin matrix after vascular injury, effectively disrupting the repair mechanism and allowing endothelial invasion<sup>159</sup>. MT1-MMP may also be involved in migration of the endothelial cells into the ECM, by proteolytic degradation of the extracellular matrix proteins, and by processing of various adhesion molecules<sup>160</sup>. The formation and stabilization of the newly formed capillary tubes may also be dependent on MT1-MMP activity, as demonstrated by impaired capillary formation in knockout models and RNA interference experiments<sup>161</sup>. Finally, MT1-MMP is able to release vascular endothelial growth factor A (VEGF-A) by shedding, again promoting neovascularization<sup>162</sup>.

### **MMP-15**

MT2-MMP (MMP-15) was first described in 1995 as the second member of the membrane-anchored MMP subfamily<sup>163</sup>. MT2-MMP is an ubiquitously expressed enzyme with largely overlapping substrate specificity with MT1-MMP<sup>151</sup>. Although the physiological function of this protease is not as well described as for MT1-MMP, some studies have indicated a role in follicle rupture during ovulation<sup>164</sup>, generation of tubular structures during

angiogenesis<sup>165</sup> and has anti-apoptotic properties<sup>166</sup>. MT2-MMP is also capable of activating the MMP-2 zymogen, but contrary to MT1-MMP the activation mechanism is not dependent on the presence of TIMP-2, but rather on interaction with the hemopexin-like domain of MMP-2<sup>167</sup>.

Involvement of MT2-MMP in pathology is still unclear, but considering the similarity with MT1-MMP a role in cancer is expected. Indeed, MT2-MMP is present in many investigated tumours, such as glioblastoma<sup>168</sup>, non-small cell lung carcinoma<sup>169</sup>, ovarian<sup>170</sup> and breast carcinoma<sup>171</sup>, and seems to correlate to tumour invasiveness<sup>168</sup>.

#### **MMP-16 and MMP-24**

The two latest additions to the membrane-spanning MMP family are MT3-MMP (MMP-16), first described in 1997<sup>172</sup> and MT5-MMP (MMP-24), first described in 1999<sup>173</sup>. These enzymes are still poorly described in literature. The crystal structure of MT3-MMP has been elucidated, and shows extensive homology to MT1-MMP<sup>174</sup>. MT3-MMP activity is regulated by an autoproteolytic shedding process where a soluble form of the enzyme is released from the cell surface, and the active enzyme shows a high affinity to TIMP-3, as opposed to TIMP-1. Although originally anticipated to be a brain-specific enzyme<sup>175</sup>, MT5-MMP is possibly involved in remodelling events in endometrial lesions, and endometriosis<sup>176</sup>.

Like the other MT-MMPs, both MT3- and MT5-MMP are capable of activating proMMP-2<sup>177,178</sup>.

#### **MMP-17 and MMP-25**

The final two MT-MMPs are structurally different from the other four with respect to their interaction with the cell membrane. MT4-MMP (MMP-17)<sup>179</sup> and MT6-MMP (MMP-25)<sup>180</sup> are linked to the cell membrane via a glycosyl-phosphatidyl-inositol (GPI) anchor, as opposed to containing a membrane-spanning domain. This anchor moiety is linked to the hemopexin-like domain by a 35-45 amino acid long hydrophilic linker, or stem. After production of the enzyme this stem region is linked to a short hydrophobic tail, which is exchanged for a GPI anchor in the endoplasmatic reticulum<sup>181</sup>. The stem region further contains 2 or 3 cysteine residues, which probably have a function in formation of complexes, as demonstrated by the presence of ~120 kDa and ~180 kDa isoforms of MT6-MMP that are dissociated in the mature 57 kDa form of the enzyme under reducing conditions<sup>182</sup>. The GPI anchor gives the possibility of interaction of these proteases with lipid raft microstructures, and the possibility of internalization and recycling of the enzymes<sup>181</sup>. The TIMP-inhibition profile of the GPI anchored MT-MMPs is different than for the membrane-anchored MT-MMPs. While the latter are relatively resistant to inhibition by TIMP-1 due to incompatibility of the Thr<sub>98</sub> residue with the S'1 selectivity pocket, MT4- and MT6-MMP are effectively inhibited by TIMP-1, as well as by TIMP-2 and TIMP-3<sup>183,184</sup>. GPI anchored MT-MMPs can be shed from various cells in exosomes, possibly leading to paracrine transfer to other cells<sup>185</sup>.

Both MT4- and MT6-MMP are capable of degrading ECM protein, albeit MT4-MMP in a limited fashion with cleavage demonstrated for gelatin, fibrin and fibrinogen, while MT6-



MMP can cleave a wider range of ECM constituents including fibronectin, type IV collagen and proteoglycans<sup>186</sup>. In vitro experiments have revealed a multitude of possible substrates (including TNF alpha, indicating sheddase activity), but the physiological relevance is unclear (reviewed in<sup>181</sup>). Interestingly, MT4-MMP is not able to activate proMMP-2 even in vitro, making this the only MT-MMP that lacks this trait<sup>187</sup>. MT6-MMP does activate proMMP-2, but generates a different form of the active enzyme than the other MT-MMPs, indicating that the interaction between MMP-2 and MT6-MMP is unique<sup>188</sup>. This activation mechanism is possibly dependent on the tight junction protein claudin-5, since cells that do not produce this protein are incapable of proMMP-2 activation by MT6-MMP<sup>189</sup>. MT4-MMP has been described as an activator of the aggrecanase ADAMTS-4<sup>190</sup>. Both GPI-anchored MT-MMPs are highly expressed in a wide variety of cancer cells ranging from breast carcinoma to glioma and colon cancers (reviewed in<sup>181</sup>), but the clinical relevance of the presence of these proteins in malignant cells is not yet clear.

### 1.2.5 Matrilysins

#### **MMP-7**

MMP-7 (matrilysin) was originally described as PUMP-1 (putative uterine metalloproteinase-1) in 1988, and was long considered a third member of the stromelysin family (MMP-11 was not known yet), although it appeared only distantly related to the other stromelysins<sup>125</sup>. The pump-1 gene identified from rat tumour cDNA cloning experiments was confirmed to code for a secreted metalloprotease in rat uterus<sup>191</sup>, and later dubbed MMP-7. The mmp-7 gene contains a AP-1 promoter region, leaving it sensitive to upregulation by cytokines and growth factors<sup>192</sup>. MMP-7 is the smallest human MMP (28 kDa zymogen, 19 kDa mature active form) since it lacks the C-terminal hemopexin-like domain. This 'minimal domain structure' means the activated enzyme is comprised of only the zinc-binding catalytic domain and results in an inability of MMP-7 to degrade intact collagens, again demonstrating the importance of the hemopexin-like domain in substrate recognition<sup>193</sup>. MMP-7 is however capable of degradation a wide array of other ECM components such as gelatin, fibronectin, laminin and elastin. MMP-7 is capable of cleaving the prodomain of the gelatinases MMP-2 and -9<sup>194</sup>, but the relevance under physiological conditions seems debatable considering the alternative, well-described activation pathway of proMMP-2. Finally, MMP-7 is a possible sheddase, with potential to liberate TNF $\alpha$ , Fas ligand, heparin binding epidermal growth factor (HB-EGF), E-cadherin and  $\beta$ 4-integrin<sup>195</sup>. The original biological function of MMP-7 was the involvement in involution of the endometrium after pregnancy, but as later discovered, an MMP-unique function of MMP-7 seems to be the role in innate immunity. MMP-7 knockout mice exhibit decreased resistance to bacterial gastrointestinal infection, and show decreased clearance of *E. coli* from the small intestine. MMP-7 is constitutively produced in the mucosal epithelium, and may exert its function in mice by cleavage of pro- $\alpha$ -defensins yielding peptides with antibacterial properties. Co-localization of MMP-7 and pro- $\alpha$ -defensins in specialized epithelial Paneth cells seems to confirm this function<sup>196</sup>. The role in mucosal host defense is further confirmed by the finding that exposure of cultured mucosal epithelial tissue or cells

to pathogenic bacteria such as *E. coli* or *Pseudomonas aeruginosa* causes an increased production of MMP-7, and that germ-free bred mice show no expression of MMP-7 in the unchallenged gastro-intestinal tract<sup>197</sup>. The strong induction of MMP-7 by bacterial challenge is an epithelium-specialized function, since it does not occur in other cell types expressing MMP-7, and is exclusive for MMP-7 since no other MMPs are upregulated<sup>198</sup>.

MMP-7 seems to be important in wound repair, since MMP-7 null mice show severe defects in epithelial wound healing which is probably due to a disrupted re-epithelialization<sup>199</sup>.

MMP-7 further enables migration of neutrophils through the epithelium during inflammation, since in MMP-7 null mice show accumulation of neutrophils in the interstitium without crossing of these cells over the epithelium, resulting in reduced mortality<sup>200</sup>. The mechanism underlying this effect is probably the creation of a chemotactic gradient by cleavage of the proteoglycan syndecan-1 at the epithelial cell surface, causing liberation of the syndecan-bound chemokine KC that is produced by the epithelial cells after injury.

The role of MMP-7 in cancer is well described in literature. MMP-7 seems to occupy a unique place in the MMP-cancer association, since it is one of the few MMPs that is actually produced by cancerous cells themselves, as opposed to the stroma under stimulation of the malignant cells<sup>195</sup>. As with other MMPs, MMP-7 has been identified in a wide range of tumours, and correlates with the aggressiveness of the tumour. In a malignant state, the activation of MMP-2 and -9 by MMP-7 may have an important function, and lead to increased invasiveness of the tumour<sup>201,202</sup>. MMP-7, like MMP-3 may further promote tumour invasion by shedding of E-cadherin, leading to decreased cellular adhesion. Tumour growth is likely associated with MMP-7 sheddase activity, since the release of soluble HB-EGF by MMP-7 promotes cellular proliferation<sup>203</sup>. MMP-7 is further capable of cleaving all six members of the insuline-like growth factor binding protein (IGF-BP) family, leading to increased availability of free IGF which again promotes cancer cell growth and survival<sup>204</sup>. The activation of ADAM-28 is attributed to MMP-7<sup>205</sup>, leading to increased degradation of IGF-BP3.

### **MMP-26**

Recently, a novel matrilysin-like enzyme was identified in an endometrial tumour and named MMP-26 (matrilysin-2, endometase)<sup>206,207</sup>. Like MMP-7, this is a minimal domain MMP missing the hemopexin-like C-terminus. This protease seems to have a more limited substrate specificity compared to MMP-7, not being able to degrade collagens, laminin and elastin<sup>208</sup>. MMP-26 is unique from other MMPs since it is the only MMP described so far that does not have a functional cysteine switch mechanism keeping the pro-enzyme in its latent conformation. This functional loss of the cysteine switch is attributed to the presence of a histidine residue n-terminal of the cysteine, a feature only observed in MMP-26<sup>209</sup> [marchenko 2001]. Basal expression of MMP-26 is low except in endometrium, but is increased in many carcinoma cell lines<sup>210</sup>. The physiological function of MMP-26 remains to be elucidated to date.

### 1.2.6 Macrophage metalloelastase

MMP-12 was first described in 1981<sup>211</sup> as murine metalloelastase and later identified as a member of the MMP family<sup>212</sup>. In 1993 an orthologue in human was found<sup>213</sup>. MMP-12 was identified as an elastolytic metalloproteinase produced by alveolar macrophages, which lead to the trivial name (murine) macrophage metalloelastase (MME) or human macrophage elastase (HME). MMP-12 is expressed as a 54 kDa inactive zymogen, and is activated to a 45 kDa active enzyme by removal of the propeptide sequence. This mature enzyme can be further truncated to a 22 kDa active form involving processing of the C-terminal sequence mediated by serine protease or autocatalytic cleavage<sup>213</sup>. This autolytic removal of the c-terminal domain is possible in many other MMPs, but occurs very slow in contrast to MMP-12 which is readily processed to the smaller form. Expression of MMP-12 is limited to macrophages, and is not observed in blood monocytes. As the name reveals, a major substrate for MMP-12 is elastin, but MMP-12 is capable of degrading other ECM constituents (but not gelatin)<sup>214</sup> and many non-matrix proteins *in vitro*<sup>215</sup>.

MMP-12 null mice show normal development in absence of inflammatory stress, but litter size is smaller, presumably due to placenta abnormalities during gestation. Macrophages obtained from knockout mice retain only a small fraction of their elastolytic activity, indicating that MMP-12 is indeed the most important elastin degrading enzyme (in mice). MMP-12 is a vital factor in penetration of macrophages through the basement membrane, as demonstrated by a complete inhibition of this migration in macrophages from MMP-12 null mice both *in vitro* as *in vivo*<sup>216</sup>.

MMP-12 seems to play an interesting role in cancer which is different from other MMPs. MMP-12 is the primary protease responsible for proteolytic liberation of angiostatin from plasminogen. Angiostatin is a 38 kDa protein with anti-angiogenic properties by the selective inhibition of endothelial proliferation. MMP-2 and -9 are also capable of degradation of plasminogen *in vitro*, but were found to have a minor to non-existing contribution in the production of angiostatin in an animal model<sup>217,218</sup>. This is another example of a protective effect of MMP activity in tumorigenesis, and is an indication that knowledge of the actual biochemical mechanism of the involvement of the protease is indispensable and cannot be replaced by mere association of expression levels in tumours.

Since human MMP-12 was first cloned from alveolar macrophages it is not surprising that the role of MMP-12 in lung diseases has been extensively studied. The hypothetical involvement of MMP-12 in fibrotic processes in the lung is obvious, but studies concerning the role of MMP-12 in emphysema are diffuse. Some authors find no upregulation of MMP-12 in macrophages of patients compared to control groups, but rather of other macrophage-derived MMPs<sup>219</sup>, while others state that macrophage elastase is an absolute requirement for the development of emphysema after cigarette smoke exposure in a knockout animal model<sup>220-222</sup>. The interspecies difference may have caused bias with respect to the importance of MMP-12 since, although the failure of MMP-12 null mice to develop emphysema makes a more compelling point than determination of expression levels, MMP-12 seems to be the major MMP present in murine macrophages, while in human several other MMPs are produced<sup>198</sup>. A polymorphism in the MMP-12 gene has

been described to have predictive value in lung function decline in COPD<sup>223</sup>. Elevated MMP-12 has been described in induced sputum of COPD patients<sup>224</sup>, but a recent study has found a slight increase of MMP-12 only in stage 0 of the disease<sup>225</sup>, which could imply involvement of MMP-12 in the early development of COPD. Proteolytic fragments of elastin have been implied as chemotactic factors in macrophage recruitment<sup>226</sup>, giving a possible explanation of an early role of MMP-12, with the severe tissue destruction at later stages being mainly caused by other macrophage-derived proteases.

## References

1. Stocker,W. & Bode,W. Structural features of a superfamily of zinc-endopeptidases: the metzincins. *Curr. Opin. Struct. Biol.* **5**, 383-390 (1995).
2. Nagase,H. & Woessner,J.F., Jr. Matrix metalloproteinases. *J. Biol. Chem.* **274**, 21491-21494 (1999).
3. White,J.M. ADAMs: modulators of cell-cell and cell-matrix interactions. *Current Opinion in Cell Biology* **15**, 598-606 (2003).
4. Tang,B.L. ADAMTS: a novel family of extracellular matrix proteases. *Int. J. Biochem. Cell Biol.* **33**, 33-44 (2001).
5. Nakahama,K. *et al.* Cloning and sequencing of Serratia protease gene. *Nucleic Acids Res.* **14**, 5843-5855 (1986).
6. Bode,W., Gomis-Ruth,F.X., Huber,R., Zwilling,R. & Stocker,W. Structure of astacin and implications for activation of astacins and zinc-ligation of collagenases. *Nature* **358**, 164-167 (1992).
7. Butler,P.E., McKay,M.J. & Bond,J.S. Characterization of meprin, a membrane-bound metalloendopeptidase from mouse kidney. *Biochem. J.* **241**, 229-235 (1987).
8. Bode,W., Gomis-Ruth,F.X. & Stockler,W. Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'. *FEBS Lett.* **331**, 134-140 (1993).
9. Browner,M.F., Smith,W.W. & Castelhana,A.L. Matrilysin-inhibitor complexes: common themes among metalloproteases. *Biochemistry* **34**, 6602-6610 (1995).
10. Woessner,J.F., Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.* **5**, 2145-2154 (1991).
11. Gross,J. & Lapiere,C.M. Collagenolytic activity in amphibian tissues: a tissue culture assay. *Proc. Natl. Acad. Sci. U. S. A* **48**, 1014-1022 (1962).
12. Van Wart,H.E. & Birkedal-Hansen,H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl. Acad. Sci. U. S. A* **87**, 5578-5582 (1990).
13. Nagase,H. Activation mechanisms of matrix metalloproteinases. *Biol. Chem.* **378**, 151-160 (1997).
14. Murphy,G. & Knauper,V. Relating matrix metalloproteinase structure to function: why the "hemopexin" domain? *Matrix Biol.* **15**, 511-518 (1997).
15. Bode,W. A helping hand for collagenases: the haemopexin-like domain. *Structure.* **3**, 527-530 (1995).
16. Bode,W. *et al.* Structural properties of matrix metalloproteinases. *Cell Mol. Life Sci.* **55**, 639-652 (1999).
17. Vincenti,M.P. The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specific expression. *Methods Mol. Biol.* **151**, 121-148 (2001).
18. Biswas,C. *et al.* The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Res.* **55**, 434-439 (1995).
19. Ra,H.J. & Parks,W.C. Control of matrix metalloproteinase catalytic activity. *Matrix Biol.* **26**, 587-596 (2007).
20. Pei,D. & Weiss,S.J. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature* **375**, 244-247 (1995).
21. Cao,J., Rehemtulla,A., Bahou,W. & Zucker,S. Membrane type matrix metalloproteinase 1 activates pro-gelatinase A without furin cleavage of the N-terminal domain. *J. Biol. Chem.* **271**, 30174-30180 (1996).
22. Gomez,D.E., Alonso,D.F., Yoshiji,H. & Thorgeirsson,U.P. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur. J. Cell Biol.* **74**, 111-122 (1997).
23. Sternlicht,M.D. & Werb,Z. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.* **17**, 463-516 (2001).

24. Gruber,B.L. *et al.* Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J. Clin. Invest* **84**, 1657-1662 (1989).
25. Suzuki,K., Enghild,J.J., Morodomi,T., Salvesen,G. & Nagase,H. Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry* **29**, 10261-10270 (1990).
26. Pardo,A. & Selman,M. MMP-1: the elder of the family. *Int. J. Biochem. Cell Biol.* **37**, 283-288 (2005).
27. McCawley,L.J. & Matrisian,L.M. Matrix metalloproteinases: they're not just for matrix anymore! *Curr. Opin. Cell Biol.* **13**, 534-540 (2001).
28. D'Armiento,J., Dalal,S.S., Okada,Y., Berg,R.A. & Chada,K. Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell* **71**, 955-961 (1992).
29. D'Armiento,J. *et al.* Collagenase expression in transgenic mouse skin causes hyperkeratosis and acanthosis and increases susceptibility to tumorigenesis. *Mol. Cell Biol.* **15**, 5732-5739 (1995).
30. Imai,K. *et al.* Bone growth retardation in mouse embryos expressing human collagenase 1. *Am. J. Physiol Cell Physiol* **293**, C1209-C1215 (2007).
31. Vincenti,M.P. & Brinckerhoff,C.E. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res.* **4**, 157-164 (2002).
32. Vincenti,M.P., Clark,I.M. & Brinckerhoff,C.E. Using inhibitors of metalloproteinases to treat arthritis. Easier said than done? *Arthritis Rheum.* **37**, 1115-1126 (1994).
33. Benbow,U. *et al.* A novel host/tumor cell interaction activates matrix metalloproteinase 1 and mediates invasion through type I collagen. *J. Biol. Chem.* **274**, 25371-25378 (1999).
34. Brinckerhoff,C.E., Rutter,J.L. & Benbow,U. Interstitial collagenases as markers of tumor progression. *Clin. Cancer Res.* **6**, 4823-4830 (2000).
35. Muller,M. *et al.* Matrix metalloproteinases and diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is a predictor of wound healing. *Diabet. Med.* **25**, 419-426 (2008).
36. Iredale,J.P. *et al.* Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. *J. Clin. Invest* **102**, 538-549 (1998).
37. Maeda,S. *et al.* Determination of interstitial collagenase (MMP-1) in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **54**, 970-975 (1995).
38. Peake,N.J. *et al.* Levels of matrix metalloproteinase (MMP)-1 in paired sera and synovial fluids of juvenile idiopathic arthritis patients: relationship to inflammatory activity, MMP-3 and tissue inhibitor of metalloproteinases-1 in a longitudinal study. *Rheumatology. (Oxford)* **44**, 1383-1389 (2005).
39. Balbin,M. *et al.* Collagenase 2 (MMP-8) expression in murine tissue-remodeling processes. Analysis of its potential role in postpartum involution of the uterus. *J. Biol. Chem.* **273**, 23959-23968 (1998).
40. Hasty,K.A. *et al.* Human neutrophil collagenase. A distinct gene product with homology to other matrix metalloproteinases. *J. Biol. Chem.* **265**, 11421-11424 (1990).
41. Schettler,A., Thorn,H., Jockusch,B.M. & Tschesche,H. Release of proteinases from stimulated polymorphonuclear leukocytes. Evidence for subclasses of the main granule types and their association with cytoskeletal components. *Eur. J. Biochem.* **197**, 197-202 (1991).
42. Dozier,S., Escobar,G.P. & Lindsey,M.L. Matrix metalloproteinase (MMP)-7 activates MMP-8 but not MMP-13. *Med. Chem.* **2**, 523-526 (2006).
43. Knauper,V. *et al.* Fragmentation of human polymorphonuclear-leucocyte collagenase. *Biochem. J.* **291** ( Pt 3), 847-854 (1993).
44. Weiss,S.J., Peppin,G., Ortiz,X., Ragsdale,C. & Test,S.T. Oxidative autoactivation of latent collagenase by human neutrophils. *Science* **227**, 747-749 (1985).
45. Desrochers,P.E., Mookhtiar,K., Van Wart,H.E., Hasty,K.A. & Weiss,S.J. Proteolytic inactivation of alpha 1-proteinase inhibitor and alpha 1-antichymotrypsin by oxidatively activated human neutrophil metalloproteinases. *J. Biol. Chem.* **267**, 5005-5012 (1992).
46. Gutierrez-Fernandez,A. *et al.* Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. *Cancer Res.* **68**, 2755-2763 (2008).
47. Korpi,J.T. *et al.* Collagenase-2 (matrix metalloproteinase-8) plays a protective role in tongue cancer. *Br. J. Cancer* **98**, 766-775 (2008).

48. Mok,W., Boucher,Y. & Jain,R.K. Matrix metalloproteinases-1 and -8 improve the distribution and efficacy of an oncolytic virus. *Cancer Res.* **67**, 10664-10668 (2007).
49. Stadlmann,S. *et al.* Cytokine-regulated expression of collagenase-2 (MMP-8) is involved in the progression of ovarian cancer. *Eur. J. Cancer* **39**, 2499-2505 (2003).
50. Stenman,M. *et al.* Collagenases (MMP-1, -8 and -13) and trypsinogen-2 in fluid from benign and malignant ovarian cysts. *Tumour. Biol.* **24**, 9-12 (2003).
51. Herman,M.P. *et al.* Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling. *Circulation* **104**, 1899-1904 (2001).
52. Molloy,K.J. *et al.* Unstable carotid plaques exhibit raised matrix metalloproteinase-8 activity. *Circulation* **110**, 337-343 (2004).
53. Freije,J.M. *et al.* Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. *J. Biol. Chem.* **269**, 16766-16773 (1994).
54. Leeman,M.F., Curran,S. & Murray,G.I. The structure, regulation, and function of human matrix metalloproteinase-13. *Crit Rev. Biochem. Mol. Biol.* **37**, 149-166 (2002).
55. Knauper,V., Lopez-Otin,C., Smith,B., Knight,G. & Murphy,G. Biochemical characterization of human collagenase-3. *J. Biol. Chem.* **271**, 1544-1550 (1996).
56. Barmina,O.Y. *et al.* Collagenase-3 binds to a specific receptor and requires the low density lipoprotein receptor-related protein for internalization. *J. Biol. Chem.* **274**, 30087-30093 (1999).
57. Balbin,M. *et al.* Expression and regulation of collagenase-3 (MMP-13) in human malignant tumors. *APMIS* **107**, 45-53 (1999).
58. Burrage,P.S., Mix,K.S. & Brinckerhoff,C.E. Matrix metalloproteinases: role in arthritis. *Front Biosci.* **11**, 529-543 (2006).
59. Skotnicki,J.S., DiGrandi,M.J. & Levin,J.I. Design strategies for the identification of MMP-13 and Tace inhibitors. *Curr. Opin. Drug Discov. Devel.* **6**, 742-759 (2003).
60. Hu,Y. *et al.* Potent, selective, and orally bioavailable matrix metalloproteinase-13 inhibitors for the treatment of osteoarthritis. *Bioorg. Med. Chem.* **13**, 6629-6644 (2005).
61. Templeton,N.S. & Stetler-Stevenson,W.G. Identification of a basal promoter for the human Mr 72,000 type IV collagenase gene and enhanced expression in a highly metastatic cell line. *Cancer Res.* **51**, 6190-6193 (1991).
62. Deryugina,E.I. *et al.* MT1-MMP initiates activation of pro-MMP-2 and integrin alphavbeta3 promotes maturation of MMP-2 in breast carcinoma cells. *Exp. Cell Res.* **263**, 209-223 (2001).
63. Nguyen,M., Arkell,J. & Jackson,C.J. Activated protein C directly activates human endothelial gelatinase A. *J. Biol. Chem.* **275**, 9095-9098 (2000).
64. Murphy,G. *et al.* Assessment of the role of the fibronectin-like domain of gelatinase A by analysis of a deletion mutant. *J. Biol. Chem.* **269**, 6632-6636 (1994).
65. Chakrabarti,S. & Patel,K.D. Matrix metalloproteinase-2 (MMP-2) and MMP-9 in pulmonary pathology. *Exp. Lung Res.* **31**, 599-621 (2005).
66. Nguyen,M., Arkell,J. & Jackson,C.J. Human endothelial gelatinases and angiogenesis. *Int. J. Biochem. Cell Biol.* **33**, 960-970 (2001).
67. Nagase,H. Cell surface activation of progelatinase A (proMMP-2) and cell migration. *Cell Res.* **8**, 179-186 (1998).
68. Schonbeck,U., Mach,F. & Libby,P. Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. *J. Immunol.* **161**, 3340-3346 (1998).
69. McQuibban,G.A. *et al.* Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo. *Blood* **100**, 1160-1167 (2002).
70. Corry,D.B. *et al.* Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nat. Immunol.* **3**, 347-353 (2002).
71. Dean,R.A. & Overall,C.M. Proteomics discovery of metalloproteinase substrates in the cellular context by iTRAQ labeling reveals a diverse MMP-2 substrate degradome. *Mol. Cell Proteomics.* **6**, 611-623 (2007).

72. Dean,R.A. *et al.* Identification of candidate angiogenic inhibitors processed by matrix metalloproteinase 2 (MMP-2) in cell-based proteomic screens: disruption of vascular endothelial growth factor (VEGF)/heparin affinity regulatory peptide (pleiotrophin) and VEGF/Connective tissue growth factor angiogenic inhibitory complexes by MMP-2 proteolysis. *Mol. Cell Biol.* **27**, 8454-8465 (2007).
73. Esparza,J., Kruse,M., Lee,J., Michaud,M. & Madri,J.A. MMP-2 null mice exhibit an early onset and severe experimental autoimmune encephalomyelitis due to an increase in MMP-9 expression and activity. *FASEB J.* **18**, 1682-1691 (2004).
74. Sopata,I. & Dancewicz,A.M. Presence of a gelatin-specific proteinase and its latent form in human leucocytes. *Biochim. Biophys. Acta* **370**, 510-523 (1974).
75. Mattu,T.S. *et al.* O-glycan analysis of natural human neutrophil gelatinase B using a combination of normal phase-HPLC and online tandem mass spectrometry: implications for the domain organization of the enzyme. *Biochemistry* **39**, 15695-15704 (2000).
76. Ogata,Y., Enghild,J.J. & Nagase,H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. *J. Biol. Chem.* **267**, 3581-3584 (1992).
77. Maeda,H., Okamoto,T. & Akaike,T. Human matrix metalloprotease activation by insults of bacterial infection involving proteases and free radicals. *Biol. Chem.* **379**, 193-200 (1998).
78. Bergers,G. *et al.* Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat. Cell Biol.* **2**, 737-744 (2000).
79. Lemjabbar,H. *et al.* Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. *Am. J. Respir. Crit Care Med.* **159**, 1298-1307 (1999).
80. Dubois,B., Arnold,B. & Opdenakker,G. Gelatinase B deficiency impairs reproduction. *J. Clin. Invest* **106**, 627-628 (2000).
81. Vu,T.H. *et al.* MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* **93**, 411-422 (1998).
82. Engsig,M.T. *et al.* Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. *J. Cell Biol.* **151**, 879-889 (2000).
83. Van den Steen,P.E. *et al.* Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit Rev. Biochem. Mol. Biol.* **37**, 375-536 (2002).
84. Crocker,S.J., Pagenstecher,A. & Campbell,I.L. The TIMPs tango with MMPs and more in the central nervous system. *J. Neurosci. Res.* **75**, 1-11 (2004).
85. Devarajan,P., Johnston,J.J., Ginsberg,S.S., Van Wart,H.E. & Berliner,N. Structure and expression of neutrophil gelatinase cDNA. Identity with type IV collagenase from HT1080 cells. *J. Biol. Chem.* **267**, 25228-25232 (1992).
86. Opdenakker,G., Masure,S., Grillet,B. & Van Damme,J. Cytokine-mediated regulation of human leukocyte gelatinases and role in arthritis. *Lymphokine Cytokine Res.* **10**, 317-324 (1991).
87. Baruch,R.R. *et al.* Altered matrix metalloproteinase expression associated with oncogene-mediated cellular transformation and metastasis formation. *Cell Biol. Int.* **25**, 411-420 (2001).
88. Kjeldsen,L., Johnsen,A.H., Sengelov,H. & Borregaard,N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J. Biol. Chem.* **268**, 10425-10432 (1993).
89. Bjorklund,M. & Koivunen,E. Gelatinase-mediated migration and invasion of cancer cells. *Biochim. Biophys. Acta* **1755**, 37-69 (2005).
90. Klein,G., Vellenga,E., Fraaije,M.W., Kamps,W.A. & de Bont,E.S. The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia. *Crit Rev. Oncol. Hematol.* **50**, 87-100 (2004).
91. Maisi,P. *et al.* Soluble membrane-type 1 matrix metalloproteinase (MT1-MMP) and gelatinase A (MMP-2) in induced sputum and bronchoalveolar lavage fluid of human bronchial asthma and bronchiectasis. *APMIS* **110**, 771-782 (2002).
92. Mautino,G., Oliver,N., Chanez,P., Bousquet,J. & Capony,F. Increased release of matrix metalloproteinase-9 in bronchoalveolar lavage fluid and by alveolar macrophages of asthmatics. *Am. J. Respir. Cell Mol. Biol.* **17**, 583-591 (1997).
93. Liu,Z. *et al.* The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. *Cell* **102**, 647-655 (2000).



94. Itoh,T. *et al.* Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. *Cancer Res.* **58**, 1048-1051 (1998).
95. Risau,W. Mechanisms of angiogenesis. *Nature* **386**, 671-674 (1997).
96. Levi,E. *et al.* Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1. *Proc. Natl. Acad. Sci. U. S. A* **93**, 7069-7074 (1996).
97. Somiari,S.B. *et al.* Circulating MMP2 and MMP9 in breast cancer -- potential role in classification of patients into low risk, high risk, benign disease and breast cancer categories. *Int. J. Cancer* **119**, 1403-1411 (2006).
98. Sier,C.F.M. *et al.* Enhanced urinary gelatinase activities (matrix metalloproteinases 2 and 9) are associated with early-stage bladder carcinoma: A comparison with clinically used tumor markers. *Clinical Cancer Research* **6**, 2333-2340 (2000).
99. Maatta,M., Talvensaaari-Mattila,A., Turpeenniemi-Hujanen,T. & Santala,M. Matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) in differential diagnosis between low malignant potential (LMP) and malignant ovarian tumours. *Anticancer Res.* **27**, 2753-2758 (2007).
100. Takemura,M. *et al.* Malignant cell-specific gelatinase activity in human endometrial carcinoma. *Cancer* **70**, 147-151 (1992).
101. Forsyth,P.A. *et al.* Gelatinase-A (MMP-2), gelatinase-B (MMP-9) and membrane type matrix metalloproteinase-1 (MT1-MMP) are involved in different aspects of the pathophysiology of malignant gliomas. *Br. J. Cancer* **79**, 1828-1835 (1999).
102. Nawrocki,B. *et al.* Expression of matrix metalloproteinases and their inhibitors in human bronchopulmonary carcinomas: quantitative and morphological analyses. *Int. J. Cancer* **72**, 556-564 (1997).
103. Pyke,C. *et al.* Localization of messenger RNA for Mr 72,000 and 92,000 type IV collagenases in human skin cancers by in situ hybridization. *Cancer Res.* **52**, 1336-1341 (1992).
104. Martinez,A. *et al.* Matrix metalloproteinase-2 cleavage of adrenomedullin produces a vasoconstrictor out of a vasodilator. *Biochem. J.* **383**, 413-418 (2004).
105. Chin,J.R., Murphy,G. & Werb,Z. Stromelysin, a connective tissue-degrading metalloendopeptidase secreted by stimulated rabbit synovial fibroblasts in parallel with collagenase. Biosynthesis, isolation, characterization, and substrates. *J. Biol. Chem.* **260**, 12367-12376 (1985).
106. Matrisian,L.M., Glaichenhaus,N., Gesnel,M.C. & Breathnach,R. Epidermal growth factor and oncogenes induce transcription of the same cellular mRNA in rat fibroblasts. *EMBO J.* **4**, 1435-1440 (1985).
107. Matrisian,L.M. *et al.* The mRNA coding for the secreted protease transin is expressed more abundantly in malignant than in benign tumors. *Proc. Natl. Acad. Sci. U. S. A* **83**, 9413-9417 (1986).
108. Moilanen,M. *et al.* Tumor-associated trypsinogen-2 (trypsinogen-2) activates procollagenases (MMP-1, -8, -13) and stromelysin-1 (MMP-3) and degrades type I collagen. *Biochemistry* **42**, 5414-5420 (2003).
109. Jin,X. *et al.* Matriptase activates stromelysin (MMP-3) and promotes tumor growth and angiogenesis. *Cancer Sci.* **97**, 1327-1334 (2006).
110. Saus,J. *et al.* The complete primary structure of human matrix metalloproteinase-3. Identity with stromelysin. *J. Biol. Chem.* **263**, 6742-6745 (1988).
111. McDonnell,S. & Matrisian,L.M. Stromelysin in tumor progression and metastasis. *Cancer Metastasis Rev.* **9**, 305-319 (1990).
112. Witty,J.P., Wright,J.H. & Matrisian,L.M. Matrix metalloproteinases are expressed during ductal and alveolar mammary morphogenesis, and misregulation of stromelysin-1 in transgenic mice induces unscheduled alveolar development. *Mol. Biol. Cell* **6**, 1287-1303 (1995).
113. Lochter,A. *et al.* Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J. Cell Biol.* **139**, 1861-1872 (1997).
114. Matsuno,H. *et al.* Stromelysin-1 (MMP-3) in synovial fluid of patients with rheumatoid arthritis has potential to cleave membrane bound Fas ligand. *J. Rheumatol.* **28**, 22-28 (2001).

115. Lijnen,H.R., Arza,B., Van Hoef,B., Collen,D. & Declerck,P.J. Inactivation of plasminogen activator inhibitor-1 by specific proteolysis with stromelysin-1 (MMP-3). *J. Biol. Chem.* **275**, 37645-37650 (2000).
116. Arza,B., Hoylaerts,M.F., Felez,J., Collen,D. & Lijnen,H.R. Prostromelysin-1 (proMMP-3) stimulates plasminogen activation by tissue-type plasminogen activator. *Eur. J. Biochem.* **267**, 6378-6384 (2000).
117. Green,M.J. *et al.* Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. *Rheumatology. (Oxford)* **42**, 83-88 (2003).
118. Dahlen,B., Shute,J. & Howarth,P. Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax* **54**, 590-596 (1999).
119. Silence,J., Lupu,F., Collen,D. & Lijnen,H.R. Persistence of atherosclerotic plaque but reduced aneurysm formation in mice with stromelysin-1 (MMP-3) gene inactivation. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1440-1445 (2001).
120. Fray,M.J., Dickinson,R.P., Huggins,J.P. & Occlleston,N.L. A potent, selective inhibitor of matrix metalloproteinase-3 for the topical treatment of chronic dermal ulcers. *J. Med. Chem.* **46**, 3514-3525 (2003).
121. Yoshiyama,Y., Asahina,M. & Hattori,T. Selective distribution of matrix metalloproteinase-3 (MMP-3) in Alzheimer's disease brain. *Acta Neuropathol.* **99**, 91-95 (2000).
122. Mercapide,J., Lopez,D.C., Castresana,J.S. & Klein-Szanto,A.J. Stromelysin-1/matrix metalloproteinase-3 (MMP-3) expression accounts for invasive properties of human astrocytoma cell lines. *Int. J. Cancer* **106**, 676-682 (2003).
123. Wiesen,J.F. & Werb,Z. The role of stromelysin-1 in stromal-epithelial interactions and cancer. *Enzyme Protein* **49**, 174-181 (1996).
124. Breathnach,R., Matrisian,L.M., Gesnel,M.C., Staub,A. & Leroy,P. Sequences coding for part of oncogene-induced transin are highly conserved in a related rat gene. *Nucleic Acids Res.* **15**, 1139-1151 (1987).
125. Muller,D. *et al.* The collagenase gene family in humans consists of at least four members. *Biochem. J.* **253**, 187-192 (1988).
126. Sirum,K.L. & Brinckerhoff,C.E. Cloning of the genes for human stromelysin and stromelysin 2: differential expression in rheumatoid synovial fibroblasts. *Biochemistry* **28**, 8691-8698 (1989).
127. Brinckerhoff,C.E., Sirum-Connolly,K.L., Karmilowicz,M.J. & Auble,D.T. Expression of stromelysin and stromelysin-2 in rabbit and human fibroblasts. *Matrix Suppl* **1**, 165-175 (1992).
128. Barksby,H.E. *et al.* Matrix metalloproteinase 10 promotion of collagenolysis via procollagenase activation: implications for cartilage degradation in arthritis. *Arthritis Rheum.* **54**, 3244-3253 (2006).
129. Murphy,G., Cockett,M.I., Ward,R.V. & Docherty,A.J. Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP). *Biochem. J.* **277** ( Pt 1), 277-279 (1991).
130. Saarialho-Kere,U.K., Pentland,A.P., Birkedal-Hansen,H., Parks,W.C. & Welgus,H.G. Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. *J. Clin. Invest* **94**, 79-88 (1994).
131. Madlener,M. & Werner,S. cDNA cloning and expression of the gene encoding murine stromelysin-2 (MMP-10). *Gene* **202**, 75-81 (1997).
132. Vaalamo,M., Karjalainen-Lindsberg,M.L., Puolakkainen,P., Kere,J. & Saarialho-Kere,U. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. *Am. J. Pathol.* **152**, 1005-1014 (1998).
133. Salmela,M.T. *et al.* Collagenase-1 (MMP-1), matrilysin-1 (MMP-7), and stromelysin-2 (MMP-10) are expressed by migrating enterocytes during intestinal wound healing. *Scand. J. Gastroenterol.* **39**, 1095-1104 (2004).
134. Krampert,M. *et al.* Activities of the matrix metalloproteinase stromelysin-2 (MMP-10) in matrix degradation and keratinocyte organization in wounded skin. *Mol. Biol. Cell* **15**, 5242-5254 (2004).
135. Bord,S., Horner,A., Hembry,R.M. & Compston,J.E. Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) expression in developing human bone: potential roles in skeletal development. *Bone* **23**, 7-12 (1998).

136. Basset,P. *et al.* A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature* **348**, 699-704 (1990).
137. Bassi,D.E., Mahloogi,H. & Klein-Szanto,A.J. The proprotein convertases furin and PACE4 play a significant role in tumor progression. *Mol. Carcinog.* **28**, 63-69 (2000).
138. Matziari,M., Dive,V. & Yiotakis,A. Matrix metalloproteinase 11 (MMP-11; stromelysin-3) and synthetic inhibitors. *Med. Res. Rev.* **27**, 528-552 (2007).
139. Pei,D., Majmudar,G. & Weiss,S.J. Hydrolytic inactivation of a breast carcinoma cell-derived serpin by human stromelysin-3. *J. Biol. Chem.* **269**, 25849-25855 (1994).
140. Motrescu,E.R. *et al.* Matrix metalloproteinase-11/stromelysin-3 exhibits collagenolytic function against collagen VI under normal and malignant conditions. *Oncogene* (2008).
141. Noel,A. *et al.* Identification of structural determinants controlling human and mouse stromelysin-3 proteolytic activities. *J. Biol. Chem.* **270**, 22866-22872 (1995).
142. Rio,M.C. From a unique cell to metastasis is a long way to go: clues to stromelysin-3 participation. *Biochimie* **87**, 299-306 (2005).
143. Noel,A. *et al.* Demonstration in vivo that stromelysin-3 functions through its proteolytic activity. *Oncogene* **19**, 1605-1612 (2000).
144. Mari,B.P. *et al.* Stromelysin-3 is induced in tumor/stroma cocultures and inactivated via a tumor-specific and basic fibroblast growth factor-dependent mechanism. *J. Biol. Chem.* **273**, 618-626 (1998).
145. Boulay,A. *et al.* High cancer cell death in syngeneic tumors developed in host mice deficient for the stromelysin-3 matrix metalloproteinase. *Cancer Res.* **61**, 2189-2193 (2001).
146. Sato,H. *et al.* A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* **370**, 61-65 (1994).
147. Lehti,K., Valtanen,H., Wickstrom,S.A., Lohi,J. & Keski-Oja,J. Regulation of membrane-type-1 matrix metalloproteinase activity by its cytoplasmic domain. *J. Biol. Chem.* **275**, 15006-15013 (2000).
148. Yana,I. & Weiss,S.J. Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. *Mol. Biol. Cell* **11**, 2387-2401 (2000).
149. Toth,M. *et al.* Complex pattern of membrane type 1 matrix metalloproteinase shedding. Regulation by autocatalytic cells surface inactivation of active enzyme. *J. Biol. Chem.* **277**, 26340-26350 (2002).
150. Barbolina,M.V. & Stack,M.S. Membrane type 1-matrix metalloproteinase: substrate diversity in pericellular proteolysis. *Semin. Cell Dev. Biol.* **19**, 24-33 (2008).
151. d'Ortho,M.P. *et al.* Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. *Eur. J. Biochem.* **250**, 751-757 (1997).
152. Holmbeck,K. *et al.* MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* **99**, 81-92 (1999).
153. Zhou,Z. *et al.* Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. *Proc. Natl. Acad. Sci. U. S. A* **97**, 4052-4057 (2000).
154. Ellerbroek,S.M., Wu,Y.I., Overall,C.M. & Stack,M.S. Functional interplay between type I collagen and cell surface matrix metalloproteinase activity. *J. Biol. Chem.* **276**, 24833-24842 (2001).
155. Golubkov,V.S. *et al.* Membrane type-1 matrix metalloproteinase (MT1-MMP) exhibits an important intracellular cleavage function and causes chromosome instability. *J. Biol. Chem.* **280**, 25079-25086 (2005).
156. Golubkov,V.S., Chekanov,A.V., Doxsey,S.J. & Strongin,A.Y. Centrosomal pericentrin is a direct cleavage target of membrane type-1 matrix metalloproteinase in humans but not in mice: potential implications for tumorigenesis. *J. Biol. Chem.* **280**, 42237-42241 (2005).
157. Knauper,V. *et al.* Cellular activation of proMMP-13 by MT1-MMP depends on the C-terminal domain of MMP-13. *FEBS Lett.* **532**, 127-130 (2002).
158. Holopainen,J.M. *et al.* Activation of matrix metalloproteinase-8 by membrane type 1-MMP and their expression in human tears after photorefractive keratectomy. *Invest Ophthalmol. Vis. Sci.* **44**, 2550-2556 (2003).
159. Hiraoka,N., Allen,E., Apel,I.J., Gyetko,M.R. & Weiss,S.J. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. *Cell* **95**, 365-377 (1998).

160. Galvez,B.G., Matias-Roman,S., Albar,J.P., Sanchez-Madrid,F. & Arroyo,A.G. Membrane type 1-matrix metalloproteinase is activated during migration of human endothelial cells and modulates endothelial motility and matrix remodeling. *J. Biol. Chem.* **276**, 37491-37500 (2001).
161. Robinet,A. *et al.* Elastin-derived peptides enhance angiogenesis by promoting endothelial cell migration and tubulogenesis through upregulation of MT1-MMP. *J. Cell Sci.* **118**, 343-356 (2005).
162. Sounni,N.E. *et al.* Up-regulation of vascular endothelial growth factor-A by active membrane-type 1 matrix metalloproteinase through activation of Src-tyrosine kinases. *J. Biol. Chem.* **279**, 13564-13574 (2004).
163. Takino,T., Sato,H., Shinagawa,A. & Seiki,M. Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library. MT-MMPs form a unique membrane-type subclass in the MMP family. *J. Biol. Chem.* **270**, 23013-23020 (1995).
164. Ogiwara,K., Takano,N., Shinohara,M., Murakami,M. & Takahashi,T. Gelatinase A and membrane-type matrix metalloproteinases 1 and 2 are responsible for follicle rupture during ovulation in the medaka. *Proc. Natl. Acad. Sci. U. S. A* **102**, 8442-8447 (2005).
165. Lafleur,M.A., Handsley,M.M., Knauper,V., Murphy,G. & Edwards,D.R. Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs). *J. Cell Sci.* **115**, 3427-3438 (2002).
166. Abraham,R. *et al.* Identification of MMP-15 as an anti-apoptotic factor in cancer cells. *J. Biol. Chem.* **280**, 34123-34132 (2005).
167. Morrison,C.J. *et al.* Cellular activation of MMP-2 (gelatinase A) by MT2-MMP occurs via a TIMP-2-independent pathway. *J. Biol. Chem.* **276**, 47402-47410 (2001).
168. Zhang,J., Sarkar,S. & Yong,V.W. The chemokine stromal cell derived factor-1 (CXCL12) promotes glioma invasiveness through MT2-matrix metalloproteinase. *Carcinogenesis* **26**, 2069-2077 (2005).
169. Atkinson,J.M. *et al.* Membrane type matrix metalloproteinases (MMPs) show differential expression in non-small cell lung cancer (NSCLC) compared to normal lung: correlation of MMP-14 mRNA expression and proteolytic activity. *Eur. J. Cancer* **43**, 1764-1771 (2007).
170. Davidson,B. *et al.* Expression of membrane-type 1, 2, and 3 matrix metalloproteinases messenger RNA in ovarian carcinoma cells in serous effusions. *Am. J. Clin. Pathol.* **115**, 517-524 (2001).
171. Ueno,H. *et al.* Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human invasive breast carcinomas. *Cancer Res.* **57**, 2055-2060 (1997).
172. Matsumoto,S., Katoh,M., Saito,S., Watanabe,T. & Masuho,Y. Identification of soluble type of membrane-type matrix metalloproteinase-3 formed by alternatively spliced mRNA. *Biochim. Biophys. Acta* **1354**, 159-170 (1997).
173. Llano,E. *et al.* Identification and characterization of human MT5-MMP, a new membrane-bound activator of progelatinase a overexpressed in brain tumors. *Cancer Res.* **59**, 2570-2576 (1999).
174. Lang,R. *et al.* Crystal structure of the catalytic domain of MMP-16/MT3-MMP: characterization of MT-MMP specific features. *J. Mol. Biol.* **336**, 213-225 (2004).
175. Pei,D. Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. *J. Biol. Chem.* **274**, 8925-8932 (1999).
176. Gaetje,R. *et al.* Expression of membrane-type 5 matrix metalloproteinase in human endometrium and endometriosis. *Gynecol. Endocrinol.* **23**, 567-573 (2007).
177. Zhao,H. *et al.* Differential inhibition of membrane type 3 (MT3)-matrix metalloproteinase (MMP) and MT1-MMP by tissue inhibitor of metalloproteinase (TIMP)-2 and TIMP-3 regulates pro-MMP-2 activation. *J. Biol. Chem.* **279**, 8592-8601 (2004).
178. Wang,X., Yi,J., Lei,J. & Pei,D. Expression, purification and characterization of recombinant mouse MT5-MMP protein products. *FEBS Lett.* **462**, 261-266 (1999).
179. Pendas,A.M. *et al.* Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution. *J. Biol. Chem.* **272**, 4281-4286 (1997).
180. Pei,D. Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. *Cell Res.* **9**, 291-303 (1999).
181. Sohail,A. *et al.* MT4-(MMP17) and MT6-MMP (MMP25), A unique set of membrane-anchored matrix metalloproteinases: properties and expression in cancer. *Cancer Metastasis Rev.* **27**, 289-302 (2008).

182. Sun,Q. *et al.* MMP25 (MT6-MMP) is highly expressed in human colon cancer, promotes tumor growth, and exhibits unique biochemical properties. *J. Biol. Chem.* **282**, 21998-22010 (2007).
183. Wang,Y., Johnson,A.R., Ye,Q.Z. & Dyer,R.D. Catalytic activities and substrate specificity of the human membrane type 4 matrix metalloproteinase catalytic domain. *J. Biol. Chem.* **274**, 33043-33049 (1999).
184. English,W.R., Velasco,G., Stracke,J.O., Knauper,V. & Murphy,G. Catalytic activities of membrane-type 6 matrix metalloproteinase (MMP25). *FEBS Lett.* **491**, 137-142 (2001).
185. Itoh,Y. *et al.* Membrane type 4 matrix metalloproteinase (MT4-MMP, MMP-17) is a glycosylphosphatidylinositol-anchored proteinase. *J. Biol. Chem.* **274**, 34260-34266 (1999).
186. Kang,T. *et al.* Subcellular distribution and cytokine- and chemokine-regulated secretion of leukolysin/MT6-MMP/MMP-25 in neutrophils. *J. Biol. Chem.* **276**, 21960-21968 (2001).
187. English,W.R. *et al.* Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor- $\alpha$  convertase activity but does not activate pro-MMP2. *J. Biol. Chem.* **275**, 14046-14055 (2000).
188. Nie,J. & Pei,D. Direct activation of pro-matrix metalloproteinase-2 by leukolysin/membrane-type 6 matrix metalloproteinase/matrix metalloproteinase 25 at the asn(109)-Tyr bond. *Cancer Res.* **63**, 6758-6762 (2003).
189. Miyamori,H. *et al.* Claudin promotes activation of pro-matrix metalloproteinase-2 mediated by membrane-type matrix metalloproteinases. *J. Biol. Chem.* **276**, 28204-28211 (2001).
190. Gao,G. *et al.* ADAMTS4 (aggrecanase-1) activation on the cell surface involves C-terminal cleavage by glycosylphosphatidylinositol-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 (2004).
191. Woessner,J.F., Jr. & Taplin,C.J. Purification and properties of a small latent matrix metalloproteinase of the rat uterus. *J. Biol. Chem.* **263**, 16918-16925 (1988).
192. Gaire,M. *et al.* Structure and expression of the human gene for the matrix metalloproteinase matrilysin. *J. Biol. Chem.* **269**, 2032-2040 (1994).
193. Quantin,B., Murphy,G. & Breathnach,R. Pump-1 cDNA codes for a protein with characteristics similar to those of classical collagenase family members. *Biochemistry* **28**, 5327-5334 (1989).
194. Wilson,C.L. & Matrisian,L.M. Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions. *Int. J. Biochem. Cell Biol.* **28**, 123-136 (1996).
195. Ii,M., Yamamoto,H., Adachi,Y., Maruyama,Y. & Shinomura,Y. Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Exp. Biol. Med. (Maywood.)* **231**, 20-27 (2006).
196. Wilson,C.L. *et al.* Regulation of intestinal  $\alpha$ -defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* **286**, 113-117 (1999).
197. Lopez-Boado,Y.S., Wilson,C.L. & Parks,W.C. Regulation of matrilysin expression in airway epithelial cells by *Pseudomonas aeruginosa* flagellin. *J. Biol. Chem.* **276**, 41417-41423 (2001).
198. Parks,W.C. & Shapiro,S.D. Matrix metalloproteinases in lung biology. *Respir. Res.* **2**, 10-19 (2001).
199. Dunsmore,S.E. *et al.* Matrilysin expression and function in airway epithelium. *J. Clin. Invest* **102**, 1321-1331 (1998).
200. Li,Q., Park,P.W., Wilson,C.L. & Parks,W.C. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* **111**, 635-646 (2002).
201. Wang,F.Q., So,J., Reierstad,S. & Fishman,D.A. Matrilysin (MMP-7) promotes invasion of ovarian cancer cells by activation of progelatinase. *Int. J. Cancer* **114**, 19-31 (2005).
202. Wang,F., Reierstad,S. & Fishman,D.A. Matrilysin over-expression in MCF-7 cells enhances cellular invasiveness and pro-gelatinase activation. *Cancer Lett.* **236**, 292-301 (2006).
203. Yu,W.H., Woessner,J.F., Jr., McNeish,J.D. & Stamenkovic,I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev.* **16**, 307-323 (2002).
204. Nakamura,M. *et al.* Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. *Biochem. Biophys. Res. Commun.* **333**, 1011-1016 (2005).

205. Mochizuki,S., Shimoda,M., Shiomi,T., Fujii,Y. & Okada,Y. ADAM28 is activated by MMP-7 (matrilysin-1) and cleaves insulin-like growth factor binding protein-3. *Biochem. Biophys. Res. Commun.* **315**, 79-84 (2004).
206. de Coignac,A.B. *et al.* Cloning of MMP-26. A novel matrilysin-like proteinase. *Eur. J. Biochem.* **267**, 3323-3329 (2000).
207. Uria,J.A. & Lopez-Otin,C. Matrilysin-2, a new matrix metalloproteinase expressed in human tumors and showing the minimal domain organization required for secretion, latency, and activity. *Cancer Res.* **60**, 4745-4751 (2000).
208. Park,H.I. *et al.* Identification and characterization of human endometase (Matrix metalloproteinase-26) from endometrial tumor. *J. Biol. Chem.* **275**, 20540-20544 (2000).
209. Marchenko,G.N. & Strongin,A.Y. MMP-28, a new human matrix metalloproteinase with an unusual cysteine-switch sequence is widely expressed in tumors. *Gene* **265**, 87-93 (2001).
210. Marchenko,G.N. *et al.* Characterization of matrix metalloproteinase-26, a novel metalloproteinase widely expressed in cancer cells of epithelial origin. *Biochem. J.* **356**, 705-718 (2001).
211. Banda,M.J. & Werb,Z. Mouse macrophage elastase. Purification and characterization as a metalloproteinase. *Biochem. J.* **193**, 589-605 (1981).
212. Shapiro,S.D. *et al.* Molecular cloning, chromosomal localization, and bacterial expression of a murine macrophage metalloelastase. *J. Biol. Chem.* **267**, 4664-4671 (1992).
213. Shapiro,S.D., Kobayashi,D.K. & Ley,T.J. Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. *J. Biol. Chem.* **268**, 23824-23829 (1993).
214. Banda,M.J., Clark,E.J. & Werb,Z. Selective proteolysis of immunoglobulins by mouse macrophage elastase. *J. Exp. Med.* **157**, 1184-1196 (1983).
215. Chandler,S., Cossins,J., Lury,J. & Wells,G. Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. *Biochem. Biophys. Res. Commun.* **228**, 421-429 (1996).
216. Shipley,J.M., Wesselschmidt,R.L., Kobayashi,D.K., Ley,T.J. & Shapiro,S.D. Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. *Proc. Natl. Acad. Sci. U. S. A* **93**, 3942-3946 (1996).
217. Dong,Z., Kumar,R., Yang,X. & Fidler,I.J. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* **88**, 801-810 (1997).
218. Houghton,A.M. *et al.* Macrophage elastase (matrix metalloproteinase-12) suppresses growth of lung metastases. *Cancer Res.* **66**, 6149-6155 (2006).
219. Finlay,G.A. *et al.* Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. *Am. J. Respir. Crit Care Med.* **156**, 240-247 (1997).
220. Hautamaki,R.D., Kobayashi,D.K., Senior,R.M. & Shapiro,S.D. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science* **277**, 2002-2004 (1997).
221. Matute-Bello,G. *et al.* Essential role of MMP-12 in Fas-induced lung fibrosis. *Am. J. Respir. Cell Mol. Biol.* **37**, 210-221 (2007).
222. Matute-Bello,G. *et al.* MMP-12 Mediates Fibrosis after Lung Injury in Mice. *Proc. Am. Thorac. Soc.* **5**, 361-362 (2008).
223. Joos,L. *et al.* The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum. Mol. Genet.* **11**, 569-576 (2002).
224. Demedts,I.K. *et al.* Elevated MMP-12 protein levels in induced sputum from patients with COPD. *Thorax* **61**, 196-201 (2006).
225. Ilumets,H. *et al.* Matrix metalloproteinases -8, -9 and -12 in smokers and patients with stage 0 COPD. *Int. J. Chron. Obstruct. Pulmon. Dis.* **2**, 369-379 (2007).
226. Senior,R.M., Griffin,G.L. & Mecham,R.P. Chemotactic activity of elastin-derived peptides. *J. Clin. Invest* **66**, 859-862 (1980).
227. Seals,D.F. & Courtneidge,S.A. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* **17**, 7-30 (2003).
228. Primakoff,P., Hyatt,H. & Tredick-Kline,J. Identification and purification of a sperm surface protein with a potential role in sperm-egg membrane fusion. *J. Cell Biol.* **104**, 141-149 (1987).

229. Blobel,C.P., Myles,D.G., Primakoff,P. & White,J.M. Proteolytic processing of a protein involved in sperm-egg fusion correlates with acquisition of fertilization competence. *J. Cell Biol.* **111**, 69-78 (1990).
230. Blobel,C.P. *et al.* A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. *Nature* **356**, 248-252 (1992).
231. Wolfsberg,T.G. *et al.* ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* **169**, 378-383 (1995).
232. Eto,K. *et al.* Functional classification of ADAMs based on a conserved motif for binding to integrin alpha 9beta 1: implications for sperm-egg binding and other cell interactions. *J. Biol. Chem.* **277**, 17804-17810 (2002).
233. Zhang,X.P., Kamata,T., Yokoyama,K., Puzon-McLaughlin,W. & Takada,Y. Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin alphavbeta3. *J. Biol. Chem.* **273**, 7345-7350 (1998).
234. Lu,D. *et al.* Integrin binding characteristics of the disintegrin-like domain of ADAM-15. *Thromb. Haemost.* **96**, 642-651 (2006).
235. Lu,X., Lu,D., Scully,M.F. & Kakkar,V.V. Structure-activity relationship studies on ADAM protein-integrin interactions. *Cardiovasc. Hematol. Agents Med. Chem.* **5**, 29-42 (2007).
236. Chen,H. & Sampson,N.S. Mediation of sperm-egg fusion: evidence that mouse egg alpha6beta1 integrin is the receptor for sperm fertilinbeta. *Chem. Biol.* **6**, 1-10 (1999).
237. Rubinstein,E., Ziyat,A., Wolf,J.P., Le Naour,F. & Boucheix,C. The molecular players of sperm-egg fusion in mammals. *Semin. Cell Dev. Biol.* **17**, 254-263 (2006).
238. Jury,J.A., Frayne,J. & Hall,L. The human fertilin alpha gene is non-functional: implications for its proposed role in fertilization. *Biochem. J.* **321 ( Pt 3)**, 577-581 (1997).
239. Cho,C. *et al.* Fertilization defects in sperm from mice lacking fertilin beta. *Science* **281**, 1857-1859 (1998).
240. Tomczuk,M. *et al.* Role of multiple beta1 integrins in cell adhesion to the disintegrin domains of ADAMs 2 and 3. *Exp. Cell Res.* **290**, 68-81 (2003).
241. Iba,K. *et al.* The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to beta1 integrin-dependent cell spreading. *J. Cell Biol.* **149**, 1143-1156 (2000).
242. Gaultier,A., Cousin,H., Darribere,T. & Alfandari,D. ADAM13 disintegrin and cysteine-rich domains bind to the second heparin-binding domain of fibronectin. *Journal of Biological Chemistry* **277**, 23336-23344 (2002).
243. Blobel,C.P. & White,J.M. Structure, function and evolutionary relationship of proteins containing a disintegrin domain. *Curr. Opin. Cell Biol.* **4**, 760-765 (1992).
244. Kang,Q., Cao,Y. & Zolkiewska,A. Metalloprotease-disintegrin ADAM 12 binds to the SH3 domain of Src and activates Src tyrosine kinase in C2C12 cells. *Biochem. J.* **352 Pt 3**, 883-892 (2000).
245. Galliano,M.F. *et al.* Binding of ADAM12, a marker of skeletal muscle regeneration, to the muscle-specific actin-binding protein, alpha -actinin-2, is required for myoblast fusion. *J. Biol. Chem.* **275**, 13933-13939 (2000).
246. Izumi,Y. *et al.* A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J.* **17**, 7260-7272 (1998).
247. Zhu,G.Z., Lin,Y., Myles,D.G. & Primakoff,P. Identification of four novel ADAMs with potential roles in spermatogenesis and fertilization. *Gene* **234**, 227-237 (1999).
248. Schlomann,U. *et al.* The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. *J. Biol. Chem.* **277**, 48210-48219 (2002).
249. Howard,L., Maciewicz,R.A. & Blobel,C.P. Cloning and characterization of ADAM28: evidence for autocatalytic pro-domain removal and for cell surface localization of mature ADAM28. *Biochem. J.* **348 Pt 1**, 21-27 (2000).
250. Milla,M.E. *et al.* Specific sequence elements are required for the expression of functional tumor necrosis factor-alpha-converting enzyme (TACE). *J. Biol. Chem.* **274**, 30563-30570 (1999).

251. Anders,A., Gilbert,S., Garten,W., Postina,R. & Fahrenholz,F. Regulation of the alpha-secretase ADAM10 by its prodomain and proprotein convertases. *FASEB J.* **15**, 1837-1839 (2001).
252. Black,R.A. *et al.* A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* **385**, 729-733 (1997).
253. Moss,M.L. *et al.* Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature* **385**, 733-736 (1997).
254. Yoshida,S., Setoguchi,M., Higuchi,Y., Akizuki,S. & Yamamoto,S. Molecular cloning of cDNA encoding MS2 antigen, a novel cell surface antigen strongly expressed in murine monocytic lineage. *Int. Immunol.* **2**, 585-591 (1990).
255. Yoshiyama,K., Higuchi,Y., Kataoka,M., Matsuura,K. & Yamamoto,S. CD156 (human ADAM8): expression, primary amino acid sequence, and gene location. *Genomics* **41**, 56-62 (1997).
256. Kataoka,M. *et al.* Structure of the murine CD156 gene, characterization of its promoter, and chromosomal location. *J. Biol. Chem.* **272**, 18209-18215 (1997).
257. Choi,S.J., Han,J.H. & Roodman,G.D. ADAM8: a novel osteoclast stimulating factor. *J. Bone Miner. Res.* **16**, 814-822 (2001).
258. Amour,A. *et al.* The enzymatic activity of ADAM8 and ADAM9 is not regulated by TIMPs. *FEBS Lett.* **524**, 154-158 (2002).
259. Schlomann,U., Rathke-Hartlieb,S., Yamamoto,S., Jockusch,H. & Bartsch,J.W. Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): implications for neuron-glia interactions during neurodegeneration. *J. Neurosci.* **20**, 7964-7971 (2000).
260. Sriraman,V. *et al.* Regulated expression of ADAM8 (a disintegrin and metalloprotease domain 8) in the mouse ovary: evidence for a regulatory role of luteinizing hormone, progesterone receptor, and epidermal growth factor-like growth factors. *Biol. Reprod.* **78**, 1038-1048 (2008).
261. Naus,S. *et al.* Identification of candidate substrates for ectodomain shedding by the metalloprotease-disintegrin ADAM8. *Biol. Chem.* **387**, 337-346 (2006).
262. Naus,S. *et al.* Ectodomain shedding of the neural recognition molecule CHL1 by the metalloprotease-disintegrin ADAM8 promotes neurite outgrowth and suppresses neuronal cell death. *J. Biol. Chem.* **279**, 16083-16090 (2004).
263. Kelly,K. *et al.* Metalloprotease-disintegrin ADAM8: expression analysis and targeted deletion in mice. *Dev. Dyn.* **232**, 221-231 (2005).
264. King,N.E. *et al.* Expression and regulation of a disintegrin and metalloproteinase (ADAM) 8 in experimental asthma. *Am. J. Respir. Cell Mol. Biol.* **31**, 257-265 (2004).
265. Higuchi,Y., Yasui,A., Matsuura,K. & Yamamoto,S. CD156 transgenic mice. Different responses between inflammatory types. *Pathobiology* **70**, 47-54 (2002).
266. Matsuno,O. *et al.* Role of ADAM8 in experimental asthma. *Immunol. Lett.* **102**, 67-73 (2006).
267. Matsuno,O. *et al.* Elevated soluble ADAM8 in bronchoalveolar lavage fluid in patients with eosinophilic pneumonia. *Int. Arch. Allergy Immunol.* **142**, 285-290 (2007).
268. Foley,S.C. *et al.* Increased expression of ADAM33 and ADAM8 with disease progression in asthma. *J. Allergy Clin. Immunol.* **119**, 863-871 (2007).
269. Gomez-Gavero,M. *et al.* Expression and regulation of the metalloproteinase ADAM-8 during human neutrophil pathophysiological activation and its catalytic activity on L-selectin shedding. *J. Immunol.* **178**, 8053-8063 (2007).
270. Ainola,M. *et al.* Involvement of ADAM8 in osteoclastogenesis and pathological bone destruction. *Ann. Rheum. Dis.* (2008).



### 1.3 ADAM proteinases

The “a disintegrin and metalloproteinases” (ADAMs) or MDCs (metalloproteinase-like, disintegrin-like, cysteine-rich proteins) are membrane-anchored metzincins of the adamalysin subfamily which also contains the interesting biological close relative, the class III snake venom metalloproteases<sup>1</sup>. These proteases have a similar domain structure to the membrane-type MMPs (see figure 1), but with two distinct differences. The ADAM proteases do not contain the characteristic hemopexin-like domain, but instead have three additional domains; the cysteine-rich domain, the EGF-like repeat domain and the disintegrin domain from which the family derives its name. While the biological function of the EGF-like domain has not yet been fully elucidated, the cysteine-rich and disintegrin domains enable the cell surface bound ADAMs to interact with ECM proteins and ligands on neighbouring cells. This important function in intercellular interaction was already clear from the identification of the first members of the ADAM as key players in the fertilisation process, specifically in the binding and fusion between egg and sperm cells. The first adamalysins described in mammalia were found on guinea pig sperm and dubbed fertilin  $\alpha$  and  $\beta$  (or PH-30  $\alpha$  and  $\beta$ )<sup>2-4</sup>, and were renamed ADAM-1 and -2 after identification of several other homologous genes<sup>5</sup>.

#### Disintegrin-integrin binding

The presence of a disintegrin domain in ADAMs is unique among cell-surface proteins, and can mediate cell-cell and cell-matrix interaction by binding integrins. The major integrin involved in interaction with ADAMs has been identified as integrin  $\alpha 9\beta 1$  which can interact with a conserved  $RX_6DEVF$  sequence in the disintegrin domain<sup>1</sup>, although structural modelling of a snake venom disintegrin-metalloprotease has demonstrated that this motif is shielded by the cysteine-rich domain and may be inaccessible for integrin binding<sup>6</sup>.

Many other integrins are capable of binding the disintegrin domain<sup>7</sup>. The consensus recognition site of disintegrins, first identified as integrin antagonists in snake venom, is dependent on the presence of Arg-Gly-Asp (RGD) or Lys-Gly-Asp (KGD) sequences, but surprisingly only ADAM-15 contains the RGD sequence in its disintegrin domain, and this recognition site enables interaction with a broader range of integrins such as  $\alpha v\beta 3$ ,  $\alpha v\beta 5$  and  $\alpha 5\beta 1$ <sup>8,9</sup>. Other ADAMs (e.g. ADAM-9 and -23) are capable of binding these integrins through interaction with a Glu-Cys-Asp (ECD) sequence in the disintegrin domain, an observation that corroborates the pivotal importance of especially the aspartic acid residue in disintegrin-integrin interaction site<sup>10</sup>.

Although the binding potential of the ADAM proteins to integrins is relatively well characterized, the biological relevance and scope is not yet clear<sup>10</sup>. The only physiological process where the functionality is well documented remains the fertilization process, with the two fertilin-ADAMs forming a hetero-dimer capable of binding integrin  $\alpha 6\beta 1$  on the surface of the egg<sup>11</sup>, but this model is under debate<sup>12</sup>. The fact that in human the fertilin  $\alpha$ /ADAM-1 gene encodes a non-functional protein is a strong indication that this model for ADAM involvement in egg-sperm fusion in mammalia may need to be reconsidered<sup>13</sup>.

Gene deletion experiments have demonstrated an important role for ADAM-2 in fertilization since fertilin  $\beta$  null mice are infertile<sup>14</sup>, but ADAM-2 is not indispensable in the sperm-egg fusion process since sperm from these knockout mice can still fuse with oocytes, albeit at a ~50% decreased fusion rate. The observed sterility of fertilin  $\beta$   $-/-$  mice is postulated to be due to disruption of transit of the sperm through the female reproductive tract<sup>15</sup>, which may be an indication that ADAM function during fertilization has a hitherto unknown mechanism<sup>12</sup>.

#### **Interaction via the cysteine-rich domain**

Besides the disintegrin-integrin interaction, ADAMs are capable of binding several ECM constituents. The cysteine-rich domain may have an important function in binding heparan sulfate proteoglycan such as syndecans, a phenomenon described for ADAM-12<sup>16</sup>. Studies involving the *Xenopus*-specific ADAM-13 have further revealed an important role for the cysteine-rich domain in interaction with fibronectin<sup>17</sup>. An early observation on the structure of metalloprotease-disintegrins revealed the presence of a hydrophobic stretch in the cysteine-rich domain of several ADAMs which shows high sequence similarity to viral fusion peptides<sup>18</sup>, which may implicate a role for the cysteine-rich domain in membrane fusion. This hypothesis has however not been confirmed by experimental data<sup>1</sup>.

#### **Function of the cytoplasmic domain**

The cytoplasmic domain of the individual ADAM proteases is highly variable in length (40-250 amino acids) and sequence, but the majority of ADAMs contain cytoplasmic tails with PxxP motifs, which indicates binding sites for SH3 (Src-homology 3) domain containing proteins<sup>19</sup>. This structural feature enables interaction of the cytoplasmic domain with a large range of intracellular proteins and may be important in regulation of ADAM localization by interaction with cytoskeletal proteins such as  $\alpha$ -actinin-2<sup>20</sup>. ADAM function may also be regulated by interaction of the cytoplasmic tail with intracellular signalling proteins, as demonstrated by the activation of ADAM-9 by phorbol esters via interaction with PKC  $\delta$ <sup>21</sup>.

The cytoplasmic domain of some ADAMs further contains putative phosphorylation sites for serine-threonine or tyrosine kinases, which may be involved in regulation of ADAM function, or supply binding sites for SH2 domain containing proteins<sup>1</sup>.

#### **ADAM metalloprotease function**

Perhaps the best studied function of the ADAM proteins is the catalytic activity exhibited by the metalloprotease domain. The metalloprotease domain is highly homologous with the MMP catalytic domain, but interestingly not all ADAMs contain the consensus HExxHxxGxxH zinc-binding metalloprotease active site (see table 2), which leads to the conclusion that from the 22 human ADAMs only 12 (possibly) may exert endopeptidase activity. Interestingly, in mice an additional subfamily of possibly catalytically active ADAMs (ADAM-24-26, -34 and 36-40) has been described and named testases, referencing the testis-specific expression pattern<sup>22</sup>. In human no orthologues of the testases have been identified to date.

**Table 2:** Overview of the 22 identified human ADAM proteases. (t)MDC: (transmembrane) metalloprotease-like, disintegrin-like, cysteine-rich protein.

‡: although full length ADAM-1 contains a metalloprotease domain with active site sequence, this domain is removed in the mature processed form<sup>1</sup>.

ADAM	Alternative names	MP active site?	Remarks
1	Fertilin $\alpha$ , PH-30 $\alpha$	yes ‡	Non-functional pseudogene Testis specific
2	Fertilin $\beta$ , PH-30 $\beta$ Cancer/testis antigen 15	no	Testis specific
3	Cyritestin, tMDC I	no	Non-functional pseudogene Testis specific
6	tMDC IV	no	Testis specific
7	EAP-1, Sperm maturation-related glycoprotein GP-83	no	
8	Cell surface antigen MS2, CD156a	yes	
9	MDC9, Meltrin $\gamma$ Myeloma cell metalloproteinase	yes	
10	Kuzbanian protein homolog, CD156c	yes	
11	MDC	no	Brain specific
12	Meltrin $\alpha$	yes	
15	Metargidin, MDC-15	yes	
17	TACE, CD156b Snake venom-like protease	yes	
18	tMDC III, ADAM-27	no	Testis specific
19	Meltrin $\beta$ , MADDAM Metalloprotease and disintegrin dendritic antigen marker	yes	
20	None	yes	Testis specific

21	ADAM-31	no	Testis specific
22	MDC 2	no	
28	MDC-L, ADAM-23	yes	
29	Cancer/testis antigen 73	no	Testis specific
30	None	yes	Testis specific
32	None	no	Testis specific
33	none	yes	

The ADAM metalloprotease domain is shielded off by a prodomain in the inactive zymogen in a similar structural conformation as in MMPs involving a cysteine switch mechanism. The primary activation pathway of the ADAM zymogens involves removal of the prodomain by proprotein convertases (PCs) such as furin in the trans-Golgi network as demonstrated by strongly diminished activation of ADAM-9 and -15 in the presence of early-secretory pathway inhibitors brefeldin A and monensin, increased activation of ADAM-10 after overexpression of PC-7 and blocked processing of proADAM-10, -12 and -19 after mutational modification of the furin Rx(R/K)R recognition site (discussed in <sup>1</sup>). Some ADAMs may require autoproteolytic processing for activation, as demonstrated for ADAM-8<sup>23</sup> and -28<sup>24</sup>. Interestingly, the prodomain may act as a chaperone and is required for proper folding of the ADAM protein, and especially the metalloprotease domain. Removal of the prodomain prior to folding yields an inactive form of ADAM-17<sup>25</sup>. For ADAM-10 a similar effect has been observed in cells expressing the protease without the prodomain, an effect that could be reversed by cotransfection of the prodomain<sup>26</sup>.

Although, similar to the MMPs, the catalytically active ADAMs are capable of degradation of ECM proteins such as collagens and fibronectin *in vitro*, the physiological relevance of this observation is not clear. The endopeptidase activity for which the ADAMs are best known is the so-called shedding; liberation of biologically active proteins from their membrane-anchored proforms. This function of the ADAMs was first described simultaneously by two groups in 1997, identifying a disintegrin-metalloprotease as an important contributor to the production of soluble TNF $\alpha$  from the membrane-bound precursor<sup>27,28</sup>. The responsible protease was named TACE (TNF-alpha converting enzyme) and gene-silencing experiments demonstrated a dominant, but not exclusive role for TACE since TNF $\alpha$  production was significantly diminished but not completely shut down in the knock-out mice.

Considering the scope of this thesis only the ADAMs containing the metalloprotease active site will be discussed in further detail.

**ADAM-8**

ADAM-8 (CD156) was first described in 1990 as antigen MS2 in mice<sup>29</sup>, and was later confirmed as a member of the human ADAM family in 1997<sup>30</sup>. ADAM-8 is a transmembrane glycoprotein primarily expressed as a 120 kDa zymogen by immune cells, but is also found in neurons and oligodendrocytes in the central nervous system. The promoter region of the *adam-8* gene contains response elements for lipopolysaccharide (LPS) and cytokines such as interferon  $\gamma$ , interleukin-6 and TNF  $\alpha$ <sup>31</sup>.

ProADAM-8 does not contain the furin cleavage recognition site and depends on autoproteolytic removal of the prodomain for activation, yielding a 90 kDa mature enzyme. The mature cell-surface ADAM-8 may be further processed by autoproteolytic shedding yielding a 60 kDa soluble remnant form of the protein missing the metalloprotease domain<sup>23</sup>. ADAM-8 may also exist as a soluble, catalytically active form which is presumably released from the cell by autoproteolytic shedding<sup>32</sup>. Contrary to the MMP and MT-MMP families, ADAM-8 catalytic activity is not inhibited by any of the four known TIMPs *in vitro*<sup>33</sup>.

The biological function of ADAM-8 is far from clear, but the protein has been associated with neuron-glia interactions in the central nervous system<sup>34</sup>, cell-cell fusion in osteoclast differentiation (an effect presumably mediated by the remnant form)<sup>32</sup> and recent work has shown a possible role in the ovulation process involving hormonal regulation of ADAM-8<sup>35</sup>. ADAM-8 sheddase activity *in vivo* is still rather poorly characterized, but *in vitro* experiments involving cleavage assays of 10-mers containing known recognition sites for ectodomain shedding have revealed multiple possible substrates such as  $\beta$ -amyloid precursor protein (APP), the low affinity IgG (CD16) and IgE (CD23) receptors, L-selectin, P-selectin glycoprotein ligand, transforming growth factor  $\alpha$  (TGF  $\alpha$ ) and TNF  $\alpha$ <sup>36</sup>. ADAM-8 has also been implicated in shedding of the neural adhesion molecule CHL-1 (close homologue of L-1), yielding a biologically active fragment that enhances neurite outgrowth and suppresses neuronal cell death, indicating an important role in the development of the central nervous system<sup>37</sup>.

Knock-out experiments in mice have identified ADAM-8 as a non-essential protein during development since ADAM-8 null mice show no pathological defects<sup>38</sup>. This finding may be explained by a rather large redundancy with other ADAM proteases or by a specific role for ADAM-8 not in development but rather in processes such as inflammation, a hypothesis that seems to be substantiated by the responsiveness of ADAM-8 expression to cytokine stimulation.

ADAM-8 has been associated with development of asthma following the observation that ADAM-8 is highly upregulated in experimental ovalbumine-induced asthma models in mice, showing strong induction in peribronchial and perivascular inflammatory cells and bronchiolar epithelial cells<sup>39</sup>. In an asthma model with transgenic mice producing a soluble form of ADAM-8 a protective effect of sADAM-8 was found, presumably related to suppressed trafficking of leukocyte migration<sup>40</sup>, which may be mediated by shedding of vascular cell adhesion molecule 1 (VCAM-1) by ADAM-8<sup>41</sup>. The amount of clinical evidence linking ADAM-8 to development of pulmonary diseases is limited, but soluble

ADAM-8 and VCAM-1 are increased in BAL fluid of patients suffering from eosinophilic pneumonia<sup>42</sup> and ADAM-8 mRNA is increased in asthma patients with a positive correlation with disease severity<sup>43</sup>.

ADAM-8 is expressed in human neutrophils where it is stored in granules, and is transported to the cell surface upon stimulation. The cell surface exposed ADAM-8 is then rapidly released into the extracellular space by a metalloprotease activity dependent process. These *in vitro* findings have been confirmed in neutrophils retrieved from synovial fluid from patients suffering from rheumatoid arthritis, again indicating a possible role for ADAM-8 activity in inflammatory processes<sup>44</sup>.

ADAM-8 expression is increased in neutrophils that are in contact with endothelial cells, and since L-selectin is a possible substrate ADAM-8, and ADAM-8 colocalizes with L-selectin on the neutrophil surface ADAM-8 mediated L-selectin shedding may play an important role in regulation of neutrophil rolling and trans-endothelial extravasation<sup>44</sup>.

Since ADAM-8 is involved in osteoclast differentiation it is not surprising that several studies have focussed on the role of this protease in the development of pathological bone destruction. ADAM-8 expression is positively associated with tissue destruction in rheumatoid arthritis, and ADAM-8 protein is mainly localized at the edge of the eroded cartilage and bone (the so-called pannus)<sup>45</sup>. A similar induction of ADAM-8 at the edge of healthy and eroded tissue is described in loosening hip replacements<sup>46</sup>.

ADAM-8 has further, as most metzincins, been associated with various malignant processes. In pancreatic duct adenocarcinoma, ADAM-8 expression and protein level is correlated with the invasiveness of the tumour and with reduced survival rate<sup>47</sup>, a phenomenon that may involve tumour hypoxia<sup>48</sup>. The same correlation has been described for primary brain tumours and ADAM-8 activity, as measured by conversion of a peptide substrate, is higher in cells obtained from gliomas with higher invasive potential<sup>49</sup>.

Overexpression of ADAM-8 has also been associated with poor prognosis in lung cancer, with high expression significantly more common in stage IIIb/IV adenocarcinomas compared to lower stages. The soluble isoform of the protein has been suggested as a useful diagnostic serum marker for lung cancer<sup>50</sup>. ADAM-8 overexpression has finally been described in prostate cancer<sup>51</sup> and renal cell carcinoma<sup>52,53</sup>.

### ADAM-9

ADAM-9 or meltrin  $\gamma$  was first identified in 1996 as MDC-9 from breast carcinoma<sup>54</sup> and as myeloma cell metalloproteinase in myeloma<sup>55</sup>. ADAM-9 is ubiquitously produced as a 110 kDa glycosylated zymogen which can be activated in the medial Golgi apparatus by prodomain removal by proprotein convertase activity to the 84 kDa mature form that is found on the cell surface. The cell membrane anchored form can be further truncated by proteolytic processing to a 47 kDa soluble form which contains the metalloprotease domain and retains its catalytic activity as demonstrated by the ability to cleave insulin B-chain *in vitro*. Purified soluble ADAM-9 is further capable of cleaving peptides containing the membrane-proximal cleavage region of  $\beta$ -APP, proTNF $\alpha$ , the p75 TNF receptor and c-kit ligand-1 (KL-1), but not IL-6 receptor, the p55 TNF receptor, transforming growth factor  $\alpha$  and L-selectin<sup>56</sup>. Recombinant ADAM-9 is capable of degradation of fibronectin *in vitro*,

but the physiological relevance is not proven<sup>57</sup>. Like ADAM-8, ADAM-9 activity is not inhibited by any of the four TIMPs<sup>33</sup>.

As with ADAM-8, knockout models have demonstrated that ADAM-9 is not vital for development and survival since ADAM-9 null mice show no major abnormalities<sup>58</sup>. ADAM-9 is capable of processing  $\beta$ -APP, and is widely considered to be one of the three  $\alpha$ -secretases from the ADAM family (next to ADAM-10 and -17)<sup>59</sup>. The ADAM-9 knockout mice do however show no aberrant APP processing, indicating functional compensation by or redundancy with other ADAMs. Similarly, shedding of HB-EGF which is known to be decreased in cells overexpressing a mutant form of ADAM-9<sup>21</sup> is unaffected by ADAM-9 knock-out.

In rat kidney, ADAM-9 is mainly found in the basolateral surface of the tubular cells. The expression in glomerular epithelia is restricted to areas in contact with the underlying basement membrane indicating an important role in cell-cell and cell-matrix interaction<sup>60</sup> which may be mediated by binding of the disintegrin domain to  $\beta$ -1 integrins which colocalize with the renal ADAM-9 distribution<sup>61</sup>.

The soluble isoform of ADAM-9 has been reported as an important player in tumour metastasis in the liver. After shedding from the cell surface of activated hepatic stellate cells, sADAM-9 is hypothesized to bind to  $\alpha 6\beta 4$  and  $\alpha 2\beta 1$  integrins on the surface of carcinoma cells and promote stroma-tumour interaction. Addition of the soluble ADAM-9 to Matrigel invasion assays showed an increased invasive potential in several cancer cell lines. This effect is blocked by the broad-range metalloprotease inhibitor 1,10-phenantroline, indicating a possible role for an active metalloprotease, although it remains unclear whether this is activity of ADAM-9 itself. Histological examination of tumour sections obtained from liver metastases revealed that ADAM-9 expression was highest at the invasive front, and in regions of tumour-stroma contact while the tumour cells and hepatocytes themselves were negative for ADAM-9<sup>62</sup>.

ADAM-9 expression is higher in malignant prostate tumours, and expression in carcinoma cell culture is increased in response to oxidative stress. This effect is likely mediated by an androgen receptor (AR) involving mechanism, since AR-negative prostate cancer cells do not exhibit increased expression after exposure to radical oxygen species (ROS), and preincubation of the cells with the anti-androgen bicalutamide abrogates the higher expression of ADAM-9<sup>63</sup>. By blocking ADAM-9 production apoptotic cell death could be induced in the cultured prostate cancer cells, indicating a role for ADAM-9 in tumour survival<sup>64</sup>. Concurrently, high tumour ADAM-9 expression has been identified as a significant prognostic marker for relapse in prostate cancer<sup>65</sup>.

Since ADAM-9 is a putative  $\alpha$ -secretase of  $\beta$ -APP this protease has gained some interest from the Alzheimer's disease research field (although less than the two other  $\alpha$ -secretases in the ADAM family). Alpha secretases supposedly have a protective effect in the development of Alzheimer's disease, which is amongst others characterized by accumulation of neurotoxic amyloid-beta peptides ( $A\beta$ ) and plaque formation in the brain. These peptide fragments are the result of sequential  $\beta$ - and  $\gamma$ -secretase processing of APP, while  $\alpha$ -secretases have a cleavage site within  $A\beta$  leading to degradation of the neurotoxic peptides. Decreased sheddase activity of the ADAM  $\alpha$ -secretases may therefore play an

important role in the development of the disease<sup>66</sup>. The physiological relevance of ADAM-9 as an  $\alpha$ -secretase has however been debated in recent years, especially after findings that APP cleavage is unaltered in ADAM-9 knockout models, and the consensus seems to point to a minor role of ADAM-9 in APP processing *in vivo*<sup>67,68</sup>.

### ADAM-10

ADAM-10 was first isolated in 1995 as a membrane-bound metalloproteinase capable of cleaving myelin basic protein<sup>69</sup>, later found to be expressed by various cell types and named MADM (mammalian disintegrin-metalloproteinase), and have significant sequence homology with several other earlier discovered mammalian disintegrin-metalloproteinases such as meltrin- $\alpha$  (ADAM-12) and MS2 (ADAM-8) in 1996<sup>70</sup>. MADM/ADAM-10 was discovered to be a mammalian counterpart of the *Drosophila* protein Kuzbanian which plays an important role in neurological development by proteolytic activation of the Notch-receptor and shedding of the Notch receptor ligand delta<sup>71</sup>.

ADAM-10 is produced as an inactive zymogen which is activated by proprotein convertases in the trans-Golgi network to its 56-58 kDa glycosylated mature form<sup>72</sup>. Contrary to most other metzincins, the cysteine switch mechanism which normally links the prodomain to the catalytic centre keeping the enzyme inactive seems to be non-functional in ADAM-10<sup>73</sup>. ADAM-10 catalytic activity is inhibited by TIMP-1 and -3<sup>74</sup>.

ADAM-10 is one of the better characterized ADAMs to date, with many studies focussing on the proteolytic function of this enzyme. ADAM-10 is capable of degradation of type IV collagen *in vitro*<sup>75</sup> and has been identified as a possible sheddase of cell-surface bound proteins such as epidermal growth factor receptor ligands EGF and betacellulin<sup>76</sup>, ephrin-A2<sup>77</sup>, cellular prion precursor protein<sup>78</sup>, chemokines CX3CL1 and CXCL16<sup>79,80</sup> adhesion molecule L1<sup>81</sup> and many others, although some caution has to be taken in interpreting these results since most target substrates have been identified *in vitro*, an many ADAM-10 mediated shedding events in cell lines are validated by incubation with supposedly ADAM-10/ADAM-17 selective hydroxamate-based inhibitors (like TAPI, TNF alpha protease inhibitor) that were later shown to actually be broad-range metzincin inhibitors. ADAM-10 is believed to be primarily involved in the constitutive shedding of cytokines, chemokines and their receptors, while ADAM-17 mediated shedding is more responsive to stimuli<sup>82</sup>. The release of TNF $\alpha$  from cells was thought to be (partially) mediated by ADAM-10 but recent insights have demonstrated that ADAM-10 probably plays a minor role<sup>83</sup>.

The best studied shedding activity mediated by ADAM-10 lies within the Notch signalling pathway. Notch signalling is a highly conserved, ancient pathway that is essential in intercellular contact and cell fate determination in all stages of neural development. The membrane-bound Notch receptor is activated by either membrane-bound ligands delta or serrate/jagged on adjacent cells leading to an intracellular signalling cascade involving a series of proteolytic cleavages (S1-3), internalization of the processed Notch receptor, transport to the nucleus and finally activation of gene transcription. The precise outcome of Notch receptor activation is highly dependent on other extra- and intracellular signals and can lead to inhibition of neuronal cell and oligodendrocyte differentiation, or promotion of differentiation of glial progenitor cells (reviewed in<sup>84</sup>).



Although the role of the invertebrate ADAM-10 homologues Kuzbanian (*Drosophila*) and Sup-17 (*C. elegans*) in Notch signalling is clear<sup>85,86</sup>, involving proteolytic activation of the Notch receptor-ligand complex (stage S2), attempts to identify ADAM-10 as a key player in mammalian Notch signalling have yielded variable results. Some studies point to ADAM-17 as the essential protease for Notch processing, at least by proteolytic cleavage assays using ADAM-17 *in vitro*<sup>87</sup> and cotransfection of DTACE (the *Drosophila* ADAM-17 homologue) in delta-expressing *Drosophila* S2 cells, but ADAM-17 knockout models show little similarity to Notch knockouts while ADAM-10 deficient mice die early in embryogenesis showing major defects in development of the central nervous system and the vascular system with similar histological abnormalities observed in complex Notch deficiency induced in mice<sup>88,89</sup>.

This apparent discrepancy between *in vitro* substrate specificity and biological relevance is also apparent from the unclear role of ADAM-10 as an APP  $\alpha$ -secretase. ADAM-10 is widely expressed in neuron in the CNS<sup>90</sup>, is capable of cleaving APP-derived peptides *in vitro* and overexpression of ADAM-10 increases the release of cleaved APP from HEK 293 cells, while transfection with a non-functional ADAM-10 mutant inhibited APP processing<sup>91</sup>. On the other hand most preserved embryonic fibroblast cell lines obtained from ADAM-10 knockout mice show no decreased or altered APP processing compared to control, although two out of 17 lines do have severe APP processing deficiencies<sup>88</sup>.

Several shedding events possibly mediated by ADAM-10 have been associated with cancer pathology. Proteolytic removal of the extracellular domain neuronal cell adhesion receptor L1-CAM by ADAM-10 promotes metastasis in colon cancer<sup>92</sup>, ADAM10 may be involved in metastasis by shedding of CD44 promoting migration of cancer cells through the ECM by interaction with hyaluronic acid<sup>93</sup> and ADAM-10 mediated shedding of EGF receptor ligands may promote tumour growth. ADAM-10 activity may be associated with poor prognosis in breast cancer by shedding of membrane-anchored HER2 receptor leaving a fragment with constitutive kinase activity leading to ligand independent cell growth and resistance to apoptotic signals<sup>94</sup>.

ADAM-10 proteolytic activity has been associated with a protective effect in inflammatory processes by shedding of the receptor for advance glycation products (RAGE), yielding a soluble form which acts as a decoy receptor for RAGE ligands<sup>95</sup>. ADAM-10 may on the other hand stimulate inflammatory processes in allergic response by shedding of the low affinity IgE receptor CD23 yielding the soluble form that is described to stimulate differentiation of germinal B-cells into plasma cells, cytokine release by monocytes and IgE production by B-cells<sup>96</sup>. ADAM-10 is involved in shedding of FAS ligand resulting in a soluble form which can have both pro- and anti-apoptotic effects, depending on the microenvironment. sFAS-L concentration in serum correlates with progression of inflammatory diseases such as arthritis and colitis indicating a possible role for ADAM-10 activity in these diseases<sup>97,98</sup>.

### **ADAM-12**

ADAM-12 was first described in mouse in 1995 as meltrin  $\alpha$  as a disintegrin-metalloproteinase involved in myoblast fusion<sup>99</sup>. The human homologue was cloned 3 years

later and experiments revealed a peculiarity of this ADAM compared to most other members of the family with respect to the existence of an alternative splicing variant yielding a soluble form of the enzyme (ADAM12-S)<sup>100</sup>. For most other ADAMs soluble forms have been described that are the result of autoproteolytic ectodomain shedding, but for most of these proteins the biological relevance is unclear since many experiments demonstrating these soluble forms have been performed with cell lines expressing recombinant ADAMs. Soluble ADAM-12 has a very similar ectodomain structure compared to the membrane-anchored form but lacks the transmembrane and cytoplasmic domains, but instead possesses a unique 33 amino acid C-terminus. Both the membrane-anchored and the soluble ADAM-12 are expressed as inactive zymogens that are activated by proprotein convertase cleavage of the prodomain<sup>101</sup>. Interestingly, the prodomain remains attached to the activated enzyme after PC activation, giving sADAM-12 a four-leaf clover like structure<sup>102</sup>. ADAM-12 expression is relatively resistant to transcriptional upregulation, only TGF $\beta$  has been reported as an inducer in hepatic stellate cells<sup>103,104</sup>.

Interaction of the disintegrin domain of ADAM-12 and integrins  $\alpha 9\beta 1$  and  $\alpha 7\beta 1$  may be involved in cell differentiation during muscle development<sup>105</sup>. ADAM-12 has been suggested to be involved in modulation of the cytoskeleton by altering  $\beta 1$  integrins on the ECM, which causes reorganisation of the actin-integrin binding between cytoskeleton and ECM<sup>106</sup>. A second mechanism by which ADAM-12 can reorganize the cytoskeleton is mediated by interaction with actinin-1 and -2<sup>20</sup>. ADAM-12 knockout mice show only limited abnormal muscular development, which could be an indication that ADAM-12 function overlaps with and can be compensated by other (ADAM) proteins<sup>107</sup>. Overexpression of ADAM-12 improves muscle regeneration after injury<sup>108</sup>, and induces adipogenesis due to enhanced adipocyte proliferation. ADAM-12 efficient mice are resistant against high-fat diet-induced obesity<sup>109</sup>.

ADAM-12 is capable of degrading several ECM proteins such as fibronectin, type IV collagen and gelatin *in vitro*<sup>110</sup>, but the physiological relevance is probably minor<sup>111</sup>. The membrane-bound isoform of ADAM-12 is reported in ectodomain shedding of several substrates. ADAM-12 is capable of cleaving insulin-like growth factor binding proteins (IGFBP) 3 and 5 *in vitro*<sup>112</sup>, HB-EGF<sup>113</sup>, the oxytocinase placental leucine aminopeptidase<sup>114</sup>, EGF and betacellulin<sup>115</sup> and notch ligand delta-like 1<sup>116</sup>.

ADAM-12 is highly expressed in placenta, and sADAM-12 levels in serum are markedly increased during pregnancy. Interestingly, this rise in serum sADAM-12 is not observed in women carrying trisomy-18 and -21 fetuses, making ADAM-12 a possible prenatal marker for Down syndrome<sup>117</sup>.

ADAM-12 has been associated with various pathological states. As expected from the postulated biological function, ADAM-12 is implicated in development of musculoskeletal disorders. Polymorphisms in ADAM-12 have been associated with osteoarthritis susceptibility<sup>118</sup>, and although ADAM-12 seems to play a role in muscle regeneration, prolonged high expression of ADAM-12 is associated with muscular dystrophy<sup>119</sup>. In heart muscle tissue ADAM-12 is increased in patients with hypertrophic cardiomyopathy, and ADAM-12 may contribute to tissue remodelling observed in heart failure by shedding of HB-EGF<sup>113,120</sup>.

The role of ADAM-12 in cancer has been extensively reviewed<sup>121</sup> and ADAM-12 overexpression is observed in many tumours, ranging from breast and lung cancer to glioblastoma and osteoclastoma. An interesting difference with the majority of MMPs and ADAMs is that in most tumours, the cancerous cells themselves and not the stroma are the main source of ADAM-12 in the tumour<sup>122</sup>. ADAM-12 is often hypothesized to stimulate tumour growth, as demonstrated by a strongly reduced tumour progression in ADAM-12 deficient mice with experimental prostate cancer<sup>123</sup>, but anti-ADAM-12 antibodies have a stimulating effect on breast and gastric carcinoma proliferation *in vitro*<sup>124,125</sup>. The latter effect may be effectuated by ADAM-12 induced apoptosis-resistance in tumour cells<sup>126</sup>. *Adam-12* is a candidate breast cancer gene, with two possibly functional polymorphisms in the metalloprotease and disintegrin domains showing strong association with development of breast cancer<sup>127</sup>.

The existence of a soluble variant makes ADAM-12 a possibly interesting biomarker compared to the membrane-bound ADAMs since sADAM-12 can be found in a functional form in biofluids such as serum and urine. A positive correlation has already been demonstrated between breast cancer progression and urinary level of sADAM-12<sup>110</sup>, and urine levels of patients with bladder cancer are higher than in healthy individuals<sup>128</sup>. ADAM-12 has recently been comprehensively reviewed<sup>129</sup>.

### **ADAM-15**

ADAM-15 was first described in 1996 as metargidin, referring to its RGD-motif in the disintegrin domain unique within the ADAM proteases<sup>130</sup>. ADAM-15 is produced by many cell types as an inactive glycosylated zymogen with the prodomain linked to the catalytic domain via a cysteine switch. The prodomain contains a proprotein convertase recognition site, so the activation process is presumed to involve PC mediated removal of the prodomain, yielding the 85 kDa mature protease.

ADAM-15 deficient mice are viable and fertile, but show reduced neovascularization after hypoxia-induced retinopathy, and growth of implanted melanoma is reduced in null mice compared to wild type, which could indicate inhibited tumour angiogenesis, but histological examination of the tumours revealed no difference in vascularization<sup>131</sup>.

The consensus integrin binding site RGD in disintegrins was first found in snake venom derived disintegrin, but until the discovery of ADAM-15 no similar site was found in the mammalian ADAMs. Since ADAM-15 does contain this sequence it is expected to have a much broader binding selectivity towards integrins, and therefore more possibilities for cellular adhesion to neighbouring cells and the ECM than the other ADAMs, where the interaction is preferentially mediated through RGD independent interaction with integrin  $\alpha 9\beta 1$ <sup>132</sup>. The ADAM-15 disintegrin domain containing the RGD sequence is found to have a strong affinity towards integrin  $\alpha v\beta 3$  after expression as a recombinant fusion protein with glutathione S-transferase<sup>8</sup>. Recombinant ADAM-15 is capable of binding  $\alpha v\beta 3$  on a monocytic cell line, and  $\alpha 5\beta 1$  on a T-cell line<sup>133</sup>. The putative role in intercellular adhesion is confirmed by the observation that overexpression of ADAM-15 leads to increased adhesion in cultured NIH3T3 cells<sup>134</sup>. In concurrence with this result is the observation that overexpression of ADAM-15 inhibits wound healing in monolayers of intestinal epithelial

cells by increasing cellular adhesion and inhibition of migration<sup>135</sup>. RGD-mediated integrin  $\alpha\beta3$  binding has been postulated to inhibit cellular adhesion and motility by blocking integrin  $\alpha\beta3$  so contact with ECM proteins such as vitronectin, as demonstrated by reduced migration of ovarian cancer cells when overexpressing ADAM-15<sup>136</sup>. This anti-invasive function of ADAM-15 was also found in melanoma cells, and is possibly linked to p38 kinase activation<sup>137</sup>.

The pro-adhesive properties of ADAM-15 may provide the protein with a protective or homeostatic function in degenerative diseases such as osteoarthritis. ADAM-15 deficiency accelerates cartilage destruction, while overexpression has a protective effect<sup>138</sup>. The disintegrin domain of ADAM-15 has further been demonstrated to inhibit migration of airway smooth muscle cells through binding to  $\beta1$ -integrins<sup>139</sup>.

Integrin binding mediated by ADAM-15 is a possible player in inflammatory processes such as inflammatory bowel disorder. ADAM-15 expression is higher in diseased area, and histological examination shows binding of ADAM-15 expressing endothelia and crypt epithelia to leukocytes. Interestingly, immunocytochemistry shows mainly the active, mature form to be present in these cells. In regenerating tissue, ADAM-15 positive epithelia cells are in close contact with integrin-positive myofibroblasts<sup>140</sup>.

ADAM-15 may play a role in cardiovascular disease. ADAM-15 is expressed in endothelium, and is capable of binding of platelets by interaction with platelet integrin  $\alpha(\text{IIb})\beta3$ . Platelets are further activated by adhesion to ADAM-15 and thrombus formation, leading to a possible role in thrombotic diseases<sup>141</sup>. ADAM-15 expression in arterial cells is markedly higher in atherosclerotic tissue than in healthy control. ADAM-15-integrin binding may play a modulating role in formation of neo-intima<sup>142</sup>.

Although most interest in biological function of ADAM-15 has been directed towards the integrin binding capacity, the metalloprotease domain does contain the consensus zinc-binding sequence, and is thus expected to be proteolytically active. Purified ADAM-15 is capable of proteolytic degradation of type IV collagen and gelatin<sup>143</sup>. Experiments with recombinant ADAM-15 and a peptide library have demonstrated broad substrate specificity in vitro, with a pattern similar to that of ADAM-8. Transfection experiments in HEK 293 cells exposed to recombinant ADAM-15 did reveal cleavage of the low affinity IgE receptor CD23<sup>144</sup>. Only recently the first report of a possible biologically relevant substrate was published. ADAM-15 was found to be the sheddase responsible for E-cadherin, yielding a soluble form that interacts with, and stabilizes HER2 and HER3 receptors. This finding may be a first clue of a role for ADAM-15 in the development of breast cancer<sup>145</sup>.

#### **ADAM-17**

ADAM-17 or TNF alpha converting enzyme (TACE) is undoubtedly the ADAM that has attracted the most interest in research right from its discovery as the protease responsible for the release of soluble TNF $\alpha$  from cells in 1997<sup>27,28</sup>. The shedding of TNF $\alpha$  had already been described earlier, and was reported to be inhibited by metalloprotease inhibitors<sup>146,147</sup>, but with the identification of ADAM-17 as the principle TNF sheddase the first physiological substrate for ADAM-mediated shedding was discovered.

TACE is constitutively produced by many cell types as an inactive zymogen with a cysteine switch motif, and is activated by prodomain removal by proprotein convertase proteolysis. The prodomain of ADAM-17 appears to be essential for correct folding of the metalloprotease domain in proADAM-17 and may have a chaperone function in transport through the subcellular structures<sup>25</sup>. After activation in the late Golgi apparatus, active ADAM-17 may be sequestered in lipid rafts, an observation supported by the increased processing of TNF after cholesterol depletion and after incubation of ADAM-17 expressing cells with high-density lipoproteins<sup>148,149</sup>.

One interesting observation is that recombinant soluble ADAM-17 is highly sensitive to salt (NaCl), with complete inhibition observed at concentration where no structural or conformational changes in the protein occur. This effect has been attributed to disturbance of the electrostatic interaction between protease and substrate, but the physiological relevance is puzzling. Perhaps this effect is a safety precaution regulating the proteolytic activity of TACE after its shedding from the cell surface, but experimental proof is not available<sup>25</sup>. ADAM-17 activity is inhibited by the endogenous metalloprotease inhibitor TIMP-3, but not by TIMP-1, -2 and -4<sup>150</sup>.

ADAM-17 proteolytic activity is essential during embryonic development. Mice with a targeted deletion lacking the zinc-binding region in the catalytic domain generally die perinatally, an effect that seems independent on decreased TNF processing. The mice exhibit anatomical abnormalities like open eyelids, missing conjunctival sac and attenuated corneas. Surviving mice display lower body weight, epithelial dysgenesis in many tissues, perturbed hair coats caused by disorganization of the hair follicles, and irregular pigmentation<sup>151</sup>.

ADAM-17 (together with the closely related ADAM-10) is not a typical member of the ADAM family when examining the catalytic domain: the catalytic domain is much longer than in most adamalysins, leading to two unique protuberances in the structure of the catalytic pocket. The S'3 pocket is very deep and merges with the S'1 pocket, and the characteristic Ca<sup>2+</sup> binding motif is not found in TACE<sup>152</sup>.

ADAM-17 sheddase activity is a well-studied phenomenon, with the liberation of soluble TNF $\alpha$  being the first substrate. ADAM-17 (as ADAM-10) cleaves proTNF at Ala<sub>76</sub>-Val<sub>77</sub> and is the dominant TNF sheddase, since genetic deletion of the zinc-binding sequence in mice results in monocytes that are incapable of releasing soluble TNF $\alpha$ <sup>27</sup>. Other ADAMs may compensate for decreased ADAM-17 activity as ADAM-9, -10 and -19 are capable of processing of proTNF *in vitro*, but the physiological relevance of this redundancy seems limited, especially under stimulating conditions<sup>83</sup>. ADAM-10 has been postulated to be responsible for the constitutive release of TNF $\alpha$ , while after stimulation ADAM-17 takes over the shedding from the cell surface<sup>82</sup>.

ProTNF $\alpha$  de novo production in monocytes can be induced with LPS but for TNF release (a measure for enhanced ADAM-17 activity) stimulation by phorbol esters is necessary, indicating a PKC $\delta$  mediated mechanism involving phosphorylation of the cytoplasmic tail of TACE leading to activation or translocation of the enzyme<sup>25</sup>. Other studies have found that the cytoplasmic tail is not necessary for phorbol ester induced shedding<sup>153</sup>, again contradicting a possible role for intracellular phosphorylation. The effect of phorbol ester

stimulation on ADAM-17 mediated shedding is intriguing: shedding is induced within minutes after exposure, but prolonged stimulation results in downregulation of ADAM-17 involving internalization of the cell surface bound TACE, a process which appears to be dependent of metalloprotease activity<sup>154</sup>.

Besides TNF $\alpha$ , ADAM-17 is also capable of shedding of soluble TNF receptors p55 and p75 TNF-R<sup>151</sup>. Shedding of p55 TNF-R is inducible by hydrogen peroxide<sup>155</sup>. ADAM-17 is implicated in shedding of L-selectin<sup>151</sup>, and ADAM-17 relocalizes to L-selectin on the neutrophil cell surface after interaction with endothelial E-selectin<sup>156</sup>.

ADAM-17 may function as an  $\alpha$  secretase<sup>157</sup>, but as with ADAM-9 and -10 the physiological relevance and role in development of Alzheimer's disease is under debate. There is some additional evidence identifying ADAM-17 as a relevant  $\alpha$  secretase, since ADAM-17 activation downstream of muscarinic receptor 1 stimulation has been described to decrease the accumulation of neurotoxic A $\beta$  peptides, a hallmark of  $\alpha$  secretase activity<sup>158</sup>. The activation pathway of ADAM-17 via muscarinic receptor activation is also implicated in processing of cellular prion protein<sup>159</sup>.

The *adam17* <sup>$\Delta$ Zn/ $\Delta$ Zn</sup> mouse model bears striking similarity with transforming growth factor  $\alpha$  (TGF $\alpha$ ) deficient phenotype. The role of ADAM-17 in TGF $\alpha$  shedding was confirmed by a strong decrease in soluble TGF $\alpha$  production in the ADAM-17 mutant mice<sup>151</sup>. The anatomical abnormalities in heart valves observed in ADAM-17 deficient mice may also arise from decreased shedding of another EGFR ligand, HB-EGF which is also a possible substrate for ADAM-17<sup>160,161</sup>.

Since TNF $\alpha$  is an important proinflammatory mediator, ADAM-17 activity has been implicated in many diseases involving inflammation such as rheumatoid arthritis, Crohn's disease and inflammatory bowel disorder. An interesting finding is that inhibition of ADAM-17 by conditional knockout has a strong protective effect in endotoxin shock in mice, and significantly reduces mortality. This observation may find use in the clinic where septic shock is still a condition with high mortality<sup>162</sup>.

ADAM-17 mediated TNF processing may be important in atherosclerosis, since atherosclerotic plaques express both ADAM-17 and substrates TNF $\alpha$  and p55 TNF-R. Shedding is stimulated by microparticles, small vesicles that are present in atherosclerotic lesions as a result of cellular apoptosis<sup>163</sup>.

ADAM-17 expression is upregulated in many tumours<sup>164</sup>. ADAM-17 mediated shedding of EGFR ligand TGF $\alpha$  and amphiregulin may be involved in invasion of breast cancer cells, and high expression of both ADAM-17 and TGF  $\alpha$  correlates with poor prognosis<sup>165</sup>. Experiments with renal carcinoma cells have demonstrated ADAM-17 may be an essential factor in tumour formation since cells are unable to form solid tumours and loose their invasive potential after ADAM-17 silencing<sup>166</sup>. ADAM-17 (and -10) may be involved in early steps in the malignant transformation and tumour growth. ADAM-17 is shown to be a major sheddase of the natural killer cell receptor ligand MICA, thereby inhibiting the immunological clearance of transformed cells<sup>167</sup>.

**ADAM-19**

ADAM-19 or meltrin  $\beta$  was first cloned in human in 2000 as a disintegrin-metalloprotease involved in  $1\alpha,25$ -dihydroxy vitamin  $D_3$  induced differentiation in primary monocytes and named MADDAM (metalloprotease and disintegrin dendritic antigen marker). Expression was found to be maintained in monocytes differentiating to dendritic cells, while macrophages do not produce MADDAM<sup>168</sup>. The authors also identified the novel protein as a human homologue of the earlier discovered murine meltrin  $\beta$  (ADAM-19)<sup>99,169</sup>. ADAM-19 is closely related to ADAM-12 (meltrin  $\alpha$ ) and both proteins appear to be involved during development in mesenchymal cells, with ADAM-19 expression observed mainly in areas where peripheral neural cell lineages are formed. ADAM-19 expression is also found in the developing lung, intestine, heart and skeletal muscle, while ADAM-12 gene activity is found mainly in mesenchymal cells giving rise to bone, visceral organs and skeletal muscle<sup>170</sup>. Knockout experiments revealed ADAM-19 to be essential for heart development with ADAM-19 null mice dying perinatally with severe cardiac abnormalities, mainly in the ventricular septum and the heart valves<sup>171</sup>. ADAM-19 may exist as an alternative splice variant missing the disintegrin and metalloprotease domain, dubbed meltrin  $\beta$  mini. This isoform is capable of initiating neural outgrowth in mouse neuronal cells, implicating a possible role for this alternative form in the development of the nervous system<sup>172</sup>.

ADAM-19 is widely expressed in adult mammals, with high expression in bone, lung and heart. ADAM-19 is expressed as an inactive zymogen which is activated by cleavage of either one of two furin-recognition sites locate between the pro- and metalloprotease domain<sup>173</sup>. Indications exist that autoproteolytic truncation of ADAM-19 by cleavage within the cysteine-rich domain is necessary for proteolytic activity<sup>174</sup>.

ADAM-19 is catalytically active and is capable of cleaving  $\alpha 2$  macroglobulin in vitro, but was not able to cleave proteins such as type I and IV collagen, gelatin, casein and laminin, nor was cleavage of tested synthetic fluorogenic MMP and TACE substrates observed<sup>175</sup>. Later studies using a soluble recombinant ADAM-19 revealed a wide substrate specificity, with potential targets for proteolysis including myelin basic protein, insulin B-chain TNF $\alpha$ , TRANCE (TNF related activation induced cytokine) and kit ligand-1, but not TNF receptor-55 and -75 and IL6-receptor. The proteolytic activity of recombinant ADAM-19 is not inhibited by TIMP-1, -2 and -3. Cell-based shedding assays show increased release of TRANCE (TNF related activation induced cytokine) in COS-7 cells overexpressing ADAM-19, and kit ligand-1 shedding seems to be negatively regulated by ADAM-19<sup>176</sup>. The proteolytic activity towards TRANCE, a member of the TNF family is interesting since TRANCE signalling is associated with dendritic cell survival by inhibiting apoptosis<sup>177</sup>.

ADAM-19 has been implicated in shedding of neuregulin (NRG), with a preference for the beta-isoform, with observed shedding of NRG  $\beta 1$  and  $\beta 4$ , but not  $\alpha 2$ . Overexpression of ADAM-19 increases shedding, while shedding is markedly decreased in ADAM-19 deficient mutants. The shedding of NRGs seems in line with the essential role of ADAM-19 in the development of heart and central nervous system<sup>178</sup>. The sheddase activity of ADAM-19 towards NRG has been attributed to presence of the protease in lipid rafts within the neurons, an localization which is not common in the ADAMs which are mostly membrane-anchored<sup>179</sup>. Recently the NRG-processing activity of ADAM-19 has been

demonstrated to occur within the Golgi apparatus, and not the cell surface, again hinting at a unique localization of ADAM-19 mediated proteolysis<sup>180</sup>. The role of ADAM-19 in the developing heart and the shedding of NRG  $\beta$  is not exclusive, and may be compensated by other members of the ADAM family, as demonstrated in a study investigating the role of individual ADAM proteases. Apparently there is significant redundancy between the individual family members, which may complicate interpretation of results of individual knockout models<sup>181</sup>. ADAM-19 has recently been described having  $\alpha$ -secretase activity towards APP, since overexpression increased release of the cleaved APP fragment increased while RNA interference decreased shedding. This may implicate a (partial) role for ADAM-19 in the processing of APP<sup>182</sup>.

High ADAM-19 expression has been associated with invasive potential of primary brain tumours, with detectable proteolytic activity present on tumours with the highest expression<sup>49</sup>. ADAM-19 expression is high in renal cell carcinoma<sup>52</sup> and ADAM-19 expression is significantly higher in patients with chronic allograft nephropathy compared to healthy graft recipients, but is also increased in acute rejection and in non-allograft related diseases. Interestingly, ADAM-19 colocalizes with CD4+ T-cells (T-helper cells) indicating a role in the allograft rejection process<sup>183</sup>. ADAM-19 may be involved in development of fibrotic lesions in kidney disease. Expression is markedly higher in many renal structures in patients compared to healthy adults, and may be related to infiltration of macrophages<sup>184</sup>.

#### **ADAM-20 and -30**

These two proteins are interesting with respect to being the only human testis-specific ADAMs with the metalloprotease active site sequence. Unfortunately, almost no research has been aimed at elucidating the biological roles of these proteases. ADAM-20 was first described in 1998, and has high sequence similarity to the fertilins (ADAM-1 and -2) and surprisingly, ADAM-9<sup>185</sup>. Since mature ADAM-1 loses its metalloprotease domain during processing and is considered non-functional in human, ADAM-20 was hypothesized to be the functional equivalent of ADAM-1. ADAM-30 was cloned in 1999<sup>186</sup>, but the publication describing the cDNA sequence remains the only study published to date. Considering the expression pattern these two ADAMs may be functional homologues of the murine testases, although no evidence for this is published.

#### **ADAM-28**

ADAM-28 was first described in 1999 as eMDC II<sup>187</sup> in human and macaque epididymis, and as MDC-L in human lymphocytes<sup>188</sup>. There is some confusion about the identity of ADAM-23 and ADAM-28, there is some consensus that they correspond to the same protein, but why the annotation ADAM-28 is preferred is unclear. ADAM-23 was described as a disintegrin-metalloprotease (MDC-3) without the zinc-binding catalytic site sequence, and thus probably does not have proteolytic activity<sup>189</sup>, while ADAM-28 does contain the zinc-binding site.

ADAM-28 is expressed as a 115 kDa zymogen that is activated to an 88 kDa mature form that can be detected on the cell surface. A smaller, soluble variant of ADAM-28 has been



observed. Interestingly, ADAM-28 lacks the furin recognition site, and may depend on autolytic activation, or activity of other metalloproteases (MMP-7) for prodomain removal<sup>24,190</sup>.

Purified ADAM-28 has proteolytic activity *in vitro*, and is able to cleave myelin basic protein, but not ECM constituents like collagen I-IV, fibronectin, and laminin. The substrate specificity *in vitro* seems to overlap largely with ADAM-8 and ADAM-15, as demonstrated by screening against a library of synthetic peptides, and ADAM-28 is capable of cleavage of the low affinity IgE receptor CD23<sup>144</sup>. The soluble form of ADAM-28 is capable of *in vitro* cleavage of insulin-like growth factor binding protein-3<sup>190</sup>. ADAM-28 catalytic activity is not inhibited by TIMP-1, and only weakly inhibited by TIMP-2<sup>191</sup>. ADAM-28 can bind integrin  $\alpha 4\beta 1$  by interaction with its disintegrin domain<sup>192,193</sup>.

The expression of ADAM-28 in the epididymis suggests a role in reproduction. The soluble splice variant was found in mouse epididymus, but no functional role could be attributed to this protein in sperm<sup>194</sup>. ADAM-28 may have a function in development of dental tissue, since expression in the developing tooth, and appears to be associated with tooth root hypoplasia in patients<sup>195</sup>. The mechanism behind this biological effect is postulated to be a survival promotion mediated through ADAM-28 induced proliferation of dental papilla mesenchymal cells, while silencing of ADAM-28 results in apoptosis of the dentals mesenchymal cells<sup>196</sup>. Some studies have included ADAM-28 in screening for overexpressed protein in cancer. Expression of ADAM-28 was found to be high in non-small cell lung carcinoma, and correlated with proliferation and metastasis<sup>197</sup>. ADAM-28 expression in breast cancer is also high, and proteolytic activity of ADAM-28 in shedding of IGFBP-3 may contribute to cell proliferation in breast cancer<sup>198</sup>.

### **ADAM-33**

ADAM-33 was first described in 2002 as a novel disintegrin-metalloprotease in mouse and human<sup>199</sup>. ADAM-33 is quite unique within the metzincin family in the fact that within 6 months after its discovery, even before ADAM-33 was well characterized at the protein level, it was already associated with a disease state<sup>200</sup>. ADAM-33 is now widely regarded as an asthma susceptibility gene and many studies have tried to find associations of ADAM33 polymorphisms with asthma and bronchial hyperresponsiveness.

ADAM-33 is widely expressed, except in liver. An interesting structural peculiarity is that ADAM-33 lacks SH3 binding sites in its cytoplasmic domain, which may indicate that the role in intracellular signalling is different than for other ADAMs<sup>199</sup>. ADAM-33 is produced as a 123 kDa glycosylated zymogen, and may be activated by cleavage of one of three putative furin recognition site between the pro- and metalloprotease domain resulting in a 100 kDa mature enzyme that can be detected at the cell surface. ADAM-33 contains the zinc-binding metalloprotease active site, and recombinant ADAM-33 has been demonstrated to possess proteolytic activity and soluble ADAM-33 is capable of cleaving  $\alpha 2$ -macroglobuline<sup>201</sup>. The disintegrin domain of recombinant ADAM-33 has affinity towards integrin  $\alpha 9\beta 1$ , but not  $\alpha 4\beta 1$  and  $\alpha 4\beta 7$  (implicated in ADAM-28 mediated interaction with lymphocytes)<sup>202</sup>, but later studies have revealed a possible mechanism for

inhibition of cell migration by ADAM-33 mediated interaction with integrins  $\alpha 4\beta 1$  and  $\alpha 5\beta 1$ <sup>203</sup>.

An *in vitro* cleavage specificity screening of recombinant ADAM-33 has revealed limited substrate specificity compared to other ADAMs. Only four peptides, based on the putative cleavage sites of kit ligand-1, APP, TRANCE and insulin B-chain were cleaved while broadly recognized sheddase cleavage sites such as in EGF, HB-EGF, proTNF $\alpha$ , p55 and p75 TNF receptors and TGF $\alpha$  were not hydrolyzed by ADAM-33<sup>204</sup>. The cleavage sites of the four recognized peptides are similar to those observed in ADAM-19, an observation already expected from the phylogenetic tree identifying this ADAM and *Xenopus* ADAM-13 as having the highest sequence homology<sup>199</sup>. The catalytic activity is quite different from ADAM-17, and can be explained by marked differences in the catalytic pocket as demonstrated by crystallographic resolution of the structure of both proteins<sup>205</sup>. Cellular shedding of KL-1 was evaluated by cotransfection of full length ADAM-33 into COS-7 cells. After transfection KL-1 shedding was slightly higher, and transfection of a non-functional ADAM-33 mutant slightly decreased KL-1 release. A similar experiment with APP shedding resulted in no increase in soluble  $\alpha$ APP release from the cells, leading to the conclusion that ADAM-33 is not a relevant  $\alpha$  secretase *in vivo*. ADAM-33 activity is inhibited *in vitro* by TIMP-3 albeit at much higher concentration than ADAM-17<sup>204</sup>.

As expected from the genetic association with asthma, most research efforts involving ADAM-33 have been focussed on the biological and pathophysiological function in the lung. Pulmonary ADAM-33 expression has been found in cells of mesenchymal origin like smooth muscle cells and fibroblasts, but expression in epithelia has been subject to debate. Early reports show no expression in airway epithelia, while the consensus now seems to be that epithelia does indeed produce ADAM-33<sup>43,206</sup>, but expression in epithelia is sensitive to gene silencing by hypermethylation of the promoter region<sup>206</sup>. ADAM-33 is found in smooth muscle tissue and airway epithelia while its presence is limited in the immune system, which may be an indication that the role of ADAM-33 lies more in tissue remodelling than in the allergic component of asthma<sup>207</sup>. ADAM-33 may be expressed as a number of isoforms as the result of alternative splicing. Originally ADAM-33 was already described as having a  $\beta$ -isoform as result of a 26 amino acid deletion in the region linking the cysteine-rich region to the EGF-like domain<sup>199</sup>, but a study investigating the production of ADAM-33 isoforms by fibroblasts from a healthy volunteer has revealed a large number of isoforms may be expressed in fibroblasts, with 6 forms missing the metalloprotease domain. The alternative mRNA sequences are partially translated into protein as demonstrated by Western blot with at least 5 specific bands corresponding to protein of smaller molecular size are visualized. Several of these bands in the 50-60 kDa region are hypothesized to correspond to the isoform lacking the metalloprotease domain, although no validation for this hypothesis is supplied<sup>208</sup>. Later investigation of the different splice variant expressed in biopsies of asthmatic lung revealed a similar result showing transcripts corresponding to many isoforms being present, with a minority actually containing the metalloprotease domain region<sup>209</sup>. These findings, combined with the observation that the alternatively spliced ADAM-33 $\beta$  appears to be insensitive to proteolytic activation of the zymogen may indicate a limited functionality for the catalytic domain *in vivo*<sup>210</sup>.

The 55 kDa isoform of ADAM-33 has been detected in BAL fluid of patients, and levels were higher in patients with moderate and severe asthma. The level of ADAM-33 correlated inversely with the measured FEV<sub>1</sub> % predicted, which associates ADAM-33 production in the lung with airway obstruction<sup>211</sup>. Expression of ADAM-33 in airway smooth muscle cells obtained from asthma patients was found to be higher than in healthy control, and the overexpression could be reversed by incubation of the cells with interferon  $\gamma$ <sup>212</sup>. Expression of ADAM-33, like ADAM-8 was found to increase with disease severity in asthma patients<sup>43</sup>.

Knockout experiments in mice have demonstrated that ADAM-33 is not essential for development and survival, ADAM-33 null mice show no morphological abnormalities and are fertile. Interestingly, the knockout mice response in allergen sensitization/challenge experiments is normal compared to wild type and pharmacological bronchoconstriction gives a normal response. These results have demonstrated that, at least in mouse, the role of ADAM-33 in allergic airway disease may be limited, although the model may not correspond perfectly to asthma in human<sup>213</sup>.

The genetic association of ADAM-33 with asthma and bronchial hyperresponsiveness has been extensively studied, with group worldwide attempting to reproduce the association found in 2002 in other populations. Many groups do find polymorphisms that are associated<sup>214-216</sup>, while others fail<sup>217,218</sup>, and there seems to be a rather high heterogeneity in SNPs that appear to be responsible for the association, a trait that is observed more often in population-wide genetic screening. In recent years, several reviews have been published summarizing the state of events<sup>219,220</sup>.

Since the physiological function of ADAM-33 is not clear to date, interpretation of the effect of individual SNPs on protein function are complicated. In theory both dysfunction of the metalloprotease domain (shedding of EGFR ligand and inflammatory cytokines) and the disintegrin domain (cellular adhesion and migration) are possible causes of smooth muscle remodelling observed in asthma. Much evidence points to a minor role of the metalloprotease domain, but most studies have found expression of metalloprotease domain containing ADAM-33 low, while on the protein level no distinction is made. More research on the biological role of ADAM-33 and elucidation of its endogenous substrate (if any) are necessary to explain the genetic association found in many studies.

Although almost all research on ADAM-33 has been focussed on asthma, there appears to be an association of an ADAM-33 polymorphism in the cytoplasmic and development of chronic obstructive pulmonary disease (COPD), with increased airway hyperresponsiveness and higher infiltration of inflammatory cells<sup>221</sup>.

Two recent studies have found an association of ADAM-33 with psoriasis susceptibility, with the second study reproducing two out of three SNPs identified in the first, making a strong case for involvement of ADAM-33 in psoriasis<sup>222,223</sup>.

## References

1. Seals,D.F. & Courtneidge,S.A. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* **17**, 7-30 (2003).
2. Primakoff,P., Hyatt,H. & Tredick-Kline,J. Identification and purification of a sperm surface protein with a potential role in sperm-egg membrane fusion. *J. Cell Biol.* **104**, 141-149 (1987).
3. Blobel,C.P., Myles,D.G., Primakoff,P. & White,J.M. Proteolytic processing of a protein involved in sperm-egg fusion correlates with acquisition of fertilization competence. *J. Cell Biol.* **111**, 69-78 (1990).
4. Blobel,C.P. *et al.* A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. *Nature* **356**, 248-252 (1992).
5. Wolfsberg,T.G. *et al.* ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* **169**, 378-383 (1995).
6. Takeda,S., Igarashi,T., Mori,H. & Araki,S. Crystal structures of VAP1 reveal ADAMs' MDC domain architecture and its unique C-shaped scaffold. *EMBO J.* **25**, 2388-2396 (2006).
7. Eto,K. *et al.* Functional classification of ADAMs based on a conserved motif for binding to integrin alpha 9beta 1: implications for sperm-egg binding and other cell interactions. *J. Biol. Chem.* **277**, 17804-17810 (2002).
8. Zhang,X.P., Kamata,T., Yokoyama,K., Puzon-McLaughlin,W. & Takada,Y. Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin alphavbeta3. *J. Biol. Chem.* **273**, 7345-7350 (1998).
9. Lu,D. *et al.* Integrin binding characteristics of the disintegrin-like domain of ADAM-15. *Thromb. Haemost.* **96**, 642-651 (2006).
10. Lu,X., Lu,D., Scully,M.F. & Kakkar,V.V. Structure-activity relationship studies on ADAM protein-integrin interactions. *Cardiovasc. Hematol. Agents Med. Chem.* **5**, 29-42 (2007).
11. Chen,H. & Sampson,N.S. Mediation of sperm-egg fusion: evidence that mouse egg alpha6beta1 integrin is the receptor for sperm fertilinbeta. *Chem. Biol.* **6**, 1-10 (1999).
12. Rubinstein,E., Ziyat,A., Wolf,J.P., Le Naour,F. & Boucheix,C. The molecular players of sperm-egg fusion in mammals. *Semin. Cell Dev. Biol.* **17**, 254-263 (2006).
13. Jury,J.A., Frayne,J. & Hall,L. The human fertilin alpha gene is non-functional: implications for its proposed role in fertilization. *Biochem. J.* **321 ( Pt 3)**, 577-581 (1997).
14. Cho,C. *et al.* Fertilization defects in sperm from mice lacking fertilin beta. *Science* **281**, 1857-1859 (1998).
15. Tomczuk,M. *et al.* Role of multiple beta1 integrins in cell adhesion to the disintegrin domains of ADAMs 2 and 3. *Exp. Cell Res.* **290**, 68-81 (2003).
16. Iba,K. *et al.* The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to beta1 integrin-dependent cell spreading. *J. Cell Biol.* **149**, 1143-1156 (2000).
17. Gaultier,A., Cousin,H., Darribere,T. & Alfandari,D. ADAM13 disintegrin and cysteine-rich domains bind to the second heparin-binding domain of fibronectin. *Journal of Biological Chemistry* **277**, 23336-23344 (2002).
18. Blobel,C.P. & White,J.M. Structure, function and evolutionary relationship of proteins containing a disintegrin domain. *Curr. Opin. Cell Biol.* **4**, 760-765 (1992).
19. Kang,Q., Cao,Y. & Zolkiewska,A. Metalloprotease-disintegrin ADAM 12 binds to the SH3 domain of Src and activates Src tyrosine kinase in C2C12 cells. *Biochem. J.* **352 Pt 3**, 883-892 (2000).

20. Galliano, M.F. *et al.* Binding of ADAM12, a marker of skeletal muscle regeneration, to the muscle-specific actin-binding protein, alpha-actinin-2, is required for myoblast fusion. *J. Biol. Chem.* **275**, 13933-13939 (2000).
21. Izumi, Y. *et al.* A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J.* **17**, 7260-7272 (1998).
22. Zhu, G.Z., Lin, Y., Myles, D.G. & Primakoff, P. Identification of four novel ADAMs with potential roles in spermatogenesis and fertilization. *Gene* **234**, 227-237 (1999).
23. Schlomann, U. *et al.* The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. *J. Biol. Chem.* **277**, 48210-48219 (2002).
24. Howard, L., Maciewicz, R.A. & Blobel, C.P. Cloning and characterization of ADAM28: evidence for autocatalytic pro-domain removal and for cell surface localization of mature ADAM28. *Biochem. J.* **348 Pt 1**, 21-27 (2000).
25. Milla, M.E. *et al.* Specific sequence elements are required for the expression of functional tumor necrosis factor-alpha-converting enzyme (TACE). *J. Biol. Chem.* **274**, 30563-30570 (1999).
26. Anders, A., Gilbert, S., Garten, W., Postina, R. & Fahrenholz, F. Regulation of the alpha-secretase ADAM10 by its prodomain and proprotein convertases. *FASEB J.* **15**, 1837-1839 (2001).
27. Black, R.A. *et al.* A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* **385**, 729-733 (1997).
28. Moss, M.L. *et al.* Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature* **385**, 733-736 (1997).
29. Yoshida, S., Setoguchi, M., Higuchi, Y., Akizuki, S. & Yamamoto, S. Molecular cloning of cDNA encoding MS2 antigen, a novel cell surface antigen strongly expressed in murine monocytic lineage. *Int. Immunol.* **2**, 585-591 (1990).
30. Yoshiyama, K., Higuchi, Y., Kataoka, M., Matsuura, K. & Yamamoto, S. CD156 (human ADAM8): expression, primary amino acid sequence, and gene location. *Genomics* **41**, 56-62 (1997).
31. Kataoka, M. *et al.* Structure of the murine CD156 gene, characterization of its promoter, and chromosomal location. *J. Biol. Chem.* **272**, 18209-18215 (1997).
32. Choi, S.J., Han, J.H. & Roodman, G.D. ADAM8: a novel osteoclast stimulating factor. *J. Bone Miner. Res.* **16**, 814-822 (2001).
33. Amour, A. *et al.* The enzymatic activity of ADAM8 and ADAM9 is not regulated by TIMPs. *FEBS Lett.* **524**, 154-158 (2002).
34. Schlomann, U., Rathke-Hartlieb, S., Yamamoto, S., Jockusch, H. & Bartsch, J.W. Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): implications for neuron-glia interactions during neurodegeneration. *J. Neurosci.* **20**, 7964-7971 (2000).
35. Sriraman, V. *et al.* Regulated expression of ADAM8 (a disintegrin and metalloprotease domain 8) in the mouse ovary: evidence for a regulatory role of luteinizing hormone, progesterone receptor, and epidermal growth factor-like growth factors. *Biol. Reprod.* **78**, 1038-1048 (2008).
36. Naus, S. *et al.* Identification of candidate substrates for ectodomain shedding by the metalloprotease-disintegrin ADAM8. *Biol. Chem.* **387**, 337-346 (2006).
37. Naus, S. *et al.* Ectodomain shedding of the neural recognition molecule CHL1 by the metalloprotease-disintegrin ADAM8 promotes neurite outgrowth and suppresses neuronal cell death. *J. Biol. Chem.* **279**, 16083-16090 (2004).
38. Kelly, K. *et al.* Metalloprotease-disintegrin ADAM8: expression analysis and targeted deletion in mice. *Dev. Dyn.* **232**, 221-231 (2005).
39. King, N.E. *et al.* Expression and regulation of a disintegrin and metalloproteinase (ADAM) 8 in experimental asthma. *Am. J. Respir. Cell Mol. Biol.* **31**, 257-265 (2004).

40. Higuchi, Y., Yasui, A., Matsuura, K. & Yamamoto, S. CD156 transgenic mice. Different responses between inflammatory types. *Pathobiology* **70**, 47-54 (2002).
41. Matsuno, O. *et al.* Role of ADAM8 in experimental asthma. *Immunol. Lett.* **102**, 67-73 (2006).
42. Matsuno, O. *et al.* Elevated soluble ADAM8 in bronchoalveolar lavage fluid in patients with eosinophilic pneumonia. *Int. Arch. Allergy Immunol.* **142**, 285-290 (2007).
43. Foley, S.C. *et al.* Increased expression of ADAM33 and ADAM8 with disease progression in asthma. *J. Allergy Clin. Immunol.* **119**, 863-871 (2007).
44. Gomez-Gavero, M. *et al.* Expression and regulation of the metalloproteinase ADAM-8 during human neutrophil pathophysiological activation and its catalytic activity on L-selectin shedding. *J. Immunol.* **178**, 8053-8063 (2007).
45. Ainola, M. *et al.* Involvement of ADAM8 in osteoclastogenesis and pathological bone destruction. *Ann. Rheum. Dis.* (2008).
46. Mandelin, J. *et al.* Increased expression of a novel osteoclast-stimulating factor, ADAM8, in interface tissue around loosened hip prostheses. *J. Rheumatol.* **30**, 2033-2038 (2003).
47. Valkovskaya, N. *et al.* ADAM8 expression is associated with increased invasiveness and reduced patient survival in pancreatic cancer. *J. Cell Mol. Med.* **11**, 1162-1174 (2007).
48. Valkovskaya, N.V. Hypoxia-dependent expression of ADAM8 in human pancreatic cancer cell lines. *Exp. Oncol.* **30**, 129-132 (2008).
49. Wildeboer, D., Naus, S., Amy Sang, Q.X., Bartsch, J.W. & Pagenstecher, A. Metalloproteinase disintegrins ADAM8 and ADAM19 are highly regulated in human primary brain tumors and their expression levels and activities are associated with invasiveness. *J. Neuropathol. Exp. Neurol.* **65**, 516-527 (2006).
50. Ishikawa, N. *et al.* ADAM8 as a novel serological and histochemical marker for lung cancer. *Clin. Cancer Res.* **10**, 8363-8370 (2004).
51. Fritzsche, F.R. *et al.* ADAM8 expression in prostate cancer is associated with parameters of unfavorable prognosis. *Virchows Arch.* **449**, 628-636 (2006).
52. Roemer, A. *et al.* The membrane proteases adams and hepsin are differentially expressed in renal cell carcinoma. Are they potential tumor markers? *J. Urol.* **172**, 2162-2166 (2004).
53. Roemer, A. *et al.* Increased mRNA expression of ADAMs in renal cell carcinoma and their association with clinical outcome. *Oncol. Rep.* **11**, 529-536 (2004).
54. Weskamp, G., Kratzschmar, J., Reid, M.S. & Blobel, C.P. MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. *J. Cell Biol.* **132**, 717-726 (1996).
55. McKie, N. *et al.* Cloning of a novel membrane-linked metalloproteinase from human myeloma cells. *Biochem. J.* **318** ( Pt 2), 459-462 (1996).
56. Roghani, M. *et al.* Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity. *J. Biol. Chem.* **274**, 3531-3540 (1999).
57. Schwettmann, L. & Tschesche, H. Cloning and expression in *Pichia pastoris* of metalloprotease domain of ADAM 9 catalytically active against fibronectin. *Protein Expr. Purif.* **21**, 65-70 (2001).
58. Weskamp, G. *et al.* Mice lacking the metalloprotease-disintegrin MDC9 (ADAM9) have no evident major abnormalities during development or adult life. *Mol. Cell Biol.* **22**, 1537-1544 (2002).
59. Asai, M. *et al.* Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase. *Biochem. Biophys. Res. Commun.* **301**, 231-235 (2003).
60. Mahimkar, R.M., Baricos, W.H., Visaya, O., Pollock, A.S. & Lovett, D.H. Identification, cellular distribution and potential function of the metalloprotease-disintegrin MDC9 in the kidney. *J. Am. Soc. Nephrol.* **11**, 595-603 (2000).
61. Mahimkar, R.M., Visaya, O., Pollock, A.S. & Lovett, D.H. The disintegrin domain of ADAM9: a ligand for multiple beta1 renal integrins. *Biochem. J.* **385**, 461-468 (2005).

62. Mazzocca,A. *et al.* A secreted form of ADAM9 promotes carcinoma invasion through tumor-stromal interactions. *Cancer Res.* **65**, 4728-4738 (2005).
63. Shigemura,K. *et al.* Reactive oxygen species mediate androgen receptor- and serum starvation-elicited downstream signaling of ADAM9 expression in human prostate cancer cells. *Prostate* **67**, 722-731 (2007).
64. Sung,S.Y. *et al.* Oxidative stress induces ADAM9 protein expression in human prostate cancer cells. *Cancer Res.* **66**, 9519-9526 (2006).
65. Fritzsche,F.R. *et al.* ADAM9 Expression is a Significant and Independent Prognostic Marker of PSA Relapse in Prostate Cancer. *Eur. Urol.* (2007).
66. Fahrenholz,F. & Postina,R. Alpha-secretase activation--an approach to Alzheimer's disease therapy. *Neurodegener. Dis.* **3**, 255-261 (2006).
67. Deuss,M., Reiss,K. & Hartmann,D. Part-time alpha-secretases: the functional biology of ADAM 9, 10 and 17. *Curr. Alzheimer Res.* **5**, 187-201 (2008).
68. Postina,R. A closer look at alpha-secretase. *Curr. Alzheimer Res.* **5**, 179-186 (2008).
69. Howard,L. & Glynn,P. Membrane-associated metalloproteinase recognized by characteristic cleavage of myelin basic protein: assay and isolation. *Methods Enzymol.* **248**, 388-395 (1995).
70. Howard,L., Lu,X., Mitchell,S., Griffiths,S. & Glynn,P. Molecular cloning of MADM: a catalytically active mammalian disintegrin-metalloprotease expressed in various cell types. *Biochem. J.* **317 ( Pt 1)**, 45-50 (1996).
71. Rooke,J., Pan,D., Xu,T. & Rubin,G.M. KUZ, a conserved metalloprotease-disintegrin protein with two roles in Drosophila neurogenesis. *Science* **273**, 1227-1231 (1996).
72. Dallas,D.J. *et al.* Localization of ADAM10 and Notch receptors in bone. *Bone* **25**, 9-15 (1999).
73. Moss,M.L. *et al.* The ADAM10 prodomain is a specific inhibitor of ADAM10 proteolytic activity and inhibits cellular shedding events. *J. Biol. Chem.* **282**, 35712-35721 (2007).
74. Rapti,M. *et al.* The isolated N-terminal domains of TIMP-1 and TIMP-3 are insufficient for ADAM10 inhibition. *Biochem. J.* **411**, 433-439 (2008).
75. Millichip,M.I., Dallas,D.J., Wu,E., Dale,S. & McKie,N. The metallo-disintegrin ADAM10 (MADM) from bovine kidney has type IV collagenase activity in vitro. *Biochem. Biophys. Res. Commun.* **245**, 594-598 (1998).
76. Sahin,U. *et al.* Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J. Cell Biol.* **164**, 769-779 (2004).
77. Janes,P.W. *et al.* Adam meets Eph: an ADAM substrate recognition module acts as a molecular switch for ephrin cleavage in trans. *Cell* **123**, 291-304 (2005).
78. Vincent,B. *et al.* The disintegrins ADAM10 and TACE contribute to the constitutive and phorbol ester-regulated normal cleavage of the cellular prion protein. *J. Biol. Chem.* **276**, 37743-37746 (2001).
79. Abel,S. *et al.* The transmembrane CXC-chemokine ligand 16 is induced by IFN-gamma and TNF-alpha and shed by the activity of the disintegrin-like metalloproteinase ADAM10. *J. Immunol.* **172**, 6362-6372 (2004).
80. Hundhausen,C. *et al.* The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion. *Blood* **102**, 1186-1195 (2003).
81. Gutwein,P. *et al.* ADAM10-mediated cleavage of L1 adhesion molecule at the cell surface and in released membrane vesicles. *FASEB J.* **17**, 292-294 (2003).
82. Ludwig,A. *et al.* Metalloproteinase inhibitors for the disintegrin-like metalloproteinases ADAM10 and ADAM17 that differentially block constitutive and phorbol ester-inducible shedding of cell surface molecules. *Comb. Chem. High Throughput. Screen.* **8**, 161-171 (2005).
83. Zheng,Y., Saftig,P., Hartmann,D. & Blobel,C. Evaluation of the contribution of different ADAMs to tumor necrosis factor alpha (TNFalpha) shedding and of the function of the TNFalpha ectodomain in

- ensuring selective stimulated shedding by the TNFalpha convertase (TACE/ADAM17). *J. Biol. Chem.* **279**, 42898-42906 (2004).
84. Louvi,A. & Artavanis-Tsakonas,S. Notch signalling in vertebrate neural development. *Nat. Rev. Neurosci.* **7**, 93-102 (2006).
  85. Sotillos,S., Roch,F. & Campuzano,S. The metalloprotease-disintegrin Kuzbanian participates in Notch activation during growth and patterning of Drosophila imaginal discs. *Development* **124**, 4769-4779 (1997).
  86. Wen,C., Metzstein,M.M. & Greenwald,I. SUP-17, a Caenorhabditis elegans ADAM protein related to Drosophila KUZBANIAN, and its role in LIN-12/NOTCH signalling. *Development* **124**, 4759-4767 (1997).
  87. Brou,C. *et al.* A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol. Cell* **5**, 207-216 (2000).
  88. Hartmann,D. *et al.* The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Hum. Mol. Genet.* **11**, 2615-2624 (2002).
  89. Krebs,L.T. *et al.* Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev.* **14**, 1343-1352 (2000).
  90. Karkkainen,I., Rybnikova,E., Pelto-Huikko,M. & Huovila,A.P. Metalloprotease-disintegrin (ADAM) genes are widely and differentially expressed in the adult CNS. *Mol. Cell Neurosci.* **15**, 547-560 (2000).
  91. Lammich,S. *et al.* Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3922-3927 (1999).
  92. Gavert,N. *et al.* Expression of L1-CAM and ADAM10 in human colon cancer cells induces metastasis. *Cancer Res.* **67**, 7703-7712 (2007).
  93. Nagano,O. *et al.* Cell-matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca(2+) influx and PKC activation. *J. Cell Biol.* **165**, 893-902 (2004).
  94. Liu,P.C. *et al.* Identification of ADAM10 as a major source of HER2 ectodomain sheddase activity in HER2 overexpressing breast cancer cells. *Cancer Biol. Ther.* **5**, 657-664 (2006).
  95. Raucci,A. *et al.* A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). *FASEB J.* (2008).
  96. Lemieux,G.A. *et al.* The low affinity IgE receptor (CD23) is cleaved by the metalloproteinase ADAM10. *J. Biol. Chem.* **282**, 14836-14844 (2007).
  97. Tanaka,M., Itai,T., Adachi,M. & Nagata,S. Downregulation of Fas ligand by shedding. *Nat. Med.* **4**, 31-36 (1998).
  98. Schulte,M. *et al.* ADAM10 regulates FasL cell surface expression and modulates FasL-induced cytotoxicity and activation-induced cell death. *Cell Death. Differ.* **14**, 1040-1049 (2007).
  99. Yagami-Hiromasa,T. *et al.* A metalloprotease-disintegrin participating in myoblast fusion. *Nature* **377**, 652-656 (1995).
  100. Gilpin,B.J. *et al.* A novel, secreted form of human ADAM 12 (meltrin alpha) provokes myogenesis in vivo. *J. Biol. Chem.* **273**, 157-166 (1998).
  101. Loechel,F., Gilpin,B.J., Engvall,E., Albrechtsen,R. & Wewer,U.M. Human ADAM 12 (meltrin alpha) is an active metalloprotease. *J. Biol. Chem.* **273**, 16993-16997 (1998).
  102. Wewer,U.M. *et al.* ADAM12 is a four-leafed clover: the excised prodomain remains bound to the mature enzyme. *J. Biol. Chem.* **281**, 9418-9422 (2006).
  103. Le Pabic,H. *et al.* ADAM12 in human liver cancers: TGF-beta-regulated expression in stellate cells is associated with matrix remodeling. *Hepatology* **37**, 1056-1066 (2003).



104. Le Pabic,H. *et al.* Involvement of the serine/threonine p70S6 kinase in TGF-beta1-induced ADAM12 expression in cultured human hepatic stellate cells. *J. Hepatol.* **43**, 1038-1044 (2005).
105. Lafuste,P. *et al.* ADAM12 and alpha9beta1 integrin are instrumental in human myogenic cell differentiation. *Mol. Biol. Cell* **16**, 861-870 (2005).
106. Kawaguchi,N. *et al.* ADAM12 induces actin cytoskeleton and extracellular matrix reorganization during early adipocyte differentiation by regulating beta1 integrin function. *J. Cell Sci.* **116**, 3893-3904 (2003).
107. Kurisaki,T. *et al.* Phenotypic analysis of Meltrin alpha (ADAM12)-deficient mice: involvement of Meltrin alpha in adipogenesis and myogenesis. *Mol. Cell Biol.* **23**, 55-61 (2003).
108. Kronqvist,P. *et al.* ADAM12 alleviates the skeletal muscle pathology in mdx dystrophic mice. *Am. J. Pathol.* **161**, 1535-1540 (2002).
109. Masaki,M., Kurisaki,T., Shirakawa,K. & Sehara-Fujisawa,A. Role of meltrin {alpha} (ADAM12) in obesity induced by high- fat diet. *Endocrinology* **146**, 1752-1763 (2005).
110. Roy,R., Wewer,U.M., Zurakowski,D., Pories,S.E. & Moses,M.A. ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage. *J. Biol. Chem.* **279**, 51323-51330 (2004).
111. Jacobsen,J. *et al.* Catalytic properties of ADAM12 and its domain deletion mutants. *Biochemistry* **47**, 537-547 (2008).
112. Loechel,F., Fox,J.W., Murphy,G., Albrechtsen,R. & Wewer,U.M. ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. *Biochem. Biophys. Res. Commun.* **278**, 511-515 (2000).
113. Asakura,M. *et al.* Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat. Med.* **8**, 35-40 (2002).
114. Ito,N. *et al.* ADAMs, a disintegrin and metalloproteinases, mediate shedding of oxytocinase. *Biochem. Biophys. Res. Commun.* **314**, 1008-1013 (2004).
115. Horiuchi,K. *et al.* Substrate selectivity of epidermal growth factor-receptor ligand sheddases and their regulation by phorbol esters and calcium influx. *Mol. Biol. Cell* **18**, 176-188 (2007).
116. Dyczynska,E. *et al.* Proteolytic processing of delta-like 1 by ADAM proteases. *J. Biol. Chem.* **282**, 436-444 (2007).
117. Laigaard,J. *et al.* ADAM 12 as a first-trimester maternal serum marker in screening for Down syndrome. *Prenat. Diagn.* **26**, 973-979 (2006).
118. Valdes,A.M. *et al.* Association study of candidate genes for the prevalence and progression of knee osteoarthritis. *Arthritis Rheum.* **50**, 2497-2507 (2004).
119. Jorgensen,L.H., Jensen,C.H., Wewer,U.M. & Schroder,H.D. Transgenic overexpression of ADAM12 suppresses muscle regeneration and aggravates dystrophy in aged mdx mice. *Am. J. Pathol.* **171**, 1599-1607 (2007).
120. Fedak,P.W. *et al.* Altered expression of disintegrin metalloproteinases and their inhibitor in human dilated cardiomyopathy. *Circulation* **113**, 238-245 (2006).
121. Wewer,U.M., Engvall,E. & Albrechtsen,R. The ADAM family of proteases, proteases in biology and disease. hooper,N.M. & lendeckel,U. (eds.), pp. 123-146 (Springer, The Netherlands,2005).
122. Iba,K., Albrechtsen,R., Gilpin,B.J., Loechel,F. & Wewer,U.M. Cysteine-rich domain of human ADAM 12 (meltrin alpha) supports tumor cell adhesion. *Am. J. Pathol.* **154**, 1489-1501 (1999).
123. Peduto,L. *et al.* ADAM12 is highly expressed in carcinoma-associated stroma and is required for mouse prostate tumor progression. *Oncogene* **25**, 5462-5466 (2006).
124. Carl-McGrath,S., Lendeckel,U., Ebert,M., Roessner,A. & Rocken,C. The disintegrin-metalloproteinases ADAM9, ADAM12, and ADAM15 are upregulated in gastric cancer. *Int. J. Oncol.* **26**, 17-24 (2005).
125. Lendeckel,U. *et al.* Increased expression of ADAM family members in human breast cancer and breast cancer cell lines. *J. Cancer Res. Clin. Oncol.* **131**, 41-48 (2005).

126. Kveiborg,M. *et al.* A role for ADAM12 in breast tumor progression and stromal cell apoptosis. *Cancer Res.* **65**, 4754-4761 (2005).
127. Sjoblom,T. *et al.* The consensus coding sequences of human breast and colorectal cancers. *Science* **314**, 268-274 (2006).
128. Frohlich,C. *et al.* Molecular profiling of ADAM12 in human bladder cancer. *Clin. Cancer Res.* **12**, 7359-7368 (2006).
129. Kveiborg,M., Albrechtsen,R., Couchman,J.R. & Wewer,U.M. Cellular roles of ADAM12 in health and disease. *Int. J. Biochem. Cell Biol.* **40**, 1685-1702 (2008).
130. Kratzschmar,J., Lum,L. & Blobel,C.P. Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD integrin binding sequence. *J. Biol. Chem.* **271**, 4593-4596 (1996).
131. Horiuchi,K. *et al.* Potential role for ADAM15 in pathological neovascularization in mice. *Mol. Cell Biol.* **23**, 5614-5624 (2003).
132. Eto,K. *et al.* RGD-independent binding of integrin alpha9beta1 to the ADAM-12 and -15 disintegrin domains mediates cell-cell interaction. *J. Biol. Chem.* **275**, 34922-34930 (2000).
133. Nath,D. *et al.* Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells. *J. Cell Sci.* **112 ( Pt 4)**, 579-587 (1999).
134. Herren,B. *et al.* ADAM15 overexpression in NIH3T3 cells enhances cell-cell interactions. *Exp. Cell Res.* **271**, 152-160 (2001).
135. Charrier,L. *et al.* ADAM-15 inhibits wound healing in human intestinal epithelial cell monolayers. *Am. J. Physiol Gastrointest. Liver Physiol* **288**, G346-G353 (2005).
136. Beck,V. *et al.* ADAM15 decreases integrin alphavbeta3/vitronectin-mediated ovarian cancer cell adhesion and motility in an RGD-dependent fashion. *Int. J. Biochem. Cell Biol.* **37**, 590-603 (2005).
137. Wu,J., Zhang,L., Ma,X., Zhang,X. & Jin,J. Screening cellular proteins involved in the anti-proliferative effect of the ADAM15 disintegrin domain in murine melanoma cells. *Oncol. Rep.* **20**, 669-675 (2008).
138. Bohm,B.B. *et al.* Homeostatic effects of the metalloproteinase disintegrin ADAM15 in degenerative cartilage remodeling. *Arthritis Rheum.* **52**, 1100-1109 (2005).
139. Lu,D. *et al.* Inhibition of airway smooth muscle adhesion and migration by the disintegrin domain of ADAM-15. *Am. J. Respir. Cell Mol. Biol.* **37**, 494-500 (2007).
140. Mosnier,J.F. *et al.* ADAM15 upregulation and interaction with multiple binding partners in inflammatory bowel disease. *Lab Invest* **86**, 1064-1073 (2006).
141. Langer,H., May,A.E., Bultmann,A. & Gawaz,M. ADAM 15 is an adhesion receptor for platelet GPIIb-IIIa and induces platelet activation. *Thromb. Haemost.* **94**, 555-561 (2005).
142. Al Fakhri,N. *et al.* Increased expression of disintegrin-metalloproteinases ADAM-15 and ADAM-9 following upregulation of integrins alpha5beta1 and alphavbeta3 in atherosclerosis. *J. Cell Biochem.* **89**, 808-823 (2003).
143. Martin,J., Eynstone,L.V., Davies,M., Williams,J.D. & Steadman,R. The role of ADAM 15 in glomerular mesangial cell migration. *J. Biol. Chem.* **277**, 33683-33689 (2002).
144. Fourie,A.M., Coles,F., Moreno,V. & Karlsson,L. Catalytic activity of ADAM8, ADAM15, and MDC-L (ADAM28) on synthetic peptide substrates and in ectodomain cleavage of CD23. *J. Biol. Chem.* **278**, 30469-30477 (2003).
145. Najy,A.J., Day,K.C. & Day,M.L. The ectodomain shedding of E-cadherin by ADAM15 supports ErbB receptor activation. *J. Biol. Chem.* **283**, 18393-18401 (2008).
146. McGeehan,G.M. *et al.* Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor. *Nature* **370**, 558-561 (1994).
147. Gearing,A.J. *et al.* Processing of tumour necrosis factor-alpha precursor by metalloproteinases. *Nature* **370**, 555-557 (1994).
148. Tellier,E. *et al.* The shedding activity of ADAM17 is sequestered in lipid rafts. *Exp. Cell Res.* **312**, 3969-3980 (2006).

149. Tellier,E. *et al.* HDLs activate ADAM17-dependent shedding. *J. Cell Physiol* **214**, 687-693 (2008).
150. Amour,A. *et al.* TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3. *FEBS Lett.* **435**, 39-44 (1998).
151. Peschon,J.J. *et al.* An essential role for ectodomain shedding in mammalian development. *Science* **282**, 1281-1284 (1998).
152. Maskos,K. *et al.* Crystal structure of the catalytic domain of human tumor necrosis factor-alpha-converting enzyme. *Proc. Natl. Acad. Sci. U. S. A* **95**, 3408-3412 (1998).
153. Reddy,P. *et al.* Functional analysis of the domain structure of tumor necrosis factor-alpha converting enzyme. *J. Biol. Chem.* **275**, 14608-14614 (2000).
154. Doedens,J.R. & Black,R.A. Stimulation-induced down-regulation of tumor necrosis factor-alpha converting enzyme. *J. Biol. Chem.* **275**, 14598-14607 (2000).
155. Hino,T. *et al.* Hydrogen peroxide enhances shedding of type I soluble tumor necrosis factor receptor from pulmonary epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **20**, 122-128 (1999).
156. Schaff,U., Mattila,P.E., Simon,S.I. & Walcheck,B. Neutrophil adhesion to E-selectin under shear promotes the redistribution and co-clustering of ADAM17 and its proteolytic substrate L-selectin. *J. Leukoc. Biol.* **83**, 99-105 (2008).
157. Buxbaum,J.D. *et al.* Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem.* **273**, 27765-27767 (1998).
158. Fisher,A. M1 muscarinic agonists target major hallmarks of Alzheimer's disease--the pivotal role of brain M1 receptors. *Neurodegener. Dis.* **5**, 237-240 (2008).
159. Alfa,C.M. *et al.* M1 and M3 muscarinic receptors control physiological processing of cellular prion by modulating ADAM17 phosphorylation and activity. *J. Neurosci.* **27**, 4083-4092 (2007).
160. Jackson,L.F. *et al.* Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. *EMBO J.* **22**, 2704-2716 (2003).
161. Lee,D.C. *et al.* TACE/ADAM17 processing of EGFR ligands indicates a role as a physiological convertase. *Ann. N. Y. Acad. Sci.* **995**, 22-38 (2003).
162. Horiuchi,K. *et al.* Cutting edge: TNF-alpha-converting enzyme (TACE/ADAM17) inactivation in mouse myeloid cells prevents lethality from endotoxin shock. *J. Immunol.* **179**, 2686-2689 (2007).
163. Canault,M. *et al.* Microparticles of human atherosclerotic plaques enhance the shedding of the tumor necrosis factor-alpha converting enzyme/ADAM17 substrates, tumor necrosis factor and tumor necrosis factor receptor-1. *Am. J. Pathol.* **171**, 1713-1723 (2007).
164. Mochizuki,S. & Okada,Y. ADAMs in cancer cell proliferation and progression. *Cancer Sci.* **98**, 621-628 (2007).
165. Kenny,P.A. & Bissell,M.J. Targeting TACE-dependent EGFR ligand shedding in breast cancer. *J. Clin. Invest* **117**, 337-345 (2007).
166. Franovic,A. *et al.* Multiple acquired renal carcinoma tumor capabilities abolished upon silencing of ADAM17. *Cancer Res.* **66**, 8083-8090 (2006).
167. Waldhauer,I. *et al.* Tumor-associated MICA is shed by ADAM proteases. *Cancer Res.* **68**, 6368-6376 (2008).
168. Fritsche,J. *et al.* Molecular cloning and characterization of a human metalloprotease disintegrin--a novel marker for dendritic cell differentiation. *Blood* **96**, 732-739 (2000).
169. Inoue,D. *et al.* Cloning and initial characterization of mouse meltrin beta and analysis of the expression of four metalloprotease-disintegrins in bone cells. *J. Biol. Chem.* **273**, 4180-4187 (1998).
170. Kurisaki,T., Masuda,A., Osumi,N., Nabeshima,Y. & Fujisawa-Sehara,A. Spatially- and temporally-restricted expression of meltrin alpha (ADAM12) and beta (ADAM19) in mouse embryo. *Mech. Dev.* **73**, 211-215 (1998).

171. Zhou,H.M. *et al.* Essential role for ADAM19 in cardiovascular morphogenesis. *Mol. Cell Biol.* **24**, 96-104 (2004).
172. Kurisaki,T., Wakatsuki,S. & Sehara-Fujisawa,A. Meltrin beta mini, a new ADAM19 isoform lacking metalloprotease and disintegrin domains, induces morphological changes in neuronal cells. *FEBS Lett.* **532**, 419-422 (2002).
173. Kang,T., Zhao,Y.G., Pei,D., Sucic,J.F. & Sang,Q.X. Intracellular activation of human adamalysin 19/disintegrin and metalloproteinase 19 by furin occurs via one of the two consecutive recognition sites. *J. Biol. Chem.* **277**, 25583-25591 (2002).
174. Kang,T. *et al.* Autolytic processing at Glu586-Ser587 within the cysteine-rich domain of human adamalysin 19/disintegrin-metalloproteinase 19 is necessary for its proteolytic activity. *J. Biol. Chem.* **277**, 48514-48522 (2002).
175. Wei,P., Zhao,Y.G., Zhuang,L., Ruben,S. & Sang,Q.X. Expression and enzymatic activity of human disintegrin and metalloproteinase ADAM19/meltrin beta. *Biochem. Biophys. Res. Commun.* **280**, 744-755 (2001).
176. Chesneau,V. *et al.* Catalytic properties of ADAM19. *J. Biol. Chem.* **278**, 22331-22340 (2003).
177. Wong,B.R. *et al.* TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J. Exp. Med.* **186**, 2075-2080 (1997).
178. Shirakabe,K., Wakatsuki,S., Kurisaki,T. & Fujisawa-Sehara,A. Roles of Meltrin beta /ADAM19 in the processing of neuregulin. *J. Biol. Chem.* **276**, 9352-9358 (2001).
179. Wakatsuki,S., Kurisaki,T. & Sehara-Fujisawa,A. Lipid rafts identified as locations of ectodomain shedding mediated by Meltrin beta/ADAM19. *J. Neurochem.* **89**, 119-123 (2004).
180. Yokozeki,T. *et al.* Meltrin beta (ADAM19) mediates ectodomain shedding of Neuregulin beta1 in the Golgi apparatus: fluorescence correlation spectroscopic observation of the dynamics of ectodomain shedding in living cells. *Genes Cells* **12**, 329-343 (2007).
181. Horiuchi,K., Zhou,H.M., Kelly,K., Manova,K. & Blobel,C.P. Evaluation of the contributions of ADAMs 9, 12, 15, 17, and 19 to heart development and ectodomain shedding of neuregulins beta1 and beta2. *Dev. Biol.* **283**, 459-471 (2005).
182. Tanabe,C. *et al.* ADAM19 is tightly associated with constitutive Alzheimer's disease APP alpha-secretase in A172 cells. *Biochem. Biophys. Res. Commun.* **352**, 111-117 (2007).
183. Melenhorst,W.B. *et al.* Upregulation of ADAM19 in chronic allograft nephropathy. *Am. J. Transplant.* **6**, 1673-1681 (2006).
184. Melenhorst,W.B. *et al.* ADAM19 expression in human nephrogenesis and renal disease: associations with clinical and structural deterioration. *Kidney Int.* **70**, 1269-1278 (2006).
185. Hooft,v.H. ADAM 20 and 21; two novel human testis-specific membrane metalloproteases with similarity to fertilin-alpha. *Gene* **206**, 273-282 (1998).
186. Cerretti,D.P., DuBose,R.F., Black,R.A. & Nelson,N. Isolation of two novel metalloproteinase-disintegrin (ADAM) cDNAs that show testis-specific gene expression. *Biochem. Biophys. Res. Commun.* **263**, 810-815 (1999).
187. Jury,J.A., Perry,A.C. & Hall,L. Identification, sequence analysis and expression of transcripts encoding a putative metalloproteinase, eMDC II, in human and macaque epididymis. *Mol. Hum. Reprod.* **5**, 1127-1134 (1999).
188. Roberts,C.M., Tani,P.H., Bridges,L.C., Laszik,Z. & Bowditch,R.D. MDC-L, a novel metalloprotease disintegrin cysteine-rich protein family member expressed by human lymphocytes. *J. Biol. Chem.* **274**, 29251-29259 (1999).
189. Sagane,K., Ohya,Y., Hasegawa,Y. & Tanaka,I. Metalloproteinase-like, disintegrin-like, cysteine-rich proteins MDC2 and MDC3: novel human cellular disintegrins highly expressed in the brain. *Biochem. J.* **334** ( Pt 1), 93-98 (1998).

190. Mochizuki,S., Shimoda,M., Shiomi,T., Fujii,Y. & Okada,Y. ADAM28 is activated by MMP-7 (matrilysin-1) and cleaves insulin-like growth factor binding protein-3. *Biochem. Biophys. Res. Commun.* **315**, 79-84 (2004).
191. Howard,L., Zheng,Y., Horrocks,M., Maciewicz,R.A. & Blobel,C. Catalytic activity of ADAM28. *FEBS Lett.* **498**, 82-86 (2001).
192. Bridges,L.C. *et al.* The lymphocyte metalloprotease MDC-L (ADAM 28) is a ligand for the integrin alpha4beta1. *J. Biol. Chem.* **277**, 3784-3792 (2002).
193. Bridges,L.C., Hanson,K.R., Tani,P.H., Mather,T. & Bowditch,R.D. Integrin alpha4beta1-dependent adhesion to ADAM 28 (MDC-L) requires an extended surface of the disintegrin domain. *Biochemistry* **42**, 3734-3741 (2003).
194. Oh,J. *et al.* Molecular, biochemical, and cellular characterization of epididymal ADAMs, ADAM7 and ADAM28. *Biochem. Biophys. Res. Commun.* **331**, 1374-1383 (2005).
195. Zhao,Z., Wen,L.Y., Jin,M., Deng,Z.H. & Jin,Y. ADAM28 participates in the regulation of tooth development. *Arch. Oral Biol.* **51**, 996-1005 (2006).
196. Zhao,Z., Tang,L., Deng,Z., Wen,L. & Jin,Y. Essential role of ADAM28 in regulating the proliferation and differentiation of human dental papilla mesenchymal cells (hDPMCs). *Histochem. Cell Biol.* (2008).
197. Ohtsuka,T. *et al.* ADAM28 is overexpressed in human non-small cell lung carcinomas and correlates with cell proliferation and lymph node metastasis. *Int. J. Cancer* **118**, 263-273 (2006).
198. Mitsui,Y. *et al.* ADAM28 is overexpressed in human breast carcinomas: implications for carcinoma cell proliferation through cleavage of insulin-like growth factor binding protein-3. *Cancer Res.* **66**, 9913-9920 (2006).
199. Yoshinaka,T. *et al.* Identification and characterization of novel mouse and human ADAM33s with potential metalloprotease activity. *Gene* **282**, 227-236 (2002).
200. Van Eerdewegh,P. *et al.* Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* **418**, 426-430 (2002).
201. Garlisi,C.G. *et al.* Human ADAM33: protein maturation and localization. *Biochem. Biophys. Res. Commun.* **301**, 35-43 (2003).
202. Bridges,L.C., Sheppard,D. & Bowditch,R.D. ADAM disintegrin-like domain recognition by the lymphocyte integrins alpha4beta1 and alpha4beta7. *Biochem. J.* **387**, 101-108 (2005).
203. Huang,J., Bridges,L.C. & White,J.M. Selective modulation of integrin-mediated cell migration by distinct ADAM family members. *Mol. Biol. Cell* **16**, 4982-4991 (2005).
204. Zou,J. *et al.* Catalytic activity of human ADAM33. *J. Biol. Chem.* **279**, 9818-9830 (2004).
205. Orth,P. *et al.* Crystal structure of the catalytic domain of human ADAM33. *J. Mol. Biol.* **335**, 129-137 (2004).
206. Yang,Y. *et al.* Epigenetic mechanisms silence a disintegrin and metalloprotease 33 expression in bronchial epithelial cells. *J. Allergy Clin. Immunol.* **121**, 1393-9, 1399 (2008).
207. Umland,S.P. *et al.* Human ADAM33 messenger RNA expression profile and post-transcriptional regulation. *Am. J. Respir. Cell Mol. Biol.* **29**, 571-582 (2003).
208. Powell,R.M., Wicks,J., Holloway,J.W., Holgate,S.T. & Davies,D.E. The splicing and fate of ADAM33 transcripts in primary human airways fibroblasts. *Am. J. Respir. Cell Mol. Biol.* **31**, 13-21 (2004).
209. Haitchi,H.M. *et al.* ADAM33 expression in asthmatic airways and human embryonic lungs. *Am. J. Respir. Crit Care Med.* **171**, 958-965 (2005).
210. Umland,S.P. *et al.* Mouse ADAM33: two splice variants differ in protein maturation and localization. *Am. J. Respir. Cell Mol. Biol.* **30**, 530-539 (2004).
211. Lee,J.Y. *et al.* A disintegrin and metalloproteinase 33 protein in patients with asthma: Relevance to airflow limitation. *Am. J. Respir. Crit Care Med.* **173**, 729-735 (2006).

212. Ito, I. *et al.* Downregulation of a disintegrin and metalloproteinase 33 by IFN-gamma in human airway smooth muscle cells. *J. Allergy Clin. Immunol.* **119**, 89-97 (2007).
213. Chen, C., Huang, X. & Sheppard, D. ADAM33 is not essential for growth and development and does not modulate allergic asthma in mice. *Mol. Cell Biol.* **26**, 6950-6956 (2006).
214. Howard, T.D. *et al.* Association of a disintegrin and metalloprotease 33 (ADAM33) gene with asthma in ethnically diverse populations. *J. Allergy Clin. Immunol.* **112**, 717-722 (2003).
215. Kedda, M.A., Duffy, D.L., Bradley, B., O'Hehir, R.E. & Thompson, P.J. ADAM33 haplotypes are associated with asthma in a large Australian population. *Eur. J. Hum. Genet.* **14**, 1027-1036 (2006).
216. van Diemen, C.C. *et al.* A disintegrin and metalloprotease 33 polymorphisms and lung function decline in the general population. *Am. J. Respir. Crit Care Med.* **172**, 329-333 (2005).
217. Lind, D.L. *et al.* ADAM33 is not associated with asthma in Puerto Rican or Mexican populations. *Am. J. Respir. Crit Care Med.* **168**, 1312-1316 (2003).
218. Wang, P. *et al.* Lack of association between ADAM33 gene and asthma in a Chinese population. *Int. J. Immunogenet.* **33**, 303-306 (2006).
219. Holgate, S.T. *et al.* The genetics of asthma: ADAM33 as an example of a susceptibility gene. *Proc. Am. Thorac. Soc.* **3**, 440-443 (2006).
220. Saglani, S. & Bush, A. The early-life origins of asthma. *Curr. Opin. Allergy Clin. Immunol.* **7**, 83-90 (2007).
221. Gosman, M.M. *et al.* A disintegrin and metalloprotease 33 and chronic obstructive pulmonary disease pathophysiology. *Thorax* **62**, 242-247 (2007).
222. Lesueur, F. *et al.* ADAM33, a new candidate for psoriasis susceptibility. *PLoS. ONE.* **2**, e906 (2007).
223. Siroux, V. *et al.* Replication of association between ADAM33 polymorphisms and psoriasis. *PLoS. ONE.* **3**, e2448 (2008).

## 1.4 ADAMs with a thrombospondin type I-like motif (ADAMTS)

The latest discovered subfamily of the metzincins are the ADAMTS proteases whose first members were cloned in mouse in 1997, and were immediately associated with inflammation<sup>1</sup>. The human ADAMTS family comprises 19 secreted disintegrin-metalloproteases (ADAMTS-1 to -20, with -11 being identified as a literature alias of ADAMTS-5) with a similar domain structure to the membrane-anchored ADAMs but with some marked structural differences in the C-terminal region. ADAMTS proteins lack the EGF-like domain (and naturally the transmembrane and cytoplasmic regions), but contain a central thrombospondin type I-like repeat (TSR) between the disintegrin domain and the cysteine-rich domain. The cysteine-rich domain is followed by a spacer region of unknown function, and (with the exception of ADAMTS-4) a variable number of additional TSR's (see figure 1)<sup>2</sup>. The TSR motifs have a putative function in protease-substrate interaction and association with the extracellular matrix, as demonstrated for the interaction of ADAMTS-1 with sulphated glycosaminoglycans like heparan<sup>3</sup>.

ADAMTS's are expressed as inactive zymogens that can be activated intracellularly, a process that is likely dependent on proprotein convertase activity in the late-Golgi network since all ADAMTS's contain the furin recognition site within their prodomain<sup>4</sup>. Autocatalytic processing has been described in the C-terminal domain, effectively releasing several of the TSR's from the protein<sup>5</sup>.

The ADAMTS's can be roughly divided into four functional groups: the hyaluronanases or aggrecanases (ADAMTS-1, -4, -5, -8, -9 and -20), the pro-collagen N-endopeptidases (ADAMTS-2, -3 and -14), ADAMTS-13 as a unique member and a heterogeneous group of proteases with unknown function. The biochemical and biological characterization is limited for the majority of ADAMTS's especially when looking at the most recently described members. Although several studies were published on the biological and pathological role of the pro-collagen peptidases, which are implied in processing of pro-collagens prior to fibril formation and therefore have an important role in formation of connective tissue<sup>6,7</sup>, most research effort so far has been directed to only a few submembers: the aggrecanases ADAMTS-1, -4 and -5 and the putative von Willebrand-factor cleaving protein ADAMTS-13.

### 1.4.1 Aggrecanases

ADAMTS-1 or METH-1 was the first identified human ADAMTS in 1998<sup>8</sup>. ADAMTS-1 is a proteolytically active enzyme as demonstrated by the  $\alpha$ 2-macroglobulin trapping assay<sup>9</sup>, and is capable of degradation of aggrecan by cleavage in the chondroitin sulphate attachment domain<sup>10</sup>. ADAMTS-1 knock-out mice are viable, but are considerably smaller than wildtype, with growth retardation continuing after birth. The smaller size of the null mice is contributed mainly to disfunction in fat metabolism, since the knockouts are extremely lean, although no histological abnormalities are visible in adipocytes (besides the smaller size). ADAMTS-1 knockout mice show abnormalities in the structure of the adrenal medulla, but no functional deficiency is observed. The mice further exhibit abnormal development of the kidney, with enlarged calices and fibrotic interstitial lesions, indicating

a role for ADAMTS-1 in ECM turnover during development<sup>11,12</sup>. This role in ECM homeostasis is confirmed by the observation that ADAMTS-1 is markedly downregulated in the liver of cirrhotic rats<sup>13</sup>. ADAMTS-1 has been implicated in ECM turnover during bone formation<sup>14,15</sup>, and may play a role during follicular development and ovulation<sup>16,17</sup>, a function that is corroborated by the observed sub-fertility in ADAMTS-1 null mice. ADAMTS-1 inhibits fibroblast-growth factor-2 VEGF mediated vascularization, an effect presumably mediated by autocatalytically processed fragments, while full-length ADAMTS-1 has sheddase activity towards HB-EGF and amphiregullin and may promote angiogenesis in tumours<sup>18</sup>. ADAMTS-1 expression is lower in patients suffering from asthma<sup>19</sup> and non-small-cell lung carcinoma<sup>20</sup>.

Although ADAMTS-1 is capable of aggrecan processing, this process is attributed more to ADAMTS-4 (aggrecanase-1) and -5 (aggrecanase-2) activity, with ADAMTS-1 only fulfilling a minor role *in vivo*<sup>21</sup>. ADAMTS-4 was first described in 1999<sup>22</sup>, as was ADAMTS-5/ADAMTS-11<sup>23,24</sup>. Since the specific Glu<sup>373</sup>-Ala<sup>374</sup> cleavage of aggrecan mediated by the ADAMTS's is observed in cultured explants undergoing cartilage destruction<sup>25,26</sup> and the resulting aggrecan fragments are detectable in synovial fluid of patients with arthritis<sup>27</sup> the role of ADAMTS-4 and -5 in arthritic diseases is well investigated. ADAMTS-4 expression is sensitive to pro-inflammatory factors such as IL-1, TGF $\beta$  and TNF $\alpha$ , but ADAMTS-5 is not, or to a much lesser extent (<sup>28</sup> and references therein), which is indicative that ADAMTS-5 functions as a constitutive aggrecanase while ADAMTS-4 functions more under inflammation. IL-1 stimulation may induce production of a 37 kDa variant form of ADAMTS-4 in chondrocytes which is capable of aggrecan degradation<sup>29</sup>, while a truncated form resulting from C-terminal processing yields an inactive form, presumably due to disrupted substrate binding<sup>30</sup>. Another interesting alternative processing pathway has been described in *in situ*, extracellular activation by the proprotein convertase PACE4 in cultured cartilage explants<sup>31</sup>.

Findings in knockout experiments investigating the major aggrecanase in murine osteoarthritis however contradicted the role of ADAMTS-4 under inflammatory stimuli as observed during arthritis, with knockout yielding no protective effect<sup>32</sup>, while the ADAMTS-5 null mice exhibited a decreased cartilage destruction<sup>33,34</sup>. The relevance of these observations in human arthritic diseases is not entirely clear, since indications exist that the cytokine-sensitivity of ADAMTS-4 is not present in murine cells, making the translation of the knockout results difficult<sup>35</sup>, but recent characterization of the proteolytic profile of both aggrecanases have provided indication that ADAMTS-5 is indeed the major aggrecanase in arthritis, since the aggrecanolytic activity is around a 1000-fold higher than of ADAMTS-4 under physiological conditions<sup>36</sup>, but this may not be true under inflammatory conditions observed in arthritis. The consensus seems to be that both aggrecanases have a significant effect in the pathological cartilage destruction observed in human osteo- and rheumatoid arthritis<sup>28,37</sup>, but recent evidence from double-knockout mice has shown that aggrecanase activity from ADAMTS-4 and -5 may have redundancy with another, yet unidentified protease which has a different substrate specificity under inflammatory conditions. Neither ADAMTS-4 nor -5 was found to be essential in both



development and cartilage turnover, indicating that the role of these aggrecanases may be more limited than found in earlier studies<sup>38</sup>.

#### **1.4.2 ADAMTS-13**

The most studied ADAMTS to date is perhaps ADAMTS-13 or the van Willebrand factor cleaving protease. This protein was first identified in 2001, and immediately associated with processing of ultralarge multimeric form of von Willebrand factor (vWF)<sup>39,40</sup>.

In the phylogenetic tree ADAMTS-13 stand apart from the other family members, mainly due to the presence of C-terminal CUB (Complement C1r/C1s, Uegf (EGF-related sea urchin protein) and BMP-1 (bone morphogenic protein-1)) domains which are presumed to play a role in protein-protein interaction with other CUB-containing proteins<sup>2</sup>. The prodomain of ADAMTS-13 is unusually short and, contrary to other ADAMs is not necessary for accurate folding of the metalloprotease domain. Removal of the prodomain by proprotein convertases is furthermore not required for proteolytic activity, since proADAMTS-13 is well capable of processing of vWF<sup>41</sup>.

Defects in vWF processing are the underlying pathology in thrombotic thrombocytopenic purpura (TTP), a rare syndrome involving microangiopathic haemolytic anaemia and thrombocytopenia, and may lead to neurological dysfunction, tissue infarction and renal failure. The mortality is high (90%) when untreated, but the reduced processing of the ultralarge vWF multimers can be compensated by plasma exchange essentially supplementing the patient with active vWF cleaving protease (ADAMTS-13)<sup>42,43</sup>. The inactivity of ADAMTS-13 in TTP may be congenital, or the result of an acquired auto-immune response targeting ADAMTS-13. The pathophysiology of TTP and the role of ADAMTS-13 has recently been extensively reviewed<sup>44</sup>.

## References

1. Kuno, K. *et al.* Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. *J. Biol. Chem.* **272**, 556-562 (1997).
2. Jones, G.C. & Riley, G.P. ADAMTS proteinases: a multi-domain, multi-functional family with roles in extracellular matrix turnover and arthritis. *Arthritis Res. Ther.* **7**, 160-169 (2005).
3. Kuno, K. & Matsushima, K. ADAMTS-1 protein anchors at the extracellular matrix through the thrombospondin type I motifs and its spacing region. *J. Biol. Chem.* **273**, 13912-13917 (1998).
4. Wang, P. *et al.* Proprotein convertase furin interacts with and cleaves pro-ADAMTS4 (Aggrecanase-1) in the trans-Golgi network. *J. Biol. Chem.* **279**, 15434-15440 (2004).
5. Flannery, C.R. *et al.* Autocatalytic cleavage of ADAMTS-4 (Aggrecanase-1) reveals multiple glycosaminoglycan-binding sites. *J. Biol. Chem.* **277**, 42775-42780 (2002).
6. Colige, A. *et al.* Cloning and characterization of ADAMTS-14, a novel ADAMTS displaying high homology with ADAMTS-2 and ADAMTS-3. *J. Biol. Chem.* **277**, 5756-5766 (2002).
7. Li, S.W. *et al.* Transgenic mice with inactive alleles for procollagen N-proteinase (ADAMTS-2) develop fragile skin and male sterility. *Biochem. J.* **355**, 271-278 (2001).
8. Vazquez, F. *et al.* METH-1, a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity. *J. Biol. Chem.* **274**, 23349-23357 (1999).
9. Kuno, K., Terashima, Y. & Matsushima, K. ADAMTS-1 is an active metalloproteinase associated with the extracellular matrix. *J. Biol. Chem.* **274**, 18821-18826 (1999).
10. Kuno, K. *et al.* ADAMTS-1 cleaves a cartilage proteoglycan, aggrecan. *FEBS Lett.* **478**, 241-245 (2000).
11. Shindo, T. *et al.* ADAMTS-1: a metalloproteinase-disintegrin essential for normal growth, fertility, and organ morphology and function. *J. Clin. Invest.* **105**, 1345-1352 (2000).
12. Yokoyama, H. *et al.* A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1 null mutant mice develop renal lesions mimicking obstructive nephropathy. *Nephrol. Dial. Transplant.* **17 Suppl 9**, 39-41 (2002).
13. Diamantis, I., Luthi, M., Hosli, M. & Reichen, J. Cloning of the rat ADAMTS-1 gene and its down regulation in endothelial cells in cirrhotic rats. *Liver* **20**, 165-172 (2000).
14. Lind, T., McKie, N., Wendel, M., Racey, S.N. & Birch, M.A. The hyalactan degrading ADAMTS-1 enzyme is expressed by osteoblasts and up-regulated at regions of new bone formation. *Bone* **36**, 408-417 (2005).
15. Rehn, A.P., Birch, M.A., Karlstrom, E., Wendel, M. & Lind, T. ADAMTS-1 increases the three-dimensional growth of osteoblasts through type I collagen processing. *Bone* **41**, 231-238 (2007).
16. Brown, H.M., Dunning, K.R., Robker, R.L., Pritchard, M. & Russell, D.L. Requirement for ADAMTS-1 in extracellular matrix remodeling during ovarian folliculogenesis and lymphangiogenesis. *Dev. Biol.* **300**, 699-709 (2006).
17. Espey, L.L. *et al.* Ovarian expression of a disintegrin and metalloproteinase with thrombospondin motifs during ovulation in the gonadotropin-primed immature rat. *Biol. Reprod.* **62**, 1090-1095 (2000).
18. Liu, Y.J., Xu, Y. & Yu, Q. Full-length ADAMTS-1 and the ADAMTS-1 fragments display pro- and antimetastatic activity, respectively. *Oncogene* **25**, 2452-2467 (2006).
19. Paulissen, G. *et al.* Expression of ADAMs and their inhibitors in sputum from patients with asthma. *Mol. Med.* **12**, 171-179 (2006).
20. Rocks, N. *et al.* Expression of a disintegrin and metalloprotease (ADAM and ADAMTS) enzymes in human non-small-cell lung carcinomas (NSCLC). *Br. J. Cancer* **94**, 724-730 (2006).
21. Little, C.B. *et al.* ADAMTS-1-knockout mice do not exhibit abnormalities in aggrecan turnover in vitro or in vivo. *Arthritis Rheum.* **52**, 1461-1472 (2005).
22. Tortorella, M.D. *et al.* Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science* **284**, 1664-1666 (1999).

23. Hurskainen,T.L., Hirohata,S., Seldin,M.F. & Apte,S.S. ADAM-TS5, ADAM-TS6, and ADAM-TS7, novel members of a new family of zinc metalloproteases. General features and genomic distribution of the ADAM-TS family. *J. Biol. Chem.* **274**, 25555-25563 (1999).
24. Abbaszade,I. *et al.* Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family. *J. Biol. Chem.* **274**, 23443-23450 (1999).
25. Sandy,J.D., Neame,P.J., Boynton,R.E. & Flannery,C.R. Catabolism of aggrecan in cartilage explants. Identification of a major cleavage site within the interglobular domain. *J. Biol. Chem.* **266**, 8683-8685 (1991).
26. Lark,M.W. *et al.* Cell-mediated catabolism of aggrecan. Evidence that cleavage at the "aggrecanase" site (Glu373-Ala374) is a primary event in proteolysis of the interglobular domain. *J. Biol. Chem.* **270**, 2550-2556 (1995).
27. Sandy,J.D., Flannery,C.R., Neame,P.J. & Lohmander,L.S. The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. *J. Clin. Invest* **99**, 1512-1516 (1992).
28. Bondeson,J., Wainwright,S., Hughes,C. & Caterson,B. The regulation of the ADAMTS4 and ADAMTS5 aggrecanases in osteoarthritis: a review. *Clin. Exp. Rheumatol.* **26**, 139-145 (2008).
29. Powell,A.J., Little,C.B. & Hughes,C.E. Low molecular weight isoforms of the aggrecanases are responsible for the cytokine-induced proteolysis of aggrecan in a porcine chondrocyte culture system. *Arthritis Rheum.* **56**, 3010-3019 (2007).
30. Kashiwagi,M. *et al.* Altered proteolytic activities of ADAMTS-4 expressed by C-terminal processing. *J. Biol. Chem.* **279**, 10109-10119 (2004).
31. Malfait,A.M. *et al.* Proprotein convertase activation of aggrecanases in cartilage in situ. *Arch. Biochem. Biophys.* (2008).
32. Glasson,S.S. *et al.* Characterization of and osteoarthritis susceptibility in ADAMTS-4-knockout mice. *Arthritis Rheum.* **50**, 2547-2558 (2004).
33. Glasson,S.S. *et al.* Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* **434**, 644-648 (2005).
34. Stanton,H. *et al.* ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature* **434**, 648-652 (2005).
35. Zwerina,J. *et al.* TNF-induced structural joint damage is mediated by IL-1. *Proc. Natl. Acad. Sci. U. S. A* **104**, 11742-11747 (2007).
36. Gendron,C. *et al.* Proteolytic activities of human ADAMTS-5: comparative studies with ADAMTS-4. *J. Biol. Chem.* **282**, 18294-18306 (2007).
37. Song,R.H. *et al.* Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum.* **56**, 575-585 (2007).
38. Rogerson,F.M. *et al.* Evidence of a novel aggrecan-degrading activity in cartilage: Studies of mice deficient in both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum.* **58**, 1664-1673 (2008).
39. Tsai,H.M. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. *Blood* **87**, 4235-4244 (1996).
40. Soejima,K. *et al.* A novel human metalloprotease synthesized in the liver and secreted into the blood: possibly, the von Willebrand factor-cleaving protease? *J. Biochem.* **130**, 475-480 (2001).
41. Majerus,E.M., Zheng,X., Tuley,E.A. & Sadler,J.E. Cleavage of the ADAMTS13 propeptide is not required for protease activity. *J. Biol. Chem.* **278**, 46643-46648 (2003).
42. Moake,J.L. *et al.* Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *N. Engl. J. Med.* **307**, 1432-1435 (1982).
43. Zheng,X. *et al.* Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J. Biol. Chem.* **276**, 41059-41063 (2001).
44. Sadler,J.E. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. *Blood* **112**, 11-18 (2008).

## 1.5 Scope of the thesis

This thesis describes the development of novel methods for activity-based proteomics based on the selective interaction of active metalloproteinases such as matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinases (ADAMs) with newly synthesized inhibitor molecules.

The general introduction (**chapter 1**) gives an overview of the current literature on the biological and pathophysiological role of the MMPs, the ADAMs and the recently discovered subfamily of ADAMs with a thrombospondin repeat (ADAMTSs).

Excessive metalloproteinase activity is a possible underlying cause for the development of many diseases ranging from inflammatory conditions to pathological tissue remodelling and cancer the profiling of metalloproteinase activity could provide a valuable diagnostic tool and shed light on the complicated role of the MMPs and ADAMs in health and disease. As metalloproteinase activity is highly regulated in vivo, determination or profiling of the actual active isoforms that are present could provide a better picture of the (patho)-physiological situation. Several analytical techniques for distinguishing active from non-active, either in pro-protease, or zymogen form or inactivated by endogenous inhibitors, have been described in literature. **Chapter 2** gives an overview of the traditional methods for activity determination based on monitoring of substrate conversion and expands to the recently developed field of activity-based proteomics (ABP).

ABP methodology uses small molecular weight inhibitors as ligands and roughly can be divided into two approaches; using inhibitor probes labelled with either biotin or a fluorophore for tagging and visualisation of active proteinases or by using immobilized reversible inhibitors for activity-based solid phase extraction.

In **chapter 3** we describe the first approach where the base structure of a successful reversible hydroxamate-based inhibitor was modified to include a photoreactive moiety for crosslinking of the probe to the active metalloproteinase and a biotin group for visualisation and pulldown using biotin-streptavidin interaction. A similar probe with an incorporated fluorescent moiety was tested alongside the biotinylated probe and the probe is optimized by transfer of the photocrosslinking group from the P'2 to the P'1 position in the molecule.

In **chapter 4** we test a library of reversible peptide-based hydroxamates as inhibitors against three model metalloproteinases, MMP-9, MMP-12 and ADAM-17. The best performing inhibitors are immobilized on Sepharose beads and used for activity-based enrichment of metalloproteinases. This approach is used to enrich active endogenous ADAM-17 from a complex proteome (a total cell lysate of A549 lung carcinoma cells).

Solid phase extraction has the advantage of being susceptible to automation, and this approach is described in **chapter 5**. By packing the immobilized inhibitor beads into a cartridge suitable for the PROSPEKT automated solid phase extraction module, and coupling the automated extraction to inline tryptic digestion and LC-MS analysis we show a system for fully automated activity-based analysis of metalloproteinases. The enrichment platform is coupled to a chip-based LC interface for improved robustness and the system is tested with the model proteinase MMP-12.

In **chapter 6** we summarize the results and give future perspectives. A preliminary approach for the use of the inhibitor probes for in-vivo imaging using a <sup>99m</sup>-technetium label is discussed and the challenging task of proper pre-analytical sample handling to preserve the activity status of the proteinases in the samples is discussed.