Critical Reviews in Biochemistry and Molecular Biology, 42:113–185, 2007

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230701340019

informa
healthcare

The Biochemical, Biological, and Pathological Kaleidoscope of Cell Surface Substrates Processed by Matrix Metalloproteinases

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ABSTRACT Matrix metalloproteinases (MMPs) constitute a family of more than 20 endopeptidases. Identification of specific matrix and non-matrix components as MMP substrates showed that, aside from their initial role as extracellular matrix modifiers, MMPs play significant roles in highly complex processes such as the regulation of cell behavior, cell-cell communication, and tumor progression. Thanks to the comprehensive examination of the expanded MMP action radius, the initial view of proteases acting in the soluble phase has evolved into a kaleidoscope of proteolytic reactions connected to the cell surface. Important classes of cell surface molecules include adhesion molecules, mediators of apoptosis, receptors, chemokines, cytokines, growth factors, proteases, intercellular junction proteins, and structural molecules. Proteolysis of cell surface proteins by MMPs may have extremely diverse biological implications, ranging from maturation and activation, to inactivation or degradation of substrates. In this way, modification of membrane-associated proteins by MMPs is crucial for communication between cells and the extracellular milieu, and determines cell fate and the integrity of tissues. Hence, insights into the processing of cell surface proteins by MMPs and the concomitant effects on physiological processes as well as on disease onset and evolution, leads the way to innovative therapeutic approaches for cancer, as well as degenerative and inflammatory diseases.

KEYWORDS ectodomain shedding, receptor, membrane, cancer, immunity, autoimmunity

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INTRODUCTION

Matrix metalloproteinases (MMPs) constitute a family of over 20 different endopeptidases characterized by a conserved Zn^{2+}-binding motif **H**EXX**H**XXGXX**H** in the catalytic domain and a number of conserved protein domains. The three histidines in this motif are responsible for binding the catalytic Zn^{2+} ion (Figure 1) (Nagase and Woessner, 1999; Brinckerhoff and Matrisian, 2002). Expression of most MMPs is under transcriptional regulation, e.g., by growth factors, hormones, cytokines and oncogenic transformation. MMPs are neutral endopeptidases produced as secreted or membrane-bound pro-enzymes or zymogens, which become activated by removal of the NH_2-terminal propeptide. The interaction of a conserved cysteine in the propeptide with the catalytic Zn^{2+} ion seals the catalytic site and results in the latency of the pro-enzyme (Figure 1) (Visse and Nagase, 2003). Removal of the propeptide, for example by proteolysis, alters this coordination with Zn^{2+}. Due to the subsequent conformational change, the Zn^{2+} ion becomes available for the binding of a hydrolytic water molecule and of the substrate. Therefore, the MMP activation mechanism was named the 'cysteine switch mechanism' (Van Wart and Birkedal-Hansen, 1990). This activation can be mediated by proteases and other MMPs, or chemically by means of organomercurials, urea, some detergents and also by reactive oxygen species. In addition, some MMPs are activated intracellularly by furins (Nagase and Woessner, 1999).

Examples of 'archetypical' MMPs are the collagenases, which are active against fibrillar collagens, and the stromelysins, which cleave non-collagen components of the extracellular matrix (ECM) (Folgueras *et al.*, 2004). They contain a COOH-terminal hemopexin domain (Figure 1), which contributes to substrate specificity, and to interactions with endogenous inhibitors and cargo receptors (Piccard *et al.*, 2007). This hemopexin domain is absent in the smallest MMPs, the matrilysins. In addition, the gelatinases, active on denaturated collagens, incorporate three fibronectin type II repeats for the binding of gelatin, and MMP-9 is the only MMP to possess a Ser/Thr/Pro-rich O-glycosylated domain, which forms an attachment site for multiple O-linked sugars (Van den Steen *et al.*, 2006). In addition to the secreted MMPs, six human membrane-bound MMPs (MT-MMPs) exist. These are linked to the cell surface through a COOH-terminal transmembrane domain (MT1-, MT2-, MT3- and MT5-MMP) or a glycosyl phosphatidylinositol (GPI) anchor (MT4- and MT6-MMP) (Visse and Nagase, 2003).

Once switched on, MMP proteolytic activity is under tight control by specific inhibitors, primarily the tissue inhibitors of metalloproteinases (TIMPs) (Brew *et al.*, 2000). In plasma, the general protease inhibitor, α_2-macroglobulin, is the predominant MMP inhibitor (Baker *et al.*, 2002), whereas the TIMPs are considered to be the key inhibitors in tissue. In humans, four different TIMPs (TIMP-1 to -4) have been characterized that form non-covalent 1:1 complexes with MMPs. TIMP-1, TIMP-2, and TIMP-4 are present in the extracellular environment in a soluble form, whereas TIMP-3 is insoluble, sequestered by the ECM (Gomez *et al.*, 1997). Although TIMPs bind tightly to most MMPs, some important differences exist in the inhibition profile (Table 1). For instance, TIMP-1 is known to inhibit most soluble MMPs, but it is a poor inhibitor for MT1-MMP, MT2-MMP, MT3-MMP, MT5-MMP, and MMP-19. Binding affinity to MMP-9 is high for TIMP-1, but low for TIMP-2 and TIMP-3, whereas TIMP-2, TIMP-3, and TIMP-4 bind with high affinity to

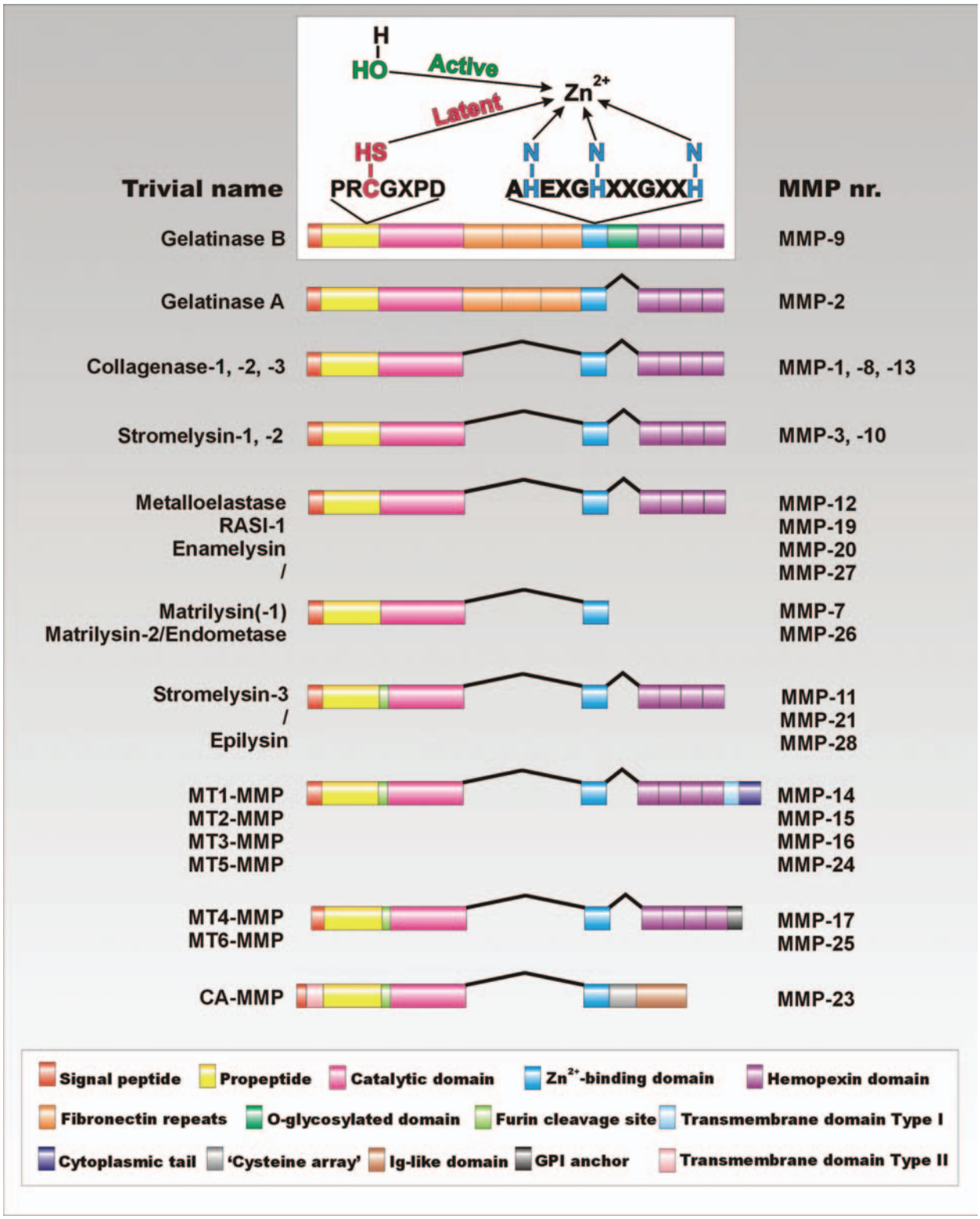

FIGURE 1 Domain structure of the human MMPs. An archetypal MMP contains a signal peptide for secretion, a propeptide, a catalytic domain with a conserved Zn^{2+}-binding motif and a COOH-terminal domain. The hemopexin domain is absent in the smallest MMPs, the matrilysins, whereas the gelatinases incorporate three fibronectin type II repeats for the binding of gelatin, and MMP-9 is the only MMP to possess a Ser/Thr/Pro-rich O-glycosylated domain. Some MMPs are attached to the cell surface through a COOH-terminal transmembrane domain or a GPI anchor. The interaction of a conserved cysteine in the propeptide with the catalytic Zn^{2+} ion seals the catalytic site and results in the latency of the pro-enzyme. MMPs are activated according to the 'cysteine switch mechamism' in which removal of the propeptide frees the catalytic Zn^{2+} ion, allowing it to bind a hydrolytic water ion and the substrate. CA-MMP, cysteine array-MMP; GPI, glycosyl phosphatidylinositol; Ig, Immunoglobulin; RASI-1, rheumatoid arthritis synovial inflammation-1. Based on (Van den Steen *et al.*, 2002), (Folgueras *et al.*, 2004) and (Nagase *et al.*, 2006).

TABLE 1 **TIMP characteristics and inhibition profiles**

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Localization	Soluble	Soluble/cell surface	ECM	Soluble/cell surface
MW (kDa)	28	21	24/27	22
MMPs inhibited	Most MMPs, best inhibited:MMP-9	Many MMPs, best inhibited: MMP-2	Many MMPs, best inhibited: MMP-2	Many MMPs, best inhibited: MMP-2
MMPs poorly inhibited	MT1-MMP MT2-MMP MT3-MMP MT5-MMP MMP-19	None	None	None
ADAMs inhibited	ADAM-10	None	ADAM-10 ADAM-12 ADAM-17 ADAM-19 ADAMTS-4 ADAMTS-5	none

MMP-2 (Gomez *et al.*, 1997; Nagase *et al.*, 2006). In addition, TIMP-3 inhibits members of the 'a disentegrin and metalloproteinase (ADAM)-family', including ADAM-10/Kuzbanian, ADAM-12, ADAM-17/tumor necrosis factor-α (TNF-α)-converting enzyme (TACE) and ADAM-19, as well as the aggrecan-degrading enzymes 'a disentegrin and metalloproteinase with thrombospondin-like motif' (ADAMTS)-4 and ADAMTS-5, and TIMP-1 inhibits ADAM-10 (Baker *et al.*, 2002). As a consequence, the TIMP inhibition profile of a particular cleavage can already shed some light on the identity of the protease in charge.

Thanks to their structural diversity and broad substrate range, MMPs play a part in multiple physiological and pathological processes (Sternlicht and Werb, 2001). As it is more challenging to study membrane-bound molecules, the initial identification of MMP substrates included mainly soluble proteins. However, due to the fast development and fine-tuning of powerful biochemical techniques, insights into MMP cleavage of membrane-bound substrates grew in parallel with the appreciation of its relative importance in physiology as well as pathology. This review contains a survey of known membrane-bound substrates of every MMP, or more specifically of all proteins with a transmembrane domain that have been shown to be the subject of modification by one or several MMPs. In addition, we will discuss the cleavage of some important cell surface proteins that do not contain a transmembrane domain but are always attached to the cell surface by other means of anchoring. The consequences of proteolysis of cell surface proteins by MMPs will be stressed to generate, from scattered information, a clear view on the importance of this process. Consequently, the substrates are grouped in functional classes in the context of the physiological or pathological roles they play with or without proteolysis. To facilitate comprehensive reading, all substrate molecules are grouped in a master table which includes the modifying MMPs, the context of cleavage, known cleavage sites, the biological effect of proteolysis and the physiopathological implications (Table 2). In many instances, various names, abbreviations and acronyms have been given to these substrates. For clarity, these are provided once at the beginning of each section (see also the list of abbreviations).

1. PROTEOLYTIC MODIFICATION OF CELL SURFACE PROTEINS IN CANCER DEVELOPMENT AND EVOLUTION

Tumorigenesis and cancer progression originate from at least seven fundamental alterations in cellular physiology: 1) production of autocrine growth signals; 2) unsensitivity to growth-inhibitory signals; 3) escape from apoptosis; 4) loss of senescence; 5) sustained angiogenesis; 6) tissue invasion; and 7) metastasis (Hanahan and Weinberg, 2000). MMPs have an impact on the microenvironment of tumors. Moreover, the expression and activity of specific MMPs is elevated in almost every kind of cancer. Some MMPs are synthesized principally by tumor cells (*e.g.*, MMP-7), whereas other MMPs (*e.g.*, MMP-2 and -9) are rather produced by stromal cells in the tumors, like fibroblasts, inflammatory cells, and endothelial cells (Egeblad and Werb,

TABLE 2 **Proteolysis of cell surface proteins by MMPs in physiological and pathological processes**

Substrate[1]	CD[2]	MA[3]	MMP	Cleavage sites	Substrate modulation[4]	Effects on physiology/pathology	Reference
				ADHESION MOLECULES			
pro-α_v-integrin	CD51	TMD	MT1-MMP	Asp891-Leu 892 after Cys852 *In vitro*	Maturation	Activation of $\alpha_v\beta_3$, leading to increased tumor cell adhesion and migration on vitronectin; Cross-talk between $\alpha_v\beta_3$ and $\alpha_2\beta_1$ integrins, leading to increased tumor cell adhesion to type I collagen	(Ratnikov *et al.*, 2002; Deryugina *et al.*, 2002; Baciu *et al.*, 2003)
pro-α_3-integrin	CD49c	TMD	MT1-MMP	*In vitro*	Maturation	ND	(Baciu *et al.*, 2003)
pro-α_5-integrin	CD49e	TMD	MT1-MMP	*In vitro*	Maturation	No influence on $\alpha_5\beta_1$ integrin function	(Baciu *et al.*, 2003)
pro-β_3-integrin	CD61	TMD	MT1-MMP	*In vitro*	Maturation	Activation of $\alpha_v\beta_3$, leading to increased tumor cell adhesion to vitronectin	(Deryugina *et al.*, 2000)
pro-β_4-integrin	CD104	TMD	MMP-7	*In vitro*	Inactivation	Reduced binding of $\alpha_6\beta_4$ to laminin, reducing tumor cell adhesion and migration	(von Bredow *et al.*, 1997; Abdel-Ghany *et al.*, 2001)
tTG	/	CSL	MT1-MMP MMP-2	Pro375-Val376 Arg458-Ala459 His461-Leu462 *In vitro*	Degradation	Suppression of tumor cell adhesion and migration on fibronectin; Stimulation of tumor cell migration on collagen matrices	(Belkin *et al.*, 2001; Belkin *et al.*, 2004)
			MT2,3-MMP	*In vitro*	Degradation	ND	(Belkin *et al.*, 2001; Belkin *et al.*, 2004)
CD44	CD44	TMD	MT1-MMP	Gly192-Tyr193 Gly233-Ser234 (Ser249-Gln250) *In vitro + in vivo*	Inactivation	Stimulation of tumor cell migration and invasion	(Kajita *et al.*, 2001; Nakamura *et al.*, 2004)
			MT2,3,5-MMP	Gly233-Ser234 *In vitro*	Inactivation	ND (Possible stimulation of tumor cell migration and invasion)	(Suenaga *et al.*, 2005)
ICAM-1	CD54	TMD	MMP-9	Between Arg441 and the TMD	Inactivation	Protection of tumor cells against their elimination by cytotoxic T cells and NK cells	(Fiore *et al.*, 2002; Sultan *et al.*, 2004)
L-selectin	CD62L	TMD	MMP-1 MMP-3	*In vitro*	ND	ND (Possible effect on leukocyte rolling, transendothelial migration, activation and T-cell reentry into the peripheral lymph nodes after activation)	(Preece *et al.*, 1996)
				APOPTOSIS MEDIATORS			
FasL	CD178	TMD	MMP-7	Glu110-Leu111 Glu113-Leu114 Ser126-Leu127 Glu142-Leu143 *In vitro + in vivo*	sEffector* with lower activity**	Apoptosis induction in epithelial cells* but reduced apoptosis induction in tumor cells**	(Tanaka *et al.*, 1998; Powell *et al.*, 1999; Mitsiades *et al.*, 2001; Vargo-Gogola *et al.*, 2002a)

				In vitro		Protection of neuron cultures from Aβ-induced neuronal cell apoptosis**, possibly preventing neuronal loss in Alzheimer's disease	(Ethell *et al.*, 2002)
			MMP-3	Glu110-Leu111 Glu113-Leu114 Ser126-Leu127 *In vitro*		Reduced intra-articular apoptosis** leading to synovial proliferation in rheumatoid arthritis	(Matsuno *et al.*, 2001)
Fas	CD95	TMD	MMP-7	Glu19-Leu20 Asn32-Leu33 *In vitro*	Down-regulation	Reduced oligomerization of Fas receptors leading to reduced sensitivity of tumor cells to Fas-mediated apoptosis	(Strand *et al.*, 2004)
Death receptor-6	/	TMD	MT1-MMP	*In vitro*	ND	ND	(Overall *et al.*, 2004)
					RECEPTORS		
FGFR-1	CD331	TMD	MMP-2	Val368-Met369 *In vitro*	sInhibitor	Inhibition of FGF-mediated stimulation of tumor cell growth	(Levi *et al.*, 1996)
HER2	CD340	TMD	ND MMP	*In vitro*	Potentiation	Enhanced oncogenic potential including promotion of excessive and autonomous tumor cell growth, increased tumor cell survival, neovascularisation and invasiveness	(Codony-Servat *et al.*, 1999)
uPAR	CD87	GPI	MMP-12	Thr86-Tyr87 *In vitro* + *ex vivo*	Inactivation + Activation	- Destruction of the binding site for u-PA and vitronectin, decreasing cell-surface associated u-PA activity (= inactivation) and reducing tumor-induced angiogenesis + generation of uPAR molecules with chemotactic activities (= activation) - Destruction of u-PA-dependent invasion, proliferation and morphogenesis of endothelial cells, leading to vascular alterations in systemic sclerosis patients	(Koolwijk *et al.*, 2001; Andolfo *et al.*, 2002; D'Alessio *et al.*, 2004; Serrati *et al.*, 2006)
			MMP-3	Thr86-Tyr87 *In vitro*	Inactivation + Activation	Destruction of the binding site for u-PA and vitronectin, decreasing cell-surface associated u-PA activity (= inactivation) and reducing tumor-induced angiogenesis + generation of cell-surface activated uPAR molecules with chemotactic activities (= activation)	(Andolfo *et al.*, 2002)
			MT6-MMP	Thr86-Tyr87 Tyr87-Ser88 Ser88-Arg89 Arg89-Ser90 *In vitro*			

(Continued on next page)

TABLE 2 **Proteolysis of cell surface proteins by MMPs in physiological and pathological processes *(Continued)***

Substrate[1]	CD[2]	MA[3]	MMP	Cleavage sites	Substrate modulation[4]	Effects on physiology/pathology	Reference
			MMP-19	Thr86-Tyr87 Tyr87-Ser88 Arg89-Ser90 *In vitro*			
			MMP-2,-9 MMP-8,-13 MT1-MMP	*In vitro*	ND	ND	(Andolfo *et al.*, 2002)
Semaphorin 4D	CD100	TMD	MT1-MMP	*In vitro* + *in vivo*	sEffector	Promotion of tumor-induced angiogenesis	(Basile *et al.*, 2007)
LR	/	TMD	*X*MMP-11	Ala115-Phe116 Pro133-Ile134 *In vitro*	ND (sEffector)	ND (Possible enhancement of tumor cell invasion and angiogenesis)	(Amano *et al.*, 2005b)
*X*LR	/	TMD	*X*MMP-11	Ala115-Phe116 Pro133-Ile134 *In vitro* + *in vivo*	ND (sEffector)	ND (Possible promotion of larval epithelial cell apoptosis needed for intestinal metamorphosis in *Xenopus laevis*)	(Amano *et al.*, 2005a, 2005b)
			MMP-2,-3–9 MT1-MMP	*In vitro*	ND	ND	(Amano *et al.*, 2005b)
EMMPRIN	CD147	TMD	MT1-MMP MT2-MMP	Asn98-Ile99 Pro93-Met94 *In vitro*	Inactivation + sEffector	Downregulation of cellular EMPRINN functions, possible diffusion and amplification of EMPRINN activities on adjacent or more distal cells, promoting tumor growth, metastasis, angiogenesis and multidrug resistance	(Egawa *et al.*, 2006)
			MMP-1 MMP-2	*In vitro*	Inactivation + sEffector	Amplification cascade of MMP activity, leading to enhanced ECM degradation in atherosclerotic plaques, promoting plaque growth and plaque destabilization	(Haug *et al.*, 2004)
LRP	CD91	TMD	MT1,2,3,4-MMP	*In vitro*	Inactivation	Suppression of clearance of ECM-degrading proteases, promoting tissue remodelling by migrating tumor cells	(Rozanov *et al.*, 2004a)
PAR1	/	TMD	MMP-1	Arg41-Ser42 *In vitro* + *in vivo*	Activation	Promotion of cancer cell migration and invasion + induction of endothelial cell activation	(Boire *et al.*, 2005; Pei, 2005; Goerge *et al.*, 2006)
IL-2Rα	CD25	TMD	MMP-9	*In vitro*	Inactivation	Reduced proliferation of tumor-infiltrating cytotoxic T cells, leading to tumor immune escape	(Sheu *et al.*, 2001)
CD14	CD14	GPI	MMP-12	*In vitro* + *in vivo*	Inactivation + sEffector	Reduced innate host defence activities such as impairment of LPS uptake and reduced LPS-induced TNF-α production by alveolar macrophages	(Senft *et al.*, 2005)
			MMP-1	*In vitro*	ND	ND	(Bryniarski *et al.*, 2003)
					CHEMOKINES		
Fractalkine	/	TMD	MMP-2	*In vitro*	sEffector + sInhibitor	ND	(Overall and Dean, 2006)

CYTOKINES							
RANKL	CD254	TMD	MMP-7	Met145-Met146 *In vitro* + *in vivo*	sEffector	Promotion of prostate cancer-induced osteolysis	(Lynch *et al.*, 2005)
			MT1-MMP			Downregulation of local osteoclastogenesis and bone resorption	(Hikita *et al.*, 2006)
			MMP-3	*In vitro*	ND	ND	(Lynch *et al.*, 2005)
			MT2,3,5-MMP	Met145-Met146 *In vitro*	ND	ND	(Hikita *et al.*, 2006)
pro-TNF-α	/	TMD	MMP-1	Ala74-Gln75 Ala76-Val77 *In vitro*	sEffector	Induction of inflammation in response to bacteria and pathogen-associated molecular patterns; Excess TNF-α in Crohn's disease, multiple sclerosis, rheumatoid arthritis and septic shock	(Gearing *et al.*, 1994; Gearing *et al.*, 1995; Chandler *et al.*, 1996; d'Ortho *et al.*, 1997; English *et al.*, 2000; Mohan *et al.*, 2002)
			MMP-2,-3	*In vitro*			
			MMP-9	Ala74-Gln75			
			MT4-MMP	*In vitro*			
			MT1-MMP	Ala76-Val77			
			MT2-MMP	before Leu113 *In vitro*			
			MMP-7	Ala76-Val77 *In vitro* + *ex vivo*		Induction of MMP-3, leading to spontaneous resorption of herniated discs	(Haro *et al.*, 2000a; Haro *et al.*, 2000b; Mohan *et al.*, 2002)
			MMP-12	*In vitro* + *in vivo*		Induction of acute cigarette smoke-provoked inflammation	(Churg *et al.*, 2003)
pro-IL-1β	/	CSL	MMP-2,-3,-9	*In vitro*	Activation	Induction of inflammation; Stimulation of tumor invasion and angiogenesis	(Schönbeck *et al.*, 1998)
IL-1β	/	S	MMP-1,-3,-9	*In vitro*	Degradation	Downregulation of the pro-inflammatory, metastatic and angiogenic effects of active IL-1β	(Ito *et al.*, 1996)
			MMP-2	Glu25-Leu26 *In vitro*			
KitL	/	TMD	MMP-9	*In vivo*	sEffector	Maintenance of leukocyte homeostasis in the blood; Stimulation of proliferation of quiescent smooth muscle cells, leading to intimal hyperplasia	(Heissig *et al.*, 2002; Hollenbeck *et al.*, 2004)

(Continued on next page)

TABLE 2 **Proteolysis of cell surface proteins by MMPs in physiological and pathological processes *(Continued)***

Substrate[1]	CD[2]	MA[3]	MMP	Cleavage sites	Substrate modulation[4]	Effects on physiology/pathology	Reference
pro-TGF-β	/	CSL	MMP-2,-3,-9 MT1-MMP	*In vitro*	Activation	Tumor suppression in early stages of tumorigenesis, but stimulation of angiogenesis and metastasis in later stages of cancer development	(Yu and Stamenkovic, 2000)
					GROWTH FACTORS		
HB-EGF	/	TMD	MMP-7	*In vitro* + *in vivo*	sEffector	- Regulation of postpartum uterine and mammary gland involution and maintenance of lactation - Pathological vasoconstriction and growth of smooth muscle cells; Induction of tumor cell growth and angiogenesis	(Iwamoto and Mekada, 2000; Roelle *et al.*, 2003; Razandi *et al.*, 2003; Milenkovic *et al.*, 2003; Hao *et al.*, 2004; Lucchesi *et al.*, 2004; Ongusaha *et al.*, 2004)
			MMP-2	*In vitro* + *in vivo*		- Signal transduction induced by GnRH and estradiol	
			MMP-9	*In vitro* + *in vivo*		- Signal transduction induced by GnRH and estradiol; Proliferation of glia cells in PVR	
			MMP-3	Glu151-Asn152 *In vitro*		- Induction of tumor cell growth and angiogenesis	(Iwamoto and Mekada, 2000; Ongusaha *et al.*, 2004)
					PROTEASES		
MT1-MMP	/	TMD	MT1-MMP	Gly284-Gly285 Ala255-Ile256 *In vitro*	Inactivation + mInhibitor	Downregulation of MT1-MMP-mediated promotion of tumor invasion and metastasis	(Toth *et al.*, 2002)
			MT3-MMP	*In vitro*	Inactivation	Downregulation of MT1-MMP activity in smooth muscle cells, gliomas or injured blood vessels	(Shofuda *et al.*, 2001)
ADAMTS-4	/	CSL	MT4-MMP	Lys694-Phe685 Thr581-Phe582 *In vitro*	sEffector	Degradation of aggrecan in rheumatoid arthritis	(Gao *et al.*, 2002; Gao *et al.*, 2004)
			MMP-9,-13	*In vitro*	Activation	ND	(Tortorella *et al.*, 2005)

INTERCELLULAR JUNCTION PROTEINS

E-cadherin	CD324	TMD	MMP-7	*In vitro* + *in vivo*	sInhibitor	Induction of tumor cell invasion and metastasis; rounding of apoptotic cells and tumor cells, promoting tumor cell exit from the epithelium; repair of injured lung epithelium	(Steinhusen *et al.*, 2001; Davies *et al.*, 2001; Noe *et al.*, 2001; McGuire *et al.*, 2003)
			MMP-3	*In vitro*	sInhibitor	Induction of tumor cell invasion and metastasis; promotion of epithelial-mesenchymal transition; rounding of apoptotic cells and tumor cells, promoting tumor cell exit from the epithelium	(Lochter *et al.*, 1997; Steinhusen *et al.*, 2001; Noe *et al.*, 2001)
			MT1-MMP	*In vitro*	Degradation	Disruption of cell-cell attachments during renal ischemia, possibly causing intra-tubular obstructions in acute renal failure	(Covington *et al.*, 2006)
N-cadherin	CD325	TMD	MT1-MMP	*In vitro*	Degradation	Disruption of cell-cell attachments during renal ischemia, possibly causing intra-tubular obstructions in acute renal failure	(Covington *et al.*, 2006)
			MT5-MMP	*In vitro*	ND	ND (Possible role in mechanisms of synaptic regulation)	(Monea *et al.*, 2006)
VE-cadherin	CD144	TMD	MMP-7	*In vitro*	ND	Acceleration of endothelial cell proliferation	(Ichikawa *et al.*, 2006)
Occludin	/	IM	MMP-2	*In vitro*	Degradation	BRB disruption leading to retinal edema in PDR	(Giebel *et al.*, 2005)
			MMP-9	*In vitro* + *in vivo*	Degradation	BRB disruption leading to retinal edema in PDR + disruption of corneal epithelial barrier function, causing ocular irritation and visual morbidity in KS Disruption of endothelial tight junctions during renal ischemia and possible degradation of the vascular basement membrane, leading to acute renal failure	(Giebel *et al.*, 2005; Pflugfelder *et al.*, 2005; Caron *et al.*, 2005)
			MMP-3	*In vitro* + *in vivo*	Degradation	BBB disruption in neuroinflammation	(Gurney *et al.*, 2006)
			MMP-7	*In vitro*	Degradation	Estrogen-mediated increase of paracellular permeability in vaginal-cervical epithelia	(Gorodeski, 2007)
Claudin-5	/	IM	MMP-3	*In vitro* + *in vivo*	Degradation	BBB disruption in neuroinflammation	(Gurney *et al.*, 2006)

(Continued on next page)

TABLE 2 **Proteolysis of cell surface proteins by MMPs in physiological and pathological processes *(Continued)***

Substrate[1]	CD[2]	MA[3]	MMP	Cleavage sites	Substrate modulation[4]	Effects on physiology/pathology	Reference
				STRUCTURAL PROTEINS			
Betaglycan	/	TMD	MT1-MMP MT3-MMP	*In vitro*	sInhibitor	Inhibition of TGF-β-induced tumor angiogenesis	(Velasco-Loyden *et al.*, 2004; Bandyopadhyay *et al.*, 2005)
MUC1	CD227	TMD	MT1-MMP	*In vitro*	ND	Possible role in the defense of epithelial surfaces like uterine, lung and intestinal epithelia; Possible influence on tumor cell proliferation, metastasis and immune evasion	(Thathiah and Carson, 2004)
Syndecan-1	CD138	TMD	MT1,3-MMP	Gly245-Leu246 *In vitro*	Inactivation	Enhanced tumor cell migration on collagen	(Endo *et al.*, 2003)
			MMP-7	*In vitro* + *in vivo*	sEffector	Release of a syndecan-1/KC complex from the mucosal surface of injured lungs, forming a chemokine gradient that directs neutrophils to the site of injury	(Li *et al.*, 2002)
			MMP-9	*In vitro*	Inactivation	ND	(Brule *et al.*, 2006)
Syndecan-3	/	TMD	ND MMP	*In vitro* + *In vivo*	Inactivation	Abrogation of Schwann cell adhesion to α4(V) collagen *in vitro* and in the peripheral nerve tissue of newborn rats during the myelin-forming process in Schwann cells	(Asundi *et al.*, 2003)
Syndecan-4	/	TMD	MMP-9	*In vitro*	Inactivation	ND	(Brule *et al.*, 2006)
BP180	/	TMD	MMP-9	*In vitro*	Degradation	Disruption of BP180-mediated anchoring of dermis and epidermis (*in vitro*), possibly leading to blister formation in BP	(Liu *et al.*, 1998)
MBP	/	IM	MMP-9	Phe90-Lys91 Ser110-Leu111 Phe114-Ser115 Asp133-Tyr134 *In vitro* + *in vivo*	Degradation	Demyelination and generation of encephalitogenic peptides, causing neuroinflammation in multiple sclerosis	(Proost *et al.*, 1993; Chandler *et al.*, 1995; Asahi *et al.*, 2001)
			MMP-1,-2,-3 MMP-7,-12	*In vitro*	Degradation	ND	(Chandler *et al.*, 1995; Chandler *et al.*, 1996)
NG2 proteoglycan	/	TMD	MMP-9	*In vitro* + *in vivo*	Degradation	Removal of NG2 proteoglycan-mediated inhibition of oligodendrocyte maturation and differentiation, thus promoting remyelination after CNS injury	(Larsen *et al.*, 2003)

β dystroglycan	/	TMD	MMP-2,-9	*In vitro + in vivo*	Degradation	BBB breakdown, CNS infiltration by leukocytes and development of multiple sclerosis disease symptoms	(Agrawal *et al.*, 2006)
APP	/	TMD	MMP-2	Lys687-Leu688* Glu668-Val669** *In vitro*	sEffector	Release of sAPPα*, preventing Aβ formation in Alzheimer's disease; or release of sAPPβ**, promoting Aβ formation; inhibition of MMP-2 by sAPP	(Miyazaki *et al.*, 1993, 1994; LePage *et al.*, 1995)
			MMP-3	Glu668-Val669 Glu674-Phe675 *In vitro*	ND	ND	(Rapala-Kozik *et al.*, 1998)
			MT1-MMP	Asn579-Met580# *In vitro*	sEffector	Release of sAPPtrc#, which displaces the MMP-2 inhibitory activity of APP or sAPP, promoting MMP-2-catalyzed ECM degradation and tumor cell migration; or release of sAPPα##, reducing Aβ formation in Alzheimer's disease	(Higashi and Miyazaki, 2003b)
			MT3-MMP	Ala463-Met464 Asn579-Met580# His622-Ser623 His685-Gln686## *In vitro*	sEffector		(Ahmad *et al.*, 2006)
			MT5-MMP	ND *In vitro*	ND	ND	(Ahmad *et al.*, 2006)
Soluble Aβ	/	S	MMP-3	*In vitro*	Degradation	Possible reduction of the accumulation of extracellular Aβ peptides in toxic amyloid plaques during Alzheimer's disease	(White *et al.*, 2006)
			MMP-2	Lys16-Leu17 Leu34-Met35 Met35-Val36 *In vitro + in vivo*			(Roher *et al.*, 1994; White *et al.*, 2006; Yin *et al.*, 2006)
			MMP-9	Lys16-Leu17 Phe20-Ala21 Asp23-Val24 Ala30-Ile31 Gly33-Leu34 Leu34-Met35 Gly37-Gly38 *In vitro + in vivo*			(Backstrom *et al.*, 1996; Yan *et al.*, 2006; Yin *et al.*, 2006)
Aβ fibrils	/	S	MMP-2,-9	Phe20-Ala21 Ala30-Ile31 *In vitro*	Degradation	Degradation of Aβ fibrils in amyloid plaques, possibly contributing to the clearance of plaques from Alzheimer's diseased brains	(Yan *et al.*, 2006)

Aβ, β-amyloid protein;APP, amyloid precursor protein; BBB, blood-brain barrier; BP, bullous pemphigoid; BRB, blood-retinal barrier; CNS, central nervous system; GnRH, gonadotropin-releasing hormone; KS, keratitis sicca; PDR, proliferative diabetic retinopathy; PVR, proliferative vitreoretinopathy; *X*, *Xenopus*.

[1]Substrate: for the meaning of the acronyms, see the list of abbreviations.

[2]CD: Cluster of differentiation of human (glyco)proteins.

[3]MA: type of cell membrane association: TMD, transmembrane domain; CSL, cell surface localization; GPI, glycosyl phosphatidylinositol anchor; S, soluble; IM, integral membrane protein.

[4]Substrate modulation: mInhibitor, membrane-bound inhibitor; sInhibitor, release of soluble inhibitor; sEffector, release of soluble effector; ND, not defined.

2002). In earlier studies, the MMPs were claimed to be important in migration, invasion and metastasis mainly by the degradation of basement membranes and structural components in the ECM. However, more recent findings show that proteolysis of a panacea of essential secreted and membrane-bound molecules, like growth factor precursors and receptors, tyrosine kinase receptors (TKRs), cytokines and chemokines, cell adhesion molecules, and other proteases, modifies the tumor microenvironment. These modifications will affect tumor progression at all levels (Nelson *et al.*, 2000; McCawley and Matrisian, 2001). In this first part we will discuss proteolytic modifications of cell surface proteins by MMPs and their concomitant effects on cancer evolution (see Figure 2). Insight into the paradoxical consequences of proteolysis of cell surface proteins by MMPs might be of paramount importance in the design of new cancer therapies based on MMP inhibition.

1.1 Promotion or Inhibition of Cancer Cell Proliferation

MMPs stimulate tumor cell proliferation by catalysing the release of growth factors from the ECM, by the activation of membrane-bound growth factor receptors or by cleavage of membrane-bound substrates, e.g., integrins, that assist in the induction of cell proliferation. Conversely, MMPs might also negatively regulate cancer-cell growth by releasing pro-apoptotic molecules like Fas ligand (FasL) and TNF-α, or by activating transforming growth factor-β (TGF-β), which has a tumor suppressing effect in early phases of oncogenesis (Egeblad and Werb, 2002).

1.1.1 Fibroblast Growth Factor Receptor-1 (FGFR-1)

The fibroblast growth factors (FGFs) constitute a family of twenty structurally related polypeptides with a common high affinity to heparin. Their biological functions are broad and range from the induction of cellular proliferation to tissue regeneration, neurite outgrowth, and angiogenesis (Powers *et al.*, 2000). FGFs elicit their biological response by binding to four different cell surface TKRs, which are typically composed of an extracellular part with three immunoglobulin (Ig)-like domains, a single transmembrane domain and a bipartite TKR domain. Regulation of FGF biological activity may be achieved through several mechanisms, including binding to high and low affinity receptors on the cell surface, release of FGF from the ECM by heparanases or other proteases or binding to a carrier protein that can deliver FGFs to their receptors. In addition, FGF activity might be regulated by the release of the entire ectodomain of the FGF receptor-1 (FGFR-1, basic fibroblast growth factor receptor-1 (bFGF-R), fms-like tyrosine kinase 2, c-fgr, CD331) into the circulation and the ECM (Levi *et al.*, 1996; Powers *et al.*, 2000). MMP-2 is able to free the entire FGFR-1 ectodomain from an immobilized FGFR1 ectodomain-alkaline phosphatase (FRAP) fusion protein *in vitro* by hydrolyzing the Val368-Met369 bond, eight amino acids upstream of the transmembrane domain (Figure 3). By this truncation, the ligand binding site is released as a soluble ectodomain retaining its FGF binding ability (Levi *et al.*, 1996). FGFs may stimulate tumor cell growth in at least one of the following ways: 1) by acting as mitogens for the tumor cells themselves; 2) by promoting angiogenesis for the growing tumor and 3) by inhibiting apoptosis and allowing tumor cells to keep growing beyond normal constraints (Powers *et al.*, 2000). In this regard, release of the FGFR-1 ectodomain by MMP-2 would counter these mechanisms in two ways. On the one hand, the released FGFR-1 ectodomain might bind extracellular FGF and in this manner diminish the biological availability and growth promoting activity of FGF. On the other hand, treatment of FGFR-1 overexpressing cells with MMP-2 clearly reduces binding of the FGF mitogen (Levi *et al.*, 1996). Thus, investigating the release of the FGFR-1 ectodomain by MMP-2 or by related MMPs *in vivo* may provide new insights in tumor cell growth inhibition.

1.1.2 Heparin-Binding Epidermal Growth Factor-Like Growth Factor (HB-EGF)

The heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF, also called diphtheria toxin receptor [DT-R]) is a member of the EGF family of growth factors, which encompasses the receptor family of a number of structurally homologous mitogens like EGF, transforming growth factor-α (TGF-α) and amphiregulin (AR) (Raab and Klagsbrun, 1997; Iwamoto and Mekada, 2000). HB-EGF binds to the EGF receptor (EGFR/HER1/ErbB1) as well as to HER4/ErbB4, inducing homo- or heterodimerisation of the monomeric receptors with consequent tyrosine phosphorylation in the cytoplasmic domains, resulting in the activation of several signal tranduction pathways (Zwick *et al.*, 1999; Herbst, 2004). HB-EGF is synthesized as a transmembrane protein (mHB-EGF) with the ectodomain

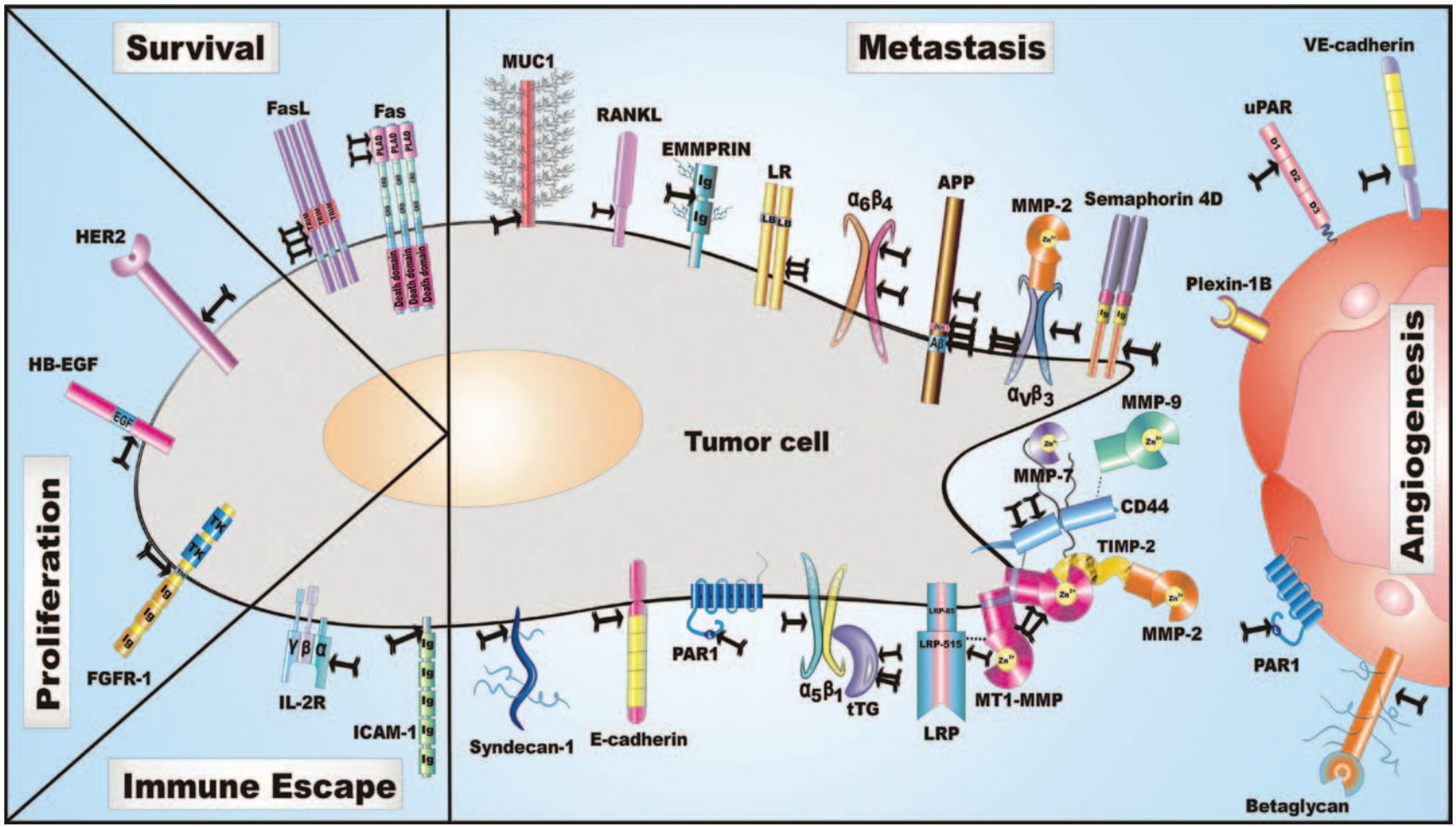

FIGURE 2 Proteolytic modification of cell surface proteins by MMPs in cancer development and evolution. All cell surface-associated molecules cleaved by MMPs during cancer development are presented on the cell membrane of a migrating tumor cell. These susbstrates are grouped according to the most affected cancer mechanism. Ectodomain cleavage of growth factor receptors may amplify the promotion of cell proliferation (HER2) or on the contrary release a soluble decoy receptor that binds soluble growth factors, thus decreasing their binding to intact growth-promoting receptors (FGFR-1). Proteolysis of FasL and Fas disturbs trimerization, which attenuates apoptosis induction, allowing the tumor cells to survive and proliferate. Formation of new vessels is crucial for the survival of a growing tumor. Cleavage of proteins on the endothelial cell membranes by MMPs may have a pro-angiogenic (VE-cadherin, PAR1) as well as an anti-angiogenic effect (uPAR, Betaglycan). Furthermore, alternation of adhesion and anti-adhesion is required as the cell migrates during the processes of invasion and metastasis. MMPs promote metastasis by proteolysis of adhesion molecules (integrin precursors, tTG, CD44, E-cadherin), cytokines (RANKL), receptors (PAR1, EMMPRIN, LRP) and structural proteins (syndecan-1, APP). Many of these cleavages are mediated by MT1-MMP, which colocalizes with CD44 at the ruffling edge of migrating tumor cells. CD44 forms a platform to cluster MMPs, which stimulate migration by cleaving substrates on the cell surface and by degrading the ECM. Finally, cleavage of the transmembrane proteins, IL-2Rα and ICAM-1 on transformed leukocytes, allows tumor cells to escape assaults from the immune system. Arrows indicate cleavages by MMPs. For details of the substrate acronyms, see the list of abbreviations.

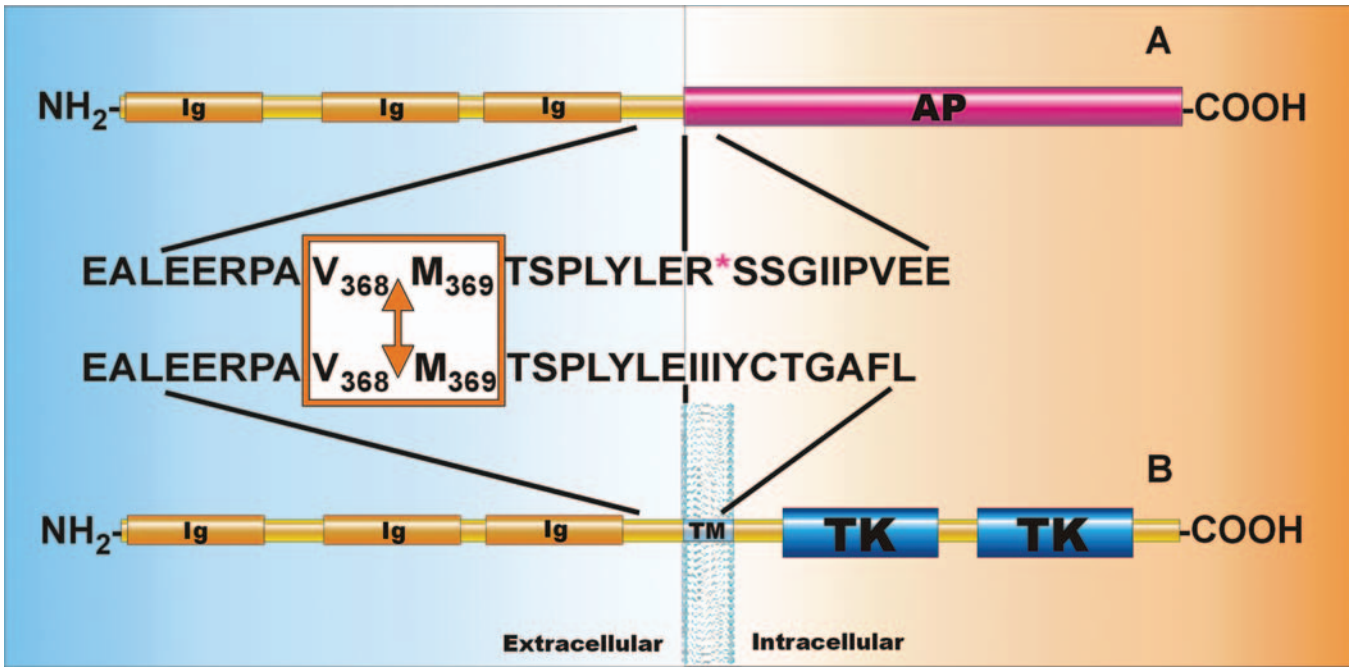

FIGURE 3 **Cleavage of the human FGFR-1 ectodomain-AP fusion protein by MMP-2 and comparison with murine FGFR-1. MMP-2 cleaves the FGFR-1 ectodomain from an immobilized FGFR1 ectodomain-alkaline phosphatase (FRAP) fusion protein *in vitro* at the Val368-Met369 bond, eight amino acids upstream of the transmembrane domain (A). This truncation releases a soluble ectodomain that retains FGF binding ability, thus decreasing FGF binding sites on the tumor cell and competing with the remaining intact FGFR-1. Murine FGFR-1 is shown for comparison (B). Arrow, cleavage site of MMP-2; AP, alkaline phosphatase; Ig, immunoglobulin domain; TK, tyrosine kinase domain; TM, transmembrane domain; R*, start of the AP. Adapted from (Levi *et al.*, 1996).**

containing a heparin-binding and an EGF-like domain. mHB-EGF can be cleaved at the plasma membrane to yield soluble HB-EGF (sHB-EGF) (Raab and Klagsbrun, 1997; Iwamoto and Mekada, 2000). mHB-EGF is not only a precursor for sHB-EGF but is a bioactive molecule itself, which inhibits growth of neighboring cells. As a contrast, sHB-EGF is a potent mitogen for a number of cells including smooth muscle cells, epithelial cells and keratinocytes. Therefore, release of HB-EGF causes the conversion of a juxtacrine growth inhibitor into a paracrine/autocrine growth factor, with an opposite impact on cell growth (Iwamoto and Mekada, 2000; Higashiyama, 2004). mHB-EGF proteolysis is probably mediated by metalloproteinases as it is prevented by the broad spectrum metalloproteinase inhibitor batimastat (Prenzel *et al.*, 1999). In cell cultures, MMP-3 cleaves mHB-EGF in the juxtamembrane domain at the Glu151-Asn152 site (Suzuki *et al.*, 1997; Wu *et al.*, 2004), whereas MMP-7, MMP-2 and MMP-9 and some proteases of the ADAM family [ADAM-9, ADAM-10, ADAM-12, and ADAM-17/TACE] mediate mHB-EGF cleavage *in vivo* under specific circumstances (Higashiyama, 2004; Hao *et al.*, 2004).

HB-EGF plays an important role in a multitude of biological processes and diseases. According to the concerned process, the mHB-EGF proteolysis is induced by varying stimuli and mediated by different metalloproteinases. Release of sHB-EGF allows cross-talking between G protein-coupled receptors (GPCRs) and TKRs in accordance with the triple membrane-passing signal mechanism (TMPS) for the transactivation of TKRs (Figure 4). In the TMPS, GPCRs are activated by an extracellular ligand and subsequently induce the activation of metalloproteinases through the initiation of several signaling cascades. Finally, metalloproteinase activity releases sHB-EGF that activates its TKR, the EGFR (Higashiyama, 2004; Shah and Catt, 2004a). Several studies showed enhanced HB-EGF gene expression in tumors compared to normal tissue (Raab and Klagsbrun, 1997). sHB-EGF expression increased tumor growth rate, colony-forming ability, and activation of the cyclin D1 promotor, as well as induction of vascular endothelial growth factor (VEGF) *in vitro*. In addition, sHB-EGF induced the expression and activity of MMP-3 and MMP-9, leading to enhanced cell migration. *In vivo* sHB-EGF enhanced tumorigenesis and angiogenesis. Hence, release of sHB-EGF by MMPs may be an interesting step for therapeutical intervention (Ongusaha *et al.*, 2004; Miyamoto *et al.*, 2004).

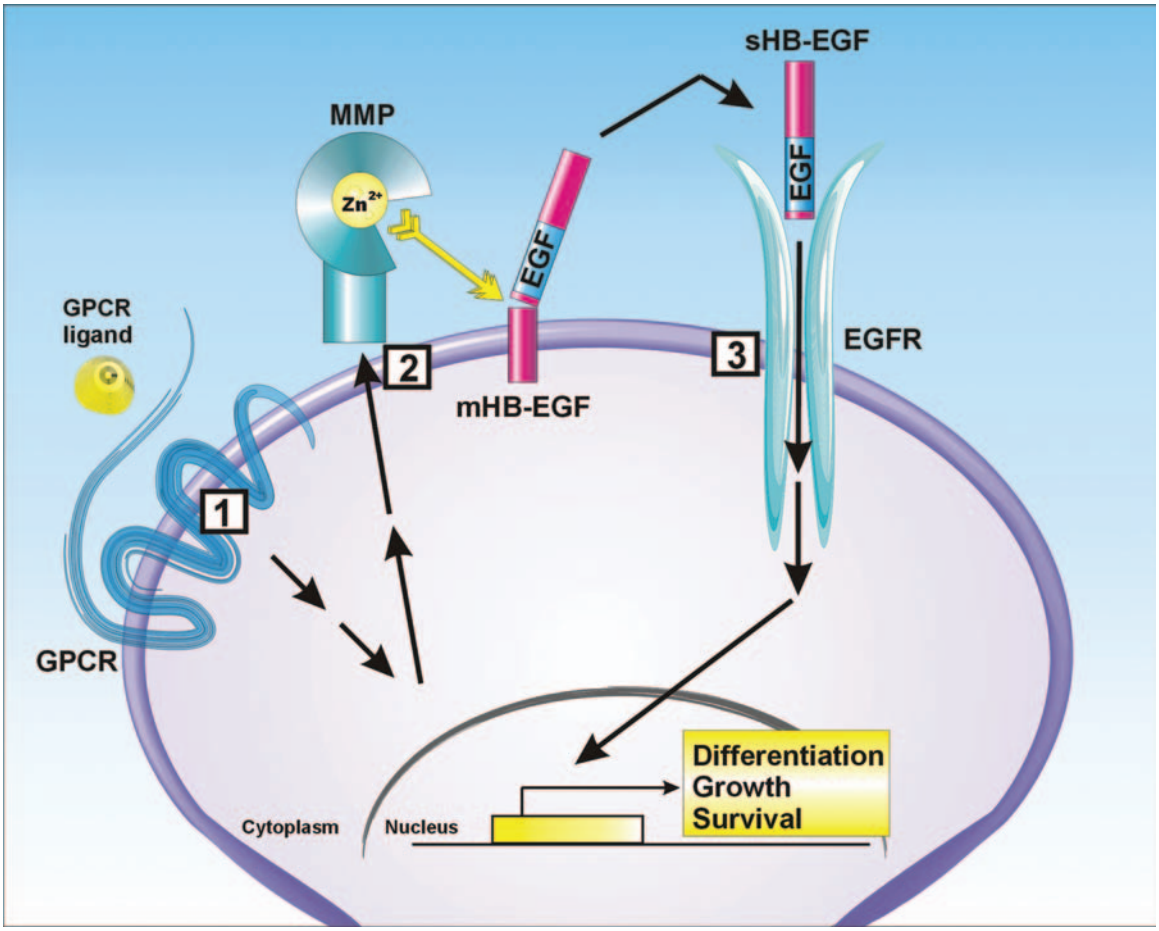

FIGURE 4 **Release of sHB-EGF by MMPs allows cross-talking between G protein-coupled receptors (GPCRs) and tyrosine kinase receptors (TKRs) in accordance with the triple membrane-passing signal mechanism (TMPS). In the TMPS, GPCRs are first activated by an extracellular ligand (1) and subsequently induce the production of metalloproteinases through the initiation of several signaling cascades (2). Finally, metalloproteinase activity releases sHB-EGF that activates its TKR, the EGFR, which induces signal transduction to stimulate cell differentiation, growth and survival (3). Arrow, cleavage by an MMP. Adapted from (Shah and Catt, 2004a).**

Besides stimulating tumor cell proliferation, sHB-EGF induces proliferation of Müller glial cells, which is a common feature of several diseases of the sensory retina. During proliferative vitreoretinopathy (PVR), Müller cells proliferate continuously, migrate onto retinal surfaces, and participate in the formation of periretinal cellular membranes (Rentsch, 1973; Bringmann and Reichenbach, 2001). Proliferation of Müller cells in PVR seems to be stimulated by binding of ATP to P2Y purine receptors (GPCRs) and requires the transactivation of two TKRs. Activation of P2Y receptors by ATP leads to an increase in intracellular Ca^{2+} concentration and may cause a release of platelet-derived growth factor (PDGF) from the cells. Released PDGF activates the PDGF-α receptor (1st TKR), which causes MMP-9 production. MMP-9 then releases sHB-EGF, which in turn activates the EGFR (2nd TKR). Finally, the activated EGFRs and PDGF-α receptors induce proliferative activity in Müller cells by initiating several kinase signaling pathways. As a neutralizing antibody against MMP-9 reversed the mitogenic effect of ATP, inhibition of MMP-9 in the pathway may be a useful tool to suppress uncontrolled intraocular proliferation in PVR (Milenkovic *et al.*, 2003; Shah and Catt, 2004a).

Besides the stimulating effects on tumor progression and PVR, proteolysis of mHB-EGF by MMPs plays a part in cardiovascular diseases (see Section 2.1.1) and reproductive endocrinology (see Section 5.2), which will be discussed in later chapters.

1.1.3 Tyrosine Kinase-Type Cell Surface Receptor HER2

The EGFR family comprises four TKRs of which tyrosine kinase-type cell surface receptor HER2 (HER2, receptor tyrosine-protein kinase erbB-2, p185erbB2, c-ErbB2, NEU proto-oncogene [neu], MLN 19, CD340)

is the most oncogenic, as it is active in the absence of a stimulating ligand. In addition, HER2 in heterodimeric receptors is highly mitogenic because it decelerates growth factor dissociation from its partner receptor, prolonging the duration of intracellular signaling. In addition, endocytosis of HER2-containing complexes is relatively slow and these complexes tend to recycle back to the cell surface, rather than being degraded in the lysosomes. HER2, a 185 kDa transmembrane glycoprotein receptor, underlies many altered functions of tumor cells, including excessive growth, invasive behavior and attraction of blood vessels (Mosesson and Yarden, 2004). It is overexpressed in 25% to 30% of breast cancers, and it has been associated with high risks of relapse and death (Slamon *et al.*, 1987), although the prognostic value of HER2 has been the matter of some controversy (Ross *et al.*, 2003). Blockage of HER2 with the humanized anti-HER2 monoclonal antibody trastuzumab is an example of successful immunotherapy for HER-2-positive breast cancer patients, as it has been demonstrated to reduce the risk of recurrence by roughly 50% in five randomized clinical trials (Piccart-Gebhart, 2006).

The HER2 ectodomain is released and detected in the serum of cancer patients. High serum levels of HER2 ectodomain correlate with a poor prognosis and decreased responsiveness to therapy in patients with advanced breast cancer. This may be due to the enhanced signaling activity of the remaining cell-associated part of HER2. The cleavage is inhibited by the metalloproteinase inhibitors batimastat and TNF-α protease inhibitor (TAPI), as well as by TIMP-1, which inhibits soluble MMPs (Codony-Servat *et al.*, 1999). In addition, trastuzumab inhibits basal and induced HER2 cleavage, preceding antibody-induced receptor downmodulation (Molina *et al.*, 2001). Further identification of the metalloproteinase in charge could lead to new options for the therapy of patiens with breast cancer and high levels of HER2 shedding, for example by combining MMP inhibition with trastuzumab therapy.

1.2 Survival of Cancer Cells or Induction of Apoptosis

Evasion of apoptosis permits survival of tumor cells in spite of genetic instability. Low levels of oxygen and nutrients, host defence against the tumor by the immune system, anti-cancer treatments and local *in vivo* changes in the ECM with effects on invasion and metastasis alter the protease load in the tumor environment (Reed, 1999). Proteolysis by MMPs can have apoptotic as well as anti-apoptotic effects.

1.2.1 Fas Ligand (FasL)

An important effector in apoptosis is the Fas/FasL system, which is involved in three types of immune-associated killing: 1) elimination of virally infected cells and tumor cells by cytotoxic T cells (CTLs) and natural killer (NK) cells; 2) maintenance of immune privilege or survival of tumor cells; and 3) regulation of lymphocyte development and maintenance of peripheral immune homeostasis. Fas ligand (FasL, tumor necrosis factor ligand superfamily member 6, CD95 L, apoptosis antigen ligand (APTL), APO-1 L, CD178) is a ~40 kDa type II transmembrane protein of the TNF family of death factors. It is expressed on activated T lymphocytes like CTLs and tumor infiltrating lymphocytes (TILs), on macrophages and NK cells and on cells within immune privileged tissues including the eye, testis, uterus, and placenta (Linkermann *et al.*, 2003). After trimerization FasL induces apoptosis by binding to a trimer of its receptor Fas (tumor necrosis factor receptor superfamily member 6, apoptosis-mediating surface antigen FAS, APO-1, FASLG receptor, CD95). FasL can be released form the cell surface by MMP-3 and MMP-7. However, the effects of soluble FasL (sFasL) on apoptosis and tumor progression seem to vary. Apoptosis may be induced in epithelial cells through cleavage of membrane-bound FasL to functional sFasL by MMP-7. Furthermore, this cleavage turns out to be crucial for apoptosis of prostate epithelium after castration, as prostate involution is significantly reduced in MMP-7 deficient mice (Powell *et al.*, 1999). In contrast with apoptosis induction in epithelial cells, FasL shedding by MMP-7 has an anti-apoptotic effect in tumor cells, protecting them from chemotherapeutic drug toxicity (Mitsiades *et al.*, 2001). This protection of tumor cells may be explained by the fact that the ability of sFasL to induce apoptosis is significantly lower than that of its cell surface precursor (Tanaka *et al.*, 1998). A possible explanation for the paradoxical effects of FasL in both cell types might be the higher sensivity of epithelial cells to apoptotic signals, while FasL confers a kind of immune privilege to tumors by inducing apoptosis in infiltrating lymphocytes. Tumor cells indeed express lower levels of Fas and increased levels of FasL, which allows them to 'counterattack' Fas-bearing immune cells (Kim *et al.*, 2004). Acute overexpression of MMP-7 *in vitro*

as well as *in vivo* induces apoptosis, whereas chronic or repeated exposure to MMP-7 can select for tumor cells that are less sensitive to death-inducing stimuli (Fingleton *et al.*, 2001; Vargo-Gogola *et al.*, 2002b). Another potential explanation for the mentioned discrepancies in sFasL activity came up after the identification of novel MMP-7 cleavage sites in murine and human FasL. Mutational analysis showed that MMP-7 cleaves human and murine FasL at an ELAELR sequence, close to the transmembrane domain (Figure 5), followed by a secondary cleavage at the COOH-terminally located SL sites. Strong indications exist that cleavage of human FasL also occurs at another ELR sequence in the trimerization domain. As trimerization of FasL has been shown to be important for its activity, cleavage within the trimerization domain may play a significant role in the regulation of sFasL function, releasing less active FasL. Human sFasL peptides generated after MMP-7 cleavage at ELAELR contain 13 or 16 additional amino acids compared to the peptides formed after proteolysis at the SL site. The entire extracellular portion of FasL was shown to be pro-apoptotic *in vitro*, whereas sFasL produced by cleavage at the SL site was inactive in the same assay (Hohlbaum *et al.*, 2000). This implies that the amino acid sequence between the SL cleavage site and the transmembrane domain contributes to the pro-apoptotic activity of sFasL. Therefore, truncation at the SL site might release inactive sFasL peptides (Vargo-Gogola *et al.*, 2002a). Differential FasL proteolysis by MMP-7 might thus lead to the release of sFasL molecules with diverse biological activities.

1.2.2 Fas

MMP-7 expression also contributes to another strategy by which tumor cells can resist Fas-induced apoptosis. As a matter of fact, Fas too is cleaved by MMP-7, between Glu19-Leu20 and Asn32-Leu33 (Figure 6) (Strand *et al.*, 2004). These cleavages remove 19 or 32 amino acids from the extracellular NH_2-terminus of the Fas molecule and delete part of a domain for self-association termed 'preligand assembly domain' (PLAD). The PLAD domain has been shown to facilitate oligomerization of Fas receptors before ligand binding. Preassembly of Fas receptors might be crucial for the regulation of Fas signaling (Siegel *et al.*, 2000). Fas proteolysis by MMP-7 indeed results in attenuated apoptosis induction (Strand *et al.*, 2004). MMP-7 is produced by the tumor cells themselves at early stages of tumor development. Therefore, the impact of this MMP on apoptosis resistance provides a potential target for new combination therapies in which MMP inhibition may significantly augment the efficacy of conventional chemotherapy (Poulaki *et al.*, 2001).

1.3 Regulation of Angiogenesis

Oxygen and nutrients, provided by the vascular system, are crucial for cell function and survival, obligating virtually all cells in a tissue to reside within 100 μm of a capillary blood vessel. Consequently, angiogenesis or the formation of new blood vessels, is a prerequisite for the survival of proliferating cells and is the result of subtle and often complex interactions between regulator and effector molecules (Hanahan and Weinberg, 2000; Pepper, 2001; Bergers and Benjamin, 2003).

1.3.1 Urokinase-Type Plasminogen Activator Receptor (uPAR)

The urokinase-type plasminogen activator receptor (uPAR, monocyte activation antigen Mo3, CD87) is a GPI-anchored receptor containing three homologous domains (D1, D2, and D3) (Blasi and Carmeliet, 2002). D1 is involved in the binding of the urokinase-type plasminogen activator (urokinase or u-PA) and enhances the interaction with the ECM by binding the ECM molecule vitronectin. D2 and D3 are also indispensable for high-affinity interactions (Hoyer-Hansen *et al.*, 1997a; Oda *et al.*, 1998). uPAR regulates u-PA activity–the activation of plasminogen to plasmin that degrades fibrin (Blasi and Carmeliet, 2002)–on the cell surface and is also important for the activation of signaling pathways through the interaction with several integrins (Reuning *et al.*, 2003). Besides the membrane-anchored uPAR, a soluble receptor (suPAR) is released after proteolysis of the GPI anchor by cellular phospholipase D (Figure 7) (Wilhelm *et al.*, 1999). Both uPAR and suPAR can be cleaved between the D1 and D2 domain, generating a D1-fragment and a D2D3-fragment (Blasi and Carmeliet, 2002). The D1 domain can be released by proteolytic activity of u-PA, directly or indirectly through activation of plasminogen (Hoyer-Hansen *et al.*, 1997b), or by other proteases such as chymotrypsin and elastase (Ploug and Ellis, 1994). The specificty of the cleaving protease is crucial, as uPAR fragments may or may not be chemotactically active on different cell types *in vitro*, depending on the presence or

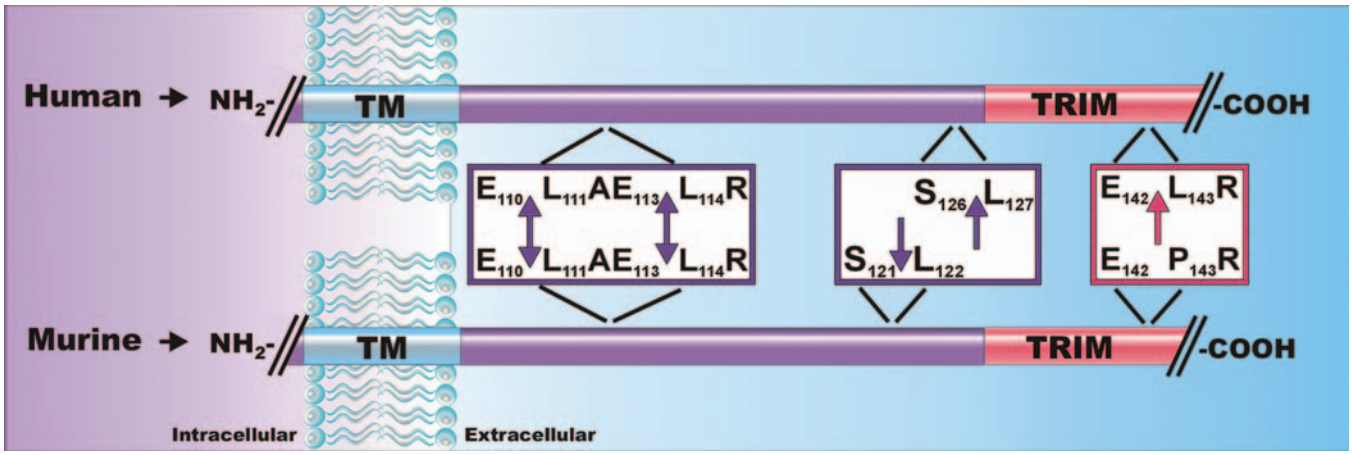

FIGURE 5 **Cleavage sites of MMP-7 in the ectodomain of human and murine FasL. MMP-7 cleaves human and murine FasL at an ELAELR sequence, close to the transmembrane domain (TM), followed by a secondary cleavage at COOH-terminally located SL sites. Human sFasL peptides generated after MMP-7 cleavage at ELAELR were shown to be pro-apoptotic *in vitro*. However, the peptides formed after proteolysis at the S_{126}-L_{127} site, which contain 13 or 16 amino acids less, were inactive in the same apoptosis assay. This might mean that the amino acid sequence between the SL cleavage site and the transmembrane domain contributes to the pro-apoptotic activity of sFasL. Cleavage of human FasL almost certainly occurs at an additional ELR sequence, E_{142}-L_{143}R, in the trimerization domain (TRIM). As trimerization of FasL has been shown to be important for its activity, cleavage within the trimerization domain may release less active FasL. Arrows indicate the cleavage sites of MMP-7. Adapted from (Vargo-Gogola *et al.*, 2002a).**

absence of a specific NH_2-terminal peptide, SRSRY, on the D2D3-fragment (Fazioli *et al.*, 1997). Inhibition of MMPs in a three-dimensional fibrin matrix, used as an experimental angiogenesis model, caused enhanced formation of capillary-like tubular structures and showed that the cleavage between D1 and D2 can also be performed by MMPs. The first MMP to be identified as uPAR sheddase was MMP-12 (Koolwijk *et al.*, 2001), but also several other MMPs were able to release the D1 domain of suPAR *in vitro*: MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, MMP-19, MT1-MMP, and MT6-MMP (Andolfo *et al.*, 2002). However, the most efficient MMPs for uPAR cleavage were MMP-3, MMP-12, MMP-19, and MT6-MMP. In particular, MMP-3, MMP-12, and MT6-MMP show the same specificity of cleavage having the Thr86-Tyr87 peptide bond as major cleavage site, while MMP-19 cleaves suPAR predominantly at Tyr87-Ser88 (Figure 7). uPAR cleavage by MMPs results in the exposure of the chemotactic epitope SRSRY at the NH_2-terminus of the generated D2D3-fragment and may thus generate biologically active fragments. Murine uPAR is cleaved by MMP-12

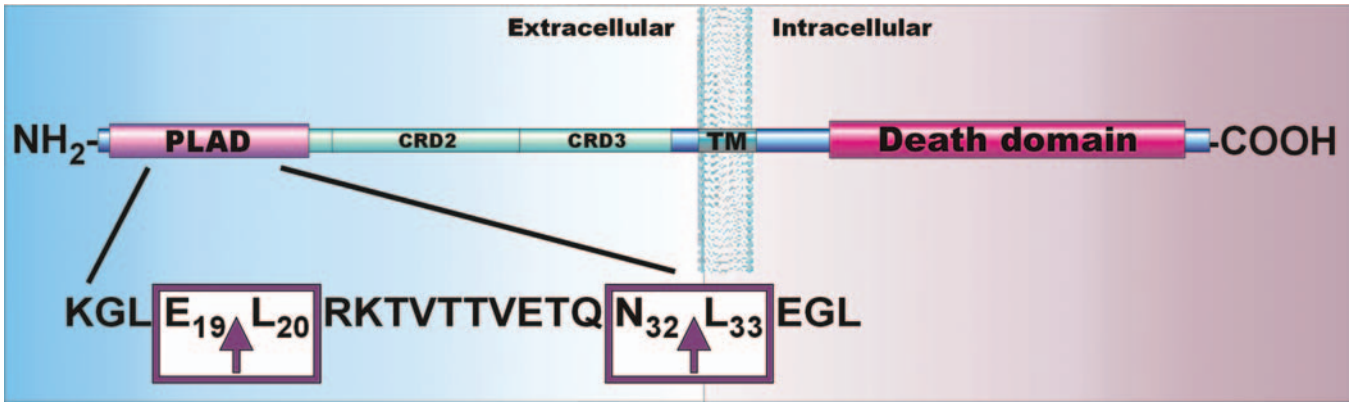

FIGURE 6 **MMP-7 cleaves the 'preligand assembly domain' (PLAD) in the Fas ectodomain. Fas is cleaved by MMP-7 between Glu19-Leu20 and Asn32-Leu33. These cleavages remove 19 or 32 amino acids from the Fas NH_2-terminus and delete part of a domain for self-association termed 'preligand assembly domain' (PLAD). The PLAD domain facilitates oligomerization of Fas receptors before ligand binding. Preassembly of Fas receptors may be crucial for the regulation of Fas signaling and proteolysis of the PLAD domain by MMP-7 indeed results in decreased sensitivity of tumor cells to Fas-mediated apoptosis. Arrows indicate the cleavage sites of MMP-7; CRD, cysteine-rich domain; TM, transmembrane domain. Adapted from (Strand *et al.*, 2004).**

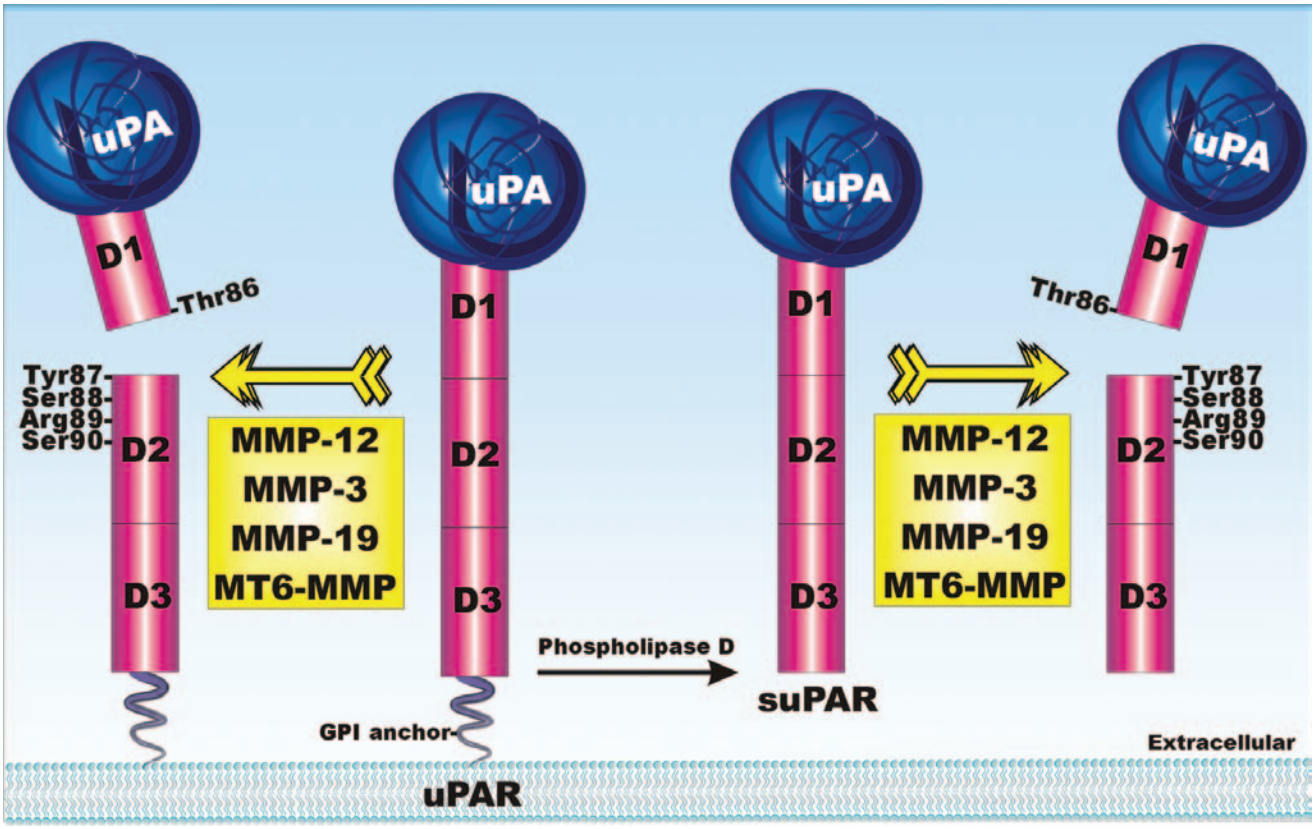

FIGURE 7 **Cleavage of uPAR and suPAR by MMPs releases the u-PA-binding domain D1. uPAR is a GPI-anchored receptor for u-PA containing 3 homologous domains (D1, D2, and D3). In addition to the membrane-anchored uPAR, a soluble receptor (suPAR) is released after cleavage of the GPI anchor by cellular phospholipase D. Both uPAR and suPAR can be cleaved between the D1 and D2 domains, generating a D1-fragment and a D2D3-fragment. Release of the D1 domain can be mediated by MMPs, but also by serine proteases such as u-PA, plasmin, chymotrypsin and elastase. The first MMP to be identified as uPAR sheddase was MMP-12 but also several other MMPs release the D1 domain of suPAR *in vitro*. However, the MMPs most efficient at uPAR cleavage are MMP-3, MMP-12, MMP-19 and MT6-MMP. In particular, MMP-3, MMP-12 and MT6-MMP show the same specificity of cleavage with the Thr86-Tyr87 peptide bond as major cleavage site, whereas MMP-19 cleaves suPAR predominantly at Tyr87-Ser88. Loss of the D1 domain results in less cellular u-PA binding and, consequently, in diminished formation of new capillary structures in an angiogenesis model.**

as well (at the Pro89-Gln90 peptide bond), despite the limited sequence homology between the linker regions (Andolfo *et al.*, 2002).

Unexpected enhanced angiogenesis through MMP inhibition might be caused by the higher availability of functional uPAR at the cell surface, resulting in increased u-PA binding and, subsequently, enhanced formation of new capillary structures. As plasmin activates several MMPs (for reviews, see: Collen, 2001; Pepper, 2001; Van den Steen *et al.*, 2001), release of the D1 fragment by an MMP might be a kind of feedback regulation. Since angiogenesis in and growth of some tumors are inhibited by competitors of uPAR, and taking into account that some MMPs (such as MMP-9) exert pro-angiogenic effects, the influence of MMP inhibition on uPAR levels and on angiogenesis certainly requires further examination.

Cleavage of uPAR by MMP-12 may also play a role in the autoimmune disease systemic sclerosis. This will be detailed in a subsequent paragraph (see Section 3.4.4).

1.3.2 Betaglycan

Betaglycan, also known as the TGF-β type III receptor, is a membrane-anchored proteoglycan whose glycosaminoglycan chains consist of heparan and chondroitin sulfate. Membrane-bound betaglycan is generally considered a positive regulator of TGF-β because it increases the binding affinity of TGF-β for its receptor II, enhancing cell responsiveness to TGF-β (Lopez-Casillas *et al.*, 1994). TGF-β controls many physiological processes and has tumor-suppressing activity in the early phases of carcinogenesis. In subsequent stages of tumor progression, the increased secretion of TGF-β by both tumor cells and stroma cells, is involved in the

enhancement of tumor invasion and mestastasis, accompanied by immunosuppression (Kim *et al.*, 2004). TGF-β upregulates MMP-9 activity, which may also amplify angiogenesis and tumor growth. Additionally, MMP-9 is capable of activating latent TGF-β (Yu and Stamenkovic, 2000; Bandyopadhyay *et al.*, 2005), thanks to the anchoring of MMP-9 and TGF-β to CD44 on the cell surface (see Section 1.4.7) (Yu and Stamenkovic, 1999).

Two soluble forms of betaglycan are relased by proteolytic cleavage. In some cell types these cleavages are induced by the tyrosine phosphatase inhibitor, pervanadate, and generate a bigger fragment of 120 kDa (sBG-120), which encompasses almost the entire extracellular domain, and a smaller 90 kDa fragment (sBG-90). The cleavage that generates sBG-90 is inhibited by TIMP-2, but not by TIMP-1, which points to an MT-MMP as the involved protease. Overexpression of MT1-MMP and MT3-MMP, but not of the other MT-MMPs, indeed releases the sBG-90 fragment. Surprisingly, MT2-MMP overexpression decreases the levels of betaglycan and of MT1-MMP (Velasco-Loyden *et al.*, 2004). In contrast with membrane-bound betaglycan, recombinant soluble betaglycan has been shown to inhibit TGF-β *in vitro* (Lopez-Casillas *et al.*, 1994). Therefore, betaglycan might function as a dual modulator of TGF-β activity: as a membrane-anchored protein it enhances TGF-β activity, whereas its soluble form causes TGF-β inhibition. TGF-β-promoted tumor-host interactions leading to enhanced angiogenesis have been shown to be effectively attenuated by the systemic administration of soluble betaglycan in a xenograft model of prostate cancer. The inhibition of tumor angiogenesis and consequently of tumor growth appears at least in part due to the inhibition of TGF-β-induced MMP-9 upregulation (Bandyopadhyay *et al.*, 2005). Further investigation will clarify if sBG-90 and sBG-120 have the same TGF-β-inhibiting activities and if proteolysis of betaglycan by MT1-MMP or MT3-MMP can reduce angiogenesis and tumor growth.

1.3.3 Vascular Endothelial Cadherin (VE-Cadherin)

Vascular endothelial-cadherin (VE-cadherin, cadherin-5, 7B4 antigen, CD144) is a member of the large cadherin family that includes Ca^{2+}-dependent cell-cell adhesion molecules responsible for cell-to-cell recognition and adhesion in solid tissues. Cadherins dimerize through the extracellular domain with other cadherin molecules on adjacent cells (*trans*-interaction). In this homotypic interaction, the intracellular domain interacts with various catenin proteins to form the cytoplasmic cell-adhesion complex (CCC), which is crucial for strong cell-cell adhesion and potent suppression of invasion. Cadherins are expressed in several types of tissues with some specificity: Epithelial (E)-cadherin is mostly present in epithelial cells, Neuronal (N)-cadherin in the nervous system, smooth muscle cells, fibroblasts and endothelial cells, and VE-cadherin is specific for the endothelium (Cavallaro and Christofori, 2004; Cavallaro *et al.*, 2006).

MMP-7 treatment of human umbilical endothelial cells (HUVECs) accelerates HUVEC proliferation and degrades VE-cadherin on the cell surface, with concomitant accumulation of β-catenin in the nucleus and an increase of MMP-7 expression. These results suggest that MMP-7-mediated cleavage of VE-cadherin releases β-catenin from the VE-cadherin/catenin complex, allowing it to translocate from the cytoplasm to the nucleus, where it can activate T-cell factor DNA binding protein, which accelerates cell proliferation and MMP-7 expression (Ichikawa *et al.*, 2006). However, MMP-7 also has another important role in angiogenesis as it cleaves plasminogen and converts it to angiostatin (Patterson and Sang, 1997), which is one of the strongest inhibitors of angiogenesis. In light of this, it is crucial to further examine the sometimes paradoxical effects of MMP-7 on angiogenesis before targetting its activity in anti-cancer therapy.

1.3.4 Semaphorin 4D

Semaphorins are secreted, transmembrane or GPI-linked proteins, defined by cysteine-rich semaphorin protein domains, that have essential roles in a variety of tissues. Functionally, semaphorins were initially characterized for their importance in the development of the nervous system and in axonal guidance. More recently, they have been found to play a role in a wide range of processes, including tissue organization during development, angiogenesis, immunoregulation, and tumor progression. A common theme in the mechanisms of semaphorin function is that they alter the cytoskeleton, *i.e.*, the organization of actin filaments and the microtubular network, through binding with their receptors. The best characterized semaphorin receptors are members of the neurophilin and plexin families (Yazdani and Terman, 2006). Plexin-1B is highly expressed in endothelial cells and promotes migration and

tubulogenesis *in vitro* as well as *in vivo* when bound by its ligand, semaphorin 4D (BB18, A8, GR3, CD100) (Basile *et al.*, 2004). Semaphorin 4D is highly expressed in head and neck squamous cell carcinomas as well as in some of the most prevalent solid tumors, including breast, prostate, colon and lung cancer tissues (Basile *et al.*, 2006).

In order to exert its pro-angiogenic functions, semaphorin 4D, a transmembrane protein, must be processed and released into a soluble form to act in a paracrine manner on endothelial cells. Semaphorin 4D is expressed on the cell surface as a homodimer (see Figure 2), which is a prerequisite for its proteolytic release from the cell surface (Elhabazi *et al.*, 2001). The shedding process can be inhibited by the metalloproteinase inhibitors EDTA, EGTA and Ilomastat/GM6001, as well as by TIMP-2, but not by TIMP-1 (Elhabazi *et al.*, 2001; Basile *et al.*, 2007). The inhibitor profile, combined with the observation that MT1-MMP, while not expressed in non-tumorigenic epithelial cell lines, was present in several head and neck squamous carcinoma cell lines, pointed to MT1-MMP as the semaphorin 4D sheddase. Basile and coworkers (2007) demonstrated that MT1-MMP was required for processing and release of semaphorin 4D from these cells, thereby inducing endothelial cell chemotaxis *in vitro* and blood vessel growth *in vivo*. As a consequence, MT1-MMP-dependent shedding of semaphorin 4D may play a critical role in tumor-induced angiogenesis, and therefore may represent new fronts of attack in the anti-angiogenic therapy of cancer.

1.4 Stimulation or Inhibition of Migration, Invasion, and Metastasis

Much like tumorigenesis, the processes of invasion and metastasis are highly complex. In its simplest form, metastasis requires the tumor cell to detach from its primary location, invade through stromal elements or existing junctions between normal cells, enter and leave blood vessels or lymphatics, and then establish a colony at the metastatic site. These steps require molecular processes at the cell surface in which contacts between the invading tumor cell and surrounding cells and stroma are repeatedly broken (anti-adhesion) and new contacts established as the tumor cell moves forward (adhesion). This alternation of adhesion and anti-adhesion can be achieved through coordinated expression of proteases and adhesion molecules (Chambers *et al.*, 2002; Hollingsworth and Swanson, 2004).

1.4.1 Mucin-1 (MUC1)

Mucin-1 (MUC1, polymorphic epithelial mucin [PEM, PEMT], episialin, tumor-associated mucin, carcinoma-associated mucin, tumor-associated epithelial membrane antigen [EMA], H23AG, peanut-reactive urinary mucin [PUM], breast carcinoma-associated antigen DF3, CD227), a transmembrane mucin, plays a key role in the inhibition of embryo implantation, in the protection of mucosal surfaces against microbial and proteolytic degradation, and in some aspects of tumor progression. It is expressed on several epithelial surfaces like uterine, lung and intestinal epithelia as well as on tumor cells. The relatively short cytoplasmic tail associates with cytoskeletal elements, cytosolic adaptor proteins and/or participates in signal transduction. The extracellular domain can be released form the cell surface, which might serve practical functions, such as the facilitation of rapid clearance of mucosa surface-associated material, or cell mobility. The mechanism controling this ectodomain release has not yet been elucidated, even though it has been postulated that alterations in pH, ionic concentration or hydration might trigger proteolytic release by specific proteases (Hollingsworth and Swanson, 2004). The major protease in this cleavage process is TACE (Thathiah *et al.*, 2003). However, according to the TIMP-inhibition profile (see Table 1), an additional MUC1 shedding activity belonging to the MT-MMP family was identified in TACE deficient cells. MT1-MMP is expressed on these cells and, in addition, MT1-MMP overexpression or deficiency causes increased or inhibited MUC1 shedding, respectively. Furthermore, MT1-MMP indeed cleaves MUC1 *in vitro* and is colocalized with MUC1 *in vivo* in human uterine epithelia (Thathiah and Carson, 2004). Cancer cells, especially from adenocarcinomas, express aberrant forms and levels of mucins, which have an impact on the biological properties of tumors in several ways (Hollingsworth and Swanson, 2004). Evidences are emerging that cell surface mucins contribute to the regulation of differentiation and proliferation of tumor cells, through ligand-receptor interactions and morphogenetic signal transduction. In addition, MUC1 expression on tumor cells causes anti-adhesion through steric hindrance by forming multiple exposed glycosylated rod-like structures and by the binding of receptors on the same cell (*cis*-interactions), preventing

interaction of these receptors with other cells. This anti-adhesion permits tumor cells to detach form the tumor mass and to invade the surrounding stroma. Invasion is subsequently enhanced, as MUC1 also has an adhesive action by binding adhesion molecules on stroma cells and endothelial cells (*trans*-interactions). Finally, MUC1 also contributes to immune evasion by forming a leukocyte-impermeable barrier around the tumor and through immunosuppressive effects on T-cell proliferative responses. Since MT1-MMP is often expressed by cancer cells, the cleavage of MUC1 may affect all these processes.

1.4.2 Epithelial Cadherin (E-Cadherin)

Epithelial cadherin (E-cadherin, uvomorulin, cadherin-1, CAM 120/80, CD324) is another member of the cadherin family and is expressed mostly in epithelial cells (see Section 1.3.3). Loss of E-cadherin-mediated cell-cell adhesion has been shown to be a prerequisite for tumor cell invasion and metastasis (Birchmeier and Behrens, 1994). Proteolytic degradation of E-cadherin by MMPs is one of the regulation mechanisms by which epithelial cell-cell adhesion can be ablated. MMP-3 and MMP-7 both cleave the 120 kDa transmembrane E-cadherin, releasing a 80 kDa soluble form (sE-cadherin) (Lochter *et al.*, 1997; Noe *et al.*, 2001). Treatment of prostate cancer cells with hepatocyte growth factor/scatter factor (HGF/SF) causes shedding of E-cadherin through the induction of MMP-7, resulting in cell scattering and a switch to a more invasive phenotype (Davies *et al.*, 2001). Released sE-cadherin induces invasion *in vitro* and inhibits cell aggregation indicating that it disturbs cell-bound E-cadherin functions in a paracrine way (Noe *et al.*, 2001). However, it is not clear whether sE-cadherin induces invasion by perturbation of cell-cell interaction and/or by engaging a signalling pathway in which free β-catenin can act as a transcriptional coactivator (Hecht and Kemler, 2000). An induction of MMP-2, MMP-9, and MT1-MMP expression was observed both at the mRNA and protein levels in the presence of sE-cadherin. ECM degradation by these MMPs might be an extra mechanism by which E-cadherin ectodomain shedding contributes to tumor invasion and metastasis formation (Nawrocki-Raby *et al.*, 2003). Besides the influence on invasion and metastasis, proteolysis of E-cadherin also plays a role in epithelial-mesenchymal transition, a conversion to an altered cellular phenotype which is associated with aggressive malignant behaviour (Cavallaro and Christofori, 2004). Induction of MMP-3 expression in mammary epithelial cells results in E-cadherin cleavage and triggers a progressive phenotypic conversion cumulating in cells that are unable to undergo lactogenic differentiation and that become invasive (Lochter *et al.*, 1997; Sternlicht *et al.*, 1999). Finally, during apoptosis, the cytoplasmic tail of E-cadherin is truncated by caspase-3, whilst a 84 kDa ectodomain fragment is released by a metalloproteinase. This simultanous cleavage of intracellular and extracellular domains might be a highly efficient mechanism to disrupt E-cadherin-dependent cell-cell contacts in apoptotic cells or tumor cells, which is a prerequisite for cell rounding and exit from the epithelium (Steinhusen *et al.*, 2001).

In acute renal failure, E-cadherin degradation by MT1-MMP leads to disruption of epithelial integrity and epithelial cell shedding (see Section 2.2.3), whereas in normal physiology, MMP-7-mediated shedding of E-cadherin is required for the repair of injured lung epithelium (McGuire *et al.*, 2003).

1.4.3 Integrin Subunit Precursors

Integrins are a diverse family of transmembrane glycoproteins that form heterodimeric receptors for ECM molecules and membrane-associated molecules of the Ig family. Every integrin is composed of a non-covalently coupled α- and β-subunit. The 18 known α-subunits and 8 β-subunits form at least 25 distinct heterodimers in human, with each pair being specific for a unique set of ligands. Integrins are crucial for cell adhesion, migration and invasion, not only through the direct physical adhesion to the ECM and to other cells, but also because they send and receive molecular signals that are essential for these processes (inside-out and outside-in signaling, respectively) (Hood and Cheresh, 2002).

The binding of integrins to ECM molecules is altered by changes in integrin expression and affinity when cancer cells become metastatic, or when endothelial cells enter the angiogenic state (Varner and Cheresh, 1996; Demetriou and Cress, 2004). Maturation of some integrin subunits requires a posttranslational cleavage of the precursor chain. The exact role of this endoproteolytical modification in integrin function is unclear, but its absence has important consequences for signal transduction pathways and leads to alterations in integrin functions such as cell adhesion to vitronectin (Berthet *et al.*, 2000). Proteolysis of pro-integrin chains is performed by proprotein convertases (PC) of the subtilisin/

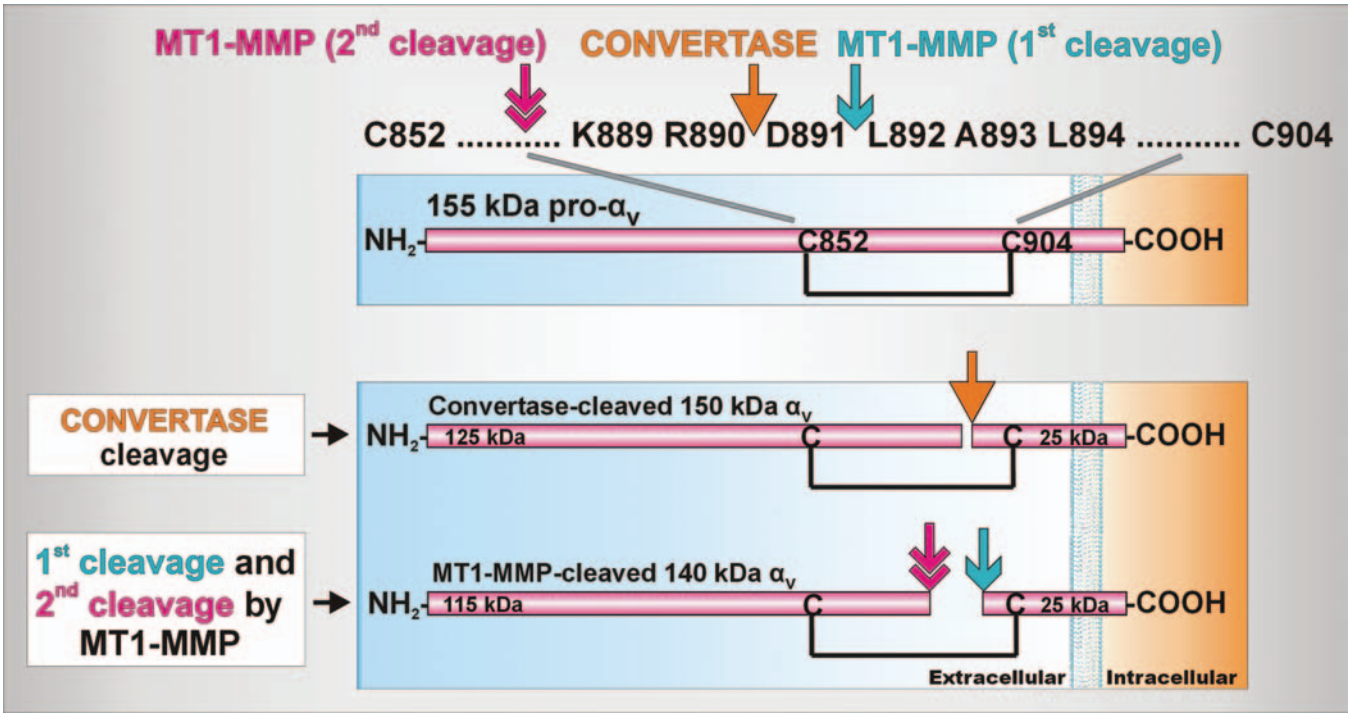

FIGURE 8 **Endoproteolytic cleavage of pro-α_v-integrin by MT1-MMP and by a proprotein convertase. Proteolysis of pro-integrin chains is performed by proprotein convertases, but also by MT1-MMP. MT1-MMP cleaves the pro-α_v chain at two different positions between the disulfide-connected Cys852 and Cys904. The first cleavage, between Asp891 and Leu892, generates a 125 kDa heavy α-chain, disulfide-bound to a 25 kDa light chain that is one residu shorter at the N-terminus in comparison with the convertase-processed chain. The second MT1-MMP cleavage removes an additional 10 kDa from the heavy α-chain and is situated downstream from the Cys852. These proteolytic modifications do not affect ligand binding of the resulting $\alpha_v\beta_3$ integrin but enhance outside-in signal transduction, which results in more efficient adhesion and migration on vitronectin. Adapted from (Ratnikov *et al.*, 2002).**

kexin-like family, but also by MT1-MMP. MT1-MMP cleaves the pro-α_v chain (integrin α-V precursor, vitronectin receptor subunit α, CD51) at two different positions between the disulfide-connected Cys852 and Cys904. The first cleavage, between Asp891 and Leu892, generates the 125 kDa heavy chain, disulfide-bound to a 25 kDa light chain that is one residu shorter at the N-terminus in comparison with the PC-processed chain (Figure 8). The putative second MT1-MMP cleavage site is situated downstream from the Cys852 and produces a 115 kDa heavy α-chain (Ratnikov *et al.*, 2002). These proteolytic modifications do not affect ligand binding of the resulting $\alpha_v\beta_3$ integrin but enhance outside-in signal transduction. As a result, cells co-expressing MT1-MMP and $\alpha_v\beta_3$ integrin show more efficient adhesion and migration on vitronectin, the ECM ligand of $\alpha_v\beta_3$ (Deryugina *et al.*, 2002).

In addition, the MT1-MMP-mediated cleavage seems to regulate cross-talk between $\alpha_v\beta_3$ and $\alpha_2\beta_1$ integrins. In MT1-MMP deficient cells, the functional activity of the collagen-binding $\alpha_2\beta_1$-integrin, which is not cleaved by MT1-MMP, is suppressed by the presence of $\alpha_v\beta_3$ integrin, resulting in diminished cell adhesion to collagen type I. Co-expression of MT1-MMP and $\alpha_v\beta_3$ restores the $\alpha_2\beta_1$-mediated collagen binding. Expression of both $\alpha_v\beta_3$ integrin and MT1-MMP is elevated in malignant tumor cells and is correlated with increased migration. Cross-talk between $\alpha_v\beta_3$ and $\alpha_2\beta_1$ integrins through MT1-MMP-mediated pro-α_v-proteolysis might thus contribute to efficient adhesion of aggressive tumor cells to type I collagen, an important substratum of the ECM (Baciu *et al.*, 2003). In addition, MT1-MMP modifies the β_3-subunit (integrin β-3 precursor, platelet membrane glycoprotein IIIa (GPIIIa), CD61) of $\alpha_v\beta_3$ integrin from a 95 kDa to a 90 kDa chain, which is also correlated with functional $\alpha_v\beta_3$ integrin activation and increased adhesion on vitronectin (Deryugina *et al.*, 2000). Moreover, after functional activation by MT1-MMP, $\alpha_v\beta_3$ integrin shows a higher affinity for the MMP-2 hemopexin domain, and binding to $\alpha_v\beta_3$ integrin concentrates MMP-2 at specific spots on the cell surface, which may again contribute to cell migration through the ECM. Hence, functional regulation of integrins by MT1-MMP plays an essential role in

efficient adhesion and directional migration of tumor cells. As a consequence, this pathway could be another interesting target in therapeutic approaches aiming at suppressing tumor cell spreading.

MT1-MMP also mediates maturation of the pro-α_3 (integrin α-3 precursor, galactoprotein B3 (GAPB3), VLA-3 α chain, FRP-2, CD49c) and pro-α_5 (integrin α-5 precursor, fibronectin receptor subunit α, integrin α-F, VLA-5, CD49e) chains to the respective mature disulfide-bound heavy and light α-chains (Baciu *et al.*, 2003). However, pro-α_5 cleavage does not affect $\alpha_5\beta_1$ integrin function. The consequences of pro-α_3 cleavage have not been investigated.

MMP-7 is another tumor-derived MMP capable of integrin subunit proteolysis. MMP-7 releases a 90 kDa fragment of the 200 kDa β_4-subunit precursor (integrin β-4 precursor, GP150, CD104), possibly through cleavage of the Tyr106-Ile107 and Gly416-Leu417 peptide bounds. The first putative cleavage site is very close to the ligand-binding domain, and cleavage might thus interfere with the binding of the $\alpha_6\beta_4$ integrin to its ligand laminin (von Bredow *et al.*, 1997). $\alpha_6\beta_4$ integrin has been most implicated in epithelial carcinogenesis (Watt, 2002). In epithelium-derived carcinoma, increased levels of β_4 integrin and loss of its polarized distribution to the basolateral membrane site of the cell have been correlated with tumor aggressiveness (Rigot *et al.*, 1999). Adhesion of breast cancer cells to endothelial cells through binding of $\alpha_6\beta_4$ integrin to a specific lung-endothelial cell adhesion molecule is critical for lung metastasis, but is totally abolished after cleavage of the β_4 integrin ectodomain by MMP-7 (Abdel-Ghany *et al.*, 2001). Hence, in this case, MMP-mediated modification of an integrin subunit reduces tumor cell adhesion and migration.

Furthermore, integrins are important players in tumor cell proliferation, apoptosis and angiogenesis, in leukocyte migration and in a whole array of pathologies (Wehrle-Haller and Imhof, 2003). As a consequence, insight in the functional regulation of these bidirectional signaling molecules by MMPs may generate new possibilities for therapeutic intervention.

1.4.4 Tissue Transglutaminase (tTG)

Another adhesion and signaling receptor being cleaved by MT-MMPs is the ubiquitously expressed cell surface-associated tissue transglutaminase (tTG, protein-glutamine γ-glutamyltransferase 2, TGase C (TGC), transglutaminase-2, TGase-H). tTG catalyzes covalent cross-linking between reactive lysine and glutamine residues of proteins and protein polymers. In addition, tTG functions as a coreceptor for β_1 and β_3 integrins and promotes integrin-dependent adhesion and cell spreading on fibronectin. Good functioning of cell surface tTG is essential for regulation and maintenance of cell-matrix interactions, as well as for the mobility of tumor and host cells. On the contrary, deregulation of tTG activity is associated with multiple human diseases (Griffin *et al.*, 2002). Overexpression of MT1-MMP by glioma and fibrosarcoma cells causes proteolytic degradation of tTG at the leading edge of motile cancer cells, leading to specific suppression of cell migration and adhesion on fibronectin (Belkin *et al.*, 2001). 80 kDa tTG degradation is mediated *in vitro* by MT1-MMP, MT2-MMP, and MT3-MMP, but not MT4-MMP, and produces fragments of ~53 kDa, ~41 kDa and ~32 kDa. MT1-MMP cleavage at Arg458-Ala459 and His461-Leu462 generates the 53 and 32 kDa fragments, while cleavage at Pro375-Val376 splits the protein in half, providing the 41 kDa fragments. Cleavage at any of these three sites abolishes receptor and enzymatic activity by separating the NH_2-terminal fibronectin-binding domain and the COOH-terminal integrin-binding domain, as well as by inactivation of the catalytic domain. tTG proteolysis suppresses cell adhesion and migration on fibronectin. Reciprocally, fibronectin protects its surface receptor, tTG, from MT1-MMP-mediated proteolysis, thereby supporting cell adhesion and mobility. In contrast, cell migration on collagen matrices is stimulated by tTG degradation. This suggests that the composition of the surrounding ECM might control the proteolysis of adhesion molecules colocalized with MT-MMPs on distinct areas of the cell surface of migrating tumor cells (Belkin *et al.*, 2001).

Additional examination shows that MMP-2 supports its activator MT1-MMP in tTG proteolysis. MMP-2 hydrolyzes cell-associated tTG very efficiently and associates predominantly with the catalytic core domain II of tTG. Furthermore, *in silico* simulations show that during the tTG-MMP-2 interaction, the catalytic site of MMP-2 is probably in very close proximity of the MMP cleavage sites. tTG, in turn, preferentially associates with the activation intermediate of MMP-2, which reduces MMP-2 activation and protects tTG against MMP-2 proteolysis (Belkin *et al.*, 2004). Hence, MMP-2 is as important as MT1-MMP in the degradation of cell surface-tTG, and the cooperation of both MMPs explains the

extensive tTG proteolysis at the normal tissue/tumor boundary. Loss of adhesive and enzymatic activities of tTG at the interface between normal and tumor tissue will reduce cell-matrix interactions and inhibit matrix cross-linking, which might cause multiple pathological alterations in host cell adhesion and mobility (Belkin *et al.*, 2004).

1.4.5 34/67 kDa Laminin Receptor (LR)

As mentioned before, the regulation of sequential tumor cell adhesion and anti-adhesion to ECM components is crucial in the complex process of tumor invasion and metastasis. Besides the modulation of integrin binding to vitronectin and type I collagen, and of tTG binding to fibronectin, MMPs also intervene in cell adhesion to laminin by modification of a major laminin binding molecule, the 34/67 kDa laminin receptor (LR, 40S ribosomal protein SA, p40, colon carcinoma laminin-binding protein, NEM/1CHD4, multidrug resistance-associated protein, MGr1-Ag). Overexpression of LR is strongly correlated with metastatic and aggressive tumor cell phenotypes (Berno *et al.*, 2005). Using thyroid hormone-dependent *Xenopus Laevis* metamorphosis as a model, the 37 kDa LR precursor was identified as a potential physiological substrate of *Xenopus* stromelysin-3 or *X*MMP-11 (Amano *et al.*, 2005b). The highly conserved 37 kDa protein is the precursor of the receptor but the exact manner by which it configures its mature 67 kDa form is not clear. It was suggested that acylation followed by homo- or heterodimerization of the 37 kDa precursor forms the mature 67 kDa laminin receptor. The heterodimer is likely to be stabilized by strong intramolecular hydrophobic interactions between fatty acids bound to the 37 kDa precursor and to an unknown galectin-3 cross-reacting molecule (Buto *et al.*, 1998). The COOH-terminal two-thirds of the LR is located extracellularly and contains a six-amino-acid laminin-binding sequence, whereas the NH_2-terminal third faces the cytoplasm preceded by a short transmembrane domain (see Figure 2). *In vitro* incubation of *X*pro-LR with other (human) MMPs showed that all tested MMPs cleaved LR, with MMP-2 being most efficient, MT1-MMP least efficient, and MMP-3 and MMP-9 cleaving with intermediate efficiencies. However, the cleavage products generated by these MMPs were distinct from those produced by MMP-11. Whereas the cleavage sites of MMP-11, Ala115-Phe116, and Pro133-Ile134, were located between the transmembrane domain and the laminin-binding sequence, cleavage by all other MMPs occurred COOH-terminally of the laminin-binding sequence. As a consequence, only MMP-11 releases LR-fragments that contain the laminin-binding site and may alter cell-laminin interactions. In addition, human LR was cleaved by MMP-11 at the two same sites as in *Xenopus* LR, which means that LR is a conserved substrate for MMP-11 in vertebrates (Amano *et al.*, 2005b). Further investigation using transgenic tadpoles overexpressing MMP-11 showed that LR is cleaved *in vivo* by MMP-11 during intestinal metamorphosis (Amano *et al.*, 2005a). Besides its physiological role in *Xenopus Laevis* development, MMP-11-mediated cleavage of LR is likely to be involved in tumor developement and cancer progression.

MMP-11, similar to LR, is an active partner of invading cancer cells (Rio, 2005). Thus, the coexistence of MMP-11, which is expressed by the fibroblasts within the tumors but not actually by the tumor cells themselves, and LR in tumors may be expected to lead to the cleavage of tumor cell surface LR. This may alter tumor cell-ECM interaction to affect tumor development and cell migration. Peptide G, an LR peptide (residues 161 to 180) containing the laminin-binding sequence (residues 173 to 178) indeed changes the conformation of laminin-1 and increases and stabilizes laminin-1 binding on tumor cells (Magnifico *et al.*, 1996). In addition, peptide G-modified laminin signals tumor cells to change their cytoskeleton to promote motility and invasion. It also induces the expression of a number of proteases characteristic of invasive cancer cells, and leads to increased gelatinolytic activity by MMP-2. Invasiveness of tumor cells conditioned by peptide G-modified laminin was shown to be MMP-2-dependent as it was significantly more inhibited by TIMP-2 than invasiveness induced by native laminin (Berno *et al.*, 2005). Full-length LR shed from malignant cells also induced conformational changes in laminin after binding. In addition, the shed LR modified production of anti-angiogenic angiostatins from plasmin *in vitro*, in this way promoting tumor-associated neoangiogenesis (Moss *et al.*, 2006). As LR-fragments released by MMP-11 contain the laminin-binding site, they might also modulate laminin conformation and enhance tumor cell invasiveness and angiogenesis. Gaining insight into the malignant potential of these soluble LR-fragments may thus be of great interest to elucidate one of the mechanisms that underlies the detrimental effect of MMP-11 in cancer progression.

1.4.6 Syndecan-1, -3, and -4

Syndecans are transmembrane heparan sulfate proteoglycans expressed on all adherent cells (see Figure 2). They are important players in tissue morphogenesis by binding a variety of ECM components such as fibronectin, thrombospondin, various collagens and growth factors via their glycosaminoglycan chains (Beauvais and Rapraeger, 2004). In addition, they can interact with the cytoskeleton through their conserved cytoplasmic domains. Syndecan expression can alter cell adhesion, migration and morphology. The syndecan family is composed of four strongly related proteins (syndecan-1, -2, -3, and -4). The intact ectodomain of each syndecan is constitutively shed from cultured cells as part of normal cell surface heparan sulfate proteoglycan turnover and this process seems to play a role in various pathophysiological events such as host defense, wound healing, arthritis and Alzheimer's disease. How this shedding is regulated remains largely unknown. The ectodomain release of syndecan-1 (CD138) and syndecan-4 (amphiglycan, ryudocan core protein) from NMuMG epithelial and SVEC4–10 endothelial cells is accelerated by various physiological agents activating several intracellular signal transduction pathways. The proteolytic activity responsible for this accelerated shedding is associated with the cell surface and can be specifically inhibited by TIMP-3, pointing to ADAMs as possible mediators (Fitzgerald *et al.*, 2000). However, as will be discussed in a later chapter, secreted MMP-7 also sheds the syndecan-1 ectodomain, releasing a syndecan-1/chemokine KC complex from the mucosal surface of injured lungs, in this way forming a chemokine gradient that directs neutrophils to the site of injury (Li *et al.*, 2002; Shapiro, 2003). HT1080 fibrosarcoma cells also show constitutive shedding of the syndecan-1 ectodomain, but here the shedding activity is inhibited by TIMP-2 and batimastat, but not by TIMP-1. Therefore, the MMP in charge here is not MMP-7, but probably the endogenous MT1-MMP. Recombinant syndecan-1 is cleaved *in vitro* by MT1-MMP as well as by MT3-MMP preferentially at the Gly245-Leu246 peptide bond (Endo *et al.*, 2003). Syndecan-1 expression is associated with inhibition of invasion and reduced migration of HT1080 fibrosarcoma cells. Treatment of these cells with MMP inhibitors increases cell surface syndecan-1 concentrations concomitant with formation of actin stress fibers, which results in further reduction of migration. In contrast, shedding of the syndecan-1 ectodomain by MT1-MMP enhances cell motility on collagen (Endo *et al.*, 2003).

In addition, the shedding of syndecan-4, and to a lesser extent that of syndecan-1, from HeLa cells and human primary macrophages was reported to be accelerated by the chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) and mediated by MMP-9. SDF-1 increases MMP-9 mRNA and MMP-9 activity in HeLa cells, whereas MMP-9 silencing by RNA interference strongly decreases syndecan-1 and syndecan-4 ectodomain shedding accelerated by SDF-1. Shedding of syndecan-4 and syndecan-1 from human primary macrophages is accelerated by SDF-1 as well, and inhibited by anti-MMP-9 antibodies (Brule *et al.*, 2006). As SDF-1 does not bind to soluble syndecan ectodomains, this shedding process by MMP-9 may be part of an autoregulatory/down-regulation cycle: (1) SDF-1 binding to syndecan-4 facilitates its presentation to its receptor CXCR4; (2) SDF-1 activates MMP-9, which removes syndecan-1 and –4 from the cell surface; (3) decreased membrane expression of syndecans down-regulates SDF-1 binding to its receptor (Charnaux *et al.*, 2006). In addition, MMP-9 cleaves off the NH_2-terminal tetrapeptide of SDF-1, which also results in loss of binding to its receptor CXCR4 (McQuibban *et al.*, 2001). As syndecan-4 promotes cell spreading in a β1-integrin-dependent fashion (Thodeti *et al.*, 2003), the role played by its MMP-9-mediated ectodomain shedding in tumor cell migration and metastasis certainly deserves further investigation.

Syndecan shedding has also been observed in physiological systems. *In vitro* release of the syndecan-3 ectodomain from rat Schwann cells, the myelin-forming cells of the peripheral nervous system, is also mediated by an MMP. These Schwann cells transiently express syndecan-3 during embryonic and early postnatal development. Inhibition of syndecan-3 cleavage by several MMP inhibitors such as batimastat/BB-94 and BB-3103 significantly enhances Schwann cell adhesion to the non-collagenous NH_2-terminal domain of α4(V) collagen, which binds syndecan-3 and mediates heparan sulfate-dependent Schwann cell adhesion. MMP-dependent syndecan-3 shedding was also observed *in vivo* in the peripheral nerve tissue of newborn rats, disappearing on day 10, the end of the myelin-forming process in Schwann cells (Asundi *et al.*, 2003). As a consequence, syndecan ectodomain shedding is cell type-specific and this illustrates the varying physiological roles of these proteoglycans in different tissues. As syndecan ectodomain release has an impact on various

pathophysiological processes, the identification of the proteolytic activity in charge might be of great use in the development of new diagnostic and therapeutic strategies.

1.4.7 CD44

CD44 (phagocytic glycoprotein 1 [PGP-1]), HUTCH-1, ECM receptor-III [ECMR-III], GP90 lymphocyte homing/adhesion receptor, hermes antigen, hyaluronate receptor, heparan sulfate proteoglycan, epican) is a ubiquitous multistructural and multifunctional cell adhesion molecule involved in cell-cell and cell-matrix interactions. This family of glycoproteins consists of many isoforms generated by different use of alternatively spliced exons and extensive glycosylation. The most abundant form is the standard hematopoietic type, CD44H, which does not have any variant insertions (Naor *et al.*, 1997). The ECM adhesion activity of CD44 is located in the NH_2-terminal globular domain that forms an important receptor for hyaluronic acid, an abundant glycosaminoglycan that fills interstitial spaces between different tissues and takes part in embryonic development, healing processes, inflammation and tumor development (Toole, 2004). CD44 also binds other ECM components such as type I collagen, fibronectin, fibrin, laminin, and chondroitin sulfate. CD44 has been shown to take part in many important processes such as lymph node homing, T-cell activation, presentation of chemokines and growth factors to traveling cells, wound healing, angiogenesis, metastasis and apoptosis (Naor *et al.*, 1997). The NH_2-terminal ligand-binding domain is followed by a stem sequence, a transmembrane domain and a cytoplasmic tail. The cytoplasmic domain interacts with the actin cytoskeleton and is important for the localization of CD44 at the ruffling edge of migrating cells. MT1-MMP is co-expressed with CD44 on migrating cells and metastatic tumor cells (Seiki, 2002; Seiki, 2003; Itoh and Seiki, 2004). MT1-MMP binds the extracellular portion of CD44H by its hemopexin domain. Hence, CD44H has a major role in ECM degradation, as it forms the connection between MT1-MMP and the actin cytoskeleton, and anchors MT1-MMP at the migrating front (Figure 9) (Mori *et al.*, 2002).

MT1-MMP, in turn, acts as a processing enzyme for CD44H, which is critical for the stimulation of cell motility, probably because it allows the cells to detach from the ECM (Kajita *et al.*, 2001). Co-expression of MT1-MMP and CD44H increases shedding of the commonly produced 65 to 70 kDa fragments of CD44 and generates two additional smaller fragments. Shedding of the 65 to 70 kDa fragments occurs constitutively and is inhibited by TIMP-3, but not by TIMP-1 or TIMP-2, suggesting the proteolytic activity is an ADAM-like protease, although this shedding is increased by MT1-MMP expression. In contrast, release of the two smaller fragments is abolished by TIMP-2 and TIMP-3, but not by TIMP-1, which is the inhibition pattern of MT1-MMP. The cleavage sites (CS) corresponding to the three major fragments are Ser249-Gln250 (CS3) for the big fragment, and Gly192-Tyr193 (CS1) and Gly233-Ser234 (CS2) for the two smaller fragments (Figure 9). These cleavage sites are also detected *in vivo*. In normal tissues, more clipping of the fragment at CS3 occurs than of those of the other sites, whereas in carcinomas CS1 fragments are significantly increased. Thus, CD44 shedding at CS1 and CS3 represents the normal physiological process, whereas increased shedding at CS1 is associated with malignant tumors (Nakamura *et al.*, 2004).

Whereas MT1-MMPs displays the most potent CD44 H shedding activity, other MT-MMPs such as MT2-, MT3-, and MT5-MMP, but not MT4- and MT6-MMP, can also cleave CD44H *in vitro* at CS2, while cleavage at CS1 was hardly detectable (Suenaga *et al.*, 2005). The interaction between the hemopexin domain and CD44 H is conserved in each MT-MMP, which suggests that CD44 is more than a receptor for ECM molecules and may also form a platform for the assembly of various MMPs with their substrates, to modulate cell migration (Figure 9) (Seiki, 2002; Suenaga *et al.*, 2005). Like MT1-MMP, MMP-9 binds directly to CD44 (Yu and Stamenkovic, 1999) and activates latent TGF-β, which contributes to tumor-induced angiogenesis (Yu and Stamenkovic, 2000). In contrast, MMP-7 attaches indirectly to the heparan sulfate chains of the CD44 isoform with a variant exon 3, which also binds substrates of MMP-7, namely mHB-EGF and osteopontin (Yu *et al.*, 2002; Seiki, 2002).

Furthermore, proteolysis of CD44 results in signal transduction to the nucleus. Ectodomain shedding of CD44 induces cleavage in the cytoplasmic portion, which releases the intracellular domain, CD44ICD, into the cytoplasm (Figure 9). CD44ICD migrates to the nucleus where it activates transcription mediated through the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-responsive element (TRE). One of the potential targets for transcriptional activation by CD44ICD is

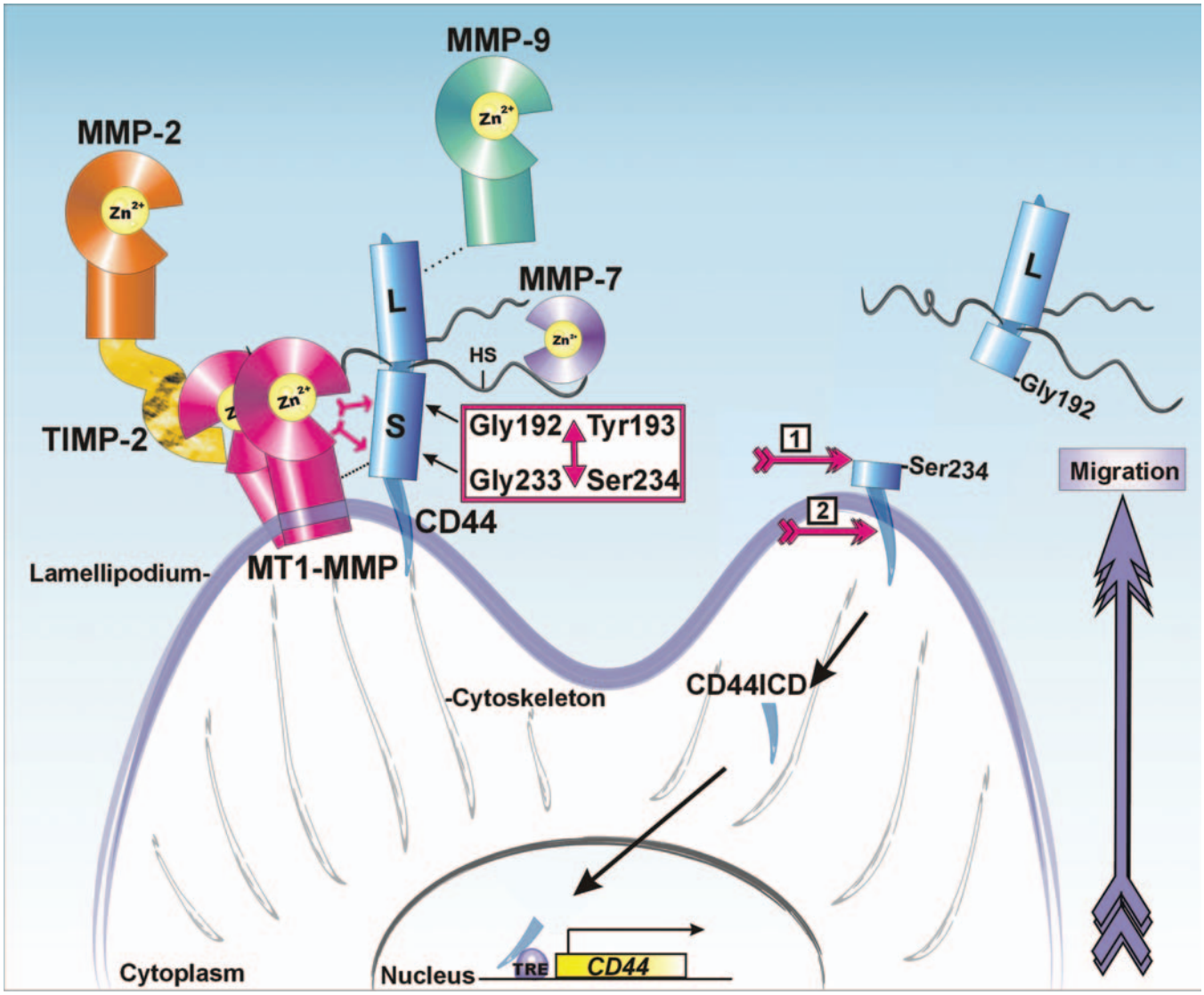

FIGURE 9 CD44 forms a platform for the assembly of various MMPs with their substrates, to modulate cell migration. The extracellular portion of CD44 binds to the MT1-MMP hemopexin domain (*thin line*), while the CD44 cytoplasmic domain interacts with the actin cytoskeleton, in this way anchoring MT1-MMP on the ruffling edge of migrating tumor cells. MT1-MMP, in turn, acts as a processing enzyme for CD44, which is critical for the stimulation of cell motility, probably because it allows the cells to detach from the ECM. Co-expression of MT1-MMP and CD44 generates two soluble fragments, resulting from proteolysis at Gly192-Tyr193 (CS1) (shown in the figure) and Gly233-Ser234 (CS2) (not shown). Furthermore, ectodomain shedding of CD44 by MT1-MMP (1) induces cleavage in the cytoplasmic portion (2), which releases the intracellular domain, CD44ICD, into the cytoplasm. CD44ICD migrates to the nucleus where it activates transcription mediated through the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-responsive element (TRE). One of the genes under control of TRE is the *CD44* gene itself, suggesting that CD44 ectodomain cleavage promotes the rapid turnover of CD44 that is required for efficient cell migration. In addition, CD44 acts as a platform to assemble various MMPs with their substrates, clustering proteolytic events that regulate cell migration. MMP-9 also interacts directly with CD44 (*dotted line*), whereas MMP-7 attaches indirectly to the heparan sulfate chains (HS) of CD44. L, ligand-binding domain; S, stem sequence. Adapted from (Seiki, 2002).

the *CD44* gene itself, suggesting that CD44 ectodomain cleavage and the subsequent intracellular signaling promote the rapid turnover of CD44 that is required for efficient cell migration (Okamoto *et al.*, 2001). As a consequence, disruption of the interaction between MT-MMPs and CD44 might be another strategy to inhibit metastasis of malignant tumors (Peterson *et al.*, 2000; Ueda *et al.*, 2003).

1.4.8 Extracellular Matrix Metalloproteinase Inducer (EMMPRIN)

A search for MMP inducing factors in tumor cells led to the identification of EMMPRIN (Extracellular matrix metalloproteinase inducer) with the numerous synonyms: basigin, leukocyte activation antigen M6 (M6), tumor cell-derived collagenase stimulatory factor (TCSF), neurothelin, OK blood group antigen, OX-47,

gp42, CE9, 5A11, 5F7, HT7, and CD147. EMMPRIN is composed of two Ig domains in the extracellular region, a single transmembrane domain and a short cytoplasmic domain containing 39 amino acids (see Figure 2). It is a highly glycosylated cell surface protein, with the different glycosylation patterns of the 28 kDa native protein accounting for a variable molecular weight, ranging between 44 and 66 kDa. Depending on the cell system, EMMPRIN can stimulate production of MMP-1, -2, -3, -9, MT1-MMP, and MT2-MMP, and only glycosylated EMMPRIN is able to induce these MMPs. The NH_2-terminal Ig domain is required for the MMP induction, but also for the formation of homo-oligomers in a *cis*-dependent manner in the plama membrane. The MMP-inducing function of EMMPRIN in part involves the molecule acting as a counter-receptor for itself, also requiring the NH_2-terminal Ig domain, but in this case the interaction is in a *trans* manner (Gabison *et al.*, 2005; Yan *et al.*, 2005).

EMMPRIN can be released from the cell surface in at least two different ways. A significant amount is released via vesicular shedding, whereby EMMPRIN is initially associated with microvesicles which are quickly degraded upon release from the cells to discharge full-length soluble EMMPRIN. The other pathway is MMP-dependent proteolytic shedding (Gabison *et al.*, 2005). In addition to inducing MMPs, EMMPRIN is cleaved and shed by MMPs or other MPs, because this shedding is inhibited by Zn^{2+} chelators (EDTA and 1,10-phenanthroline) and by the broad-spectrum MMP inhibitor GM6001 (Tang *et al.*, 2004; Haug *et al.*, 2004). In addition, MMP-1 and MMP-2 cleave EMMPRIN at the membrane-proximal region *in vitro* (Haug *et al.*, 2004). Besides release of the intact form, a new EMMPRIN fragment of 22 kDa was identified in the culture media of two tumor cell lines (HT1080 and A431), the shedding of which was enhanced by phorbol 12-myristate 13-acetate (PMA) while that of the intact form was not (Egawa *et al.*, 2006). The EMMPRIN sheddase was hypothesized to be of the MT-MMP family, in view of its expression and inhibition profiles. In addition, HT1080 and A431 cells express MT1-MMP and EMMPRIN was co-purified from cell lysates with MT1-MMP. Knockdown of MT1-MMP with siRNA indeed inhibited the shedding substantially, although MT2-MMP may also contribute to the shedding because knockdown of both MT1- and MT2-MMP produced slightly greater inhibition. The COOH-terminal amino acid of the 22 kDa fragment was identified as Asn98, and MT1-MMP also cleaved at this site in an *in vitro* digestion. Although an additional cleavage site, between Pro93 and Met94, was observed after *in vitro* incubation with MT1-MMP, the corresponding fragment was not identified in cell culture media (Egawa *et al.*, 2006). The Asn98-Ile99 cleavage site is located in the linker sequence connecting the two Ig-like domains (see Figure 2), which means that the 22 kDa fragment contains the NH_2-terminal Ig domain that is crucial for MMP induction and homophilic interactions. The purified 22 kDa fragment indeed retained MMP-inducing activity. Thus, the shedding may down-regulate the cellular functions mediated by EMMPRIN, because MT1-MMP cleaves off the essential distal Ig domain. This regulation may be particularly important at the ruffling edge of migrating tumor cells, as both proteins co-localize there (Egawa *et al.*, 2006). At the same time, the released soluble 22 kDa active fragment may act on cells either in the local tumor environment or diffuse away to act on distant cells to further stimulate MMP and EMPRINN expression and augment the migration and invasion potential of tumor cells.

The pathologic consequences of elevated EMMPRIN expression in tumor growth and metastasis were directly demonstrated using EMMPRIN-overexpressing cancer cells. MDA-MB-436 human breast cancer cells are normally slow-growing cells when they are implanted into nude mice. However, after EMMPRIN gene transfection, these cells adopted a more aggressive phenotype, exhibiting both accelerated growth and increased invasiveness, and increased MMP-2 and MMP-9 expression (Zucker *et al.*, 2001). In addition to stimulating MMP production, EMMPRIN also binds MMP-1 and retains it at the cell surface, an arrangement that may promote turnover of pericellular collagen, thereby also facilitating migration and metastasis. The role of EMMPRIN in tumor cell invasion was confirmed, as EMMPRIN function-blocking antibodies inhibited invasion through a reconstituted basement membrane. Besides its role in the stimulation of invasion, migration and metastasis, EMMPRIN induces angiogenesis via stimulation of VEGF production and multidrug resistance via upregulation of HER2-signaling and cell survival pathway activities (Gabison *et al.*, 2005; Yan *et al.*, 2005). Targeting the release of soluble EMMPRIN molecules by MMPs, may thus be of great interest to limit the expansion and migration of the tumor by restricting the diffusion of malignant EMMPRIN actions.

Besides the elevated EMMPRIN expression on tumor cells, EMMPRIN was also shown to have a broader tissue distribution, including activated T-cells, differentiated macrophages and epithelia. The presence of EMMPRIN in non-tumoral tissue suggests a role in other physiological and/or pathological situations, such as embryonic development, adult tissue homeostasis, atherosclerosis (see Section 2.1.2), arthritis and ulceration (Gabison *et al.*, 2005).

1.4.9 Low-Density Lipoprotein Receptor-Related Protein (LRP)

The low-density lipoprotein receptor (LDL-R) family consists of several related cargo transporters that also inform the cell of changes in its environment by mediating signaling responses. Low-density lipoprotein receptor-related protein 1 (LRP, α2-macroglobulin receptor (α_2-MR), apolipoprotein E receptor (ApoE-R), CD91) is a membrane-bound receptor which mediates the endocytosis of a wide variety of ligands, including lipoproteins, proteases, proteinase inhibitor complexes, ECM components, bacterial toxins, viruses, intracellular proteins and growth factors. In addition to the four clusters of ligand-binding repeats, LRP consists of a 85 kDa membrane-spanning light β-chain (LRP-85) that is non-covalently associated with a 515 kDa large extracellular α-chain (LRP-515) (Figure 10). The cytoplasmic domain, containing 100 amino acids, plays a role in signal transduction by interacting with the cytoplasmic scaffold and adaptor proteins (Strickland *et al.*, 2002; Lillis *et al.*, 2005). In view of its multiple interactions at the cell surface, LRP is a strategic relay in the control of cell behavior. Indeed, LRP not only mediates the endocytic clearance of several major contributors of cancer development, such as ECM components (fibronectin, and thrombospondin-1 and -2) and various proteolytic enzymes (tissue-type plasminogen activator [t-PA], u-PA, MMP-2, MMP-9, and MMP-13), but it is also involved in cell signalling that regulates cell migration and possibly, survival and proliferation. Its own regulation thus appears to be a crucial process, as suggested by two opposite physiopathological examples, with, respectively, enhanced and suppressed LRP expression: the cycling human endometrium (see Section 5.1) and cancer (Emonard *et al.*, 2005).

Invasive cancer cells derived from human prostate or breast tumors express lower levels of LRP, as compared to their non-invasive counterparts. Similarly, LRP expression decreases in late stages of melanocytic tumour progression and in invasive endometrial carcinoma (Emonard *et al.*, 2005). In addition, MT1-MMP, which is highly expressed by the most invasive tumor cells (Seiki, 2003; Sato *et al.*, 2005), has been shown to efficiently degrade LRP (Rozanov *et al.*, 2004a). LRP-515 associates with the MT1-MMP catalytic domain and is highly susceptible to MT1-MMP proteolysis *in vitro* (Figure 10). In cells co-expressing LRP and MT1-MMP, the levels of cellular LRP are decreased and the NH_2-terminal ligand-binding portion is released in the extracellular milieu, thereby destroying the functional activity of the receptor. Similar to MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP also degrade LRP (Rozanov *et al.*, 2004a), suggesting that LRP is likely to be susceptible to proteolysis by several individual MT-MMPs in many cancer cell types. LRP is directly involved in the capture, internalization, and clearance of MMP-2, MMP-9, and MMP-13 from the extracellular milieu, and in the translocation into the cell compartment for subsequent lysosomal degradation (Emonard *et al.*, 2005; Van den Steen *et al.*, 2006). In malignant cells, MT1-MMP activates MMP-2, and protects the active enzyme from uptake and clearance by cleaving LRP (Figure 10). Thus, LRP proteolysis by MT1-MMP contributes to maintaining high levels of proteinases such as MMP-2, MMP-9, u-PA, and t-PA in the extracellular milieu, allowing for extensive degradation of the ECM by aggressive migrating cells. In this context, inhibition of MT1-MMP would have a dual beneficial effect in countering tumor invasion and metastasis; first by reducing the activation of pro-MMP-2 and second by enhancing LRP-mediated clearance of ECM-degrading proteases.

1.4.10 MT1-MMP

MT1-MMP expression levels are closely associated with invasiveness and malignancy of tumors, suggesting that MT1-MMP is one of the critical factors for tumor invasion and metastasis. Besides degrading multiple ECM molecules, MT1-MMP contributes to the process of tumor cell metastasis by cell surface proteolysis of various biologically important molecules such as MUC1, tTG, integrins, syndecan-1, CD44, and LRP, as discussed before. Furthermore, it activates pro-MMP-2 and concentrates its proteolytic activity on the cell surface, which is an important step for cancer cells to invade into basal lamina (Sato *et al.*, 2005; Itoh and Seiki, 2006). MT1-MMP is produced as an inactive ~60 kDa zymogen that is activated by furin-like convertases, which cleave at

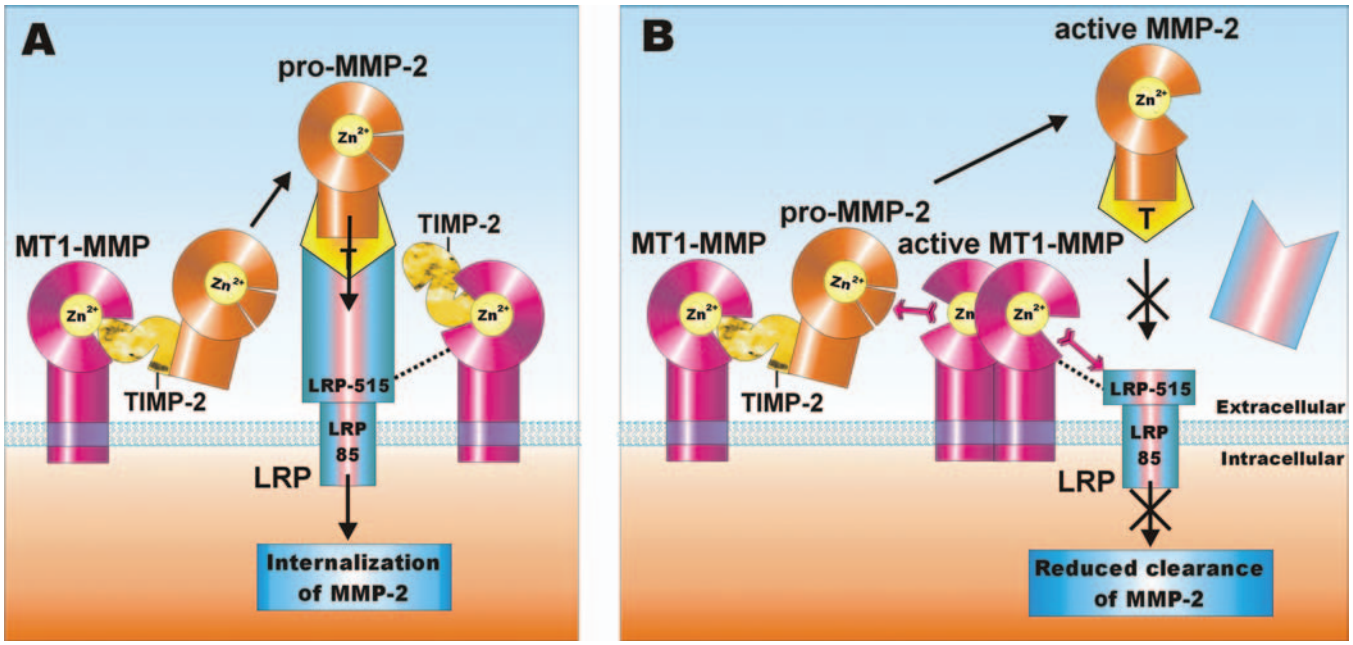

FIGURE 10 **Degradation of LRP by MT1-MMP leads to reduced internalization of MMP-2. (A) When MT1-MMP activity is inhibited by TIMP-2, no MMP-2 activation occurs. MMP-2 binds thrombospondin (T) and is cleared from the extracellular milieu after binding the scavenger receptor LRP. (B) In malignant cells, which often over-express both MT1-MMP and pro-MMP-2, MT1-MMP activates MMP-2. In addition, the 515 kDa large extracellular α-chain of LRP (LRP-515), which associates with the MT1-MMP catalytic domain (*dotted line*), is cleaved by MT1-MMP and the NH_2-terminal ligand-binding portion is released in the extracellular milieu, thereby destroying the functional activity of the receptor. Hence, active MMP-2 is protected from uptake and clearance by LRP and accumulates in the extracellular environment, where it assists MT1-MMP in degrading the ECM in front of aggressive migrating cells. LRP-85, the 85 kDa membrane-spanning light β-chain of LRP. Adapted from (Rozanov *et al.*, 2004a).**

the Arg108-Arg-Lys-Arg motif located between the propeptide and the catalytic domain. Active MT1-MMP (~57 kDa), starting at Tyr112, is then transported to the plasma membrane with the catalytic domain facing the extracellular space, where it cleaves pericellular substrates. In addition to being a sheddase, MT1-MMP is regulated by ectodomain shedding itself. Active MT1-MMP undergoes autocatalytic processing at the cell surface, leading to the formation of an inactive 44 kDa fragment and release of the entire catalytic domain (Figure 11). First, MT1-MMP cleaves itself at the Gly284-Gly285 peptide bond in the hinge region, generating the inactive 44 kDa membrane-bound fragment. The second cleavage takes place at the Ala255-Ile256 peptide bond, in the active site of MT1-MMP, near the conserved methionine turn, a structural feature of the catalytic domain of all MMPs. The released 18 kDa soluble fragment has no catalytic activity and does not bind TIMP-2 (Toth *et al.*, 2002). Hence, the autocatalytic cleavage represents a self-regulatory mechanism that evolved to terminate MT1-MMP-dependent proteolysis both at the cell surface and in the extracellular space. The remaining 44 kDa degradation product can even negatively influence enzymatic activity. For example, it was shown to compete with the full-length enzyme for collagen binding, reducing collagenolytic activity, and cellular invasion of a collagen matrix. Additionaly, it was speculated that the 44 kDa product may indirectly reduce pro-MMP-2 activation by reducing the clustering of MT1-MMP induced by collagen (Osenkowski *et al.*, 2004). Inhibition of MT1-MMP activity slows down autocatalytic enzymatic turnover and consequently the mature form of the enzyme (57 kDa) accumulates on the cell surface while the level of the inactive 44 kDa fragment is reduced. In contrast, autocatalytic processing is promoted in the absence of inhibitors or under conditions of MT1-MMP overexpression. This effect of inhibition on autocatalytic processing unveiled a new paradigm in MT1-MMP regulation because it signaled a potential side effect of MT1-MMP inhibition: enhancement rather than inhibition of activity (Osenkowski *et al.*, 2004).

To make the picture even more complex, mature MT1-MMP is also shed from the cell surface via a non-autocatalytic process that results in the release of various soluble forms (Toth *et al.*, 2002), and, as opposed to the autocatalytic processing, it generates active soluble forms. This shedding occurs *in vivo* and

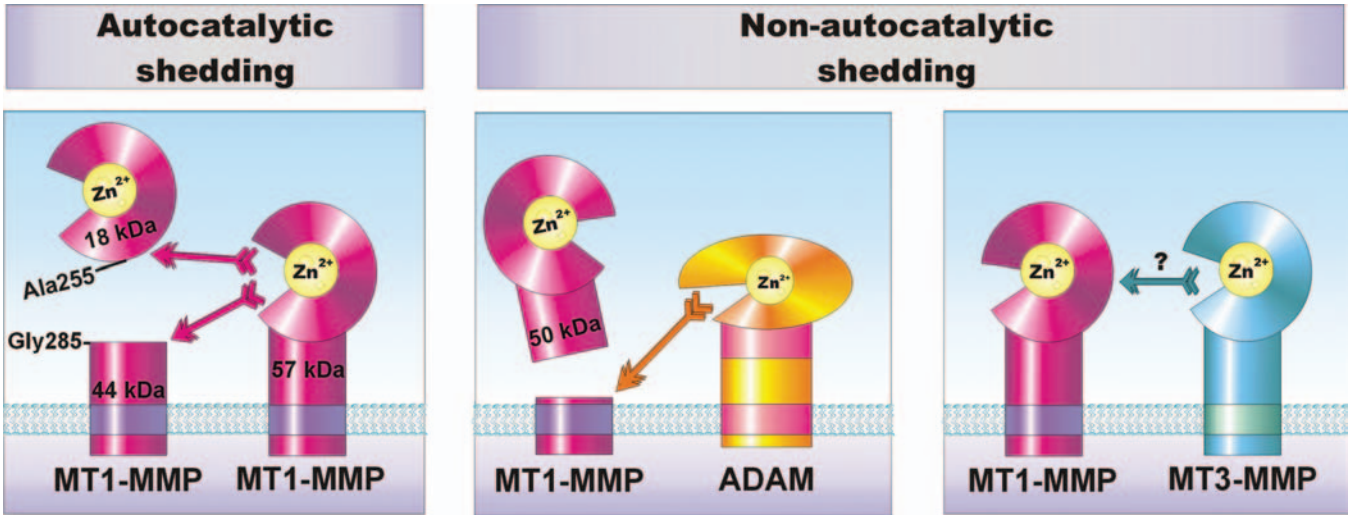

FIGURE 11 **Autocatalytic and non-autocatalytic shedding regulates MT1-MMP activity at the cell surface. Active MT1-MMP undergoes *autocatalytic processing* on the cell surface, leading to the formation of an inactive 44 kDa fragment and release of the entire catalytic domain. First, MT1-MMP cleaves itself at the Gly284-Gly285 peptide bond in the hinge region, generating the inactive 44 kDa membrane-bound fragment. The second cleavage takes place at the Ala255-Ile256 peptide bond, in the active site of MT1-MMP. The resulting 18 kDa soluble fragment has no catalytic activity. MT1-MMP is also shed from the cell surface via a *non-autocatalytic process* that results in the release of active soluble forms. This shedding is mediated by an ADAM and produces a minor form of ~25–32 kDa (not shown) and a major soluble form ~50–52 kDa, wich represents the entire ectodomain, including the catalytic domain. Finally, MT1-MMP may as well be degraded by MT3-MMP. However, no soluble products or cleavage sites of MT3-MMP have been characterized so far. Based on (Osenkowski *et al.*, 2004), (Toth *et al.*, 2006) and (Shofuda *et al.*, 2001).**

produces a minor form of ~25–32 kDa and a major soluble form ~50–52 kDa, wich represents the entire ectodomain, including the catalytic domain (Figure 11) (Toth *et al.*, 2005). The inhibition pattern of this shedding process suggests that it is mediated by an ADAM (Toth *et al.*, 2006).

A third proteolytic mechanism regulates MT1-MMP acitivity at the cell surface. While studying activities of MT1- and MT3-MMP in activated smooth muscle cells, it was shown that when MT3-MMP and MT1-MMP were coexpressed, MT1-MMP degradation was enhanced. This result supports the possibility that MT3-MMP can degrade MT1-MMP (Figure 11), providing another negative regulatory mechanism for MT1-MMP activity in cells such as smooth muscle cells and gliomas, or in tissues such as injured blood vessels and brain, where both MT-MMPs are coexpressed (Shofuda *et al.*, 2001).

Emerging evidence points to internalization as another means of controlling MT1-MMP activity at the cell surface. Classical endocytosis depends on clathrin-coated pits and involves an intracellular pathway in which lysosomes fuse with internalized vesicles, degrading their contents (Shin and Abraham, 2001). MT1-MMP is cleared from the cell surface by dynamin-dependent endocytosis in clathrin-coated pits through its cytoplasmic domain (Jiang *et al.*, 2001). This type of endocytosis was shown essential for MT1-MMP to stimulate cell migration and invasion into Matrigel (Uekita *et al.*, 2001). In addition, MT1-MMP was detected in caveolae (Annabi *et al.*, 2001; Puyraimond *et al.*, 2001). Remacle and coworkers (2003) showed that MT1-MMP is also internalized by a clathrin-independent and caveolae-dependent pathway in HT1080 cells. Caveolar traffic is required for proper MT1-MMP localization, activity and function in migrating endothelial cells (Galvez *et al.*, 2004). Interestingly, internalized MT1-MMP can be recycled to the cell surface, which could represent a rapid mechanism for relocalizing active MT1-MMP at the leading edge during cell migration (Remacle *et al.*, 2003).

Regarding the major role of MTl-MMP in tumor invasion and metastasis (Sato *et al.*, 2005; Itoh and Seiki, 2006), inhibition of its activity is an obvious therapeutic approach to block spreading of the cancer. However, further insight in the proteolytic mechanisms at the cell surface that modulate MT1-MMP activity is a prerequisite in the development of new inhibitors, as unexpected effects such as the paradoxical enhancement of MT1-MMP activity by inhibition of autocatalytic processing, may fatally alter the outcome of inhibitor therapy.

1.4.11 Protease-Activated Receptor-1 (PAR1)

Thus far, the protease-activated receptor (PAR) family comprises four members defined as PAR1 to PAR4 (Coughlin, 2000). They form a unique class of GPCRs that are characterized by a distinctive mechanism of activation. Proteolytic cleavage at specific sites in the extracellular NH_2-terminus exposes a new NH_2-terminus, which serves as a tethered ligand and binds to the second extracellular loop of the same receptor, activating it intramolecularly (Figure 12). The activated PARs initiate signal transduction across the membrane to activate intracellular G proteins that regulate pathways for cell shape changes, secretion, cell proliferation, migration, and adhesion in numerous cell types. Many serine proteases, including thrombin, factor Xa, granzyme A, cathepsin G, elastase, trypsin and plasmin cleave the PAR1 scissile bond at Arg41-Ser42. PAR1 (thrombin receptor, coagulation factor II receptor) is expressed by a wide range of tumor cells and has been shown to be upregulated in breast carcinomas and pulmonary tumors. In addition, the level of expression of PAR1 on tumor cells directly correlates with metastatic potential in both primary breast carcinoma and in established cancer cell lines (Ossovskaya and Bunnett, 2004). Neither thrombin nor other serine proteases appear to be involved in PAR1-dependent breast cancer cell motility. However, MMP inhibitors (1,10-phenanthroline and MMP-200, a hydroxamate inhibitor) block both migration and invasion of breast cancer cells *in vitro*. Only MMP-1, not MMP-2, -3, -7, or -9, cleaves and activates PAR1 upon addition to PAR1-transfected breast cancer cells. MMP-1 inhibitors reduce both cell migration *in vitro* and tumor growth in nude mice (Boire *et al.*, 2005; Pei, 2005). In addition, antagonism of either MMP-1 or PAR1 significantly attenuates tumor-induced endothelial cell activation (ECA), the transformation of the intravascular milieu to a prothrombotic, proinflammatory, and cell-adhesive state, as a result of tumor-endothelial cross-talk (Goerge *et al.*, 2006). Thus, the MMP-1/PAR1 axis functions in both ways: host-derived MMP-1 activating tumor-expressed PAR1, as well as tumor-derived MMP-1 acting on endothelial PAR1 (see Figure 2). Since both MMPs and PARs have also been shown to play important roles in cardiovascular and inflammatory diseases (Ossovskaya and Bunnett, 2004), targetting the MMP-1/PAR1 pathway with therapeutics that block MMP-1 may become an attractive approach in the treatment of a variety of invasive, proliferative, and inflammatory conditions.

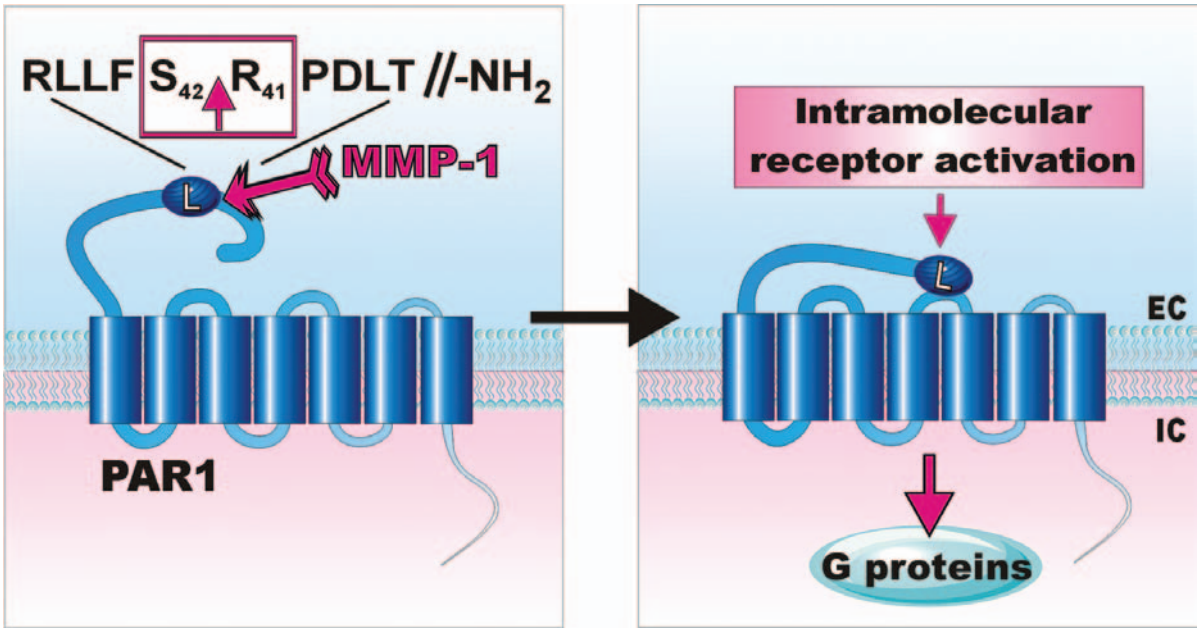

FIGURE 12 **Proteolytic cleavage by MMP-1 in the NH_2-terminus of PAR1 results in intramolecular receptor activation. Proteolytic cleavage by MMP-1 at Arg41-Ser42 in the NH_2-terminus of PAR1 exposes a new NH_2-terminus, which serves as a tethered ligand (L) and binds to the second extracellular loop of the receptor, activating it intramolecularly. Activated PAR1 initiates signal transduction across the membrane to activate intracellular G proteins that regulate pathways for cell morphology, secretion, cell proliferation, migration and adhesion in numerous cell types. EC, extracellular; IC, intracellular. Adapted from Pei (2005).**

1.4.12 Receptor Activator of Nuclear Factor κB Ligand (RANKL)

Prostate cancer deaths are primarily due to metastases that are resistant to conventional therapies. The most common site for metastasis of prostate cancer is the bone, with patients often experiencing severe bone pain, pathological fractures, leukoerythroblastic anaemia, bone deformity, hypercalcaemia, nerve-compression syndromes, and immobility (Mundy, 2002). Osteolytic lesions are produced by the interaction between tumor and bone stroma, commonly referred to as the 'vicious cycle,' whereby tumor cells in the bone can secrete factors such as parathyroid hormone related peptide (PTHrP) that stimulate osteoblast expression of the **r**eceptor **a**ctivator of **n**uclear factor κB **l**igand (RANKL). By binding to its receptor RANK, RANKL has been shown to be essential in mediating osteoclast activation (Figure 13). The osteoclasts degrade the bone matrix using a powerful array of proteases such as the MMPs and the cathepsins (Mundy, 2002). RANKL, also known as tumor necrosis factor ligand superfamily member 11, CD254, TNF-**r**elated **a**ctivation-i**n**duced **c**ytokine (TRANCE), **o**steo**p**rote**g**erin **l**igand (OPGL) and **o**steoclast **d**ifferentiation **f**actor (ODF), is a member of the TNF family. RANKL is required in osteoclast differentiation and activation; T and B cell maturation; dendritic cell survival; and might also play a role in the cardiovascular system. The interaction with its receptor RANK and its decoy receptor osteoprotegerin (OPG) forms a molecular triad which can modulate the bone system, the immune system, and the cardiovascular system (Theoleyre *et al.*, 2004).

To improve overall patient survival and to identify new therapeutic targets, the molecular mechanisms underlying prostate tumor-induced changes in the bone microenvironment need to be elucidated. Therefore, Lynch and coworkers (2005) developed a murine model of rat prostate cancer in the bone environment that mimics the osteoblastic and osteolytic changes associated with human metastatic prostate cancer. MMP-7 was identified as a proteolytic enzyme whose expression correlates with tumor-induced osteolysis. Interestingly, MMP-7 was expressed by the osteoclasts at the tumor-bone interface, and its secretion resulted in the solubilization of RANKL being presented by the osteoblasts, stromal cells or tumor cells in the bone microenvironment (Figure 13). Cleavage of RANKL by MMP-7 occurs at the residues Met145-Met146 in the stalk region of the protein, releasing an active soluble form of RANKL from the cell surface. MMP-3, but not MMP-2, MMP-9, or MMP-13, was also found to cleave RANKL, although the amount of sRANKL produced was significantly less. sRANKL released by MMP-7 was as active and efficient in osteoclast activation as full-length RANKL. The shedding of RANKL is not without significance, because it eliminates the need for close contact between RANKL-expressing cells such as osteoblasts and tumor cells, and RANK-expressing osteoclast precursor cells (Blavier and Declerck, 2005). MMP-7-mediated release of RANKL was also shown *in vivo*, as sRANKL was clearly detected in the tumor-bone interface lysates from wild-type mice, while little or no sRANKL was detected in MMP-7 deficient animals. In addition, tumor-induced osteolysis was significantly reduced in MMP-7 deficient mice (Lynch *et al.*, 2005). Hence, these results make MMP-7 an attractive therapeutic target for the control of cancer-induced bone metastasis.

Interestingly, RANKL was also reported to be solubilized by MT1-, MT2-, MT3-, and MT5-MMP, which will be discussed in a later section (see Section 3.4.2.2).

1.5 Inflammatory Processes and Immune Escape in Cancer

1.5.1 Intercellular Adhesion Molecule-1 (ICAM-1)

Intercellular adhesion molecule-1 (ICAM-1, major group rhinovirus receptor, CD54) is a transmembrane glycoprotein expressed on multiple cell types including leukocytes, epithelial cells, endothelial cells and fibroblasts. It is involved in multiple transient cellular interactions that regulate infiltration, activation and effector functions of leukocytes. Interaction of ICAM-1 with its physiological ligand lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18 or $\alpha_L\beta_2$, the prototypic β_2-integrin) (see Section 1.4.3) is crucial for leukocyte arrest on endothelial cells, stabilization of interactions between antigen-presenting cells and T lymphocytes by the so-called immune synapse, and for adhesion of cytotoxic T cells and NK cells to their target cells. Besides LFA-1, ICAM-1 binds other ligands such as Mac-1 (Complement receptor 3, CD11b/CD18 or $\alpha_M\beta_2$ integrin), rhinoviruses and malaria-infected red blood cells (Fiore *et al.*, 2002; Hopkins *et al.*, 2004). ICAM-1 expression on resting vascular endothelial cells

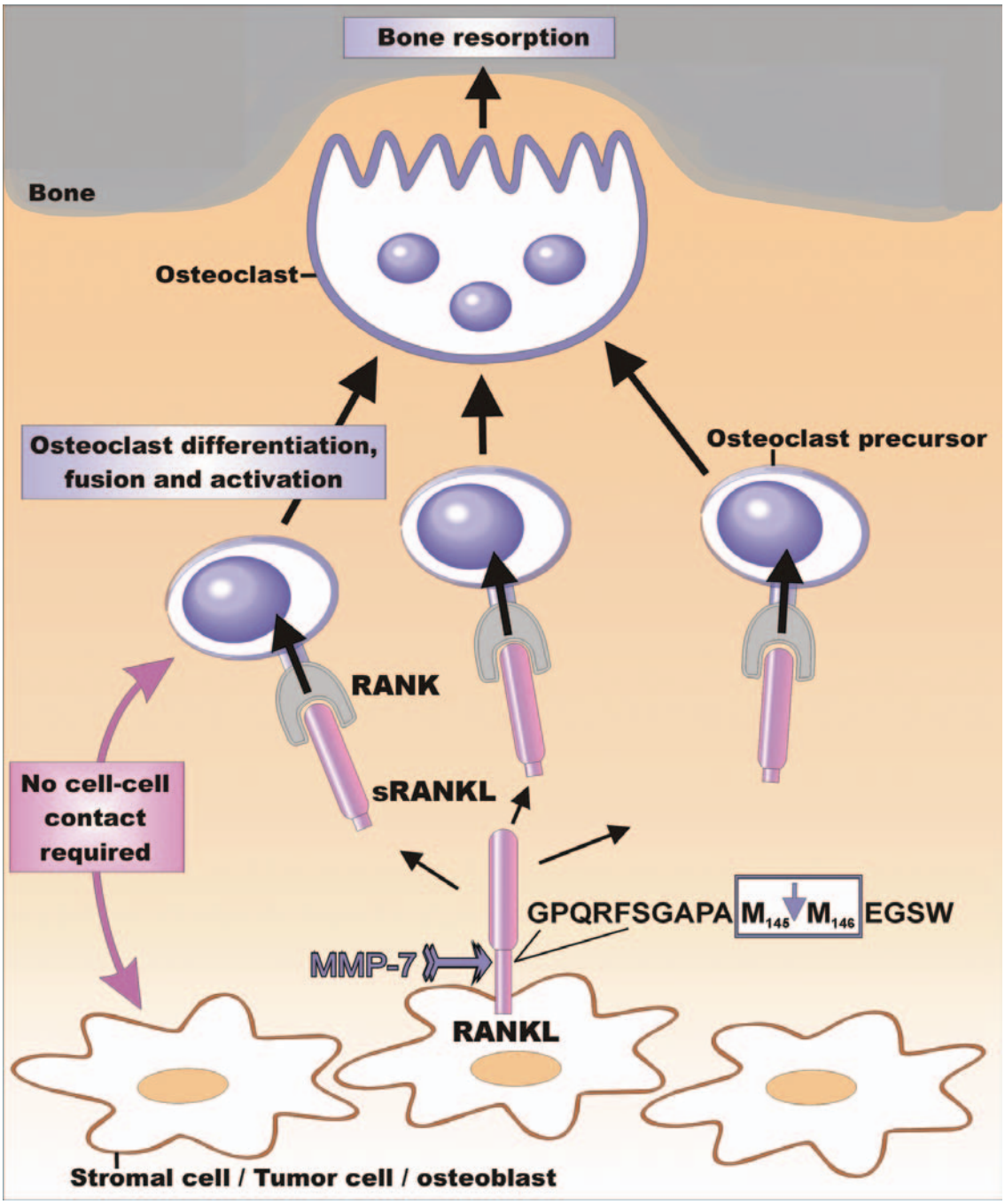

FIGURE 13 **The shedding of RANKL by MMP-7 promotes cancer-induced osteolysis. Osteolytic lesions in bone metastasis are produced by the interaction between tumor and bone stroma, whereby tumor cells in the bone can secrete factors that stimulate osteoblast expression of RANKL. By binding to its receptor RANK, RANKL has been shown to be essential in mediating osteoclast activation. The osteoclasts degrade the bone matrix using a powerful array of proteases such as the MMPs and the cathepsins. MMP-7 has been shown to mediate solubilization of RANKL on osteoblasts, stromal cells or tumor cells in the bone microenvironment. Cleavage of RANKL by MMP-7 occurs at the residues Met145-Met146 in the stalk region of the protein. sRANKL released by MMP-7 was as active and efficient at osteoclast activation as full-length RANKL. The shedding of RANKL is noteworthy because it eliminates the need for cell-cell contact between RANKL-expressing cells such as osteoblasts, stromal cells and tumor cells, and RANK-expressing osteoclast precursor cells. Based on Mundy (2002) and Blavier and Declerck (2005).**

and lymphocytes is low, whereas its expression on monocytes is moderate. Leukemia and carcinoma cells express ICAM-1 as well, and this would make them an easier target for CTLs (Cho *et al.*, 2000). However, ICAM-1 can be released from the surface of tumor cells. This cleavage inhibits cell-mediated cytotoxicity, forming a defence mechanism of primary tumor cells against CTLs and NK cells. The protease in charge of this release

is MMP-9 (Fiore *et al.*, 2002). Sequencing of sICAM-1 peptides points to a cleavage site in the hinge region between Arg441 and the transmembrane domain (see Figure 2), with the Glu442-Val443 peptide bond being the most probable cleavage site (Sultan *et al.*, 2004) in accordance with the substrate specificity of MMP-9 (Kridel *et al.*, 2001). However, the exact site of proteolysis remains to be experimentally determined. In conclusion, MMP-9 might be involved in tumor cell evasion of immune surveillance.

1.5.2 Interleukin-2 Receptor-α Chain (IL-2Rα)

Not infrequently, cancer cell spreading occurs despite the apparent presence of TILs. These autologous CTLs seem unable to display their anti-tumor capacities in the tumor microenvironment. One of the various immune escape mechanisms of cancer cells (see Figure 2) is down-regulation of the interleukin-2 Receptor-α chain (IL2-Rα, p55, TAC antigen, CD25). As IL2-Rα is an essential receptor for the proliferation of T-cells, decreased expression of this receptor *in vivo* may result in poor clonogenicity of TILs and cause immune suppression. An *in vitro* cervical cancer model shows that cervical cancer cells can induce the release of soluble IL2-Rα from encountered T-cells, a process inhibited by TIMPs. Immunohistochemical stainings show abundant expression of MMP-1, MMP-2 and MMP-9 in cervical cancer tissues, and MMP-9, and to a lesser extent MMP-2, are capable of IL-2Rα truncation *in vitro* (Sheu *et al.*, 2001). Consequently, as MMP-9 may contribute to tumor cell evasion of immune surveillance by ectodomain cleavage of ICAM-1 as well as IL-2Rα, its inhibition may be an attractive perspective in countering this type of immune escape of cancer cells.

2. MODIFICATION OF MEMBRANE PROTEINS IN DISEASES AFFECTING VASCULAR AND EPITHELIAL INTEGRITY

2.1 Shedding of MMP Substrates in Cardiovascular Diseases

2.1.1 HB-EGF

sHB-EGF is a potent chemoattractant and mitogen for vascular smooth muscle cells. Atherogenesis in the arterial wall is characterized by the formation of fibrous lesions and the proliferation of neointimal smooth muscle cells. Smooth muscle cells and macrophages in atherosclerotic plaques have indeed been reported to produce large amounts of HB-EGF. Moreover, sHB-EGF-induced proliferation of smooth muscle cells is a key step in the progressive neointimal thickening seen in the development of transplant arteriosclerosis, a major obstacle to long-term graft survival after clinical organ transplantation (Higashiyama, 2004). Release of sHB-EGF is mediated bij ADAM-12 in the heart and by MMP-7 in large mesenteric arteries as a result of ligand binding to GPCRs (see Figure 4), like some adrenoreceptors and angiotensin receptors (Hao *et al.*, 2004). The role of MMP-7 in large mesenteric arteries is taken over by MMP-2 and MMP-9 in the case of increased luminal pressure in small mesenteric resistance arteries (Lucchesi *et al.*, 2004). As the EGFR transactivation is triggered by agonists typically overexpressed in hypertension, its blockade may have therapeutical potential for simultanously inhibiting pathological vasoconstriction and growth in hypertensive disorders like vascular inflammation, atherosclerosis, left ventricular hypertrophy, and cardiac hypertrophy (Shah and Catt, 2003; Hao *et al.*, 2004; Shah and Catt, 2004b).

2.1.2 EMMPRIN

MMPs seem to play an important role in atherosclerotic plaque growth, neointima formation, and plaque disruption by inducing smooth muscle migration and proliferation (*e.g.*, after release of sHB-EGF, as discussed before), and by enhancing ECM degradation (Rouis, 2005). Deposition of low-density lipoproteins (LDLs) in the vessel wall and their oxidative modification seem to initiate, or at least accelerate, the atherosclerotic process by several mechanisms, including promotion of foam cell formation, chemotactic effects on monocytes, and mitogenic effects on smooth muscle cells. In addition, oxidized LDLs increase the expression of MMPs in endothelial cells, monocyte-derived macrophages, and smooth muscle cells (Haug *et al.*, 2004). EMMPRIN (see Section 1.4.8) regulates MMP release and activity in fibroblasts, endothelial cells, and tumor cells (Gabison *et al.*, 2005; Yan *et al.*, 2005). In addition, EMMPRIN has been shown to be expressed in macrophage-rich atheromas from human coronary arteries (Major *et al.*, 2002) and in cultured human coronary artery smooth muscle cells (HCA-SMCs) (Haug *et al.*, 2004). Oxidized LDLs significantly enhanced the release of soluble EMMPRIN (~50 kDa), as well as the release of MMP-1 and MMP-2 into HCA-SMC

supernatants. Oxidized LDL-induced release of soluble EMMPRIN was parallelled by a decrease in cell-associated EMMPRIN. These effects were antagonized by antioxidants as well as by EDTA and the MMP inhibitor GM6001. In addition, MMP-1 and MMP-2 cleaved off the cytoplasmic and transmembrane domains of EMMPRIN *in vitro*. Purified soluble EMMPRIN significantly enhanced MMP-1 and MMP-2 release by HCA-SMC. Thus, oxidized LDLs might induce an amplification cascade of increased MMP activity, enhanced MMP-dependent shedding of soluble EMMPRIN, and EMMPRIN-induced upregulation of MMP production. This cascade might accelerate ECM degradation in atherosclerotic plaques and thereby promote plaque growth and plaque destabilization (Haug *et al.*, 2004). Thus, inhibiting MMP-mediated production of soluble EMMPRIN-molecules, which may diffuse and act on multiple cells, may be an interesting therapeutic tactic to interupt or prevent the atherosclerotic cascade.

2.2 Degradation of Intercellular Junction Proteins in Inflammation, Stroke, Acute Renal Failure and Ophtalmic Pathologies

Adhesion between vertebrate cells is generally mediated by three types of adhesion junctions: 1) tight junctions, 2) adherens junctions, and 3) desmosomes. Together they constitute the intercellular junctional complex, which has an important role in defining the physiological function of a cell; that is, they define whether and how a cell will be integrated in functional structures, such as organ epithelia or stroma (Cavallaro and Christofori, 2004).

Occludin and claudins were identified as the major integral membrane proteins forming the tight junctions in epithelial and endothelial cell sheets. Tight junctions are sites of cell-cell contacts composed of a number of transmembrane and cytoplasmic proteins, assembled into a complex tethered to the cytoskeleton. Paracellular permeability, or the flow of ions and molecules between cells, is regulated by these tight junctions. Both, occludin and claudins have four transmembrane domains and their NH_2- and COOH-terminal ends are located in the cytoplasm. This conformation generates two extracellular loops that are supposed to provide the intercellular interaction sites (Figure 14). The COOH-terminal domains of occludins and claudins serve as binding sites for a complex set of signaling proteins including zonula occludens (ZO)-1, -2 and -3, kinases and phosphatases. Compared to occludin (65 kDa), however, claudins are smaller (20 to 25 kDa). Different cellular sheets have their own unique set of claudin species, and this compositional heterogeneity explains the

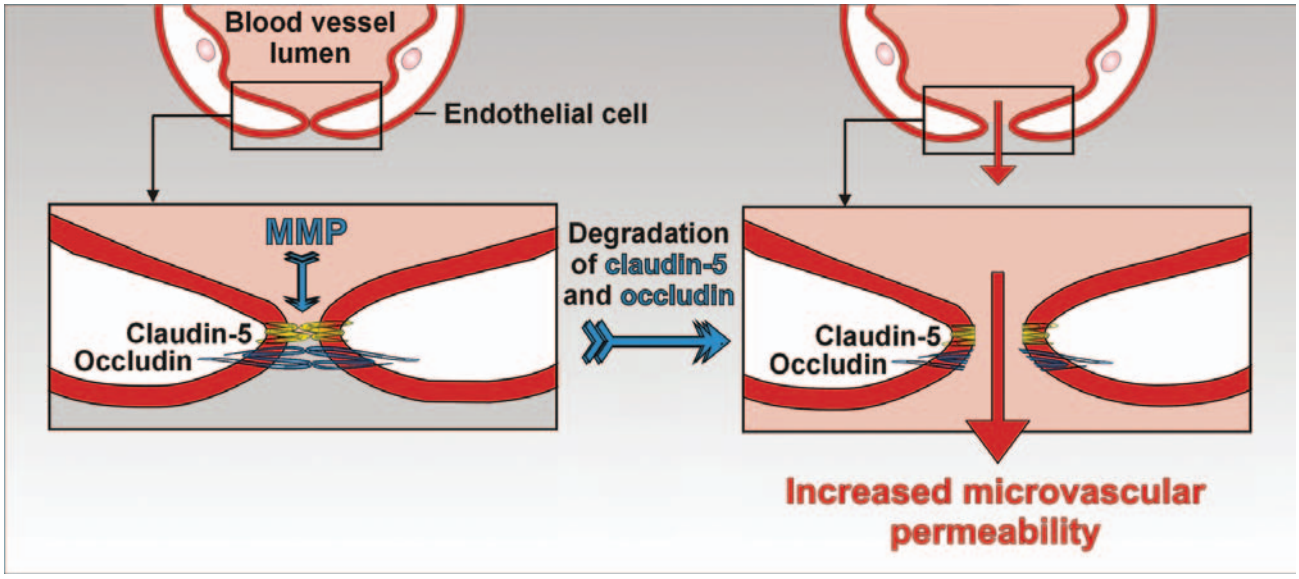

FIGURE 14 **Degradation of tight junction proteins by MMPs disrupts endothelial integrity. Occludin and claudins are the major components of tight junctions in endothelial cell sheets. Both, occludin and claudins have four transmembrane domains and their NH_2- and COOH-terminal ends are located in the cytoplasm. This conformation generates two extracellular loops that are supposed to provide the intercellular interaction sites. Degradation of intercellular junction proteins is mediated by various MMPs and increases microvascular permeability. The widening of interendothelial tight junctions allows for increased solute exchange and immune cell diapedesis, which is a hallmark of inflammation and brain damage after BBB opening.**

diversified barrier properties of tight junctions. Claudin-5 (also named transmembrane protein deleted in velo-cardio-facial syndrome [VCFS]) was shown to be a key molecule in the blood-brain barrier (BBB) in mice (Feldman *et al.*, 2005; Koval, 2006; Bazzoni, 2006; Furuse and Tsukita, 2006). Adherens junctions and desmosomes are cell-cell junctions that are formed by cadherins and additional associated proteins into which actin filaments are inserted.

Degradation of intercellular junction proteins by MMPs disrupts endothelial and/or epithelial integrity and has major consequences in inflammation, stroke, acute renal failure and ophthalmic pathologies, which will be illustrated in the following paragraphs.

2.2.1 Occludin and Claudin-5 Degradation in Inflammation

Increased microvascular permeability is a central hallmark of inflammation and allows for increased solute exchange and extravasation of leukocytes into the inflamed tissue. These inflammatory changes in microvascular permeability are correlated with the reorganization and widening of interendothelial tight junctions. MMPs have been reported to be responsible for the tight junction disruption by degradation of occludin and some claudins (Figure 14) (Alexander and Elrod, 2002). In human umbilical vein endothelial cells and in porcine brain capillary endothelial cells, the tyrosine phophatase inhibitor phenylarsine oxide (PAO) induced increased MMP activity, which was parallelled by severe disruption of cell-cell contacts and degradation of occludin. PAO-induced occludin proteolysis could be prevented by different MMP inhibitors (GM6001 and 1,10-phenantroline) (Wachtel *et al.*, 1999; Lohmann *et al.*, 2004). In addition, MMP-7 has been reported to proteolyse VE-cadherin (see Section 1.3.3). Degradation of intercellular junction proteins by MMPs thus contributes to increased endothelial permeability, a prerequisite for solute exchange and leukocyte extravasation during inflammation.

2.2.2 Occludin and Claudin-5 Degradation in the Blood-Brain Barrier

Tight junction proteins in endothelial cells such as occludin and claudins are major structural components of the BBB formed by components of the neurovascular unit (Bazzoni, 2006). Cerebral ischemia is a complex insult that involves a loss of blood flow accompanied by depletion of oxygen and essential nutrients. *In vitro* models of the BBB have indicated that hypoxia and hypoxia/reoxygenation lead to increased permeability and/or disruption of BBB tight junctions (Hawkins and Davis, 2005). Focal ischemia with reperfusion in spontaneously hypertensive rats lead to opening of the BBB and degradation of occludin and claudin-5 (see Figure 14). Treatment with an MMP inhibitor (BB-1101) prevented the opening and reversed the degradation of the tight junction proteins (Yang *et al.*, 2007). In addition, using green fluorescent protein (GFP)-tagged occludin and live cell imaging, it was shown that monocytes scroll over the brain endothelial surface toward cell-cell contacts, inducing gap formation, which is associated with local disappearance of GFP-occludin, and subsequently traverse the endothelium paracellularly. The broad spectrum MMP inhibitor BB-3103 significantly inhibited endothelial gap formation, occludin loss, and the ability of monocytes to pass the endothelium (Reijerkerk *et al.*, 2006). In addition, after lipopolysaccharide (LPS)-induced opening of the BBB, less BBB disruption was observed in MMP-3 deficient mice than in wild-type controls, as well as diminished degradation of claudin-5 and occludin (Gurney *et al.*, 2006). Hence, use of MMP inhibitors in stroke might reduce or prevent BBB damage by minimizing degradation of tight junction proteins and subsequent immune cell diapedesis.

2.2.3 Degradation of E-Cadherin, N-Cadherin and Occludin in Acute Renal Failure

Acute renal failure (ARF) is characterised by a rapid fall in glomerular filtration rate, clinically manifested as an abrupt and sustained raise in urea and creatinine in plasma. Life threatening consequences include volume overload, hyperkalemia, and metabolic acidosis. ARF is increasingly common and carries a high morbidity and mortality (Hilton, 2006). Although ischemia is a leading cause of ARF, the molecular mechanisms leading to renal injury and failure are not completely understood. In ischemia-induced ARF, a loss of epithelial integrity and shedding of epithelial cells occurs in the tubuli. After injury, both viable and non-viable cells are shed, leaving the basement membrane as the only barrier between filtrate and interstitium, which allows for backleak of the filtrate and tubular obstruction by intraluminal aggregation of cells, proteins, and glycoproteins, such as fibronectin (Bonventre and Weinberg, 2003).

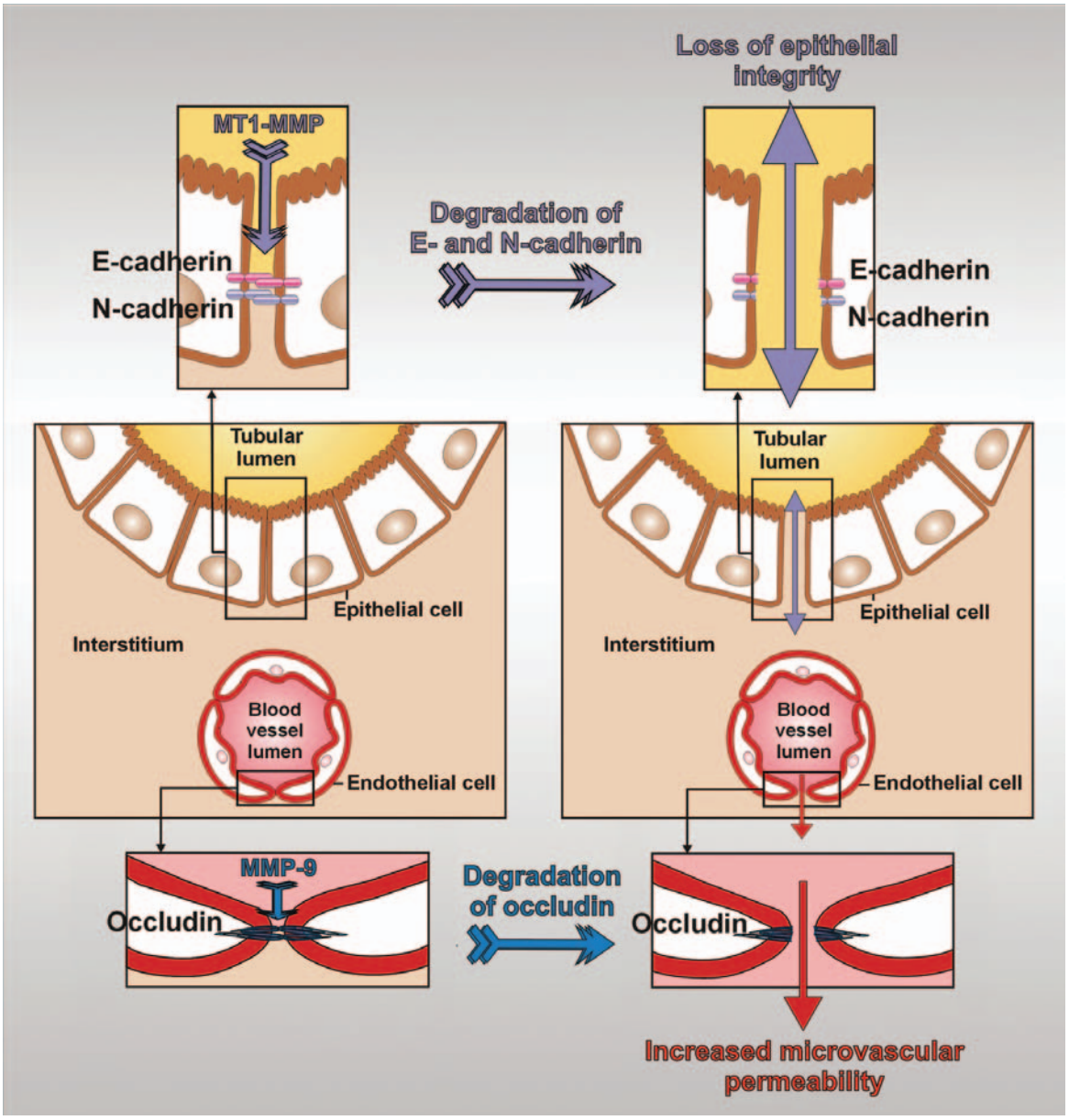

FIGURE 15 **Degradation of intercellular junctions by MMPs in acute renal failure. Cadherins in the adherens junctions and occludins in the tight junctions are key molecules in the regulation of paracellular permeability by the intercellular junction. In ischemia-induced ARF, MT1-MMP-mediated degradation of E-cadherin and N-cadherin results in disruption of epithelial integrity and shedding of epithelial cells in the tubuli, leaving the basement membrane as the only barrier between filtrate and interstitium, which allows for backleak of the filtrate and tubular obstruction by intraluminal aggregation of cells and proteins. In addition, degradation of occludin by MMP-9 leads to the disruption of intercellular endothelial junctions and concomitant cell detachment and vascular injury.**

Application of an *in vitro* model of ischemia-reperfusion resulted in selective fragmentation/loss of E-cadherin and loss of N-cadherin (neural-cadherin, cadherin-2, CD325) levels from normal rat kidney cells that could be blocked by the MMP inhibitors GM6001 and TAPI-0 (Figure 15). TIMP-3 completely blocked both cleavage and/or loss of E-cadherin and N-cadherin, whereas TIMP-2 protected full-length E-cadherin protein expression and TIMP-1 had no effect (Covington *et al.*, 2005). This implies that different MMPs may play a role in E-cadherin and N-cadherin regulation. Chemical inhibitors against a number of soluble MMPs (1, 2, 3, 8, and 9) failed to completely attenuate ischemia-induced E- and N-cadherin loss. Under ischemic conditions, there was increase in active MT1-MMP, and the role of MT1-MMP in ischemia-induced cadherin loss was confirmed by blocking MT1-MMP activity with a neutralizing antibody or by blocking MT1-MMP expression with siRNA constructs, which protected full-length E- and N-cadherin during ischemia and preserved cell-cell

contacts (Covington *et al.*, 2006). Consequently, therapeutic inhibition of MT1-MMP may preserve epithelial integrity and inhibit epithelial cell shedding in ARF. In normal physiology, MMP-7-mediated shedding of E-cadherin is required for the repair of injured lung epithelium (McGuire *et al.*, 2003). Similarly, N-cadherin cleavage also occurs under physiological conditions. Indeed, MT5-MMP was shown to produce a ~35 kDa N-cadherin degradation product in neurons, possibly contributing to mechanisms of synaptic regulation (Monea *et al.*, 2006). Therefore, targetting MT1-MMP in ARF must occur by using extremely specific inhibitors, as not to disturb any vital physiological process.

As discussed above, occludin is one of the major integral membrane proteins forming the tight junctions in endothelial cell sheets. During acute *in vivo* reversible ischemia induced in rat kidneys by vascular clamping, pro-MMP-2, pro-MMP-9, and active MMP-9 were upregulated in the endothelial cell fractions. This increase in MMP-9 during ischemia is accompanied by a lower level of occludin in endothelial fractions. This finding suggests that the induction of MMP-9 during kidney ischemia leads to an increased degradation of occludin (Caron *et al.*, 2005). Thus, ischemia in kidneys could lead to the disruption of intercellular endothelial junctions by MMP-9 and concomitant cell detachment and vascular injury (Figure 15). In addition, active MMP-9 in ischemic kidney may also contribute to vascular basement membrane degradation and increased permeability. Accordingly, blocking MMP-9 and MT1-MMP may be a way to reduce mortality in ARF.

2.2.4 Occludin Proteolysis in Ophtalmic Pathologies

Breakdown of the blood-retinal barrier (BRB) is an early feature of proliferative diabetic retinopathy (PDR) and results in vascular leakage and the developent of retinal edema (Frank, 2004). As in the BBB (see Section 2.2.2), tight junction proteins such as occludin and claudins are an integral structural component of the BRB. Both TGF-β and MMP-9 increased the permeability of retinal endothelial cells and reduced the levels of occludin (see Figure 14) (Behzadian *et al.*, 2001). Treatment of retinal microvessel endothelial cells and retinal pigment epithelial cells with MMP-2 and MMP-9 also revealed specific degradation of occludin, but not of claudin-5 (Giebel *et al.*, 2005). These results suggest that elevated expression of MMPs in the retina may facilitate an increase in vascular permeability by a mechanism involving proteolytic degradation of occludin, followed by disruption of the entire tight junction complex. Thus, a greater understanding of the role of MMPs in altering tight junction proteins in PDR may provide future targets for therapeutic intervention.

Corneal epithelial disease, termed keratoconjunctivitis sicca (KS), is a severe and sight-threatening complication of dry eye syndrome. A key clinical feature of KS is disruption of epithelial barrier function. This results in eye irritation, corneal surface irregularity, blurred and fluctuating vision, and increased risk for corneal ulceration (Pflugfelder, 1998; Pflugfelder *et al.*, 2005). Concentration and activity of MMP-9 in the tear fluid was found to be significantly increased in these eyes, as well as in an experimental murine model of dry eye. Corneal epithelial permeability increased in dry eye wild-type mice, but not in MMP-9 deficient mice. Compared to MMP-9 knockout mice, wild-type mice showed greater desquamification of differentiated apical corneal epithelial cells and this was accompanied by an increase in lower sized (50 kDa) occludin in the corneal epithelia of wild-type mice. The same effects were observed in cultured human corneal epithelial cells treated with active MMP-9 (Pflugfelder *et al.*, 2005). These observations show that increased MMP-9 activity on the ocular surface in response to dryness disrupts corneal epithelial barrier function (Figure 16). The mechanism appears to be similar to BBB and BRB disruption (see above), that is, through disruption of tight junctions by proteolysis of occludin. Accordingly, reducing barrier degradation in human KS by targeting MMP-9 may lessen the severity of clinical disease.

3. SHEDDING OF MMP SUBSTRATES IN THE MODULATION OF INFLAMMATION AND INNATE IMMUNITY

Innate immunity comprises several rapid defence mechanisms against invading microorganisms and other types of damage to the host. During inflammatory reactions, MMP-mediated cleavage of cell surface molecules leads to activation of pro-inflammatory cytokines. This is a fundamental step in the regulation of leukocyte recruitment and homeostasis. In addition, the previously mentioned modifications of mHB-EGF, E-cadherin, integrin subunit precursors, MUC1, ICAM-1 and IL-2Rα, also have major effects on immune responses and wound healing. Finally, proteolysis of cell surface

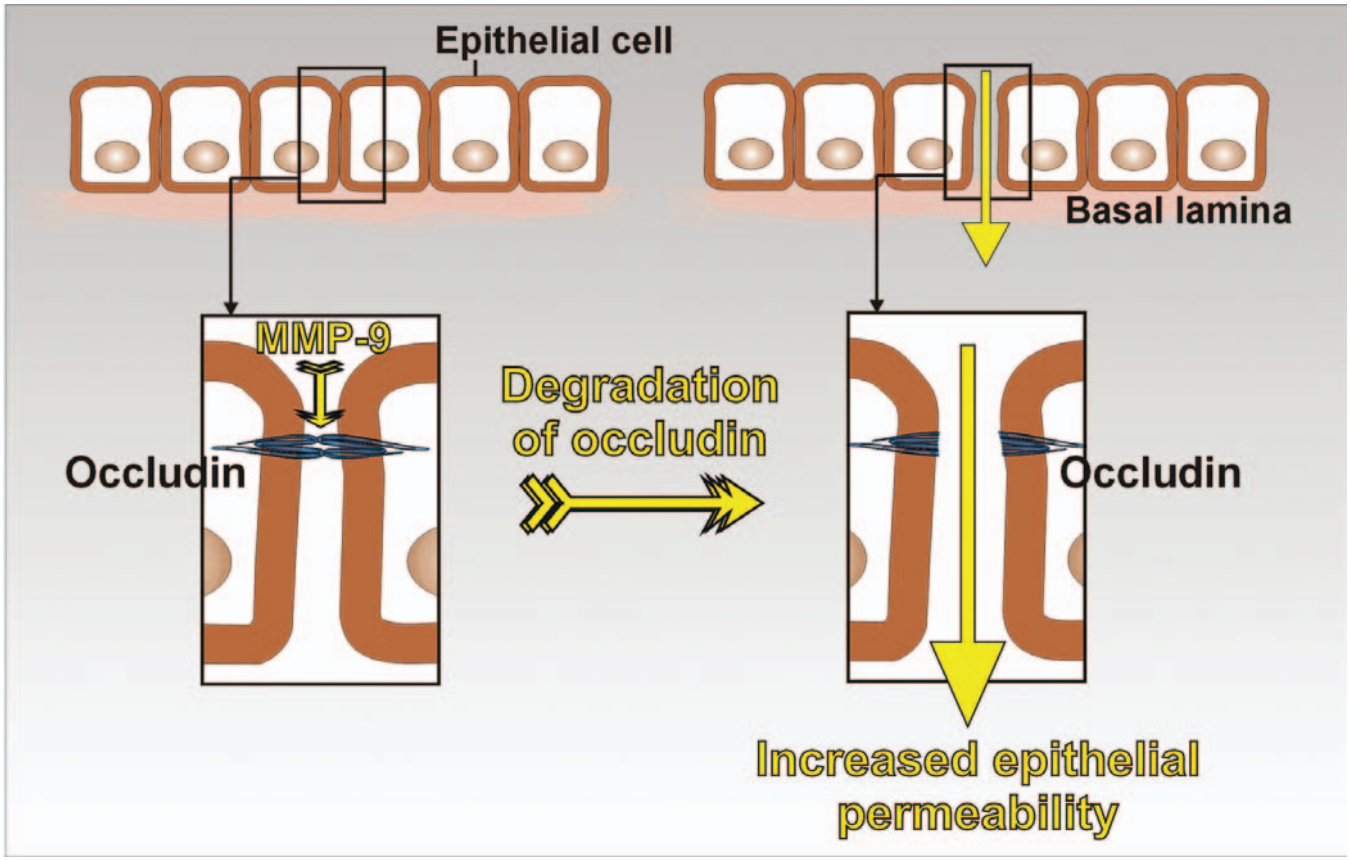

FIGURE 16 **MMP-9-mediated degradation of occludin increases corneal epithelial permeability in keratoconjunctivitis sicca. Occludins in the tight junctions between epithelial cells mediate intercellular adhesion and hence, are essential in epithelial barrier function. Increased MMP-9 activity on the ocular surface in response to dryness leads to degradation of occludin and disruption of the corneal epithelial barrier. Subsequent desquamification of apical corneal epithelial cells leads to keratoconjunctivitis sicca.**

proteins by MMPs plays a non-negligible role in various autoimmune diseases.

3.1 Activation of Membrane-Bound Pro-Inflammatory Cytokines

3.1.1 Tumor Necrosis Factor-α (TNF-α)

Tumor necrosis factor-α (TNF-α, tumor necrosis factor ligand superfamily member 2, cachectin) is a pleiotropic cytokine with potent immunomodulatory and pro-inflammatory properties. Excessive or prolonged production of TNF-α is a feature of septic shock and several important autoimmune diseases like rheumatoid arthritis, Crohn's disease and multiple sclerosis (Kollias *et al.*, 1999). TNF-α is initially expressed on T cells and macrophages as an active 26 kDa membrane-bound protein (pro-TNF-α), which is cleaved by TACE to form the 17 kDa soluble cytokine (Black *et al.*, 1997; Moss *et al.*, 1997; Mohan *et al.*, 2002). TNF-α proteolysis is inhibited *in vitro* and *in vivo* by broad-spectrum metalloproteinase inhibitors (McGeehan *et al.*, 1994; Mohler *et al.*, 1994). MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9 also cleave recombinant pro-TNF-α fusion proteins *in vitro*, as do MMP-12, MT1-MMP, MT2-MMP and MT4-MMP (Gearing *et al.*, 1994; Gearing *et al.*, 1995; Chandler *et al.*, 1996; d'Ortho *et al.*, 1997; English *et al.*, 2000). Of all these MMPs, only MMP-7 processes pro-TNF-α at the natural cleavage site, between Ala76 and Val77 (Table 3). However, this reaction shows a 30-fold lower specificity constant relative to the TACE-mediated proteolysis (Mohan *et al.*, 2002). MMP-7 and MMP-12 are both capable of TNF-α shedding from isolated macrophages. MMP-7-mediated release of TNF-α from peritoneal macrophages is essential for the induction of MMP-3 in coculture with vertebral disc cells. This MMP-3 in turn generates a macrophage chemoattractant, resulting in the macrophage infiltration that is essential for the spontaneous resorption of herniated discs (Haro *et al.*, 2000a; Haro *et al.*, 2000b). TNF-α solubilization by MMP-12 is a crucial step in acute cigarette smoke-induced inflammation, which causes emphysema and chronic obstructive pulmonary disease

(Churg *et al.*, 2003). To summarize, it may be stated that, whereas TACE is the main enzyme responsible for the inducible release of TNF-α in response to bacteria and pathogen-associated molecular patterns (the ligands of Toll-like receptors), MMP-7 and MMP-12 may cause constitutive TNF-α release from macrophages during common processes as tissue resorption and resolution in response to injury (Parks *et al.*, 2004).

3.1.2 Interleukin-1β (IL-1β)

Interleukin-1β (IL-1β, catabolin, endogenous pyrogen, granulocytic pyrogen, leukocytic pyrogen, lymphocyte activating factor, hemopoietin-1, osteoclast activating factor, mononuclear cell factor [MCF]) is a strong pro-inflammatory cytokine that induces fever, inflammation, induction of acute phase reactants, tissue destruction, and, in some cases, shock and death (Dinarello, 2000). IL-1β lacks a typical transmembrane domain and most of its precursor is stored in the cytoplasm. In addition, a small fraction of the precursor can be found in the extracellular space. We discuss its cleavage in the context of the functional analogy with the above mentioned TNF-α proteolysis. IL-1β is primarily produced by activated macrophages, monocytes and polymorphonuclear phagocytes (Delaleu and Bickel, 2004). It lacks a secretory signal peptide and, as a result, it is not secreted through the classical exocytic route, but presumably through exocytosis of secretory lysosomes, a mechanism that still requires further clarification (Andrei *et al.*, 2004; Wewers, 2004). Whereas the COOH-terminal 17 kDa segment has full biological activity (Van Damme *et al.*, 1985), the 33 kDa proform of IL-1β is inactive and its maturation requires proteolytic processing into the mature protein by the IL-1β-converting enzyme (ICE or caspase-1), an intracellular cysteine protease (Kostura *et al.*, 1989). The possibility of extracellular precursor proteolysis and *in vivo* studies with ICE deficient mice suggest the existence of ICE-independent mechanisms of IL-1β activation (Delaleu and Bickel, 2004). Indeed, various proteases cleave recombinant IL-1β *in vitro*, including bacterial enzymes, trypsin, chymotrypsin, leukocyte elastase and granzyme A. However, with the exception of granzyme A, all of these cleavages result in fragments >17 kDa. On the contrary, proteolysis by MMP-2, MMP-3, and especially by MMP-9, yields biologically active forms (Schönbeck *et al.*, 1998). In addition, prolonged incubation of mature IL-1β with MMP-3, and to a lesser extent with MMP-1, MMP-2, and MMP-9, results in degradation of the mature cytokine (Ito *et al.*, 1996). IL-1β is one of the classical inducers of these MMPs in inflammation. In addition, IL-1β is an autoregulating protein with the ability to induce its own gene expression. Thus, at sites of acute or chronic inflammation the presence of MMP-9 might result in biologically active IL-1β, whereas MMP-3 can degrade the active IL-1β form, mediating downregulation of its activities. As IL-1β plays a crucial role in multiple inflammatory and autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis (Delaleu and Bickel, 2004), and seems to be involved in tumor invasion and angiogenesis as well (Voronov *et al.*, 2003), it is a principal target for drug development (Braddock and Quinn, 2004). In this regard, understanding IL-1β activity modulation by MMPs is crucial.

3.2 Regulation of Leukocyte Recruitment, Migration and Homeostasis in Inflammation

3.2.1 Syndecan-1

The influx of inflammatory cells to skin or mucosal sites of injury is largely directed by signals from the epithelium, but how these cells generate and modulate chemotactic gradients has not yet been completely elucidated. In murine lungs, a potential mechanism for the formation of neutrophil-attracting chemotactic gradients is based on the interaction of three components of the epithelial tissue: a secreted protease (MMP-7), a cell-bound proteoglycan (syndecan-1) and a CXC chemokine (KC) (Li *et al.*, 2002). When damaged, lung epithelial cells secrete the chemokine KC (and probably other chemokines as well), which binds to preexisting syndecan-1 molecules. MMP-7 is also induced by tissue injury, secreted by wound-edge epithelia and anchored to the heparan sulfate chains of cell surface proteoglycans (Yu and Woessner, 2000). MMP-7 cleaves the syndecan-1 core protein to release the ectodomain-KC complex. The shed complex is then transported, either actively or passively, to the apical surface where it forms a chemotactic gradient which guides neutrophils to the alveolar space (Li *et al.*, 2002). Syndecan-1 ectodomain shedding by MT1-MMP results in enhanced tumor cell migration (see Section 1.4.6), while cleavage by MMP-7 is required for directing neutrophil migration to injured lung tissue. Further clarification of such chemotactic processes is important to allow control of the inflammatory process, in order to improve the removal of

micro-organisms and the repair of tissues, while limiting damage (Li *et al.*, 2002; Shapiro, 2003).

3.2.2 Leukocyte-Selectin (L-Selectin)

Leukocyte-selectin (L-selectin, lymph node homing receptor, leukocyte adhesion molecule-1 [LAM-1], leukocyte surface antigen Leu-8, TQ1, leukocyte-endothelial cell adhesion molecule-1 [LECAM-1], gp90-MEL, MEL-14 antigen, CD62L) is a member of the selectin family of adhesion molecules. L-, P-, and E-selectins each possess a C-type lectin domain, specialized to recognize specific oligosaccharides on mucins and other glycoproteins. Selectins mediate the process of reversible "rolling" in the binding of leukocytes in the bloodstream onto (activated) endothelial cells and specialized endothelial cells lining the high endothelial venules (HEVs) in lymph nodes (Gallatin *et al.*, 1983; Ley and Kansas, 2004).

L-selectin shows the unique property of being cleaved in the membrane-proximal extracellular domain with concomitant release of a soluble fragment that contains the functional lectin and EGF domains. This provides a rapid mechanism for the regulation of L-selectin levels on leukocytes, and hence, controls their ability to migrate into tissues. An extended variety of stimuli, such as chemotactic factors, phorbol ester activation and reagents that cross-link L-selectin, induce L-selectin proteolysis (Preece *et al.*, 1996). The hydroxamic acid-based MMP inhibitor, Ro 31-9790, completely prevents shedding of L-selectin from leukocytes in mice, rats, and humans. *In vitro* reduction of L-selectin-positive leukocytes is mediated by MMP-1, and to a lesser extent by MMP-3, while MMP-2 and MMP-9 have no such effect. However, lymphocytes do not express MMP-1 and MMP-3, and L-selectin levels are not affected by TIMP-1. Moreover, the L-selectin sheddase only acts in *cis* (on the same cell) and not in *trans* configuration (Preece *et al.*, 1996). This shedding is only inhibited by TIMP-3, and not by TIMP-2 (Borland *et al.*, 1999). TACE was identified as the L-selectin sheddase following PMA stimulation (Peschon *et al.*, 1998). However, multiple agents induce L-selectin shedding and have structural requirements that differ from PMA-induced shedding (Smalley and Ley, 2005). In addition, with the use of TACE deficient mice, a significant sheddding of L-selectin was still detected, and a small fraction of this shedding was not inhibited by a metalloprotease inhibitor, suggesting that there may be more than one additional sheddase (Walcheck *et al.*, 2003). Thus, separate proteolytic mechanisms of L-selectin shedding may play a role under a variety of distinctive conditions, for instance to regulate distinct antiadhesive mechanisms.

As L-selectin mediates crucial leukocyte adhesion and migration processes in inflammation, the elucidation of the functional implications of L-selectin shedding was of paramount importance. Using inhibitors of L-selectin shedding it was demonstrated that L-selectin release participates in regulating neutrophil rolling. Moreover, shedding of L-selectin limits leukocyte activation and thus may limit inflammation. Interestingly, plasma of healthy humans and mice contains approximately 1.6 μg/mL of soluble L-selectin. As sL-selectin concentrations of 0.9 μg/mL already reduce lymphocyte migration to peripheral lymphe nodes by over 30%, shedded L-selectin was suggested to regulate normal lymphocyte trafficking and possibly the inflammatory response (Smalley and Ley, 2005). Studies with transgenic mice expressing shedding-resistant L-selectin showed that L-selectin shedding has a role in minimizing reentry of T-cells into peripheral lymph nodes following activation and limits neutrophil adhesion at sites of inflammation. Mice lacking the ability to shed L-selectin on neutrophils also showed reduced neutrophil migration to inflammatory chemokines, suggesting that L-selectin shedding is required for efficient transendothelial migration (Smalley and Ley, 2005). Specification of the other sheddases in charge might contribute to further understanding and control of leukocyte mobility in inflammatory processes.

3.2.3 Kit-Ligand (KitL)

Kit ligand (c-KitL, stem cell factor [SCF], mast cell growth factor [MGF]) is a 31 kDa membrane-bound growth-stimulating cytokine, which is proteolyzed into an active soluble form (sKitL, 164 amino acids) (Heissig *et al.*, 2002). KitL exists in homodimers, and binding to its cell surface receptor c-Kit (SCF receptor or CD117) thus causes dimerization of this TKR. Dimerization of c-Kit in turn results in autophosphorylation and activation of the receptor and of downstream signal transduction proteins, involved in cell proliferation, survival and chemotaxis. KitL is found on stromal cells of the bone marrow, on vascular smooth muscle cells and on endothelial cells, whereas c-Kit is expressed on a variety of cell types, including mast cells, hematopoietic progenitor cells, melanocytes, germ cells and gastrointestinal pacemaker cells (Akin and Metcalfe, 2004).

sKitL plays a key role in the maintenance and reconsitution of the stem and progenitor cell pool. Under steady-state conditions, quiescent c-Kit$^+$ hematopoietic stem cells (HSCs) and circulating endothelial progenitors (CEPs) reside in a niche in close contact with stromal cells from the bone marrow, including osteoblasts. Membrane-bound cytokines such as KitL transmit survival signals and support the adhesion of stem cells to the stroma. Bone marrow ablation (*e.g.*, by cytotoxic agents) induces upregulation of MMP-9, which cleaves KitL. Released sKitL confers signals that enhance mobility of c-Kit$^+$ HSCs and CEPs. As a result, they can translocate into a vascular-enriched proliferative zone, which stimulates differentiation and mobilization into the peripheral blood stream (Heissig *et al.*, 2002). The net number of circulating leukocytes is dependent on the balance between production in the bone marrow, and margination and migration of leukocytes into peripheral tissues and spleen (Opdenakker *et al.*, 1998). Consequently, sKitL release by MMP-9 is crucial for the maintenance of leukocyte homeostasis in the blood, and might be a key step in the recruitment of bone marrow stem cells during cell therapy for cancer and other diseases.

In addition, MMP-9-mediated shedding of KitL also affects smooth muscle function and development of intimal hyperplasia, as this process, characterized by transformation of medial smooth muscle cells from a quiescent contractile to a synthetic proliferative phenotype, seems to be influenced by autocrine sKitL/c-Kit signal transduction (Hollenbeck *et al.*, 2004). The importance of KitL ectodomain proteolysis by MMP-9 is further confirmed by the extended variety of physiological processes affected by KitL/c-Kit signaling, such as maintenance of adult lymphopoiesis in bone marrow and thymus (Waskow *et al.*, 2002), survival, differentiation, chemotaxis, and functional activation of mast cells, melanocyte development and regulation of oogenesis, folliculogenesis, and spermatogenesis (Akin and Metcalfe, 2004).

3.3 Recognition and Clearance of Pathogens in Innate Host Defense

3.3.1 CD14

Surfactant protein-D (SP-D) and CD14 are important innate immune defense molecules that mediate clearance of pathogens and apoptotic cells from the lung. CD14 (monocyte differentiation antigen CD14, myeloid cell-specific, leucine-rich glycoprotein) is a 55 kDa pattern recognition receptor that is present on the surface of monocytes, macrophages and neutrophils. As a GPI-linked receptor, it lacks a cytoplasmic signaling domain and, therefore, it requires interaction with other receptors to elicit its biological responses. CD14 binds LPS and interacts with toll-like receptor 4 (TLR4) and myeloid differentiation protein 2 (MD-2) enhancing MAPK signaling and production of cytokines and chemokines (Antal-Szalmas, 2000). Additional biological functions mediated by CD14 include the transport of lipids, phagocytosis of bacteria, and clearance of apoptotic cells. CD14 also exists as a soluble molecule (sCD14) found in normal human serum and in culture supernatans of monocytes and cell lines. Two molecular forms of sCD14 with different origins have been characterized. First, some CD14 molecules escape GPI anchor attachment. They are stored intracellularly and released spontaneously as 55 to 56 kDa full-length molecules upon a short temperature shift at 37°C. Second, various stimuli such as PMA, interferon (IFN)-γ or LPS, induce shedding of the GPI-anchored CD14, resulting in sCD14 with a molecular mass of 48 to 49 kDa (Antal-Szalmas, 2000). SP-D plays a central role in the pulmonary host defence and is a member of the collectin (collagen-lectin) family, which form multimeric structures consisting of a collagenous NH_2-terminal domain and a globular COOH-terminal carbohydrate recognition domain that binds oligosaccharides at the surfaces of many microorganisms and mediates phagocytosis and killing by phagocytic cells.

SP-D knockout mice (SP-D$^{-/-}$) develop progressive emphysema that is characterized by chronic inflammation, accumulation of surfactant phospholipids, and infiltration with lipid-laden alveolar macrophages (Hartl and Griese, 2006). CD14 is reduced on alveolar macrophages from SP-D$^{-/-}$ mice and is associated with reduced uptake of LPS and decreased production of TNF-α after LPS stimulation. In addition, sCD14 is increased in the bronchoalveolar lavage (BAL) fluid from SP-D$^{-/-}$ mice, while MMP-9 and MMP-12 activities are enhanced in the lungs. Since treatment of macrophages with MMP-1 reduced the level of cell surface CD14 (Bryniarski *et al.*, 2003), MMP-9 and/or MMP-12 were also candidates for the proteolysis of cell surface CD14 to release sCD14 in SP-D$^{-/-}$ mice. sCD14 was indeed significantly reduced in BAL fluid from MMP-9$^{-/-}$/SP-D$^{-/-}$ and MMP-12$^{-/-}$/SP-D$^{-/-}$ mice compared with SP-D$^{-/-}$ mice.

In addition, MMP-12 treatment of RAW 264.7 cells increased sCD14 in the cell culture medium, supporting the concept that MMP-12 cleaves CD14 from the alveolar macrophage cell surface (Senft *et al.*, 2005). Since it was not investigated whether MMP-9 cleaves CD14 *in vitro*, and MMP-12 is decreased in MMP-12$^{-/-}$/SP-D$^{-/-}$ mice, the exact contribution of MMP-9 remains unclear.

In conclusion, SP-D loss results in reduced innate host defence activities, such as decreased LPS uptake and TNF-α production, through shedding of CD14 by MMP-12. In addition, upon recognition of Gram-negative bacterial LPS, sCD14 has a dual regulatory role that is concentration- and environment-dependent. Low concentrations of sCD14 seem to promote beneficial pro-inflammatory responses to LPS at local sites of infection, whereas high circulating sCD14 concentrations may help control potentially harmful systemic responses to LPS (Kitchens and Thompson, 2005). In summary, MMP-12 may be an interesting target in the regulation of microbial clearance and inflammatory processes that are important for host defense and pulmonary homeostasis. However, more investigation will be required to better understand the dual activities of sCD14 in the host during infection.

3.4 Membrane-bound MMP Substrates in Autoimmune Diseases

3.4.1 Bullous Pemphigoid

3.4.1.1 Bullous Pemphigoid Antigen-2 (BP180)

Bullous pemphigoid (BP) is an autoimmune blistering disease of the skin affecting primarily the elderly. Blister formation by detachment of the epidermis from the underlying dermis occurs within the lamina lucida of the basement membrane and is initiated by deposition of IgG autoantibodies and complement components along the basement membrane zone. These autoantibodies are directed against two major hemidesmosomal components, the 230 kDa intracellular protein BP230 (Bullous pemphigoid antigen-1 [BPAG1], Hemidesmosomal plaque protein, Dystonia musculorum protein, Dystonin) and the 180 kDa transmembrane protein BP180 (Bullous pemphigoid antigen-2 [BPAG2]/HD4/Type XVII collagen). Autoantibody deposition causes complement activation and mast cell degranulation, essential for the recruitment and infiltration of inflammatory cells with subsequent degradation of hemidesmosomal and ECM components (Liu, 2003). Proteinases and reactive free radicals from infiltrating inflammatory cells contribute to tissue damage in BP lesions. Blister fluid and fluid at lesional and perilesional regions indeed contain proteolytic enzymes such as neutrophil elastase (NE), plasmin and plasminogen activators, cathepsin G, collagenases and gelatinases, MMP-2 and MMP-9 (Liu *et al.*, 1998; Liu *et al.*, 2000 a; Liu *et al.*, 2000b). *In vitro* experiments show that NE as well as MMP-9 cleave the extracellular, collagenous domain of a recombinant BP180-Glutathion S-transferase (GST) fusion protein. NE deficient and MMP-9 deficient mice are resistant to blister formation after intracutaneous injection of BP180-specific antibodies, although these mice show deposition of autoantibodies at the dermis-epidermis junction. Moreover, blister formation is completely abolished by the α1-proteinase inhibitor (α1-PI), the major NE inhibitor (Liu *et al.*, 2000b) and *in vivo* BP180 is proteolyzed by NE, but not by MMP-9 (Liu *et al.*, 2000a). Consequently, NE is the main tissue-damaging enzyme in murine experimental BP, whereas neutrophil MMP-9 most likely contributes indirectly by inactivating α1-PI, in this way potentiating NE action. In addition, MMP-9 might collaborate with NE in the fragmentation of other proteins, in this way generating and/or maintaining the chemoattractant gradients needed for neutrophil infiltration, an essential step in the blistering process of experimental BP (Liu *et al.*, 1997; Liu *et al.*, 2000b). MMP-9 activation was shown to be plasmin-dependent and independent of MMP-3 (Liu *et al.*, 2005). NE is the BP180-cleaving enzyme in human BP as well (Verraes *et al.*, 2001). In addition, TIMP-1 is present in five-fold molar excess to MMP-9, indicating that cleavage of α1-PI by MMP-9 is less probable in human BP. However, in an *in vitro* model of BP, inhibition of MMP-9 with a specific monoclonal antibody does abolish blister formation (Shimanovich *et al.*, 2004). As a consequence, the contribution of MMP-9 to disease progression is not as clear in humans as it is in mice. This discrepancy might be explained by the differences that exist between murine and human BP. For instance, the majority of biopsies from BP patients show large numbers of eosinophils in their lesional skin, whereas in mice neutrophils are the predominant inflammatory cells. However, some patients may have neutrophil-rich or cell-poor lesions. These varying and different pathological features in human BP indicate that BP is a heterogenous disease. Subepidermal blistering can

be caused by several mechanisms and cell types, the above mentioned immunopathological cascade being one of them (Liu, 2003). Since BP180 is also an autoantibody target in several other subepidermal blistering diseases including cicatricial pemphigoid, herpes gestationis, linear IgA bullous dermatosis, and lichen planus pemphigoides (Liu, 2003), further identification of the tissue damaging-proteases in these skin autoimmune disorders might allow to develop therapies based on the use of synthetic protease inhibitors or inhibitory monoclonal antibodies.

3.4.2 Rheumatoid Arthritis

3.4.2.1 FasL

Rheumatoid arthritis is a chronic inflammatory autoimmune disease, which results in inflammation of the synovial lining and destruction of the adjacent bone and cartilage. Synovial macrophages, fibroblasts and lymphocytes are critical for the pathogenesis of this disease, in which apoptosis may play divergent roles (Liu and Pope, 2003). Insufficient intra-articular apoptosis induces proliferation of the synovial membrane (Sakai *et al.*, 1998; Okamoto *et al.*, 1998). In joints of patients with active rheumatoid arthritis, few apoptotic cells are detected, which might contribute to persistence of the disease (Pope, 2002). MMP-3 concentrations in the synovial fluid of rheumatoid arthritis patients are closely correlated with increased sFasL levels and with disease activity. Consequently, MMP-3 might contribute to the pathogenic mechanism by cleaving FasL, in this way reducing apoptosis (sFasL is a less potent apoptosis-inducer than its membrane-bound precursor, see Section 1.2.1). However, sFasL shedding was not completely blocked by an MMP inhibitor alone, indicating that further investigation is needed for detection of the other proteases involved (Matsuno *et al.*, 2001). Moreover, rheumatoid arthritis is characterized by a very heterogenous disease course with strong synovial proliferation in early disease, whereas in the later stages synovial proliferation is reduced and often replaced by connective tissue (Smith and Walker, 2004). As a consequence, stimulation of apoptosis, for instance by MMP inhibition, might have some therapeutic benefit, but requires further insight into the exact role of apoptosis in the subsequent stages of rheumatoid arthritis.

3.4.2.2 RANKL

Another important TNF family member in rheumatoid arthritis is RANKL. In the absence of RANKL or RANK, osteoclast differentiation from monocyte precursors does not occur (see Figure 13). RANKL is expressed on T-cells and fibroblasts within the synovial inflammatory tissue of patients with rheumatoid arthritis and its expression is regulated by pro-inflammatory cytokines. In animal models of arthritis, blockade of RANKL-RANK interactions, or a genetic absence of RANKL or RANK, protects against joint damage despite the presence of joint inflammation (Schett *et al.*, 2005).

As discussed before, RANKL is a transmembrane glycoprotein that can be converted to a soluble form by ectodomain shedding (see Section 1.4.12). Besides MMP-7, TACE cleaves RANKL *in vitro* (Lum *et al.*, 1999), but further *in vitro* experiments show no difference in sRANKL shedding from fibroblasts with or without TACE (Schlondorff *et al.*, 2001). However, two other RANKL shedding activities can be discerned in these fibroblast cultures. One is induced by the tyrosine phosphatase inhibitor pervanadate and its TIMP inhibition profile is similar to that of several MT-MMPs. The other is constitutive and is insensitive to any TIMP. MT1-MMP overexpression indeed causes increased RANKL release, the ectodomain being cleaved between Met145 and Met146 (Schlondorff *et al.*, 2001). In accordance with these observations, Western Blot of sRANKL shows two bands with molecular weights of 25 kDa and 24 kDa, that are produced by cleavage at Arg138-Phe139 and Met145-Met146, respectively (Hikita *et al.*, 2006). Experiments with inhibitors suggest that the upper band is produced by an ADAM and the lower band by an MMP. A large number of MMPs were shown to cleave RANKL in a RANKL shedding activity screening system, with a fusion protein. However, only MT1-MMP, MT2-MMP, MT3-MMP, and MT5-MMP cleaved full-length RANKL and generated sRANKL with the expected molecular weight. Real-time PCR showed that MT1-MMP mRNA level was much higher than that of MT2-, MT3-, and MT5-MMP in a bone marrow stromal cell line and in primary osteoblasts, suggesting that MT1-MMP is mainly involved in the production of the 24 kDa band. Reduced MT1-MMP expression in primary osteoblasts by siRNA or its deficiency in MT1-MMP knockout mouse osteoblasts reduced RANKL shedding and increased membrane-bound RANKL, which led to increased osteoclastogenic activity in the cells. Conversely, overexpression of MT1-MMP in osteoblasts suppressed osteoclastogenesis. In addition, although sRANKL produced

by MT1-MMP induced osteoclastogenesis from bone marrow macrophages, the culture medium of activated primary osteoblasts did not induce osteoclastogenesis, even when MT1-MMP was overexpressed. These results suggest that membrane-bound RANKL induces osteoclastogenesis more efficiently than sRANKL, and the ectodomain shedding of RANKL by MT1-MMP negatively regulates osteoclastogenesis, which is in accordance with a previous report (Nakashima *et al.*, 2000). Consistent with these *in vitro* observations, soft X-ray images of MT1-MMP deficient mice displayed osteoporosis and a much higher osteoclast number, while the serum level of sRANKL in the MT1-MMP knockout mice was undetectable (Hikita *et al.*, 2006). In conclusion, MT1-MMP was identified as the major endogenous RANKL sheddase in primary osteoblasts and RANKL shedding seemed to downregulate local osteoclastogenesis.

As MMP-7-released sRANKL is as efficient in osteoclast activation as its full-length precursor (see Section 1.4.12 and Figure 13), it seems paradoxical that cleavage by MT1-MMP at the same site in the ectodomain releases a less active form. However, Hikita and colleagues indeed mentioned that the concentration of the sRANKL produced by MT1-MMP in the culture media was more than tenfold lower than the concentration needed to induce osteoclastogenesis with recombinant sRANKL *in vitro*. It is possible that when the expression of RANKL is highly upregulated, MMP-released sRANKL does have substantial effects on general bone metabolism. Hence, further insight into RANKL shedding by MMPs and its concomitant biological consequences *in vivo* may be crucial in the clarification of some osteoarticular pathologies, including rheumatoid arthritis.

3.4.2.3 ADAMTS-4

Aggrecan hydrates the collagen network and thus provides cartilage with its properties of compressibility and elasticity. Degradation of aggrecan can be mediated by two proteases of the ADAMTS family: ADAMTS-4 and ADAMTS-5 (Malfait *et al.*, 2002). ADAMTS-4 (aggrecanase-1, ADMP-1) is synthesized as a protein containing a signal peptide, prodomain, catalytic domain with a Zn^{2+}-binding motif, disintegrin-like domain, thrombospondin Type I motif, a spacer region and a cysteine-rich domain (Tortorella *et al.*, 1999). The intracellular proprotein convertases (PCs), furin, PACE4 and PC5/6 efficiently remove the prodomain through cleavage at Arg212-Phe213, generating an active enzyme. Interestingly, the secreted proteases trypsin, MMP-9, and in a much lesser extent MMP-13, are also effective in removing the prodomain of ADAMTS-4, but the cleavage by MMP-9 occurs at a site other than the PC/trypsin cleavage site. The MMP-9-activated species cleaves the aggrecan peptide substrate, but not native aggrecan, suggesting that the alternative cleavage site changes substrate specificity of the enzyme (Tortorella *et al.*, 2005). In addition to NH_2-terminal activation, it has been suggested that truncation of the COOH-terminus of ADAMTS-4 by a TIMP-1-sensitive GPI-anchored MMP, MT4-MMP, is required for full catalytic activity against aggrecan (Gao *et al.*, 2002). ADAMTS-4 (p100) and MT4-MMP are first processed intracellularly by furin-mediated removal of the prodomains, followed by their association in the secretory pathway. The GPI-anchored MT4-MMP/ADAMTS-4 (p68) complex moves to the cell surface, where MT4-MMP removes the spacer domain of ADAMTS-4 (p68), generating the p53 form, which can be found in association with both chondroitin and heparan sulfate on syndecan-1. ADAMTS-4 (p40) is generated by removal of the spacer region as well as the cysteine-rich domain, and appears in the medium. Analysis with specific antibodies shows that MT4-MMP cleaves at the Lys694-Phe695 and Thr581-Phe582, to generate the p53 and the p40 form, respectively (Gao *et al.*, 2004). Modification of synthesis, furin-mediated activation and/or GPI-anchoring of MT4-MMP may thus be ways to control ADAMTS-4 activation specifically, in this way preventing the destructive aggrecanolysis seen in human joint diseases such as arthritis. Therapeutic agents interfering with the association of ADAMTS-4, MT4-MMP and syndecan-1 on the cell surface might also be promising in this cartilage-protecting approach. In addition, if MMP-9 activates ADAMTS-4 extracellularly, it will be important to establish which ECM proteins are cleaved by MMP-9-activated ADAMTS-4, and whether it has an impact on cartilage turnover in joint diseases.

3.4.3 Multiple Sclerosis

3.4.3.1 Myelin Basic Protein (MBP)

Multiple sclerosis is a chronic neurological disorder of the central nervous system (CNS), characterised by the breakdown of the BBB, perivascular infiltration of inflammatory cells and demyelination. Extracellular

proteases, such as some MMPs, plasmin and plasminogen activators form an amplification cascade in this autoimmune disease by: 1) increasing the permeability of the BBB; 2) demyelination through degradation of myelin basic protein (MBP, myelin A1 protein, myelin membrane encephalitogenic protein), a major component of the myelin sheath and one of the most abundant proteins of the CNS; 3) release of antigenic peptides which contribute to autoimmunity; and 4) facilitating infiltration and migration of immune cells through the ECM and the basal membrane (Opdenakker and Van Damme, 1994; Chandler *et al.*, 1995; Cuzner and Opdenakker, 1999; Opdenakker and Van Damme, 2002; Opdenakker *et al.*, 2003). Various MMPs cause MBP degradation *in vitro*, the most active enzymes on this substrate being MMP-2 and MMP-12, followed by MMP-3; and by MMP-1, MMP-7, and MMP-9 with comparable but lesser activity (Proost *et al.*, 1993; Chandler *et al.*, 1995; Chandler *et al.*, 1996). MMP-9 activity is detected in the cerebrospinal fluid of patients with multiple sclerosis and other neurological inflammatory disorders (Gijbels *et al.*, 1992). Furthermore, young MMP-9 deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis (Dubois *et al.*, 1999). MMP-9 generates encephalitogenic peptides by cleavage of human MBP at four different sites *in vitro*: between Phe90-Lys91, Ser110-Leu111, Phe114-Ser115, and Asp133-Tyr134 (Proost *et al.*, 1993). Furthermore, *in vivo* MBP degradation is also exerted by MMP-9, as MBP proteolysis after transient focal ischemia is significantly reduced in MMP-9 knockout mice compared with wild-types (Asahi *et al.*, 2001).

3.4.3.2 NG2 Proteoglycan

Conversely, besides these disease-promoting roles in multiple sclerosis, MMP-9 also has a beneficial role after demyelinating CNS injury. MMP-9 knockout mice are impaired in myelin reformation after lysolecithin-induced demyelination. This might be explained by the role of MMP-9 in the clearance of injury-induced deposits of NG2 proteoglycan (chondroitin sulfate proteoglycan NG2, melanoma-associated chondroitin sulfate proteoglycan), an inhibitory transmembrane proteoglycan that retards the maturation and differentiation of oligodendrocytes needed for remyelination. Consequently, MMP-9 is needed for an efficient remyelination and in this way also has a reparative function in multiple sclerosis (Larsen *et al.*, 2003).

3.4.3.3 β-Dystroglycan

Dystroglycan (dystrophin-associated glycoprotein 1) exists as an extracellular highly glycosylated α-subunit and a non-covalently associated transmembrane β-subunit, which are products derived from one gene and result from posttranslation processing of the glycoprotein. The α-subunit binds to several extracellular ligands, including laminin, agrin, perlecan and neurexin, while β-dystroglycan connects intracellularly to dystrophin, which binds to the actin cytoskeleton. Dystroglycan is expressed in many cell types and the broad range of ECM ligand partners indicates that it has an important role in the assembly and maintenance of basement membranes (Barresi and Campbell, 2006).

The 43 kDa β-subunit was shown to be processed to a 30 kDa fragment by MMPs, the inhibitor profile pointing to MMP-2, MMP-9, and MT1-MMP as possible candidates (Yamada *et al.*, 2001). Kaczmarek and coworkers observed β-dystroglycan breakdown in the brain in response to kainate in a temporal pattern parallel to increased MMP-2 and MMP-9 activities (Kaczmarek *et al.*, 2002). This temporal parallelism of MMP-2 increase and β-dystroglycan proteolysis was also observed in the superior cervical ganglion after postganglionic nerve lesion (Leone *et al.*, 2005; Paggi *et al.*, 2006). However, Agrawal and coworkers (2006) were the first to show that MMP-2 and MMP-9 cleave β-dystroglycan *in vitro* as well as *in vivo*, as they identified β-dystroglycan as a key substrate of MMP-2 and MMP-9 in EAE. This study demonstrates that leukocyte cuffing, as it occurs in human multiple sclerosis, is the consequence of the containment of leukocytes between the endothelial and the adjacent parenchymal basement membranes around the blood vessels in the CNS. Obviously, as long as the leukocytes are contained by the parenchymal basement membrane, synthesized by the astrocyte endfeet of the glia limitans, no disease symptoms occur. Gelatinases, both MMP-2 and MMP-9, cleave *in situ* β-dystroglycan in the parenchymal basement membrane and this process coincides with barrier breakdown, infiltration of the CNS parenchyma by leukocytes and development of disease symptoms. As a result, double MMP-9 and MMP-2 knockout mice are completely resistant against disease development. This cleavage is a critical event, since this MMP substrate is localized on the interface between the extracellular milieu and the astrocytes: its cleavage results in complete desintegration of the glia limitans structure and function (Agrawal *et al.*, 2006).

Proteolysis of β-dystroglycan by MMPs may also contribute to cancer growth and spread (Jing *et al.*, 2004), as well as to skeletal muscle degeneration in the muscular diseases sarcoglycanopathy and Duchenne muscular dystrophy (Matsumura *et al.*, 2005).

Administration of synthetic MMP inhibitors to rodents with EAE significantly ameliorates clinical symptoms and pathological signs (Cuzner and Opdenakker, 1999). Therefore, a major challenge in multiple sclerosis therapy development is the design of selective and specific MMP inhibitors, taking into account the multiple disease-affecting functions of some MMPs, like for instance the role of MMP-9 in demyelination as well as in remyelination (Opdenakker *et al.*, 2003).

3.4.4 Systemic Sclerosis

3.4.4.1 uPAR

Systemic sclerosis (Ssc), also called scleroderma, is a clinically heterogenous, systemic disorder which affects the connective tissue of the skin, internal organs and the walls of blood vessels. It is characterized by alterations of the microvasculature, disturbances of the immune system and massive deposition of collagen and other matrix substances in the connective tissues (Haustein, 2002; Chen *et al.*, 2003). Defective angiogenesis, resulting in tissue ischemia, is particularly prominent in the diffuse form of Ssc. As the u-PA/uPAR system is critical in angiogenesis (see Section 1.3.1), microvascular endothelial cells (MVECs) were isolated from the dermis of healthy individuals and from the dermis of patiens with diffuse Ssc to examine u-PA and uPAR levels. Compared with MVECs from healthy skin, MVECs from Ssc patiens showed higher expression of uPAR. However, in Ssc MVECs, uPAR undergoes truncation between its D1 and D2 domains (see Figure 7), which impairs u-PA binding to uPAR. These properties of Ssc MVECs were associated with poor spontaneous and u-PA-dependent invasion, proliferation, and capillary morphogenesis. The uPAR cleavage occurring in Ssc MVECs was associated with overexpression of MMP-12 and both a general hydroxamate inhibitor of MMP activity and anti-MMP-12 antibodies restored this Ssc MVEC-induced impaired functioning (D'Alessio *et al.*, 2004). In addition, fibroblasts from Ssc patients overexpress MMP-12, which cleaves uPAR of MVECs, thus contributing to the failure of Ssc-endothelial cells to induce an efficient angiogenic programme (Serrati *et al.*, 2006). The overexpression of MMP-12 by both Ssc endothelial cells and Ssc fibroblasts indicates that MMP-12 overproduction may have a critical pathogenic role in Ssc-associated vascular alterations. Hence, selective inhibitors of MMP-12 are likely to be efficient at inducing reversal of Ssc-associated lack of angiogenesis, whereas MMP-12 itself seems disease limiting in cancer by blocking angiogenesis (see Section 1.3.1).

4. PROTEOLYSIS OF CELL SURFACE PROTEINS IN NEURODEGENERATIVE DISORDERS: ALZHEIMER'S DISEASE

Alzheimer's disease is a neurodegenerative disease of the CNS associated with progressive loss of recent memory, resulting in dementia. A clinical diagnosis of Alzheimer's disease is confirmed by observing neuritic (amyloid) plaques and neurofibrillary tangles in the hippocampus, amygdala, and association neocortex (Selkoe, 2004). The plaques are formed extracellularly and are composed of the 42- and 40-residue β-amyloid proteins (Aβs). In healthy individuals, 90% of the Aβs produced by brain cells throughout life are Aβ40 peptides *versus* only 10% of Aβ42. In the cortex of mentally normal elderly patients the Aβ deposits found are almost exclusively 'diffuse' plaques that seem to represent the relatively benign precursor lesions. These diffuse plaques are composed of Aβ42, which is far more prone to aggregation than the slightly shorter and less hydrofobic Aβ40. The Aβ hypothesis predicts that gradual elevation of Aβ42 levels in brain interstitial fluid, and perhaps also inside neurons, can lead to the oligomerization of the peptide and eventually to its fibrillization, that is, amyloid formation. Such insoluble amyloid fibrils are characteristic for 'neuritic' plaques which are associated with local microglial activation, astrocytosis, and cytokine and acute phase protein release. These local inflammatory processes and other neurotoxic effects of oligomerized Aβs finally lead to extensive neuronal and synaptic dysfunction and neurotransmitter deficits, which all contribute to memory impairment (Selkoe, 2004; Walsh and Selkoe, 2004).

4.1 Amyloid Precursor Protein (APP) and β-Amyloid Proteins (Aβs)

Aβ is derived from a membrane-bound Aβ precursor protein (APP, cerebral vascular amyloid peptide (CVAP), protease nexin-II (PN-II), Alzheimer disease amyloid protein, PreA4) after sequential cleavages by

a β-secretase and a γ-secretase (Selkoe, 2004). In normal processing of APP, the most common cut is carried out by the α-secretase and occurs between Lys 687 and Leu 688, that is 12 residues NH_2-terminal to the transmembrane region, between Lys16 and Leu17 of the Aβ region (Figure 17). This cleavage creates a large, soluble ectodomain fragment (sAPPα) that is released from the cell surface and leaves a COOH-terminal fragment of 83 amino acids embedded in the membrane. APP is also cleaved between Met671 and Asp672 by a β-secretase, releasing a slightly shorter fragment of APP (sAPPβ) and leaving a membrane-embedded COOH-terminus of 99 amino acids. This 99 amino acid fragment can then be cleaved by a γ-secretase to create Aβ. In summary, proteolysis by an α-secretase releases sAPP from the cell surface, while sequential cleavages by a β- and a γ-secretase lead to Aβ formation (Selkoe, 2004).

The α-secretase is a membrane-associated metalloproteinase, the activity of which is readily inhibited by hydroxamate-based synthetic inhibitors (Higashi and Miyazaki, 2003b). Furthermore, release of sAPP is extremely diminished in fibroblasts from TACE deficient mice *vs.* control mice, making TACE a prime candidate for the α-secretase activity (Buxbaum *et al.*, 1998). MMP-2 is capable of processing APP *in vitro,* but it is not clear whether the cleavage occurs between Lys 687 and Leu 688, like an α-secretase (Miyazaki *et al.*, 1993; Miyazaki *et al.*, 1994) or between Glu668-Val669,

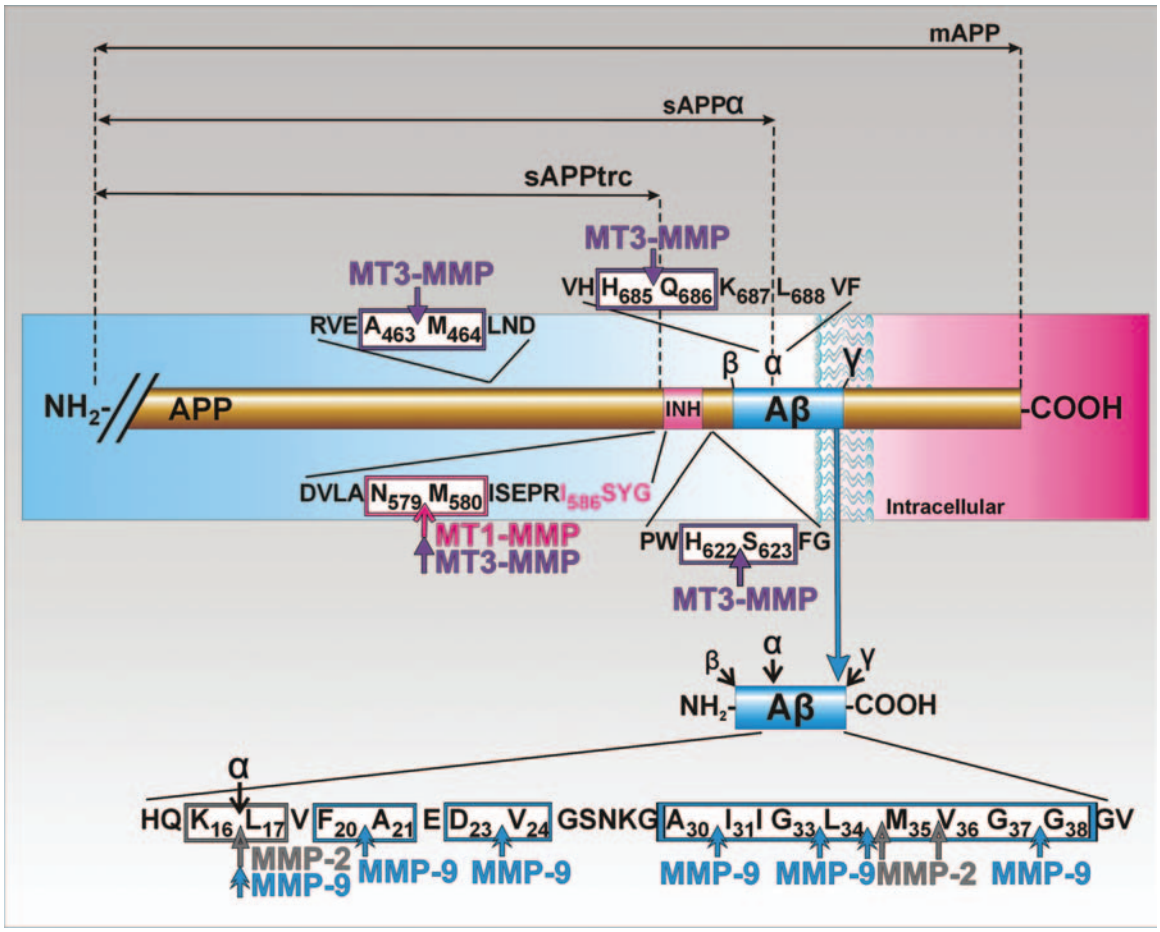

FIGURE 17 **Differential cell surface proteolysis of APP and Aβ leads to accumulation or clearance of pathogenic Aβ peptides in Alzheimer's disease. Aβ (Aβ-amyloid protein) is derived from the membrane-bound Aβ precursor protein (mAPP) after sequential cleavages by a β-secretase and a γ-secretase. However the most common processing of APP is carried out by the α-secretase and occurs between Lys 687 and Leu 688, that is between Lys16 and Leu17 of the Aβ region. This cleavage creates a large, soluble ectodomain fragment (sAPPα). A different processing of APP, between Asn579-Met580, is catalyzed by MT1-MMP and MT3-MMP, and releases a COOH-terminally truncated APP fragment (sAPPtrc) that lacks the MMP-2 inhibitor domain (INH). MT3-MMP cleaves the APP ectodomain at additional sites: at Ala463-Met464, His622-Ser623, and His685-Gln686, which is near the α-secretase cleavage site. Similar to α-secretase cleavage, proteolysis of the His685-Gln686 peptide bond destroys the Aβ-sequence, which diminishes pathologic Aβ formation and accumulation. Once released, Aβ may be cleared by MMP-2 and MMP-9, which cleave it at multiple sites. In addition, MMP-9 has been shown to degrade Aβ fibrils, by proteolytic cleavage at Phe20-Ala21 and Ala30-Ile31. Adapted from (Higashi and Miyazaki, 2003b).**

like a β-secretase (LePage *et al.*, 1995). Furthermore, *in vivo* studies with MMP-2 knockout mice suggest that MMP-2 does not play an essential role in the generation of APP fragments at physiological conditions (Itoh *et al.*, 1997). MMP-3 was also reported to cleave a synthetic APP peptide spanning the β-secretase cleavage site at the residues Glu668-Val669 and Glu674-Phe675 *in vitro*, but this was not further investigated (Rapala-Kozik *et al.*, 1998). APP binds to the ECM and contains an MMP-2-inhibitory domain, which is located in the COOH-terminal glycosylated region of the sAPPs (Miyazaki *et al.*, 1993) within the ISYGNDALMP sequence corresponding to the residues 586 to 595 of APP (Higashi and Miyazaki, 2003a). Another cell-bound MMP might thus be responsible for APP proteolysis. Cell-mediated activation of MMP-2 is indeed accompanied by a different processing of APP, between Asn579 and Met580, which is catalyzed by MT1-MMP and releases a COOH-terminally truncated APP fragment (sAPPtrc) that lacks the MMP-2 inhibitor domain (Figure 17) (Higashi and Miyazaki, 2003b).

An analogy exists in cancer biology, in which APP proteolysis at the ruffling edge of migrating cancer cells (see Figure 2) may be another mechanism by which MT1-MMP promotes migration and invasion (see Section 1.4). It may indeed be suggested that the interplay of MT1-MMP, sAPPtrc, and MMP-2 takes part in the regulation of MMP-2-catalyzed ECM degradation. On the cell surface, where the MT1-MMP concentration is low, APP is liberated mainly as sAPP that inhibits MMP-2 activity. sAPP (as well as APP) binds to the ECM, which might concentrate and enhance the inhibitory effects of sAPP, in this way protecting the ECM near the cell surface from MMP-2 degradation. In contrast, a high (local) concentration of MT1-MMP converts APP to sAPPtrc, which displaces ECM-associated APP or sAPP, thereby removing the MMP-2 inhibitory activity from the ECM. In addition, MT1-MMP activates MMP-2, which can then freely exert its proteolytic activity on the ECM (Higashi and Miyazaki, 2003b). Since autodegradation is a highly critical step in the regulation of MT1-MMP activity (see Section 1.4.10), excess expression of substrates such as APP or syndecan-1 may interfere with the autodegradation and consequently augment the MT1-MMP activation of MMP-2, further promoting MMP-2 proteolytic action.

However, MT1-MMP is not the only MT-MMP expressed in the brain as MT3-MMP expression levels are highest in the brain and localized in microglial cells in all brain tissues (Takino *et al.*, 1995; Yoshiyama *et al.*, 1998), whereas MT5-MMP is also present in all brain tissues, but most strongly expressed in cerebellum (Sekine-Aizawa *et al.*, 2001). After cotransfection, MT3-MMP and MT5-MMP, but not MT2-, MT4- and MT6-MMP, indeed caused cleavage and shedding of the APP ectodomain with a fragmentation pattern almost identical to that of MT1-MMP. MT3-MMP induced shedding of APP most efficiently, followed by MT1-MMP and MT5-MMP (Ahmad *et al.*, 2006). MT3-MMP cleaves the APP ectodomain at multiple sites that are Ala463-Met464, Asn579-Met580, His622-Ser623 and His685-Gln686, which is within the Aβ sequence (Figure 17), indicating that MT3-MMP could be an α-secretase. Since the APP fragmentation seen after cleavage by MT1-MMP, MT3-MMP and MT5-MMP was almost identical, MT1-MMP and MT5-MMP may cleave APP at the same sites as MT3-MMP (Ahmad *et al.*, 2006). As cleavage of APP by an α-secretase destroys the Aβ-sequence, shedding of APP by MT1-, MT3- and MT5-MMP might mitigate pathologic Aβ formation and accumulation.

Although familial Alzheimer's disease appears to be caused by Aβ overproduction, sporadic Alzheimer's disease (the most prevalent form) may be caused by impaired Aβ clearance. Aβ40 is a potent inducer of MMP-2, MMP-3 and MMP-9 expression *in vitro* (Deb and Gottschall, 1996). Furthermore, MMP-2 is capable of degrading Aβ40 and Aβ42 *in vitro* at the Lys16-Leu17, Leu34-Met35 and Met35-Val36 peptide bonds, although this does not lead to a complete clearing of Aβ (Roher *et al.*, 1994). Treatment of cells overexpressing APP with the metal ligand clioquinol and Cu^{2+} or Zn^{2+} resulted in an ~85 to 90% reduction of Aβ40 and Aβ42. This loss of Aβ was not caused by altered APP processing, but was mediated through upregulation of MMP-2 and MMP-3. Inhibitors of MMP-2 and MMP-3 indeed abrogated the loss of Aβ40 caused by clioquinon and Cu^{2+} (White *et al.*, 2006). However, further investigation will be necessary to determine wether MMP-2- and MMP-3-mediated cleavage is a rate-limiting step in the rapid clearance of secreted Aβ *in vitro* as well as *in vivo*. Interestingly, the human hippocampus of Alzheimer's disease patients shows increased levels of pro-MMP-9 near the amyloid plaques (Backstrom *et al.*, 1996) and the same increase was reported in amyloid-positive beagle brains (Lim *et al.*, 1997). In addition, levels of circulating MMP-9 are significantly elevated in the plasma of Alzheimer's disease patients compared

to controls (Lorenzl *et al.*, 2003). MMP-9 processes soluble Aβ40 and Aβ42 *in vitro* at multiple sites that are Lys16-Leu17; Phe20-Ala21; Asp23-Val24; Ala30-Ile31; Gly33-Leu34; Leu34-Met35 and Gly37-Gly38 (Figure 17) (Backstrom *et al.*, 1996; Yan *et al.*, 2006) However, in contrast to other proteases that also degrade sAβ such as endothelin-converting enzyme, insulin-degrading enzyme, and neprylisin, MMP-9 was the only one to degrade Aβ fibrils *in vitro*. In addition, amorphous structures suggestive of decomposed fibrils were observed after incubation with MMP-9. Fibril disruption by MMP-9 produced Aβ fragment with molecular weights corresponding to Aβ20 and Aβ30, suggesting that proteolytic cleavage at Phe20-Ala21 and Ala30-Ile31 may be important for fibril degradation. MMP-9 was also shown to degrade compact amyloid plaques in brain sections from aged APP/presenilin(PS)1 mice. Fibrillar Aβ in compact plaques is believed to be extremely resistant to degradation and clearance, but growing evidence suggests that endogenous mechanisms for plaque clearance exist, as amyloid plaque size in brains of AD patients does not invariably increase with disease duration and in aged APP/swedisch mutation(sw) mice (overpoducing APPβ), some isolated plaques even decrease in size over time. Furthermore, MMP-9 is expressed in astrocytes surrounding plaques in the brains of aged APP/PS1 mice, and its activity is specifically detected in compact plaques (Yan *et al.*, 2006). Thus, MMP-9 may contribute to clearance of plaques from amyloid-laden brains. *In vivo*, significant increases in the steady-state levels of sAβ were found in the brains of MMP-2 and MMP-9 deficient mice compared with wild-type controls. In addition, pharmacological inhibition of the MMPs with the broad-spectrum inhibitor GM6001 increased brain interstitial sAβ levels and half-life in APP/sw mice. Under these disease-free steady-state conditions, gene deletion of *mmp-2* appeared to have a greater effect on brain Aβ levels compared with *mmp-9* gene deletion (Yin *et al.*, 2006). However, it is possible that under pathological conditions, MMP-9 may play a greater role in Aβ clearance, as its expression was shown to be increased in astrocytes surrounding amyloid plaques, as mentioned above. In summary, a better understanding of the role of MMPs in the clearance of extracellular sAβ and in the degradation of Aβ fibrils in amyloid plaques, may point to alternative therapeutical approaches that reduce plaque formation and slow down the disease cascade in early stages.

4.2 FasL

The growing evidence that Aβ accumulation is a determining factor in Alzheimer's disease makes it important to elucidate the mechanism by which Aβ induces neuronal cell death. It was shown that Aβ induces neuronal cell apoptosis and that inhibition of FasL and Fas function led to a decrease in Aβ-induced neuronal apoptosis (Morishima *et al.*, 2001). As discussed before, MMP-7 releases sFasL, which is a less potent apoptosis-inducer than its membrane-bound precursor (see Section 1.2.1). Whereas treatment of neuronal cell cultures with sAβ alone increased the appearance of morphologically apoptotic cells and nuclei, addition of MMP-7 increased sFasL shedding in the culture media and compeletely protected neuron cultures from Aβ toxicity (Ethell *et al.*, 2002). Hence, FasL shedding by MMP-7 is another important MMP-mediated proteolytic process at the cell surface. Factors that affect this shedding process may play a role in the progression of Alzheimer's disease and may provide an avenue for therapeutic intervention.

5. MEMBRANE-ASSOCIATED PROTEOLYSIS IN REPRODUCTIVE ENDOCRINOLOGY

5.1 LRP

Similar to the intense tissue remodelling associated with cancer progression, the human endometrium undergoes cyclic growth and tissue remodelling throughout the reproductive life of women, with the succession of proliferative, secretory, and menstrual phases. In this rapidly changing environment, successful embryo implantation requires a tight control of the integrity of the endometrial tissue at the early and mid-secretory phase. This implies a strict control of u-PA and MMPs, which are repressed by progesterone. As discussed before (see Section 1.4.9), LRP-mediated internalization of proteases is an important regulation mechanism for proteolytic activity at the cell surface. Interestingly, the expression of LRP mRNA also varies during the menstrual cycle, with a significant increase from the proliferative to the secretory phase, when progesterone concentration is the highest, possibly promoting further repression of MMP acitivity (Emonard *et al.*, 2005). Furthermore, LRP is an important molecule during embryonic development (Herz *et al.*, 1992). LRP is highly expressed in the placenta and increased levels of soluble

LRP (sLRP) were detected in cord blood from healthy pregnancy. While sLRP release from BeWo choriocarcinoma cells is prevented by the hydroxamic acid compound, INH-38SS-PI (Quinn *et al.*, 1999), the cleavage is not mediated by MT-MMPs as it involves endoproteolysis of the membrane-spanning β-chain (LRP-85), whereas MT-MMPs cleave in the COOH-terminal part of the α-chain (LRP-515) (see Section 1.4.9 and Figure 10) (Rozanov *et al.*, 2004a). In addition, the metalloproteinase responsible for the cleavage is not induced by PMA. The BeWo cell line, which is derived from a human gestational choriocarcinoma, displays morphological and functional characteristics of both invasive cytotrophoblast and syncytiotrophoblast. The release of biologically active sLRP by trophoblast cells may have implications regarding the biology of the placenta. The pattern of LRP expression in the placenta is consistent with roles for the receptor in trophoblast invasion, a tightly regulated process that involves the coordinated activation of proteases and the transport of cholesterol. In term placenta, LRP is expressed in the syncytium, which comprises the maternal-fetal interface. Increased levels of sLRP in cord blood may reflect cellular dysfunction and increased metalloproteinase activity at this important interface (Quinn *et al.*, 1999). Hence, further investigation into the release of sLRP and its interaction with cellular LRP might be of interest to predict or monitor complications during embryonic development.

5.2 HB-EGF

Transmembrane and soluble HB-EGF have a crucial role in some events of female reproduction biology. Firstly, in blastocyst implantation, mHB-EGF, but not sHB-EGF, has been shown to promote adhesion between the blastocyst and the uterine wall, whereas sHB-EGF has been reported to induce an increase in blastocyst number and an increase in the rate of blastocyst zona pellucida hatching (Raab and Klagsbrun, 1997; Iwamoto and Mekada, 2000). Furthermore, cleavage of mHB-EGF by MMP-7 and the subsequent activation of ErbB4/HER4 by sHB-EGF appear to play a role in the regulation of postpartum uterine and lactating mammary gland involution and maintenance of lactation. CD44 plays a key role in this TMPS (by assembling MMP-7, mHB-EGF and ErbB4 in a cell surface complex (see Section 1.4.7 and Figure 9) (Yu *et al.*, 2002). Moreover, release of sHB-EGF by MMP-2 and MMP-9 is a crucial step in neuroendocrine regulation by the gonadotropin-releasing hormone (GnRH) which regulates the synthesis and secretion of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the pituitary gland. Inhibition of MMP-2 and MMP-9 by selective inhibitors (Ro28–2653) or by specific ribozymes indeed blocks transactivation of the EGFR, induced by the binding of GnRH to its receptor (a GPCR) (Roelle *et al.*, 2003; Shah *et al.*, 2004). MT1-MMP comes out as an additional player in this TMPS through the activation of MMP-2 (Shah and Catt, 2004c). Similar to GnRH, the steroid hormone estradiol (E2) also binds GPCRs, an alternative type of estrogen receptors, and rapidly stimulates signal transduction through transactivation of the EGFR. In this TMPS sHB-EGF release is also mediated by MMP-2 and MMP-9 (Razandi *et al.*, 2003). Thus, ectodomain cleavage of HB-EGF by MMPs is a crucial event in the regulation of some neuroendocrine and reproductive functions.

5.3 Occludin

In the low-resistance human vaginal-cervical epithelia, occludin is present in two main forms: the full-length 65 kDa wild-type isoform, and a truncated 50 kDa form. A shift from 65 kDa to 50 kDa can be induced by treatment with estrogen and is associated with a reversible decrease in the resistance of the tight junctions. MMPs were already described as being responsible for

TABLE 3 **pro-TNF-α cleavage sites**

Protease	Cleavage site(s)	Test system	References
TACE	Ala76-Val77	*In vitro* and in vivo	3
MMP-1	Ala74-Gln75 and Ala76-Val77	*In vitro*	1,2,3
MMP-2	Not defined	*In vitro*	1,2
MMP-3	Not defined	*In vitro*	1,2,3
MMP-7	Ala76-Val77	*In vitro* and ex vivo	1,2,3,6
MMP-9	Ala74-Gln75	*In vitro*	1,2,3
MMP-12	Ala74-Gln75 and Ala76-Val77	*In vitro* and in vivo	7,8
MT1-MMP	Ala76-Val77 and before Leu113	*In vitro*	4
MT2-MMP	Ala76-Val77 and before Leu113	*In vitro*	4
MT4-MMP	Ala74-Gln75	*In vitro*	5

1, (Gearing *et al.*, 1994); 2, (Gearing *et al.*, 1995); 3, (Mohan *et al.*, 2002); 4, (d'Ortho *et al.*, 1997);
5, (English *et al.*, 2000); 6, (Haro *et al.*, 2000b); 7, (Chandler *et al.*, 1996); 8, (Churg *et al.*, 2003).

TABLE 4 **Potential membrane-bound MMP and MP substrates**

Substrate[1]	CD	Inhibitor	Specificity[2]	Reference
ACE	CD143	EDTA, O-phen	MP	(Parvathy *et al.*, 1997)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
		TAPI-2	Zn^{2+}-dependent MP	
		TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Schwager *et al.*, 1998; Schwager *et al.*, 1999)
ANF-R	/	EDTA	MP	(Abe and Misono, 1992)
AR	/	Batimastat (BB-94)	Broad spectrum MMP and TACE	(Vecchi *et al.*, 1998; Brown *et al.*, 1998)
C1qRp	CD93	O-phen	MP	(Bohlson *et al.*, 2005)
		Not inhibited by: TAPI-1, TAPI-2 and the broad spectrum MMP inhibitors Ro-31–9790, Ro-32–7315		
CD27	CD27	GI5402	MMP-1,-3,-9,-13 and TACE	(Dekkers *et al.*, 2000)
CD30	CD30	EDTA, O-phen	MP	(Parvathy *et al.*, 1997) (Hansen *et al.*, 1995; Hooper *et al.*, 1997)
		BB2116	MMP-3, -7, -2, -9	
CD40L	CD154	EDTA	MP	(Furman *et al.*, 2004; Otterdal *et al.*, 2004)
		Ilomastat (GM6001)	Broad spectrum MMP and TACE	
		KB8301	Zn^{2+}-dependent MP	(Kato *et al.*, 1999; Jin *et al.*, 2001)
		Batimastat (BB-94)	Broad spectrum and TACE	
c-Met	/	TIMP-3	Broad spectrum MMP ADAM-10, -12, -17, -19 ADAMTS-4 and -5	(Nath *et al.*, 2001)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
		Not inhibited by TIMP-1 and TIMP-2		
CXCL16	/	Ilomastat (GM6001)	Broad spectrum MMP and TACE	(Hara *et al.*, 2006)
		MMP inhibitor III*	Broad spectrum MMP	
Desmocollin-3	/	FN-439 (MMP inhibitor I*)	MMP-1, MMP-8 > MMP-9 > MMP-3	(Weiske *et al.*, 2001)
		Not inhibited by TAPI(-1)		
Desmoglein-1	/	TAPI-0	Collagenase, gelatinase, TACE	(Dusek *et al.*, 2006)
		FN-439 (MMP inhibitor I*)	MMP-1, MMP-8 > MMP-9 > MMP-3	
		Ilomastat (GM6001)	Broad spectrum MMP and TACE	
Desmoglein-3	/	FN-439 (MMP inhibitor I*)	MMP-1, MMP-8 > MMP-9 > MMP-3	(Weiske *et al.*, 2001)
		Not inhibited by TAPI(-1)		
EGF	/	EDTA, EGTA	MP	(Dempsey *et al.*, 1997)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
FcγRIII	CD16	GI5402	MMP-1,-3,-9,-13 and TACE	(Dekkers *et al.*, 2000)
		O-phen	MP	(Bazil and Strominger, 1994) (Galon *et al.*, 1998)
		RU36156	MMP-8, MMP-9 and TACE	(Galon *et al.*, 1998; Mota *et al.*, 2004)

TABLE 4 **Potential membrane-bound MMP and MP substrates *(Continued)***

Substrate[1]	CD	Inhibitor	Specificity[2]	Reference
FcεRII	CD23	O-phen	MP	(Bailey *et al.*, 1998)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
Folate-R	/	EDTA, O-phen	MP	(Elwood *et al.*, 1991)
GHR	/	BB-3103	Broad spectrum MMP and TACE	(Amit *et al.*, 2001)
		Ro31-9790	Broad spectrum MMP and TACE	
GM-CSF-Rα	CD116	Batimastat (BB-94)	Broad spectrum MMP and TACE	(Prevost *et al.*, 2002)
GP VI	/	TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Stephens *et al.*, 2005)
		Ilomastat (GM6001)	Broad spectrum MMP and TACE	
HER2	CD340	TIMP-1	Soluble MMPs	(Codony-Servat *et al.*, 1999; Molina *et al.*, 2001)
		TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
HER4	/	Batimastat (BB-94)	Broad spectrum MMP and TACE	(Vecchi *et al.*, 1998)
IL-1RII	CD121b	Batimastat (BB-94)	Broad spectrum MMP and TACE	(Penton-Rol *et al.*, 1999)
IL-4R	CD124	O-phen	MP	(Jung *et al.*, 1999)
IL-6Rα	CD126	TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Mullberg *et al.*, 1995)
		RU36156	MMP-8, MMP-9 and TACE	(Gallea-Robache *et al.*, 1997)
		TAPI-2	Zn^{2+}-dependent MP	(Arribas *et al.*, 1996)
		O-phen	MP	
LDL-R	/	EDTA, EGTA	MP	(Begg *et al.*, 2004)
		TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	
Leukosialin	CD43	O-phen	MP	(Bazil and Strominger, 1994)
LIGHT	CD258	EDTA	MP	(Otterdal *et al.*, 2006)
		Ilomastat (GM6001)	Broad spectrum MMP and TACE	
M-CSF	/	RU36156	MMP-8, MMP-9 and TACE	(Gallea-Robache *et al.*, 1997)
Megalin	/	MMP inhibitor III*	Broad spectrum MMP	(Zou *et al.*, 2004)
		TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	
		TAPI-2	Zn^{2+}-dependent MP	
MICA	/	Batimastat-derivative	Zn^{2+}-dependent MP	(Salih *et al.*, 2002)
MICB	/	Batimastat-derivative	Zn^{2+}-dependent MP	(Salih *et al.*, 2006)
MMR-1	CD206	BB2116	MMP-3, -7, -2, -9	(Martinez-Pomares *et al.*, 1998)
N-CAM L1	CD171	BB-3103	Broad spectrum MMP and TACE	(Mechtersheimer *et al.*, 2001)
		Ro-31-9790	Broad spectrum MMP and TACE	
		TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Beer *et al.*, 1999; Gutwein *et al.*, 2005)
Nectin-1α	CD111	O-phen	MP	(Tanaka *et al.*, 2002)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
		KB-R7785	MMP-1,-2,-3,-9,-14 TACE, ADAM12	
Netrin-1	/	Ilomastat (GM6001)	Broad spectrum MMP and TACE	(Galko and Tessier-Lavigne, 2000)
		TAPI-2	Zn^{2+}-dependent MP	

(Continued on next page)

TABLE 4 **Potential membrane-bound MMP and MP substrates *(Continued)***

Substrate[1]	CD	Inhibitor	Specificity[2]	Reference
NGF-R	/	EDTA, O-phen	MP	(Diaz-Rodriguez *et al.*, 1999)
		TAPI-2	Zn^{2+}-dependent MP	
NKR BY55	CD160	O-phen	MP	(Giustiniani *et al.*, 2007)
PECAM-1	CD31	Ilomastat (GM6001)	Broad spectrum MMP and TACE	(Ilan *et al.*, 2001)
		MMP-2/MMP-9 Inhibitor I*	MMP-2, MMP-9	
		NNGH (MMP-3 Inhibitor II*)	MMP-3	
		MMP-8 Inhibitor*	MMP-8	
PSGL-1	CD162	EDTA Not inhibited by: O-phen, Batimastat and Marimastat	MP	(Davenpeck *et al.*, 2000)
Sialophorin	CD43	O-phen	MP	(Bazil and Strominger, 1994)
SorLA	/	BB-3103	Broad spectrum MMP and TACE	(Hampe *et al.*, 2000)
SRCR M130	CD163	TAPI-0	MMP-1, MMP-9, TACE	(Hintz *et al.*, 2002)
Syndecan-3	/	BB-3103	Broad spectrum MMP and TACE	(Asundi *et al.*, 2003)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	
pro-TGF-α	/	RU36156	MMP-8, MMP-9 and TACE	(Gallea-Robache *et al.*, 1997)
		TAPI-2	Zn^{2+}-dependent MP	(Arribas *et al.*, 1996)
		TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Shao *et al.*, 2004)
		O-phen	MP	(Arribas *et al.*, 1996)
		Batimastat (BB-94)	Broad spectrum MMP and TACE	(Merlos-Suarez *et al.*, 2001)
		Ilomastat (GM6001)	Broad spectrum MMP and TACE	(Yoshisue and Hasegawa, 2004; Shao *et al.*, 2004)
		Marimastat (BB-2516)	Broad spectrum MMP and TACE	
Tie-1	/	EGTA	Ca^{2+}-dependent MP	(Yabkowitz *et al.*, 1999)
		BB-24 Not inhibited by: TIMP-2	Zn^{2+}-dependent MP	
TNF-R55/60	CD120a	TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Mullberg *et al.*, 1995)
		RU36156	MMP-8, MMP-9 and TACE	(Gallea-Robache *et al.*, 1997)
		BB-2275	Zn^{2+}-dependent MP	(Williams *et al.*, 1996)
TNF-R75/80	CD120b	TAPI(-1)	Collagenase, gelatinase, ADAM-10, TACE	(Crowe *et al.*, 1995)

TABLE 4 **Potential membrane-bound MMP and MP substrates *(Continued)***

Substrate[1]	CD	Inhibitor	Specificity[2]	Reference
		RU36156	MMP-8, MMP-9 and TACE	(Gallea-Robache *et al.*, 1997)
TSHR	/	BB2116	MMP-3, -7, -2, -9	(Couet *et al.*, 1996; Misrahi and Milgrom, 1997; de Bernard *et al.*, 1999)
		Not inhibited by TIMP-1, TIMP-2		(de Bernard *et al.*, 1999)
ULBP2	/	Batimastat (BB-94)	Broad spectrum MMP and TACE	(Waldhauer and Steinle, 2006)
		MMP inhibitor III*	Broad spectrum	(Waldhauer and Steinle, 2006)
VCAM-1	CD106	Marimastat (BB-2516)	Broad spectrum MMP and TACE	(Hummel *et al.*, 2001)
VPR V2	/	O-phen	MP	(Kojro and Fahrenholz, 1995)

[1]Substrate acronyms: ACE, angiotensin-converting enzyme; ANF-R, atrial natriuretic factor receptor; C1qRp, complement component 1 q subcomponent receptor 1; AR, amphiregulin; c-Met, met proto-oncogene TKR; CXCL16, transmembrane chemokine CXCL16; FcγRIII, low affinity Ig γ Fc receptor III; FcεRII, low affinity Ig ε Fc receptor II; GHR, growth hormone receptor; GM-CSF-Rα, granulocyte-macrophage colony-stimulating factor receptor α chain; GP VI, glycoprotein VI; HER4, Tyrosine kinase-type cell surface receptor HER4; IL -R, interleukin receptor; LDL-R, low-density lipoprotein receptor; LIGHT, homologous to lymphotoxins, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes; M-CSF, macrophage-colony stimulating factor; MICA/B, MHC class I chain-related gene A/B; MMR-1, Macrophage mannose receptor-1; N-CAM L1, neural cell adhesion molecule L1; NGF-R, nerve growth factor receptor; NKR BY55, NK cell receptor BY55; NNGH, N-Isobutyl-N-(4-methoxyphenylsulfonyl)-glycylhydroxamic acid; O-phen, 1,10-phenantroline; PECAM-1, platelet endothelial cell adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand-1; -R, receptor; SorLA, sorting protein-related receptor containing LDL-R class A repeats; SRCR M130, scavenger receptor cysteine-rich type 1 protein M130; TAPI, TNF-α Protease Inhibitor; Tie-1, tyrosine kinase receptor tie-1; TSHR, thyrotropin receptor; ULBP2, UL16-binding protein 2; VCAM-1, Vascular cell adhesion molecule-1; VPR V2, V2 vasopressin receptor. Additional acronyms can be found in the list of abbreviations.

[2]Inhibitor specificities: for detailed information, the reader is referred to the reviews (Kontogiorgis *et al.*, 2005), (Sang *et al.*, 2006) and (Whittaker *et al.*, 1999).

*Additional information on the inhibitor structure and characteristics can be found at http://www.merckbiosciences.co.uk/home.asp.

occludin degradation and increased permeabilization of endothelial cell and epithelial sheets (see Section 2.2 and Figures 14 to 16). Treatment of human normal vaginal-cervical cells with natural doses of 17β-estradiol upregulated activation of MMP-7 intracellularly, in the Golgi network, and augmented secretion of activated MMP-7. This MMP-7 was shown to be necessary and sufficient to produce estrogen-mediated decrease of tight junctional resistance and extracellular modulation of occludin. Hence, MMP-7-mediated proteolysis of occludin might be an essential step in the estrogen modulation of paracellular permeability *in vivo* (Gorodeski, 2007).

6. POTENTIAL CELL SURFACE-ASSOCIATED MMP SUBSTRATES

Processing of various membrane-bound molecules is inhibited by metalloproteinase-specific inhibitors, although the involved metalloproteinase has not been identified yet. These substrates have been grouped in Table 4 with the protease inhibitor profiles and the inhibitor specificities. As discussed before, the inhibition pattern of a proteolytic process yields information on the involved enzyme(s) or protease class(es). Ectodomain shedding of a whole array of membrane-bound molecules is inhibited by the non-specific cation chelators EDTA, EGTA, and 1,10-phenantroline (*e.g.*, ANF-R, IL-4R, leukosialin). It is relevant to notice that the affinity of EDTA is higher for light metal cations, whereas 1,10-phentroline favours the binding of heavy metal cations. In addition, EGTA preferentially chelates Ca^{2+} ions, thus inhibiting Ca^{2+}-dependent proteases. Broad-spectrum MMP inhibitors, such as most hydroxamates, also inhibit TACE and other ADAMs. Hence, a shedding process inhibited by batimastat, marimastat, GM6001, *etc.* is not necessarily mediated by an MMP and requires further investigation of the protease(s) in charge (*e.g.*,desmoglein-1, HER4, megalin, VCAM-1). Along this line, inhibition by TIMPs does not guarantee that the cleaved molecule is an MMP substrate, as

TABLE 5 Cell surface MMP substrates without transmembrane domain

Substrate	Type of cell membrane association	Cleaving MMP	Reference
ADAMTS-4	Association with chondroitin and heparan sulfate chains on syndecan-1	MMP-9, MMP-13	(Tortorella *et al.*, 2005)
		MT4-MMP	(Gao *et al.*, 2002; Gao *et al.*, 2004)
C3b	Amide and ester bonds with cell surface molecules	MT1-MMP	(Rozanov *et al.*, 2004b)
C4b	Amide and ester bonds with cell surface molecules	MT1-MMP	(Rozanov *et al.*, 2004b)
Galectin-3	Binding to IgE and multiple cell surface molecules	MMP-2, MMP-9	(Ochieng *et al.*, 1994; Ochieng *et al.*, 1998)
Galectin-9	Binding to cell surface molecules	ND MMP	(Chabot *et al.*, 2002)
KiSS protein	Complex with pro-MMP-2,-9	MMP-2,-9	
		MT1-, MT3-, MT5-MMP	(Takino *et al.*, 2003)
pro-TGF-β	ECM association	MMP-2,-3,-9	
		MT1-MMP	(Yu and Stamenkovic, 2000)
tTG	Binding to integrins	MT1-MMP, MMP-2	
		MT2-, MT3-MMP	(Belkin *et al.*, 2001; Belkin *et al.*, 2004)
ApoE	Complex with pro-MMP-2	MT1-MMP, MMP-7	(Aoki *et al.*, 2005)
gC1qR	Binding to the hemopexin domain of MT1-MMP	MT1-MMP	(Rozanov *et al.*, 2002)

apoE, apolipoprotein E; **C3b**, complement component 3b; **C4b**, complement component 4b; **gC1qR**, receptor of complement component 1q.

TIMP-3 inhibits various ADAMs and some proteases of the ADAMTS family (see Table 1). However, some conclusions can be drawn from a TIMP inhibition pattern. As TIMP-2 and TIMP-4 only inhibit MMPs, inhibition or absence of inhibition by these TIMPs, respectively, identifies or rejects MMPs as the operating sheddases (*e.g.*,c-Met, tie-1, TSHR). A metalloprotease inhibited by TIMP-1 is likely to be an (MT-)MMP, as ADAM-10 is the only non-MMP to be inhibited (*e.g.*,HER2). Thus, as long as few specific MMP inhibitors exist, particular care must be taken when drawing conclusions about the identity of the sheddase. Additional cell surface molecules that are modulated by ectodomain shedding have been described (Hooper *et al.*, 1997; Blobel, 2000; Dello and Rovida, 2002; Arribas and Borroto, 2002; Garton *et al.*, 2006).

Finally, some MMP substrates without transmembrane domain are located at the cell surface by receptor binding, by a membrane vesicle, a membrane-bound proteoglycan or another transmembrane protease. For that reason, these have not all been discussed in detail in the above survey. Some examples of such cell surface-associated proteins are galectin-3 and -9 (Ochieng *et al.*, 1994; Ochieng *et al.*, 1998; Chabot *et al.*, 2002); tTG (Belkin *et al.*, 2001; Belkin *et al.*, 2004); the KiSS protein/metastin (Takino *et al.*, 2003); pro-TGF-β (Yu and Stamenkovic, 2000); ADAMTS-4 (Gao *et al.*, 2004); the complement components C3b and C4b (Rozanov *et al.*, 2004b); apolipoprotein E (Aoki *et al.*, 2005) and gC1qR, the receptor of complement component 1q (Rozanov *et al.*, 2002) (Table 5).

CONCLUSION

Due to the rapid development of innovative biochemical techniques and the expanding use of transgenic and knockout animals, it became obvious that the action radius of MMPs is not restricted to massive ECM destruction in physiological tissue remodelling and pathological tissue degradation. Identification of specific matrix– as well as non-matrix–components as MMP substrates showed that MMPs also play significant roles in highly complex processes such as the regulation of cellular behavior, cell-cell communication and tumor progression (McCawley and Matrisian, 2001). An extended variety of bioactive molecules is modified by MMPs in particular physiopathological processes (Sternlicht and Werb, 2001).

The above survey points out that MMPs do not only cleave multiple soluble substrates, but also process a

whole array of membrane-bound proteins. These proteolytic events on the cell surface may have extremely diverse biological implications (see Table 2), ranging from maturation (*e.g.*, pro-α integrin subunits), activation (*e.g.*, PAR1) and potentiation (HER2) of a cell surface component, to its inactivation (*e.g.*, syndecan-1) or even its degradation (*e.g.*, NG2 proteoglycan and β-dystroglycan). Besides the regulation of cell-bound activity, ectodomain proteolysis of substrates may also be required for the diffusion of the reaction product(s) into the extracellular environment to facilitate receptor activation on adjacent or even more distal cells (*e.g.*, pro-TNF-α and RANKL). In addition, a liberated receptor fragment might as well possess inhibitory power by acting as a soluble decoy receptor that binds soluble ligands, preventing their interaction with the cell-bound, signal-transducing receptor (*e.g.*, FGFR-1).

Through these distinct effects on the biological activity of the substrates, proteolysis of cell surface proteins by MMPs has a major impact on a multitude of physiological functions, as well as on onset and evolution of many diseases. In addition, MMP activity itself can be directly regulated by proteolysis of cell surface proteins. For instance, MMPs cleave their membrane-bound inducer, EMMPRIN, allowing the active fragment to induce MMP activity in adjacent cells or in more distal tissues. Conversely, they also modulate their own internalization and degradation by degrading their scavenger receptor, LRP, assuring sustained MMP activity. Finally, MMPs can terminate their own activity by autocatalytic proteolysis.

Inhibition of MMPs seems to be the ideal solution in many pathologies. However, the enthusiasm generated by a large number of *in vitro* and *in vivo* studies has been dramatically mitigated in recent years by the failure of MMP inhibitors to block tumor progression in clinical trials (Coussens *et al.*, 2002). This fiasco could be explained partly by the fact that inhibitors were administered only to late-stage cancer patients, whereas proteases are often involved in early stages of tumor progression. In addition, the lack of selectivity and specificity of inhibitors is a problem, as tumor invasion and metastasis require the concerted action of particular MMPs. However, as stated by Del Rosso and coworkers (2005), our understanding of protease-environment interactions is far from exhaustive. The initial, naïve view of proteases acting in the soluble phase has evolved into a kaleidoscope of images in which proteolytic reactions in tissue remodelling mostly occur at, and are modulated by, the cell surface. As a consequence, insight in the modification of cell surface proteins by MMPs and the associated implications, is essential to elucidate the cross-talk between proteases at the cell surface and with the extracellular environment. In conclusion, starting with the exploration of the cell surface could become a prerequisite in the succesful development of new MMP inhibitors and innovative therapeutic approaches for cancer and inflammatory diseases.

ABBREVIATIONS

Aβ, β-amyloid protein; **ACE,** angiotensin-converting enzyme; **ADAM,** a disentegrin and metalloproteinase; **ADAMTS,** a disentegrin and metalloproteinase with thrombospondin-like motif; **ANF-R,** atrial natriuretic factor receptor; **AP,** alkaline phosphatise; **ApoE,** apolipoprotein E; **APP,** amyloid precursor protein; **AR,** amphiregulin; **ARF,** acute renal failure; **BBB,** blood-brain barrier; **BP,** bullous pemphigoid; **BP-180,** BP antigen-2; **BRB,** blood-retinal barrier; **C1qRp,** complement component 1 q subcomponent receptor 1; **C3b/4b,** complement component 3b/4b; **CD,** cluster of differentiation of human (glyco)proteins; **CD44ICD,** intracellular domain of CD44; **c-Met**, met proto-oncogene TKR; **CNS,** central nervous system; **CTL,** cytotoxic T cell; **EAE,** experimental autoimmune encephalomyelitis; **E-cadherin,** epithelial cadherin; **ECM,** extracellular matrix; **EDTA,** ethylenediaminetetraacetic acid; **EGF(R),** epidermal growth factor (receptor); **EGTA,** ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid; **EMMPRIN,** extracellular matrix metalloproteinase inducer; **FasL,** Fas ligand; **FcγRIII,** low affinity Ig γ Fc receptor III; **FcεRII,** low affinity Ig ε Fc receptor II; **FGF,** fibroblast growth factor; **FGFR-1,** FGF receptor-1; **gC1qR,** receptor of complement component 1q; **GHR,** growth hormone receptor; **GM-CSF-Rα,** granulocyte-macrophage colony-stimulating factor receptor α chain; **GnRH,** gonadotropin-releasing hormone; **GP VI,** glycoprotein VI; **GPCR,** G protein-coupled receptor; **GPI,** glycosyl phosphatidylinositol; **HB-EGF,** heparin-binding epidermal growth factor-like growth factor; **HER2/4,** tyrosine kinase-type cell surface receptor HER2/4; **ICAM-1,** intercellular adhesion molecule-1; **ICE,** IL-1β-converting enzyme; **Ig,** immunoglobulin; **IL- (R),** interleukin- (receptor); **IL-1β,** interleukin-1β; **KitL,** Kit ligand; **KS,** keratoconjunctivitis sicca; **LDL(-R),** low density lipoprotein (receptor); **LIGHT,** homologous to

lymphotoxins, exhibits **i**nducible expression, and competes with herpes simplex virus **g**lycoprotein D for **h**erpes virus entry mediator, a receptor expressed by **T** lymphocytes; **LPS,** lipopolysaccharide; **LR,** 34/67 kDa laminin receptor; **LRP,** low-density lipoprotein receptor-related protein; **L-selectin,** leukocyte-selectin; **m-,** membrane-bound; **MBP,** myelin basic protein; **M-CSF,** macrophage-colony stimulating factor; **MEVC,** microvascular endothelial cell; **MICA/B,** MHC class I chain-related gene A/B; **MMP,** matrix metalloproteinase; **MMR-1,** macrophage mannose receptor-1; **MP,** metalloproteinase; **MT-MMP,** membrane-type MMP; **MUC1,** mucin-1; **N-cadherin,** neuronal cadherin; **N-CAM L1,** neural cell adhesion molecule L1; **NE,** neutrophil elastase; **NGF-R,** nerve growth factor receptor; **NK cell,** natural killer cell; **NKR BY55,** NK cell receptor BY55; **PAR1,** protease-activated receptor-1; **PC,** proprotein convertase; **PDGF,** platelet-derived growth factor; **PDR,** proliferative diabetic retinopathy; **PECAM-1,** platelet endothelial cell adhesion molecule-1; **PLAD,** preligand assembly domain; **PMA,** phorbol 12-myristate 13-acetate; **PSGL-1,** P-selectin glycoprotein ligand-1; **PVR,** proliferative vitreoretinopathy; **-R,** receptor; **RANK(L),** receptor activator of nuclear factor κB (ligand); **s-,** soluble; **SDF-1,** stromal cell-derived factor-1; **SorLA,** sorting protein-related receptor containing LDL-R class A repeats; **SP-D,** surfactant protein-D; **SRCR M130,** scavenger receptor cysteine-rich type 1 protein M130; **Ssc,** systemic sclerosis; **TACE,** TNF-α-converting enzyme; **TAPI,** TNF-α protease inhibitor; **TGF-α/β,** transforming growth factor-α/β; **Tie-1 ,** tyrosine kinase receptor tie-1; **TIL,** tumor infiltrating lymphocyte; **TIMP,** tissue inhibitor of metalloproteinases; **TKR,** tyrosine kinase receptor; **TMD,** transmembrane domain; **TMPS,** triple membrane-passing signal mechanism; **TNF-α,** tumor necrosis factor-α; **t-PA,** tissue-type plasminogen activator; **TSHR,** thyrotropin receptor; **tTG,** tissue transglutaminase; **ULBP2,** UL16-binding protein 2; **u-PA,** urokinase-type plasminogen activator; **uPAR,** urokinase-type plasminogen activator receptor; **VCAM-1,** vascular cell adhesion molecule-1; **VE-cadherin,** vascular endothelial-cadherin; **VEGF,** vascular endothelial growth factor; **VPR V2,** V2 vasopressin receptor; ***X,*** *Xenopus*.

ACKNOWLEDGMENTS

The present study was supported by The Fund for Scientific Research-Flanders (FWO-Vlaanderen), the Geconcerteerde OnderzoeksActies (GOA 2007–2011), The Rega Centre of Excellence (COE 05/015), the Charcot Foundation and the Belgian Foundation against Cancer. PVDS is a postdoctoral fellow of the FWO-Vlaanderen.

REFERENCES

Abdel-Ghany, M., Cheng, H.C., Elble, R.C., and Pauli, B.U. 2001. The breast cancer beta 4 integrin and endothelial human CLCA2 mediate lung metastasis. *J Biol Chem* 276:25438–25446.

Abe, T. and Misono, K.S. 1992. Proteolytic cleavage of atrial natriuretic factor receptor in bovine adrenal membranes by endogenous metalloendopeptidase. Effects on guanylate cyclase activity and ligand-binding specificity. *Eur J Biochem* 209:717–724.

Agrawal, S., Anderson, P., Durbeej, M., van Rooijen, N., Ivars, F., Opdenakker, G., and Sorokin, L.M. 2006. Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. *J Exp Med* 203:1007–1019.

Ahmad, M., Takino, T., Miyamori, H., Yoshizaki, T., Furukawa, M., and Sato, H. 2006. Cleavage of amyloid-beta precursor protein (APP) by membrane-type matrix metalloproteinases. *J Biochem (Tokyo)*. 139:517–526.

Akin, C. and Metcalfe, D.D. 2004. The biology of Kit in disease and the application of pharmacogenetics. *J Allergy Clin Immunol* 114:13–19.

Alexander, J.S. and Elrod, J.W. 2002. Extracellular matrix, junctional integrity and matrix metalloproteinase interactions in endothelial permeability regulation. *J Anat* 200:561–574.

Amano, T., Fu, L., Marshak, A., Kwak, O., and Shi, Y.B. 2005a. Spatiotemporal regulation and cleavage by matrix metalloproteinase stromelysin-3 implicate a role for laminin receptor in intestinal remodeling during Xenopus laevis metamorphosis. *Dev Dyn* 234:190–200.

Amano, T., Kwak, O., Fu, L., Marshak, A., and Shi, Y.B. 2005b. The matrix metalloproteinase stromelysin-3 cleaves laminin receptor at two distinct sites between the transmembrane domain and laminin binding sequence within the extracellular domain. *Cell Res* 15:150–159.

Amit, T., Amit, T., Hochberg, Z., Yogev-Falach, M., Youdim, M.B., Youdim, M.B., and Barkey, R.J. 2001. Shedding of growth hormone-binding protein is inhibited by hydroxamic acid-based protease inhibitors: proposed mechanism of activation of growth hormone-binding protein secretase. *J Endocrinol* 169:397–407.

Andolfo, A., English, W.R., Resnati, M., Murphy, G., Blasi, F., and Sidenius, N. 2002. Metalloproteases cleave the urokinase-type plasminogen activator receptor in the D1-D2 linker region and expose epitopes not present in the intact soluble receptor. *Thromb Haemost* 88:298–306.

Andrei, C., Margiocco, P., Poggi, A., Lotti, L.V., Torrisi, M.R., and Rubartelli, A. 2004. Phospholipases C and A2 control lysosome-mediated IL-1 beta secretion: Implications for inflammatory processes. *Proc Natl Acad Sci USA* 101:9745–9750.

Annabi, B., Lachambre, M., Bousquet-Gagnon, N., Page, M., Gingras, D., and Beliveau, R. 2001. Localization of membrane-type 1 matrix metalloproteinase in caveolae membrane domains. *Biochem J* 353:547–553.

Antal-Szalmas, P. 2000. Evaluation of CD14 in host defence. *Eur J Clin Invest* 30:167–179.

Aoki, T., Sato, D., Li, Y., Takino, T., Miyamori, H., and Sato, H. 2005. Cleavage of apolipoprotein E by membrane-type matrix metalloproteinase-1 abrogates suppression of cell proliferation. *J Biochem (Tokyo)* 137:95–99.

Arribas, J. and Borroto, A. 2002. Protein ectodomain shedding. *Chem Rev* 102:4627–4638.

Arribas, J., Coodly, L., Vollmer, P., Kishimoto, T.K., Rose-John, S., and Massague, J. 1996. Diverse cell surface protein ectodomains are

shed by a system sensitive to metalloprotease inhibitors. *J Biol Chem* 271:11376–11382.
Asahi, M., Wang, X., Mori, T., Sumii, T., Jung, J.C., Moskowitz, M.A., Fini, M.E., and Lo, E.H. 2001. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. *J Neurosci* 21:7724–7732.
Asundi, V.K., Erdman, R., Stahl, R.C., and Carey, D.J. 2003. Matrix metalloproteinase-dependent shedding of syndecan-3, a transmembrane heparan sulfate proteoglycan, in Schwann cells. *J Neurosci Res* 73:593–602.
Baciu, P.C., Suleiman, E.A., Deryugina, E.I., and Strongin, A.Y. 2003. Membrane type-1 matrix metalloproteinase (MT1-MMP) processing of pro-alphav integrin regulates cross-talk between alphavbeta3 and alpha2beta1 integrins in breast carcinoma cells. *Exp Cell Res* 291:167–175.
Backstrom, J.R., Lim, G.P., Cullen, M.J., and Tokes, Z.A. 1996. Matrix metalloproteinase-9 (MMP-9) is synthesized in neurons of the human hippocampus and is capable of degrading the amyloid-beta peptide (1–40). *J Neurosci* 16:7910–7919.
Bailey, S., Bolognese, B., Buckle, D.R., Faller, A., Jackson, S., Louis-Flamberg, P., McCord, M., Mayer, R.J., Marshall, L.A., and Smith, D.G. 1998. Hydroxamate-based inhibitors of low affinity IgE receptor (CD23) processing. *Bioorg Med Chem Lett* 8: 23–28.
Baker, A.H., Edwards, D.R., and Murphy, G. 2002. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci* 115:3719–3727.
Bandyopadhyay, A., Wang, L., Lopez-Casillas, F., Mendoza, V., Yeh, I.T., and Sun, L. 2005. Systemic administration of a soluble betaglycan suppresses tumor growth, angiogenesis, and matrix metalloproteinase-9 expression in a human xenograft model of prostate cancer. *Prostate* 63:81–90.
Barresi, R. and Campbell, K.P. 2006. Dystroglycan: from biosynthesis to pathogenesis of human disease. *J Cell Sci* 119:199–207.
Basile, J.R., Barac, A., Zhu, T., Guan, K.L., and Gutkind, J.S. 2004. Class IV semaphorins promote angiogenesis by stimulating Rho-initiated pathways through plexin-B. *Cancer Res* 64:5212–5224.
Basile, J.R., Castilho, R.M., Williams, V.P., and Gutkind, J.S. 2006. Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis. *Proc Natl Acad Sci USA* 103:9017–9022.
Basile, J.R., Holmbeck, K., Bugge, T.H., and Gutkind, J.S. 2007. MT1-MMP controls tumor-induced angiogenesis through the release of semaphorin 4D. *J Biol Chem* 282:6899–6905.
Bazil, V. and Strominger, J.L. 1994. Metalloprotease and serine protease are involved in cleavage of CD43, CD44, and CD16 from stimulated human granulocytes. Induction of cleavage of L-selectin via CD16. *J Immunol* 152:1314–1322.
Bazzoni, G. 2006. Endothelial tight junctions: permeable barriers of the vessel wall. *Thromb Haemost* 95:36–42.
Beauvais, D.M. and Rapraeger, A.C. 2004. Syndecans in tumor cell adhesion and signaling. *Reprod Biol Endocrinol* 2:3.
Beer, S., Oleszewski, M., Gutwein, P., Geiger, C., and Altevogt, P. 1999. Metalloproteinase-mediated release of the ectodomain of L1 adhesion molecule. *J Cell Sci* 112:2667–2675.
Begg, M.J., Sturrock, E.D., and van der Westhuyzen, D.R. 2004. Soluble LDL-R are formed by cell surface cleavage in response to phorbol esters. *Eur J Biochem* 271:524–533.
Behzadian, M.A., Wang, X.L., Windsor, L.J., Ghaly, N., and Caldwell, R.B. 2001. TGF-beta increases retinal endothelial cell permeability by increasing MMP-9: possible role of glial cells in endothelial barrier function. *Invest Ophthalmol Vis Sci* 42:853–859.
Belkin, A.M., Akimov, S.S., Zaritskaya, L.S., Ratnikov, B.I., Deryugina, E.I., and Strongin, A.Y. 2001. Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion. *J Biol Chem* 276:18415–18422.
Belkin, A.M., Zemskov, E.A., Hang, J., Akimov, S.S., Sikora, S., and Strongin, A.Y. 2004. Cell-surface-associated tissue transglutaminase is a target of MMP-2 proteolysis. *Biochemistry* 43:11760–11769.
Bergers, G. and Benjamin, L.E. 2003. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3:401–410.
Berno, V., Porrini, D., Castiglioni, F., Campiglio, M., Casalini, P., Pupa, S.M., Balsari, A., Menard, S., and Tagliabue, E. 2005. The 67 kDa laminin receptor increases tumor aggressiveness by remodeling laminin-1. *Endocr Relat Cancer* 12:393–406.
Berthet, V., Rigot, V., Champion, S., Secchi, J., Fouchier, F., Marvaldi, J., and Luis, J. 2000. Role of endoproteolytic processing in the adhesive and signaling functions of alphavbeta5 integrin. *J Biol Chem* 275:33308–33313.
Birchmeier, W. and Behrens, J. 1994. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta* 1198:11–26.
Black, R.A., Rauch, C.T., Kozlosky, C.J., Peschon, J.J., Slack, J.L., Wolfson, M.F., Castner, B.J., Stocking, K.L., Reddy, P., Srinivasan, S., *et al.* 1997. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* 385:729–733.
Blasi, F. and Carmeliet, P. 2002. uPAR: a versatile signalling orchestrator. *Nat Rev Mol Cell Biol* 3:932–943.
Blavier, L. and Declerck, Y.A. 2005. Considering the critical interface between tumor cells and stromal cells in the search for targets for anticancer therapy. *Cancer Cell* 7:408–409.
Blobel, C.P. 2000. Remarkable roles of proteolysis on and beyond the cell surface. *Curr Opin Cell Biol* 12:606–612.
Bohlson, S.S., Silva, R., Fonseca, M.I., and Tenner, A.J. 2005. CD93 is rapidly shed from the surface of human myeloid cells and the soluble form is detected in human plasma. *J Immunol* 175:1239–1247.
Boire, A., Covic, L., Agarwal, A., Jacques, S., Sherifi, S., and Kuliopulos, A. 2005. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell* 120:303–313.
Bonventre, J.V. and Weinberg, J.M. 2003. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol* 14:2199–2210.
Borland, G., Murphy, G., and Ager, A. 1999. Tissue inhibitor of metalloproteinases-3 inhibits shedding of L-selectin from leukocytes. *J Biol Chem* 274:2810–2815.
Braddock, M. and Quinn, A. 2004. Targeting IL-1 in inflammatory disease: new opportunities for therapeutic intervention. *Nat Rev Drug Discov* 3:330–339.
Brew, K., Dinakarpandian, D., and Nagase, H. 2000. Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim Biophys Acta* 1477:267–283.
Brinckerhoff, C.E. and Matrisian, L.M. 2002. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol* 3:207–214.
Bringmann, A. and Reichenbach, A. 2001. Role of Muller cells in retinal degenerations. *Front Biosci* 6:E72–E92.
Brown, C.L., Meise, K.S., Plowman, G.D., Coffey, R.J., and Dempsey, P.J. 1998. Cell surface ectodomain cleavage of human amphiregulin precursor is sensitive to a metalloprotease inhibitor. Release of a predominant N-glycosylated 43-kDa soluble form. *J Biol Chem* 273:17258–17268.
Brule, S., Charnaux, N., Sutton, A., Ledoux, D., Chaigneau, T., Saffar, L., and Gattegno, L. 2006. The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9. *Glycobiology* 16:488–501.
Bryniarski, K., Maresz, K., Szczepanik, M., Ptak, M., and Ptak, W. 2003. Modulation of macrophage activity by proteolytic enzymes. Differential regulation of IL-6 and reactive oxygen intermediates (ROIs) synthesis as a possible homeostatic mechanism in the control of inflammation. *Inflammation* 27:333–340.
Buto, S., Tagliabue, E., Ardini, E., Magnifico, A., Ghirelli, C., van den Brule, F., Castronovo, V., Colnaghi, M.I., Sobel, M.E., and Menard,

S. 1998. Formation of the 67-kDa laminin receptor by acylation of the precursor. *J Cell Biochem* 69:244–251.
Buxbaum, J.D., Liu, K.N., Luo, Y., Slack, J.L., Stocking, K.L., Peschon, J.J., Johnson, R.S., Castner, B.J., Cerretti, D.P., and Black, R.A. 1998. 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.
Caron, A., Desrosiers, R.R., and Beliveau, R. 2005. Ischemia injury alters endothelial cell properties of kidney cortex: stimulation of MMP-9. *Exp Cell Res* 310:105–116.
Cavallaro, U. and Christofori, G. 2004. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer* 4:118–132.
Cavallaro, U., Liebner, S., and Dejana, E. 2006. Endothelial cadherins and tumor angiogenesis. *Exp Cell Res* 312:659–667.
Chabot, S., Kashio, Y., Seki, M., Shirato, Y., Nakamura, K., Nishi, N., Nakamura, T., Matsumoto, R., and Hirashima, M. 2002. Regulation of galectin-9 expression and release in Jurkat T cell line cells. *Glycobiology* 12:111–118.
Chambers, A.F., Groom, A.C., and MacDonald, I.C. 2002. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2:563–572.
Chandler, S., Coates, R., Gearing, A., Lury, J., Wells, G., and Bone, E. 1995. Matrix metalloproteinases degrade myelin basic protein. *Neurosci Lett* 201:223–226.
Chandler, S., Cossins, J., Lury, J., and Wells, G. 1996. Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. *Biochem. Biophys Res Commun* 228:421–429.
Charnaux, N., Sutton, A., Brule, S., and Gattegno, L. 2006. Regulated shedding of syndecan ectodomains by chemokines. *ScientificWorldJournal* 6:1037–1040.
Chen, K., See, A., and Shumack, S. 2003. Epidemiology and pathogenesis of scleroderma. *Australas J Dermatol* 44:1–7.
Cho, D.H., Song, H.K., Kang, H.S., Yoon, S.R., Lee, H.G., Pyun, K.H., Lee, W.J., Kim, Y.B., and Choi, I. 2000. Ligation of ICAM-1 molecules inhibits target cell-induced granule exocytosis of IL-12-activated natural killer cells. *Cell Immunol* 199:1–7.
Churg, A., Wang, R.D., Tai, H., Wang, X., Xie, C., Dai, J., Shapiro, S.D., and Wright, J.L. 2003. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. *Am J Respir Crit Care Med* 167:1083–1089.
Codony-Servat, J., Albanell, J., Lopez-Talavera, J.C., Arribas, J., and Baselga, J. 1999. Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. *Cancer Res* 59:1196–1201.
Collen, D. 2001. Ham-Wasserman lecture: role of the plasminogen system in fibrin-homeostasis and tissue remodeling. *Hematology (Am Soc Hematol Educ Program)* 1–9.
Couet, J., Sar, S., Jolivet, A., Hai, M.T., Milgrom, E., and Misrahi, M. 1996. Shedding of human thyrotropin receptor ectodomain. Involvement of a matrix metalloprotease. *J Biol Chem* 271:4545–4552.
Coughlin, S.R. 2000. Thrombin signalling and protease-activated receptors. *Nature* 407:258–264.
Coussens, L.M., Fingleton, B., and Matrisian, L.M. 2002. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 295:2387–2392.
Covington, M.D., Bayless, K.J., Burghardt, R.C., Davis, G.E., and Parrish, A.R. 2005. Ischemia-Induced Cleavage of Cadherins in NRK Cells: Evidence for a Role of Metalloproteinases. *Am J Physiol Renal Physiol* 290:F280–F288.
Covington, M.D., Burghardt, R.C., and Parrish, A.R. 2006. Ischemia-induced cleavage of cadherins in NRK cells requires MT1-MMP (MMP-14). *Am J Physiol Renal Physiol* 290:F43–F51.
Crowe, P.D., Walter, B.N., Mohler, K.M., Otten-Evans, C., Black, R.A., and Ware, C.F. 1995. A metalloprotease inhibitor blocks shedding of the 80-kD TNF receptor and TNF processing in T lymphocytes. *J Exp Med* 181:1205–1210.
Cuzner, M.L. and Opdenakker, G. 1999. Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. *J Neuroimmunol* 94:1–14.
D'Alessio, S., Fibbi, G., Cinelli, M., Guiducci, S., Del Rosso, A., Margheri, F., Serrati, S., Pucci, M., Kahaleh, B., Fan, P., *et al.* 2004. Matrix metalloproteinase 12-dependent cleavage of urokinase receptor in systemic sclerosis microvascular endothelial cells results in impaired angiogenesis. *Arthritis Rheum* 50:3275–3285.
Davenpeck, K.L., Brummet, M.E., Hudson, S.A., Mayer, R.J., and Bochner, B.S. 2000. Activation of human leukocytes reduces surface P-selectin glycoprotein ligand-1 (PSGL-1, CD162) and adhesion to P-selectin in vitro. *J Immunol* 165:2764–2772.
Davies, G., Jiang, W.G., and Mason, M.D. 2001. Matrilysin mediates extracellular cleavage of E-cadherin from prostate cancer cells: a key mechanism in hepatocyte growth factor/scatter factor-induced cell-cell dissociation and in vitro invasion. *Clin Cancer Res* 7:3289–3297.
de Bernard, S., Misrahi, M., Huet, J.C., Beau, I., Desroches, A., Loosfelt, H., Pichon, C., Pernollet, J.C., and Milgrom, E. 1999. Sequential cleavage and excision of a segment of the thyrotropin receptor ectodomain. *J Biol Chem* 274:101–107.
Deb, S. and Gottschall, P.E. 1996. Increased production of matrix metalloproteinases in enriched astrocyte and mixed hippocampal cultures treated with beta-amyloid peptides. *J Neurochem* 66:1641–1647.
Dekkers, P.E., ten Hove, T., Lauw, F.N., Koene, H.R., Lumley, P., van Deventer, S.J., and van der Poll, T. 2000. The metalloproteinase inhibitor GI5402 inhibits endotoxin-induced soluble CD27 and CD16release in healthy humans. *Infect Immun* 68:3036–3039.
Del Rosso, M., Fibbi, G., Schmitt, M., and Mignatti, P. 2005. Proteases and extracellular environment. *Thromb Haemost* 93:190–191.
Delaleu, N. and Bickel, M. 2004. Interleukin-1 beta and interleukin-18: regulation and activity in local inflammation. *Periodontol 2000.* 35:42–52.
Dello, S.P. and Rovida, E. 2002. Transmodulation of cell surface regulatory molecules via ectodomain shedding. *Biol Chem* 383:69–83.
Demetriou, M.C. and Cress, A.E. 2004. Integrin clipping: a novel adhesion switch? *J Cell Biochem* 91:26–35.
Dempsey, P.J., Meise, K.S., Yoshitake, Y., Nishikawa, K., and Coffey, R.J. 1997. Apical enrichment of human EGF precursor in Madin-Darby canine kidney cells involves preferential basolateral ectodomain cleavage sensitive to a metalloprotease inhibitor. *J Cell Biol* 138:747–758.
Deryugina, E.I., Bourdon, M.A., Jungwirth, K., Smith, J.W., and Strongin, A.Y. 2000. Functional activation of integrin alpha V beta 3 in tumor cells expressing membrane-type 1 matrix metalloproteinase. *Int J Cancer* 86:15–23.
Deryugina, E.I., Ratnikov, B.I., Postnova, T.I., Rozanov, D.V., and Strongin, A.Y. 2002. Processing of integrin alpha(v) subunit by membrane type 1 matrix metalloproteinase stimulates migration of breast carcinoma cells on vitronectin and enhances tyrosine phosphorylation of focal adhesion kinase. *J Biol Chem* 277:9749–9756.
Diaz-Rodriguez, E., Cabrera, N., Esparis-Ogando, A., Montero, J.C., and Pandiella, A. 1999. Cleavage of the TrkA neurotrophin receptor by multiple metalloproteases generates signalling-competent truncated forms. *Eur J Neurosci* 11:1421–1430.
Dinarello, C.A. 2000. Proinflammatory cytokines. *Chest* 118:503–508.
d'Ortho, M.P., Will, H., Atkinson, S., Butler, G., Messent, A., Gavrilovic, J., Smith, B., Timpl, R., Zardi, L., and Murphy, G. 1997. Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. *Eur J Biochem* 250:751–757.
Dubois, B., Masure, S., Hurtenbach, U., Paemen, L., Heremans, H., van den, O.J., Sciot, R., Meinhardt, T., Hammerling, G., Opdenakker, G., *et al.* 1999. Resistance of young gelatinase B-deficient mice to experimental autoimmune encephalomyelitis and necrotizing tail lesions. *J Clin Invest* 104:1507–1515.
Dusek, R.L., Getsios, S., Chen, F., Park, J.K., Amargo, E.V., Cryns, V.L., and Green, K.J. 2006. The differentiation-dependent desmosomal

cadherin desmoglein 1 is a novel caspase-3 target that regulates apoptosis in keratinocytes. *J Biol Chem* 281:3614–3624.
Egawa, N., Koshikawa, N., Tomari, T., Nabeshima, K., Isobe, T., and Seiki, M. 2006. Membrane-type-1 matrix metalloproteinase (MT1-MMP/MMP-14) cleaves and releases a 22-KDA EMMPRIN fragment from tumor cells. *J Biol Chem* 281:37576–37585.
Egeblad, M. and Werb, Z. 2002. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2:161–174.
Elhabazi, A., Delaire, S., Bensussan, A., Boumsell, L., and Bismuth, G. 2001. Biological activity of soluble CD100. I. The extracellular region of CD100 is released from the surface of T lymphocytes by regulated proteolysis. *J Immunol* 166:4341–4347.
Elwood, P.C., Deutsch, J.C., and Kolhouse, J.F. 1991. The conversion of the human membrane-associated folate binding protein (folate receptor) to the soluble folate binding protein by a membrane-associated metalloprotease. *J Biol Chem* 266:2346–2353.
Emonard, H., Bellon, G., de Diesbach, P., Mettlen, M., Hornebeck, W., and Courtoy, P.J. 2005. Regulation of matrix metalloproteinase (MMP) activity by the low-density lipoprotein receptor-related protein (LRP). A new function for an "old friend". *Biochimie* 87:369–376.
Endo, K., Takino, T., Miyamori, H., Kinsen, H., Yoshizaki, T., Furukawa, M., and Sato, H. 2003. Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. *J Biol Chem* 278:40764–40770.
English, W.R., Puente, X.S., Freije, J.M., Knauper, V., Amour, A., Merryweather, A., Lopez-Otin, C., and Murphy, G. 2000. 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.
Ethell, D.W., Kinloch, R., and Green, D.R. 2002. Metalloproteinase shedding of Fas ligand regulates beta-amyloid neurotoxicity. *Curr Biol* 12:1595–1600.
Fazioli, F., Resnati, M., Sidenius, N., Higashimoto, Y., Appella, E., and Blasi, F. 1997. A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. *EMBO J* 16:7279–7286.
Feldman, G.J., Mullin, J.M., and Ryan, M.P. 2005. Occludin: structure, function and regulation. *Adv Drug Deliv Rev* 57:883–917.
Fingleton, B., Vargo-Gogola, T., Crawford, H.C., and Matrisian, L.M. 2001. Matrilysin [MMP-7] expression selects for cells with reduced sensitivity to apoptosis. *Neoplasia* 3:459–468.
Fiore, E., Fusco, C., Romero, P., and Stamenkovic, I. 2002. Matrix metalloproteinase 9 (MMP-9/gelatinase B) proteolytically cleaves ICAM-1 and participates in tumor cell resistance to natural killer cell-mediated cytotoxicity. *Oncogene* 21:5213–5223.
Fitzgerald, M.L., Wang, Z., Park, P.W., Murphy, G., and Bernfield, M. 2000. Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase. *J Cell Biol* 148:811–824.
Folgueras, A.R., Pendas, A.M., Sanchez, L.M., and Lopez-Otin, C. 2004. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int J Dev Biol* 48:411–424.
Frank, R.N. 2004. Diabetic retinopathy. *N Engl J Med* 350:48–58.
Furman, M.I., Krueger, L.A., Linden, M.D., Barnard, M.R., Frelinger, A.L., III, and Michelson, A.D. 2004. Release of soluble CD40 L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. *J Am Coll Cardiol* 43:2319–2325.
Furuse, M. and Tsukita, S. 2006. Claudins in occluding junctions of humans and flies. *Trends Cell Biol* 16:181–188.
Gabison, E.E., Hoang-Xuan, T., Mauviel, A., and Menashi, S. 2005. EMMPRIN/CD147, an MMP modulator in cancer, development and tissue repair. *Biochimie* 87:361–368.
Galko, M.J. and Tessier-Lavigne, M. 2000. Function of an axonal chemoattractant modulated by metalloprotease activity. *Science* 289:1365–1367.
Gallatin, W.M., Weissman, I.L., and Butcher, E.C. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* 304:30–34.
Gallea-Robache, S., Morand, V., Millet, S., Bruneau, J.M., Bhatnagar, N., Chouaib, S., and Roman-Roman, S. 1997. A metalloproteinase inhibitor blocks the shedding of soluble cytokine receptors and processing of transmembrane cytokine precursors in human monocytic cells. *Cytokine* 9:340–346.
Galon, J., Moldovan, I., Galinha, A., Provost-Marloie, M.A., Kaudewitz, H., Roman-Roman, S., Fridman, W.H., and Sautes, C. 1998. Identification of the cleavage site involved in production of plasma soluble Fc gamma receptor type III (CD16). *Eur J Immunol* 28:2101–2107.
Galvez, B.G., Matias-Roman, S., Yanez-Mo, M., Vicente-Manzanares, M., Sanchez-Madrid, F., and Arroyo, A.G. 2004. Caveolae are a novel pathway for membrane-type 1 matrix metalloproteinase traffic in human endothelial cells. *Mol Biol Cell* 15:678–687.
Gao, G., Plaas, A., Thompson, V.P., Jin, S., Zuo, F., and Sandy, J.D. 2004. ADAMTS4 (aggrecanase-1) activation on the cell surface involves C-terminal cleavage by glycosylphosphatidyl inositol-anchored membrane type 4-matrix metalloproteinase and binding of the activated proteinase to chondroitin sulfate and heparan sulfate on syndecan-1. *J Biol Chem* 279:10042–10051.
Gao, G., Westling, J., Thompson, V.P., Howell, T.D., Gottschall, P.E., and Sandy, J.D. 2002. Activation of the proteolytic activity of ADAMTS4 (aggrecanase-1) by C-terminal truncation. *J Biol Chem* 277:11034–11041.
Garton, K.J., Gough, P.J., and Raines, E.W. 2006. Emerging roles for ectodomain shedding in the regulation of inflammatory responses. *J Leukoc Biol* 79:1105–1116.
Gearing, A.J., Beckett, P., Christodoulou, M., Churchill, M., Clements, J., Davidson, A.H., Drummond, A.H., Galloway, W.A., Gilbert, R., and Gordon, J.L. 1994. Processing of tumour necrosis factor-alpha precursor by metalloproteinases. *Nature* 370:555–557.
Gearing, A.J., Beckett, P., Christodoulou, M., Churchill, M., Clements, J.M., Crimmin, M., Davidson, A.H., Drummond, A.H., Galloway, W.A., and Gilbert, R. 1995. Matrix metalloproteinases and processing of pro-TNF-alpha. *J Leukoc Biol* 57:774–777.
Giebel, S.J., Menicucci, G., McGuire, P.G., and Das, A. 2005. Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood-retinal barrier. *Lab Invest* 85:597–607.
Gijbels, K., Masure, S., Carton, H., and Opdenakker, G. 1992. Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders. *J Neuroimmunol* 41:29–34.
Giustiniani, J., Marie-Cardine, A., and Bensussan, A. 2007. A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. *J Immunol* 178:1293–1300.
Goerge, T., Barg, A., Schnaeker, E.M., Poppelmann, B., Shpacovitch, V., Rattenholl, A., Maaser, C., Luger, T.A., Steinhoff, M., and Schneider, S.W. 2006. Tumor-derived matrix metalloproteinase-1 targets endothelial proteinase-activated receptor 1 promoting endothelial cell activation. *Cancer Res* 66:7766–7774.
Gomez, D.E., Alonso, D.F., Yoshiji, H., and Thorgeirsson, U.P. 1997. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol* 74:111–122.
Gorodeski, G.I. 2007. Estrogen decrease in tight junctional resistance involves MMP-7—mediated remodeling of occludin. *Endocrinology* 148:218–231.
Griffin, M., Casadio, R., and Bergamini, C.M. 2002. Transglutaminases: nature's biological glues. *Biochem J* 368:377–396.
Gurney, K.J., Estrada, E.Y., and Rosenberg, G.A. 2006. Blood-brain barrier disruption by stromelysin-1 facilitates neutrophil infiltration in neuroinflammation. *Neurobiol Dis* 23:87–96.
Gutwein, P., Stoeck, A., Riedle, S., Gast, D., Runz, S., Condon, T.P., Marme, A., Phong, M.C., Linderkamp, O., Skorokhod, A., *et al.* 2005. Cleavage of L1 in exosomes and apoptotic membrane vesicles released from ovarian carcinoma cells. *Clin Cancer Res* 11:2492–2501.
Hampe, W., Riedel, I.B., Lintzel, J., Bader, C.O., Franke, I., and Schaller, H.C. 2000. Ectodomain shedding, translocation and synthesis

of SorLA are stimulated by its ligand head activator. *J Cell Sci* 113:4475–4485.
Hanahan, D. and Weinberg, R.A. 2000. The hallmarks of cancer. *Cell* 100:57–70.
Hansen, H.P., Kisseleva, T., Kobarg, J., Horn-Lohrens, O., Havsteen, B., and Lemke, H. 1995. A zinc metalloproteinase is responsible for the release of CD30 on human tumor cell lines. *Int J Cancer* 63:750–756.
Hao, L., Du, M., Lopez-Campistrous, A., and Fernandez-Patron, C. 2004. Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. *Circ Res* 94:68–76.
Hara, T., Katakai, T., Lee, J.H., Nambu, Y., Nakajima-Nagata, N., Gonda, H., Sugai, M., and Shimizu, A. 2006. A transmembrane chemokine, CXC chemokine ligand 16, expressed by lymph node fibroblastic reticular cells has the potential to regulate T cell migration and adhesion. *Int Immunol* 18:301–311.
Haro, H., Crawford, H.C., Fingleton, B., MacDougall, J.R., Shinomiya, K., Spengler, D.M., and Matrisian, L.M. 2000a. Matrix metalloproteinase-3-dependent generation of a macrophage chemoattractant in a model of herniated disc resorption. *J Clin Invest* 105:133–141.
Haro, H., Crawford, H.C., Fingleton, B., Shinomiya, K., Spengler, D.M., and Matrisian, L.M. 2000b. Matrix metalloproteinase-7-dependent release of tumor necrosis factor-alpha in a model of herniated disc resorption. *J Clin Invest* 105:143–150.
Hartl, D. and Griese, M. 2006. Surfactant protein D in human lung diseases. *Eur J Clin Invest* 36:423–435.
Haug, C., Lenz, C., Diaz, F., and Bachem, M.G. 2004. Oxidized low-density lipoproteins stimulate extracellular matrix metalloproteinase Inducer (EMMPRIN) release by coronary smooth muscle cells. *Arterioscler Thromb Vasc Biol* 24:1823–1829.
Haustein, U.F. 2002. Systemic sclerosis-scleroderma. *Dermatol Online J* 8:Number 1, 3.
Hawkins, B.T. and Davis, T.P. 2005. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 57:173–185.
Hecht, A. and Kemler, R. 2000. Curbing the nuclear activities of beta-catenin. Control over Wnt target gene expression. *EMBO Rep* 1:24–28.
Heissig, B., Hattori, K., Dias, S., Friedrich, M., Ferris, B., Hackett, N.R., Crystal, R.G., Besmer, P., Lyden, D., Moore, M.A., *et al.* 2002. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 109:625–637.
Herbst, R.S. 2004. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* 59:21–26.
Herz, J., Clouthier, D.E., and Hammer, R.E. 1992. LDL receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell* 71:411–421.
Higashi, S. and Miyazaki, K. 2003a. Identification of a region of beta-amyloid precursor protein essential for its gelatinase A inhibitory activity. *J Biol Chem* 278:14020–14028.
Higashi, S. and Miyazaki, K. 2003b. Novel processing of beta-amyloid precursor protein catalyzed by membrane type 1 matrix metalloproteinase releases a fragment lacking the inhibitor domain against gelatinase A. *Biochemistry* 42:6514–6526.
Higashiyama, S. 2004. Metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor and its pathophysiological roles. *Protein Pept Lett* 11:443–450.
Hikita, A., Yana, I., Wakeyama, H., Nakamura, M., Kadono, Y., Oshima, Y., Nakamura, K., Seiki, M., and Tanaka, S. 2006. Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappa B ligand. *J Biol Chem* 281:36846–36855.
Hilton, R. 2006. Acute renal failure. *BMJ* 333:786–790.
Hintz, K.A., Rassias, A.J., Wardwell, K., Moss, M.L., Morganelli, P.M., Pioli, P.A., Givan, A.L., Wallace, P.K., Yeager, M.P., and Guyre, P.M. 2002. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. *J Leukoc Biol* 72:711–717.
Hohlbaum, A.M., Moe, S., and Marshak-Rothstein, A. 2000. Opposing effects of transmembrane and soluble Fas ligand expression on inflammation and tumor cell survival. *J Exp Med* 191:1209–1220.
Hollenbeck, S.T., Sakakibara, K., Faries, P.L., Workhu, B., Liu, B., and Kent, K.C. 2004. Stem cell factor and c-kit are expressed by and may affect vascular SMCs through an autocrine pathway. *J Surg Res* 120:288–294.
Hollingsworth, M.A. and Swanson, B.J. 2004. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 4:45–60.
Hood, J.D. and Cheresh, D.A. 2002. Role of integrins in cell invasion and migration. *Nat Rev Cancer* 2:91–100.
Hooper, N.M., Karran, E.H., and Turner, A.J. 1997. Membrane protein secretases. *Biochem J* 321:265–279.
Hopkins, A.M., Baird, A.W., and Nusrat, A. 2004. ICAM-1: targeted docking for exogenous as well as endogenous ligands. *Adv Drug Deliv Rev* 56:763–778.
Hoyer-Hansen, G., Behrendt, N., Ploug, M., Dano, K., and Preissner, K.T. 1997a. The intact urokinase receptor is required for efficient vitronectin binding: receptor cleavage prevents ligand interaction. *FEBS Lett* 420:79–85.
Hoyer-Hansen, G., Ploug, M., Behrendt, N., Ronne, E., and Dano, K. 1997b. Cell-surface acceleration of urokinase-catalyzed receptor cleavage. *Eur J Biochem* 243:21–26.
Hummel, V., Kallmann, B.A., Wagner, S., Fuller, T., Bayas, A., Tonn, J.C., Benveniste, E.N., Toyka, K.V., and Rieckmann, P. 2001. Production of MMPs in human cerebral endothelial cells and their role in shedding adhesion molecules. *J Neuropathol Exp Neurol* 60:320–327.
Ichikawa, Y., Ishikawa, T., Momiyama, N., Kamiyama, M., Sakurada, H., Matsuyama, R., Hasegawa, S., Chishima, T., Hamaguchi, Y., Fujii, S., *et al.* 2006. Matrilysin (MMP-7) degrades VE-cadherin and accelerates accumulation of beta-catenin in the nucleus of human umbilical vein endothelial cells. *Oncol Rep* 15:311–315.
Ilan, N., Mohsenin, A., Cheung, L., and Madri, J.A. 2001. PECAM-1 shedding during apoptosis generates a membrane-anchored truncated molecule with unique signaling characteristics. *FASEB J* 15:362–372.
Ito, A., Mukaiyama, A., Itoh, Y., Nagase, H., Thogersen, I.B., Enghild, J.J., Sasaguri, Y., and Mori, Y. 1996. Degradation of interleukin 1beta by matrix metalloproteinases. *J Biol Chem* 271:14657–14660.
Itoh, T., Ikeda, T., Gomi, H., Nakao, S., Suzuki, T., and Itohara, S. 1997. Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. *J Biol Chem* 272:22389–22392.
Itoh, Y. and Seiki, M. 2004. MT1-MMP: an enzyme with multidimensional regulation. *Trends Biochem Sci* 29:285–289.
Itoh, Y. and Seiki, M. 2006. MT1-MMP: a potent modifier of pericellular microenvironment. *J Cell Physiol* 206:1–8.
Iwamoto, R. and Mekada, E. 2000. Heparin-binding EGF-like growth factor: a juxtacrine growth factor. *Cytokine Growth Factor Rev* 11:335–344.
Jiang, A., Lehti, K., Wang, X., Weiss, S.J., Keski-Oja, J., and Pei, D. 2001. Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis. *Proc Natl Acad Sci USA* 98:13693–13698.
Jin, Y., Nonoyama, S., Morio, T., Imai, K., Ochs, H.D., and Mizutani, S. 2001. Characterization of soluble CD40 ligand released from human activated platelets. *J Med Dent Sci* 48:23–27.
Jing, J., Lien, C.F., Sharma, S., Rice, J., Brennan, P.A., and Gorecki, D.C. 2004. Aberrant expression, processing and degradation of dystroglycan in squamous cell carcinomas. *Eur J Cancer* 40:2143–2151.
Jung, T., Schrader, N., Hellwig, M., Enssle, K.H., and Neumann, C. 1999. Soluble human interleukin-4 receptor is produced by activated T cells under the control of metalloproteinases. *Int Arch Allergy Immunol* 119:23–30.
Kaczmarek, L., Lapinska-Dzwonek, J., and Szymczak, S. 2002. Matrix metalloproteinases in the adult brain physiology: a link between c-Fos, AP-1 and remodeling of neuronal connections? *EMBO J* 21:6643–6648.

Kajita, M., Itoh, Y., Chiba, T., Mori, H., Okada, A., Kinoh, H., and Seiki, M. 2001. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J Cell Biol* 153:893–904.

Kato, K., Santana-Sahagun, E., Rassenti, L.Z., Weisman, M.H., Tamura, N., Kobayashi, S., Hashimoto, H., and Kipps, T.J. 1999. The soluble CD40 ligand sCD154 in systemic lupus erythematosus. *J Clin Invest* 104:947–955.

Kim, R., Emi, M., Tanabe, K., Uchida, Y., and Toge, T. 2004. The role of Fas ligand and transforming growth factor beta in tumor progression: molecular mechanisms of immune privilege via Fas-mediated apoptosis and potential targets for cancer therapy. *Cancer* 100:2281–2291.

Kitchens, R.L. and Thompson, P.A. 2005. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. *J Endotoxin Res* 11:225–229.

Kojro, E. and Fahrenholz, F. 1995. Ligand-induced cleavage of the V2 vasopressin receptor by a plasma membrane metalloproteinase. *J Biol Chem* 270:6476–6481.

Kollias, G., Douni, E., Kassiotis, G., and Kontoyiannis, D. 1999. The function of tumour necrosis factor and receptors in models of multiorgan inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. *Ann Rheum Dis* 58:I32–I39.

Kontogiorgis, C.A., Papaioannou, P., and Hadjipavlou-Litina, D.J. 2005. Matrix metalloproteinase inhibitors: a review on pharmacophore mapping and (Q) SARs results. *Curr Med Chem* 12:339–355.

Koolwijk, P., Sidenius, N., Peters, E., Sier, C.F., Hanemaaijer, R., Blasi, F., and van Hinsbergh, V.W. 2001. Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. *Blood* 97:3123–3131.

Kostura, M.J., Tocci, M.J., Limjuco, G., Chin, J., Cameron, P., Hillman, A.G., Chartrain, N.A., and Schmidt, J.A. 1989. Identification of a monocyte specific pre-interleukin 1 beta convertase activity. *Proc Natl Acad Sci USA* 86:5227–5231.

Koval, M. 2006. Claudins—key pieces in the tight junction puzzle. *Cell Commun Adhes* 13:127–138.

Kridel, S.J., Chen, E., Kotra, L.P., Howard, E.W., Mobashery, S., and Smith, J.W. 2001. Substrate hydrolysis by matrix metalloproteinase-9. *J Biol Chem* 276:20572–20578.

Larsen, P.H., Wells, J.E., Stallcup, W.B., Opdenakker, G., and Yong, V.W. 2003. Matrix metalloproteinase-9 facilitates remyelination in part by processing the inhibitory NG2 proteoglycan. *J Neurosci* 23:11127–11135.

Leone, L., De Stefano, M.E., Del Signore, A., Petrucci, T.C., and Paggi, P. 2005. Axotomy of sympathetic neurons activates the metalloproteinase-2 enzymatic pathway. *J Neuropathol Exp Neurol* 64:1007–1017.

LePage, R.N., Fosang, A.J., Fuller, S.J., Murphy, G., Evin, G., Beyreuther, K., Masters, C.L., and Small, D.H. 1995. Gelatinase A possesses a beta-secretase-like activity in cleaving the amyloid protein precursor of Alzheimer's disease. *FEBS Lett* 377:267–270.

Levi, E., Fridman, R., Miao, H.Q., Ma, Y.S., Yayon, A., and Vlodavsky, I. 1996. Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1. *Proc Natl Acad Sci USA* 93:7069–7074.

Ley, K. and Kansas, G.S. 2004. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. *Nat Rev Immunol* 4:325–335.

Li, Q., Park, P.W., Wilson, C.L., and Parks, W.C. 2002. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* 111:635–646.

Lillis, A.P., Mikhailenko, I., and Strickland, D.K. 2005. Beyond endocytosis: LRP function in cell migration, proliferation and vascular permeability. *J Thromb Haemost* 3:1884–1893.

Lim, G.P., Russell, M.J., Cullen, M.J., and Tokes, Z.A. 1997. Matrix metalloproteinases in dog brains exhibiting Alzheimer-like characteristics. *J Neurochem* 68:1606–1611.

Linkermann, A., Qian, J., and Janssen, O. 2003. Slowly getting a clue on CD95 ligand biology. *Biochem Pharmacol* 66:1417–1426.

Liu, H. and Pope, R.M. 2003. The role of apoptosis in rheumatoid arthritis. *Curr Opin Pharmacol* 3:317–322.

Liu, Z. 2003. Immunopathology of bullous pemphigoid, an autoimmune and inflammatory skin blistering disease. *Keio J Med* 52:128–133.

Liu, Z., Giudice, G.J., Zhou, X., Swartz, S.J., Troy, J.L., Fairley, J.A., Till, G.O., and Diaz, L.A. 1997. A major role for neutrophils in experimental bullous pemphigoid. *J Clin Invest* 100:1256–1263.

Liu, Z., Li, N., Diaz, L.A., Shipley, M., Senior, R.M., and Werb, Z. 2005. Synergy between a plasminogen cascade and MMP-9 in autoimmune disease. *J Clin Invest* 115:879–887.

Liu, Z., Shapiro, S.D., Zhou, X., Twining, S.S., Senior, R.M., Giudice, G.J., Fairley, J.A., and Diaz, L.A. 2000a. A critical role for neutrophil elastase in experimental bullous pemphigoid. *J Clin Invest* 105:113–123.

Liu, Z., Shipley, J.M., Vu, T.H., Zhou, X., Diaz, L.A., Werb, Z., and Senior, R.M. 1998. Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. *J Exp Med* 188:475–482.

Liu, Z., Zhou, X., Shapiro, S.D., Shipley, J.M., Twining, S.S., Diaz, L.A., Senior, R.M., and Werb, Z. 2000b. The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. *Cell* 102:647–655.

Lochter, A., Galosy, S., Muschler, J., Freedman, N., Werb, Z., and Bissell, M.J. 1997. 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.

Lohmann, C., Krischke, M., Wegener, J., and Galla, H.J. 2004. Tyrosine phosphatase inhibition induces loss of blood-brain barrier integrity by matrix metalloproteinase-dependent and -independent pathways. *Brain Res* 995:184–196.

Lopez-Casillas, F., Payne, H.M., Andres, J.L., and Massague, J. 1994. Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachment sites. *J Cell Biol* 124:557–568.

Lorenzl, S., Albers, D.S., Relkin, N., Ngyuen, T., Hilgenberg, S.L., Chirichigno, J., Cudkowicz, M.E., and Beal, M.F. 2003. Increased plasma levels of matrix metalloproteinase-9 in patients with Alzheimer's disease. *Neurochem Int* 43:191–196.

Lucchesi, P.A., Sabri, A., Belmadani, S., and Matrougui, K. 2004. Involvement of metalloproteinases 2/9 in epidermal growth factor receptor transactivation in pressure-induced myogenic tone in mouse mesenteric resistance arteries. *Circulation* 110:3587–3593.

Lum, L., Wong, B.R., Josien, R., Becherer, J.D., Erdjument-Bromage, H., Schlondorff, J., Tempst, P., Choi, Y., and Blobel, C.P. 1999. Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *J Biol Chem* 274:13613–13618.

Lynch, C.C., Hikosaka, A., Acuff, H.B., Martin, M.D., Kawai, N., Singh, R.K., Vargo-Gogola, T.C., Begtrup, J.L., Peterson, T.E., Fingleton, B., *et al.* 2005. MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. *Cancer Cell* 7:485–496.

Magnifico, A., Tagliabue, E., Buto, S., Ardini, E., Castronovo, V., Colnaghi, M.I., and Menard, S. 1996. Peptide G, containing the binding site of the 67-kDa laminin receptor, increases and stabilizes laminin binding to cancer cells. *J Biol Chem* 271:31179–31184.

Major, T.C., Liang, L., Lu, X., Rosebury, W., and Bocan, T.M. 2002. Extracellular matrix metalloproteinase inducer (EMMPRIN) is induced upon monocyte differentiation and is expressed in human atheroma. *Arterioscler Thromb Vasc Biol* 22:1200–1207.

Malfait, A.M., Liu, R.Q., Ijiri, K., Komiya, S., and Tortorella, M.D. 2002. Inhibition of ADAM-TS4 and ADAM-TS5 prevents aggrecan degradation in osteoarthritic cartilage. *J Biol Chem* 277:22201–22208.

Martinez-Pomares, L., Mahoney, J.A., Kaposzta, R., Linehan, S.A., Stahl, P.D., and Gordon, S. 1998. A functional soluble form of the murine mannose receptor is produced by macrophages in vitro and is present in mouse serum. *J Biol Chem* 273:23376–23380.

Matsumura, K., Zhong, D., Saito, F., Arai, K., Adachi, K., Kawai, H., Higuchi, I., Nishino, I., and Shimizu, T. 2005. Proteolysis of beta-dystroglycan in muscular diseases. *Neuromuscul Disord* 15:336–341.

Matsuno, H., Yudoh, K., Watanabe, Y., Nakazawa, F., Aono, H., and Kimura, T. 2001. 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.

McCawley, L.J. and Matrisian, L.M. 2001. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* 13:534–540.

McGeehan, G.M., Becherer, J.D., Bast, R.C., Jr., Boyer, C.M., Champion, B., Connolly, K.M., Conway, J.G., Furdon, P., Karp, S., Kidao, S., *et al.* 1994. Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor. *Nature* 370:558–561.

McGuire, J.K., Li, Q., and Parks, W.C. 2003. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. *Am J Pathol* 162:1831–1843.

McQuibban, G.A., Butler, G.S., Gong, J.H., Bendall, L., Power, C., Clark-Lewis, I., and Overall, C.M. 2001. Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *J Biol Chem* 276:43503–43508.

Mechtersheimer, S., Gutwein, P., Agmon-Levin, N., Stoeck, A., Oleszewski, M., Riedle, S., Postina, R., Fahrenholz, F., Fogel, M., Lemmon, V., *et al.* 2001. Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J Cell Biol* 155:661–673.

Merlos-Suarez, A., Ruiz-Paz, S., Baselga, J., and Arribas, J. 2001. Metalloprotease-dependent protransforming growth factor-alpha ectodomain shedding in the absence of tumor necrosis factor-alpha-converting enzyme. *J Biol Chem* 276:48510–48517.

Milenkovic, I., Weick, M., Wiedemann, P., Reichenbach, A., and Bringmann, A. 2003. P2Y receptor-mediated stimulation of Muller glial cell DNA synthesis: dependence on EGF and PDGF receptor transactivation. *Invest Ophthalmol Vis Sci* 44:1211–1220.

Misrahi, M. and Milgrom, E. 1997. Cleavage and shedding of the TSH receptor. *Eur J Endocrinol* 137:599–602.

Mitsiades, N., Yu, W.H., Poulaki, V., Tsokos, M., and Stamenkovic, I. 2001. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res* 61:577–581.

Miyamoto, S., Hirata, M., Yamazaki, A., Kageyama, T., Hasuwa, H., Mizushima, H., Tanaka, Y., Yagi, H., Sonoda, K., Kai, M., *et al.* 2004. Heparin-binding EGF-like growth factor is a promising target for ovarian cancer therapy. *Cancer Res* 64:5720–5727.

Miyazaki, K., Funahashi, K., Umeda, M., and Nakano, A. 1994. Gelatinase A and APP. *Nature* 368:695–696.

Miyazaki, K., Hasegawa, M., Funahashi, K., and Umeda, M. 1993. A metalloproteinase inhibitor domain in Alzheimer amyloid protein precursor. *Nature* 362:839–841.

Mohan, M.J., Seaton, T., Mitchell, J., Howe, A., Blackburn, K., Burkhart, W., Moyer, M., Patel, I., Waitt, G.M., Becherer, J.D., *et al.* 2002. The tumor necrosis factor-alpha converting enzyme (TACE): a unique metalloproteinase with highly defined substrate selectivity. *Biochemistry* 41:9462–9469.

Mohler, K.M., Sleath, P.R., Fitzner, J.N., Cerretti, D.P., Alderson, M., Kerwar, S.S., Torrance, D.S., Otten-Evans, C., Greenstreet, T., Weerawarna, K., *et al.* 1994. Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. *Nature* 370:218–220.

Molina, M.A., Codony-Servat, J., Albanell, J., Rojo, F., Arribas, J., and Baselga, J. 2001. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res* 61:4744–4749.

Monea, S., Jordan, B.A., Srivastava, S., DeSouza, S., and Ziff, E.B. 2006. Membrane localization of membrane type 5 matrix metalloproteinase by AMPA receptor binding protein and cleavage of cadherins. *J Neurosci* 26:2300–2312.

Mori, H., Tomari, T., Koshikawa, N., Kajita, M., Itoh, Y., Sato, H., Tojo, H., Yana, I., and Seiki, M. 2002. CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain. *EMBO J* 21:3949–3959.

Morishima, Y., Gotoh, Y., Zieg, J., Barrett, T., Takano, H., Flavell, R., Davis, R.J., Shirasaki, Y., and Greenberg, M.E. 2001. Beta-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. *J Neurosci* 21:7551–7560.

Mosesson, Y. and Yarden, Y. 2004. Oncogenic growth factor receptors: implications for signal transduction therapy. *Semin Cancer Biol* 14:262–270.

Moss, B.L., Taubner, L., Sample, Y.K., Kazmin, D.A., Copie, V., and Starkey, J.R. 2006. Tumor shedding of laminin binding protein modulates angiostatin production in vitro and interferes with plasmin-derived inhibition of angiogenesis in aortic ring cultures. *Int J Cancer* 118:2421–2432.

Moss, M.L., Jin, S.L., Milla, M.E., Bickett, D.M., Burkhart, W., Carter, H.L., Chen, W.J., Clay, W.C., Didsbury, J.R., Hassler, D., *et al.* 1997. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature* 385:733–736.

Mota, G., Moldovan, I., Calugaru, A., Hirt, M., Kozma, E., Galatiuc, C., Brasoveanu, L., and Boltz-Nitulescu, G. 2004. Interaction of human immunoglobulin G with CD16 on natural killer cells: ligand clearance, FcgammaRIIIA turnover and effects of metalloproteinases on FcgammaRIIIA-mediated binding, signal transduction and killing. *Scand J Immunol* 59:278–284.

Mullberg, J., Durie, F.H., Otten-Evans, C., Alderson, M.R., Rose-John, S., Cosman, D., Black, R.A., and Mohler, K.M. 1995. A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. *J Immunol* 155:5198–5205.

Mundy, G.R. 2002. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer* 2:584–593.

Nagase, H., Visse, R., and Murphy, G. 2006. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69:562–573.

Nagase, H. and Woessner, J.F. 1999. Matrix metalloproteinases. *J Biol Chem* 274:21491–21494.

Nakamura, H., Suenaga, N., Taniwaki, K., Matsuki, H., Yonezawa, K., Fujii, M., Okada, Y., and Seiki, M. 2004. Constitutive and induced CD44 shedding by ADAM-like proteases and membrane-type 1 matrix metalloproteinase. *Cancer Res* 64:876–882.

Nakashima, T., Kobayashi, Y., Yamasaki, S., Kawakami, A., Eguchi, K., Sasaki, H., and Sakai, H. 2000. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. *Biochem Biophys Res Commun* 275:768–775.

Naor, D., Sionov, R.V., and Ish-Shalom, D. 1997. CD44: structure, function, and association with the malignant process. *Adv Cancer Res* 71:241–319.

Nath, D., Williamson, N.J., Jarvis, R., and Murphy, G. 2001. Shedding of c-Met is regulated by crosstalk between a G-protein coupled receptor and the EGF receptor and is mediated by a TIMP-3 sensitive metalloproteinase. *J Cell Sci* 114:1213–1220.

Nawrocki-Raby, B., Gilles, C., Polette, M., Bruyneel, E., Laronze, J.Y., Bonnet, N., Foidart, J.M., Mareel, M., and Birembaut, P. 2003. Upregulation of MMPs by soluble E-cadherin in human lung tumor cells. *Int J Cancer* 105:790–795.

Nelson, A.R., Fingleton, B., Rothenberg, M.L., and Matrisian, L.M. 2000. Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol* 18:1135–1149.

Noe, V., Fingleton, B., Jacobs, K., Crawford, H.C., Vermeulen, S., Steelant, W., Bruyneel, E., Matrisian, L.M., and Mareel, M. 2001. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J Cell Sci* 114:111–118.

Ochieng, J., Fridman, R., Nangia-Makker, P., Kleiner, D.E., Liotta, L.A., Stetler-Stevenson, W.G., and Raz, A. 1994. Galectin-3 is a novel

substrate for human matrix metalloproteinases-2 and -9. *Biochemistry* 33:14109–14114.
Ochieng, J., Green, B., Evans, S., James, O., and Warfield, P. 1998. Modulation of the biological functions of galectin-3 by matrix metalloproteinases. *Biochim Biophys Acta* 1379:97–106.
Oda, M., Shiraishi, A., and Hasegawa, M. 1998. Analysis of the ternary complex formation of human urokinase with the separated two domains of its receptor. *Eur J Biochem* 256:411–418.
Okamoto, I., Kawano, Y., Murakami, D., Sasayama, T., Araki, N., Miki, T., Wong, A.J., and Saya, H. 2001. Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J Cell Biol* 155:755–762.
Okamoto, K., Asahara, H., Kobayashi, T., Matsuno, H., Hasunuma, T., Kobata, T., Sumida, T., and Nishioka, K. 1998. Induction of apoptosis in the rheumatoid synovium by Fas ligand gene transfer. *Gene Ther* 5:331–338.
Ongusaha, P.P., Kwak, J.C., Zwible, A.J., Macip, S., Higashiyama, S., Taniguchi, N., Fang, L., and Lee, S.W. 2004. HB-EGF is a potent inducer of tumor growth and angiogenesis. *Cancer Res* 64:5283–5290.
Opdenakker, G., Fibbe, W.E., and Van Damme, J. 1998. The molecular basis of leukocytosis. *Immunol Today* 19:182–189.
Opdenakker, G., Nelissen, I., and Van Damme, J. 2003. Functional roles and therapeutic targeting of gelatinase B and chemokines in multiple sclerosis. *Lancet Neurol* 2:747–756.
Opdenakker, G. and Van Damme, J. 1994. Cytokine-regulated proteases in autoimmune diseases. *Immunol Today* 15:103–107.
Opdenakker, G. and Van Damme, J. 2002. Chemokines and proteinases in autoimmune diseases and cancer. *Verh K Acad Geneeskd Belg* 64:105–136.
Osenkowski, P., Toth, M., and Fridman, R. 2004. Processing, shedding, and endocytosis of membrane type 1-matrix metalloproteinase (MT1-MMP). *J Cell Physiol* 200:2–10.
Ossovskaya, V.S. and Bunnett, N.W. 2004. Protease-activated receptors: contribution to physiology and disease. *Physiol Rev* 84:579–621.
Otterdal, K., Pedersen, T.M., and Solum, N.O. 2004. Release of soluble CD40 ligand after platelet activation: studies on the solubilization phase. *Thromb Res* 114:167–177.
Otterdal, K., Smith, C., Oie, E., Pedersen, T.M., Yndestad, A., Stang, E., Endresen, K., Solum, N.O., Aukrust, P., and Damas, J.K. 2006. Platelet-derived LIGHT induces inflammatory responses in endothelial cells and monocytes. *Blood* 108:928–935.
Overall, C.M. and Dean, R.A. 2006. Degradomics: systems biology of the protease web. Pleiotropic roles of MMPs in cancer. *Cancer Metastasis Rev* 25:69–75.
Overall, C.M., Tam, E.M., Kappelhoff, R., Connor, A., Ewart, T., Morrison, C.J., Puente, X., Lopez-Otin, C., and Seth, A. 2004. Protease degradomics: mass spectrometry discovery of protease substrates and the CLIP-CHIP, a dedicated DNA microarray of all human proteases and inhibitors. *Biol Chem* 385:493–504.
Paggi, P., De Stefano, M.E., and Petrucci, T.C. 2006. Synaptic remodeling induced by axotomy of superior cervical ganglion neurons: Involvement of metalloproteinase-2. *J Physiol Paris* 99:119–124.
Parks, W.C., Wilson, C.L., and Lopez-Boado, Y.S. 2004. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 4:617–629.
Parvathy, S., Oppong, S.Y., Karran, E.H., Buckle, D.R., Turner, A.J., and Hooper, N.M. 1997. Angiotensin-converting enzyme secretase is inhibited by zinc metalloprotease inhibitors and requires its substrate to be inserted in a lipid bilayer. *Biochem J* 327:37–43.
Patterson, B.C. and Sang, Q.A. 1997. Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). *J Biol Chem* 272:28823–28825.
Pei, D. 2005. Matrix metalloproteinases target protease-activated receptors on the tumor cell surface. *Cancer Cell* 7:207–208.
Penton-Rol, G., Orlando, S., Polentarutti, N., Bernasconi, S., Muzio, M., Introna, M., and Mantovani, A. 1999. Bacterial lipopolysaccharide causes rapid shedding, followed by inhibition of mRNA expression, of the IL-1 type II receptor, with concomitant up-regulation of the type I receptor and induction of incompletely spliced transcripts. *J Immunol* 162:2931–2938.
Pepper, M.S. 2001. Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. *Arterioscler Thromb Vasc Biol* 21:1104–1117.
Peschon, J.J., Slack, J.L., Reddy, P., Stocking, K.L., Sunnarborg, S.W., Lee, D.C., Russell, W.E., Castner, B.J., Johnson, R.S., Fitzner, J.N., *et al.* 1998. An essential role for ectodomain shedding in mammalian development. *Science* 282:1281–1284.
Peterson, R.M., Yu, Q., Stamenkovic, I., and Toole, B.P. 2000. Perturbation of hyaluronan interactions by soluble CD44 inhibits growth of murine mammary carcinoma cells in ascites. *Am J Pathol* 156:2159–2167.
Pflugfelder, S.C. 1998. Advances in the diagnosis and management of keratoconjunctivitis sicca. *Curr Opin Ophthalmol* 9:50–53.
Pflugfelder, S.C., Farley, W., Luo, L., Chen, L.Z., de Paiva, C.S., Olmos, L.C., Li, D.Q., and Fini, M.E. 2005. Matrix metalloproteinase-9 knockout confers resistance to corneal epithelial barrier disruption in experimental dry eye. *Am J Pathol* 166:61–71.
Piccard, H., Van den Steen, P.E., and Opdenakker, G. 2007. Hemopexin domains as multifunctional liganding modules in matrix metalloproteinases and other proteins. *J Leukoc Biol* 81:870–892.
Piccart-Gebhart, M.J. 2006. Adjuvant trastuzumab therapy for HER2-overexpressing breast cancer: what we know and what we still need to learn. *Eur J Cancer* 42:1715–1719.
Ploug, M. and Ellis, V. 1994. Structure-function relationships in the receptor for urokinase-type plasminogen activator. Comparison to other members of the Ly-6 family and snake venom alpha-neurotoxins. *FEBS Lett* 349:163–168.
Pope, R.M. 2002. Apoptosis as a therapeutic tool in rheumatoid arthritis. *Nat Rev Immunol* 2:527–535.
Poulaki, V., Mitsiades, C.S., and Mitsiades, N. 2001. The role of Fas and FasL as mediators of anticancer chemotherapy. *Drug Resist Updat* 4:233–242.
Powell, W.C., Fingleton, B., Wilson, C.L., Boothby, M., and Matrisian, L.M. 1999. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol* 9:1441–1447.
Powers, C.J., McLeskey, S.W., and Wellstein, A. 2000. Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer* 7:165–197.
Preece, G., Murphy, G., and Ager, A. 1996. Metalloproteinase-mediated regulation of L-selectin levels on leucocytes. *J Biol Chem* 271:11634–11640.
Prenzel, N., Zwick, E., Daub, H., Leserer, M., Abraham, R., Wallasch, C., and Ullrich, A. 1999. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 402:884–888.
Prevost, J.M., Pelley, J.L., Zhu, W., D'Egidio, G.E., Beaudry, P.P., Pihl, C., Neely, G.G., Claret, E., Wijdenes, J., and Brown, C.B. 2002. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and inflammatory stimuli up-regulate secretion of the soluble GM-CSF receptor in human monocytes: evidence for ectodomain shedding of the cell surface GM-CSF receptor alpha subunit. *J Immunol* 169:5679–5688.
Proost, P., Van Damme, J., and Opdenakker, G. 1993. Leukocyte gelatinase B cleavage releases encephalitogens from human myelin basic protein. *Biochem Biophys Res Commun* 192:1175–1181.
Puyraimond, A., Fridman, R., Lemesle, M., Arbeille, B., and Menashi, S. 2001. MMP-2 colocalizes with caveolae on the surface of endothelial cells. *Exp Cell Res* 262:28–36.
Quinn, K.A., Pye, V.J., Dai, Y.P., Chesterman, C.N., and Owensby, D.A. 1999. Characterization of the soluble form of the low density lipoprotein receptor-related protein (LRP). *Exp Cell Res* 251:433–441.

Raab, G. and Klagsbrun, M. 1997. Heparin-binding EGF-like growth factor. *Biochim Biophys Acta* 1333:F179-F199.

Rapala-Kozik, M., Kozik, A., and Travis, J. 1998. Effect of oxidation of beta-amyloid precursor protein on its beta-secretase cleavage. A model study with synthetic peptides and candidate beta-secretases. *J Pept Res* 52:315–320.

Ratnikov, B.I., Rozanov, D.V., Postnova, T.I., Baciu, P.G., Zhang, H., DiScipio, R.G., Chestukhina, G.G., Smith, J.W., Deryugina, E.I., and Strongin, A.Y. 2002. An alternative processing of integrin alpha(v) subunit in tumor cells by membrane type-1 matrix metalloproteinase. *J Biol Chem* 277:7377–7385.

Razandi, M., Pedram, A., Park, S.T., and Levin, E.R. 2003. Proximal events in signaling by plasma membrane estrogen receptors. *J Biol Chem* 278:2701–2712.

Reed, J.C. 1999. Mechanisms of apoptosis avoidance in cancer. *Curr Opin Oncol* 11:68–75.

Reijerkerk, A., Kooij, G., van der Pol, S.M., Khazen, S., Dijkstra, C.D., and de Vries, H.E. 2006. Diapedesis of monocytes is associated with MMP-mediated occludin disappearance in brain endothelial cells. *FASEB J* 20:2550–2552.

Remacle, A., Murphy, G., and Roghi, C. 2003. Membrane type I-matrix metalloproteinase (MT1-MMP) is internalised by two different pathways and is recycled to the cell surface. *J Cell Sci* 116:3905–3916.

Rentsch, F.J. 1973. Preretinal proliferation of glial cells after mechanical injury of the rabbit retina. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 188:79–90.

Reuning, U., Magdolen, V., Hapke, S., and Schmitt, M. 2003. Molecular and functional interdependence of the urokinase-type plasminogen activator system with integrins. *Biol Chem* 384:1119–1131.

Rigot, V., Andre, F., Lehmann, M., Lissitzky, J.C., Marvaldi, J., and Luis, J. 1999. Biogenesis of alpha6beta4 integrin in a human colonic adenocarcinoma cell line involvement of calnexin. *Eur J Biochem* 261:659–666.

Rio, M.C. 2005. From a unique cell to metastasis is a long way to go: clues to stromelysin-3 participation. *Biochimie* 87:299–306.

Roelle, S., Grosse, R., Aigner, A., Krell, H.W., Czubayko, F., and Gudermann, T. 2003. Matrix metalloproteinases 2 and 9 mediate epidermal growth factor receptor transactivation by gonadotropin-releasing hormone. *J Biol Chem* 278:47307–47318.

Roher, A.E., Kasunic, T.C., Woods, A.S., Cotter, R.J., Ball, M.J., and Fridman, R. 1994. Proteolysis of A beta peptide from Alzheimer disease brain by gelatinase A. *Biochem Biophys Res Commun* 205:1755–1761.

Ross, J.S., Fletcher, J.A., Linette, G.P., Stec, J., Clark, E., Ayers, M., Symmans, W.F., Pusztai, L., and Bloom, K.J. 2003. The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. *Oncologist* 8:307–325.

Rouis, M. 2005. Matrix metalloproteinases: a potential therapeutic target in atherosclerosis. *Curr Drug Targets Cardiovasc Haematol Disord* 5:541–548.

Rozanov, D.V., Ghebrehiwet, B., Postnova, T.I., Eichinger, A., Deryugina, E.I., and Strongin, A.Y. 2002. The hemopexin-like C-terminal domain of membrane type 1 matrix metalloproteinase regulates proteolysis of a multifunctional protein, gC1qR. *J Biol Chem* 277:9318–9325.

Rozanov, D.V., Hahn-Dantona, E., Strickland, D.K., and Strongin, A.Y. 2004a. The low density lipoprotein receptor-related protein LRP is regulated by membrane type-1 matrix metalloproteinase (MT1-MMP) proteolysis in malignant cells. *J Biol Chem* 279:4260–4268.

Rozanov, D.V., Savinov, A.Y., Golubkov, V.S., Postnova, T.I., Remacle, A., Tomlinson, S., and Strongin, A.Y. 2004b. Cellular membrane type-1 matrix metalloproteinase (MT1-MMP) cleaves C3b, an essential component of the complement system. *J Biol Chem* 279:46551–46557.

Sakai, K., Matsuno, H., Morita, I., Nezuka, T., Tsuji, H., Shirai, T., Yonehara, S., Hasunuma, T., and Nishioka, K. 1998. Potential withdrawal of rheumatoid synovium by the induction of apoptosis using a novel in vivo model of rheumatoid arthritis. *Arthritis Rheum* 41:1251–1257.

Salih, H.R., Goehlsdorf, D., and Steinle, A. 2006. Release of MICB molecules by tumor cells: mechanism and soluble MICB in sera of cancer patients. *Hum Immunol* 67:188–195.

Salih, H.R., Rammensee, H.G., and Steinle, A. 2002. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol* 169:4098–4102.

Sang, Q.X., Jin, Y., Newcomer, R.G., Monroe, S.C., Fang, X., Hurst, D.R., Lee, S., Cao, Q., and Schwartz, M.A. 2006. Matrix metalloproteinase inhibitors as prospective agents for the prevention and treatment of cardiovascular and neoplastic diseases. *Curr Top Med Chem* 6:289–316.

Sato, H., Takino, T., and Miyamori, H. 2005. Roles of membrane-type matrix metalloproteinase-1 in tumor invasion and metastasis. *Cancer Sci* 96:212–217.

Schett, G., Hayer, S., Zwerina, J., Redlich, K., and Smolen, J.S. 2005. Mechanisms of Disease: the link between RANKL and arthritic bone disease. *Nat Clin Pract Rheumatol* 1:47–54.

Schlondorff, J., Lum, L., and Blobel, C.P. 2001. Biochemical and pharmacological criteria define two shedding activities for TRANCE/OPGL that are distinct from the tumor necrosis factor alpha convertase. *J Biol Chem* 276:14665–14674.

Schönbeck, U., Mach, F., and Libby, P. 1998. 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.

Schwager, S.L., Chubb, A.J., Scholle, R.R., Brandt, W.F., Eckerskorn, C., Sturrock, E.D., and Ehlers, M.R. 1998. Phorbol ester-induced juxtamembrane cleavage of angiotensin-converting enzyme is not inhibited by a stalk containing intrachain disulfides. *Biochemistry* 37:15449–15456.

Schwager, S.L., Chubb, A.J., Scholle, R.R., Brandt, W.F., Mentele, R., Riordan, J.F., Sturrock, E.D., and Ehlers, M.R. 1999. Modulation of juxtamembrane cleavage ("shedding") of angiotensin-converting enzyme by stalk glycosylation: evidence for an alternative shedding protease. *Biochemistry* 38:10388–10397.

Seiki, M. 2002. The cell surface: the stage for matrix metalloproteinase regulation of migration. *Curr Opin Cell Biol* 14:624–632.

Seiki, M. 2003. Membrane-type 1 matrix metalloproteinase: a key enzyme for tumor invasion. *Cancer Lett* 194:1–11.

Sekine-Aizawa, Y., Hama, E., Watanabe, K., Tsubuki, S., Kanai-Azuma, M., Kanai, Y., Arai, H., Aizawa, H., Iwata, N., and Saido, T.C. 2001. Matrix metalloproteinase (MMP) system in brain: identification and characterization of brain-specific MMP highly expressed in cerebellum. *Eur J Neurosci* 13:935–948.

Selkoe, D.J. 2004. Alzheimer disease: mechanistic understanding predicts novel therapies. *Ann Intern Med* 140:627–638.

Senft, A.P., Korfhagen, T.R., Whitsett, J.A., Shapiro, S.D., and LeVine, A.M. 2005. Surfactant protein-D regulates soluble CD14 through matrix metalloproteinase-12. *J Immunol* 174:4953–4959.

Serrati, S., Cinelli, M., Margheri, F., Guiducci, S., Del Rosso, A., Pucci, M., Fibbi, G., Bazzichi, L., Bombardieri, S., Matucci-Cerinic, M., *et al.* 2006. Systemic sclerosis fibroblasts inhibit in vitro angiogenesis by MMP-12-dependent cleavage of the endothelial cell urokinase receptor. *J Pathol* 210:240–248.

Shah, B.H. and Catt, K.J. 2003. A central role of EGF receptor transactivation in angiotensin II -induced cardiac hypertrophy. *Trends Pharmacol Sci* 24:239–244.

Shah, B.H. and Catt, K.J. 2004a. GPCR-mediated transactivation of RTKs in the CNS: mechanisms and consequences. *Trends Neurosci* 27:48–53.

Shah, B.H. and Catt, K.J. 2004b. Matrix metalloproteinase-dependent EGF receptor activation in hypertension and left ventricular hypertrophy. *Trends Endocrinol Metab* 15:241–243.

Shah, B.H. and Catt, K.J. 2004c. Matrix metalloproteinases in reproductive endocrinology. *Trends Endocrinol Metab* 15:47–49.

Shah, B.H., Farshori, M.P., and Catt, K.J. 2004. Neuropeptide-induced transactivation of a neuronal epidermal growth factor receptor is mediated by metalloprotease-dependent formation of

heparin-binding epidermal growth factor. *J Biol Chem* 279:414–420.
Shao, M.X., Nakanaga, T., and Nadel, J.A. 2004. Cigarette smoke induces MUC5AC mucin overproduction via tumor necrosis factor-alpha-converting enzyme in human airway epithelial (NCI-H292) cells. *Am J Physiol Lung Cell Mol Physiol* 287:L420-L427.
Shapiro, S.D. 2003. Immunology: Mobilizing the army. *Nature* 421:223–224.
Sheu, B.C., Hsu, S.M., Ho, H.N., Lien, H.C., Huang, S.C., and Lin, R.H. 2001. A novel role of metalloproteinase in cancer-mediated immunosuppression. *Cancer Res* 61:237–242.
Shimanovich, I., Mihai, S., Oostingh, G.J., Ilenchuk, T.T., Brocker, E.B., Opdenakker, G., Zillikens, D., and Sitaru, C. 2004. Granulocyte-derived elastase and gelatinase B are required for dermal-epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. *J Pathol* 204:519–527.
Shin, J.S. and Abraham, S.N. 2001. Cell biology. Caveolae–not just craters in the cellular landscape. *Science* 293:1447–1448.
Shofuda, K.I., Hasenstab, D., Kenagy, R.D., Shofuda, T., Li, Z.Y., Lieber, A., and Clowes, A.W. 2001. Membrane-type matrix metalloproteinase-1 and -3 activity in primate smooth muscle cells. *FASEB J* 15:2010–2012.
Siegel, R.M., Frederiksen, J.K., Zacharias, D.A., Chan, F.K., Johnson, M., Lynch, D., Tsien, R.Y., and Lenardo, M.J. 2000. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 288:2354–2357.
Slamon, D.J., Clark, G.M., Wong, S.G., Levin, W.J., Ullrich, A., and McGuire, W.L. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235:177–182.
Smalley, D.M. and Ley, K. 2005. L-selectin: mechanisms and physiological significance of ectodomain cleavage. *J Cell Mol Med* 9:255–266.
Smith, M.D. and Walker, J.G. 2004. Apoptosis a relevant therapeutic target in rheumatoid arthritis? *Rheumatology (Oxford)* 43:405–407.
Steinhusen, U., Weiske, J., Badock, V., Tauber, R., Bommert, K., and Huber, O. 2001. Cleavage and shedding of E-cadherin after induction of apoptosis. *J Biol Chem* 276:4972–4980.
Stephens, G., Yan, Y., Jandrot-Perrus, M., Villeval, J.L., Clemetson, K.J., and Phillips, D.R. 2005. Platelet activation induces metalloproteinase-dependent GP VI cleavage to down-regulate platelet reactivity to collagen. *Blood* 105:186–191.
Sternlicht, M.D., Lochter, A., Sympson, C.J., Huey, B., Rougier, J.P., Gray, J.W., Pinkel, D., Bissell, M.J., and Werb, Z. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell* 98:137–146.
Sternlicht, M.D. and Werb, Z. 2001. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 17:463–516.
Strand, S., Vollmer, P., van den, A.L., Gottfried, D., Alla, V., Heid, H., Kuball, J., Theobald, M., Galle, P.R., and Strand, D. 2004. Cleavage of CD95 by matrix metalloproteinase-7 induces apoptosis resistance in tumour cells. *Oncogene* 23:3732–3736.
Strickland, D.K., Gonias, S.L., and Argraves, W.S. 2002. Diverse roles for the LDL receptor family. *Trends Endocrinol Metab* 13:66–74.
Suenaga, N., Mori, H., Itoh, Y., and Seiki, M. 2005. CD44 binding through the hemopexin-like domain is critical for its shedding by membrane-type 1 matrix metalloproteinase. *Oncogene* 24:859–868.
Sultan, S., Gosling, M., Nagase, H., and Powell, J.T. 2004. Shear stress-induced shedding of soluble intercellular adhesion molecule-1 from saphenous vein endothelium. *FEBS Lett* 564:161–165.
Suzuki, M., Raab, G., Moses, M.A., Fernandez, C.A., and Klagsbrun, M. 1997. Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site. *J Biol Chem* 272:31730–31737.
Takino, T., Koshikawa, N., Miyamori, H., Tanaka, M., Sasaki, T., Okada, Y., Seiki, M., and Sato, H. 2003. Cleavage of metastasis suppressor gene product KiSS-1 protein/metastin by matrix metalloproteinases. *Oncogene* 22:4617–4626.
Takino, T., Sato, H., Shinagawa, A., and Seiki, M. 1995. 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.
Tanaka, M., Itai, T., Adachi, M., and Nagata, S. 1998. Downregulation of Fas ligand by shedding. *Nat Med* 4:31–36.
Tanaka, Y., Irie, K., Hirota, T., Sakisaka, T., Nakanishi, H., and Takai, Y. 2002. Ectodomain shedding of nectin-1alpha by SF/HGF and TPA in MDCK cells. *Biochem Biophys Res Commun* 299:472–478.
Tang, Y., Kesavan, P., Nakada, M.T., and Yan, L. 2004. Tumor-stroma interaction: positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase-dependent generation of soluble EMMPRIN. *Mol Cancer Res* 2:73–80.
Thathiah, A., Blobel, C.P., and Carson, D.D. 2003. Tumor necrosis factor-alpha converting enzyme/ADAM 17 mediates MUC1 shedding. *J Biol Chem* 278:3386–3394.
Thathiah, A. and Carson, D.D. 2004. MT1-MMP mediates MUC1 shedding independent of TACE/ADAM17. *Biochem J* 382:363–373.
Theoleyre, S., Wittrant, Y., Tat, S.K., Fortun, Y., Redini, F., and Heymann, D. 2004. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. *Cytokine Growth Factor Rev* 15:457–475.
Thodeti, C.K., Albrechtsen, R., Grauslund, M., Asmar, M., Larsson, C., Takada, Y., Mercurio, A.M., Couchman, J.R., and Wewer, U.M. 2003. ADAM12/syndecan-4 signaling promotes beta 1 integrin-dependent cell spreading through protein kinase Calpha and RhoA. *J Biol Chem* 278:9576–9584.
Toole, B.P. 2004. Hyaluronan: from extracellular glue to pericellular cue. *Nat Rev Cancer* 4:528–539.
Tortorella, M.D., Arner, E.C., Hills, R., Gormley, J., Fok, K., Pegg, L., Munie, G., and Malfait, A.M. 2005. ADAMTS-4 (aggrecanase-1): N-terminal activation mechanisms. *Arch Biochem Biophys* 444:34–44.
Tortorella, M.D., Burn, T.C., Pratta, M.A., Abbaszade, I., Hollis, J.M., Liu, R., Rosenfeld, S.A., Copeland, R.A., Decicco, C.P., Wynn, R., *et al.* 1999. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science* 284:1664–1666.
Toth, M., Hernandez-Barrantes, S., Osenkowski, P., Bernardo, M.M., Gervasi, D.C., Shimura, Y., Meroueh, O., Kotra, L.P., Galvez, B.G., Arroyo, A.G., *et al.* 2002. Complex pattern of membrane type 1 matrix metalloproteinase shedding. Regulation by autocatalytic cells surface inactivation of active enzyme. *J Biol Chem* 277:26340–26350.
Toth, M., Osenkowski, P., Hesek, D., Brown, S., Meroueh, S., Sakr, W., Mobashery, S., and Fridman, R. 2005. Cleavage at the stem region releases an active ectodomain of the membrane type 1 matrix metalloproteinase. *Biochem J* 387:497–506.
Toth, M., Sohail, A., Mobashery, S., and Fridman, R. 2006. MT1-MMP shedding involves an ADAM and is independent of its localization in lipid rafts. *Biochem Biophys Res Commun* 350:377–384.
Ueda, J., Kajita, M., Suenaga, N., Fujii, K., and Seiki, M. 2003. Sequence-specific silencing of MT1-MMP expression suppresses tumor cell migration and invasion: importance of MT1-MMP as a therapeutic target for invasive tumors. *Oncogene* 22:8716–8722.
Uekita, T., Itoh, Y., Yana, I., Ohno, H., and Seiki, M. 2001. Cytoplasmic tail-dependent internalization of membrane-type 1 matrix metalloproteinase is important for its invasion-promoting activity. *J Cell Biol* 155:1345–1356.
Van Damme, J., De Ley, M., Opdenakker, G., Billiau, A., De Somer, P., and Van Beeumen, J. 1985. Homogeneous interferon-inducing 22 K factor is related to endogenous pyrogen and interleukin-1. *Nature* 314:266–268.
Van den Steen, P.E., Dubois, B., Nelissen, I., Rudd, P.M., Dwek, R.A., and Opdenakker, G. 2002. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit Rev Biochem Mol Biol* 37:375–536.

Van den Steen, P.E., Opdenakker, G., Wormald, M.R., Dwek, R.A., and Rudd, P.M. 2001. Matrix remodelling enzymes, the protease cascade and glycosylation. *Biochim Biophys Acta* 1528:61–73.

Van den Steen, P.E., Van, A., I, Hvidberg, V., Piccard, H., Fiten, P., Jacobsen, C., Moestrup, S.K., Fry, S., Royle, L., Wormald, M.R., *et al.* 2006. The hemopexin and O-glycosylated domains tune gelatinase B/MMP-9 bioavailability via inhibition and binding to cargo receptors. *J Biol Chem* 281:18626–18637.

Van Wart, H.E. and Birkedal-Hansen, H. 1990. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci USA* 87:5578–5582.

Vargo-Gogola, T., Crawford, H.C., Fingleton, B., and Matrisian, L.M. 2002a. Identification of novel matrix metalloproteinase-7 (matrilysin) cleavage sites in murine and human Fas ligand. *Arch Biochem Biophys* 408:155–161.

Vargo-Gogola, T., Fingleton, B., Crawford, H.C., and Matrisian, L.M. 2002b. Matrilysin (matrix metalloproteinase-7) selects for apoptosis-resistant mammary cells in vivo. *Cancer Res* 62:5559–5563.

Varner, J.A. and Cheresh, D.A. 1996. Integrins and cancer. *Curr Opin Cell Biol* 8:724–730.

Vecchi, M., Rudolph-Owen, L.A., Brown, C.L., Dempsey, P.J., and Carpenter, G. 1998. Tyrosine phosphorylation and proteolysis. Pervanadate-induced, metalloprotease-dependent cleavage of the ErbB-4 receptor and amphiregulin. *J Biol Chem*273:20589–20595.

Velasco-Loyden, G., Arribas, J., and Lopez-Casillas, F. 2004. The shedding of betaglycan is regulated by pervanadate and mediated by membrane type matrix metalloprotease-1. *J Biol Chem* 279:7721–7733.

Verraes, S., Hornebeck, W., Polette, M., Borradori, L., and Bernard, P. 2001. Respective contribution of neutrophil elastase and matrix metalloproteinase 9 in the degradation of BP180 (type XVII collagen) in human bullous pemphigoid. *J Invest Dermatol* 117:1091–1096.

Visse, R. and Nagase, H. 2003. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 92:827–839.

von Bredow, D.C., Nagle, R.B., Bowden, G.T., and Cress, A.E. 1997. Cleavage of beta 4 integrin by matrilysin. *Exp Cell Res* 236:341–345.

Voronov, E., Shouval, D.S., Krelin, Y., Cagnano, E., Benharroch, D., Iwakura, Y., Dinarello, C.A., and Apte, R.N. 2003. IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci USA* 100:2645–2650.

Wachtel, M., Frei, K., Ehler, E., Fontana, A., Winterhalter, K., and Gloor, S.M. 1999. Occludin proteolysis and increased permeability in endothelial cells through tyrosine phosphatase inhibition. *J Cell Sci* 112:4347–4356.

Walcheck, B., Alexander, S.R., St Hill, C.A., and Matala, E. 2003. ADAM-17-independent shedding of L-selectin. *J Leukoc Biol* 74:389–394.

Waldhauer, I. and Steinle, A. 2006. Proteolytic release of soluble UL16-binding protein 2 from tumor cells. *Cancer Res* 66:2520–2526.

Walsh, D.M. and Selkoe, D.J. 2004. Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* 44:181–193.

Waskow, C., Paul, S., Haller, C., Gassmann, M., and Rodewald, H.R. 2002. Viable c-Kit(W/W) mutants reveal pivotal role for c-kit in the maintenance of lymphopoiesis. *Immunity* 17:277–288.

Watt, F.M. 2002. Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J* 21:3919–3926.

Wehrle-Haller, B. and Imhof, B.A. 2003. Integrin-dependent pathologies. *J Pathol* 200:481–487.

Weiske, J., Schoneberg, T., Schroder, W., Hatzfeld, M., Tauber, R., and Huber, O. 2001. The fate of desmosomal proteins in apoptotic cells. *J Biol Chem* 276:41175–41181.

Wewers, M.D. 2004. IL-1beta: an endosomal exit. *Proc Natl Acad Sci USA* 101:10241–10242.

White, A.R., Du, T., Laughton, K.M., Volitakis, I., Sharples, R.A., Xilinas, M.E., Hoke, D.E., Holsinger, R.M., Evin, G., Cherny, R.A., *et al.* 2006. Degradation of the Alzheimer disease amyloid beta-peptide by metal-dependent up-regulation of metalloprotease activity. *J Biol Chem* 281:17670–17680.

Whittaker, M., Floyd, C.D., Brown, P., and Gearing, A.J. 1999. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem Rev* 99:2735–2776.

Wilhelm, O.G., Wilhelm, S., Escott, G.M., Lutz, V., Magdolen, V., Schmitt, M., Rifkin, D.B., Wilson, E.L., Graeff, H., and Brunner, G. 1999. Cellular glycosylphosphatidylinositol-specific phospholipase D regulates urokinase receptor shedding and cell surface expression. *J Cell Physiol* 180:225–235.

Williams, L.M., Gibbons, D.L., Gearing, A., Maini, R.N., Feldmann, M., and Brennan, F.M. 1996. Paradoxical effects of a synthetic metalloproteinase inhibitor that blocks both p55 and p75 TNF receptor shedding and TNF alpha processing in RA synovial membrane cell cultures. *J Clin Invest* 97:2833–2841.

Wu, W., Samet, J.M., Silbajoris, R., Dailey, L.A., Sheppard, D., Bromberg, P.A., and Graves, L.M. 2004. Heparin-binding epidermal growth factor cleavage mediates zinc-induced epidermal growth factor receptor phosphorylation. *Am J Respir Cell Mol Biol* 30:540–547.

Yabkowitz, R., Meyer, S., Black, T., Elliott, G., Merewether, L.A., and Yamane, H.K. 1999. Inflammatory cytokines and vascular endothelial growth factor stimulate the release of soluble tie receptor from human endothelial cells via metalloprotease activation. *Blood* 93:1969–1979.

Yamada, H., Saito, F., Fukuta-Ohi, H., Zhong, D., Hase, A., Arai, K., Okuyama, A., Maekawa, R., Shimizu, T., and Matsumura, K. 2001. Processing of beta-dystroglycan by matrix metalloproteinase disrupts the link between the extracellular matrix and cell membrane via the dystroglycan complex. *Hum Mol Genet* 10:1563–1569.

Yan, L., Zucker, S., and Toole, B.P. 2005. Roles of the multifunctional glycoprotein, emmprin (basigin; CD147), in tumour progression. *Thromb Haemost* 93:199–204.

Yan, P., Hu, X., Song, H., Yin, K., Bateman, R.J., Cirrito, J.R., Xiao, Q., Hsu, F.F., Turk, J.W., Xu, J., *et al.* 2006. Matrix metalloproteinase-9 degrades amyloid-beta fibrils in vitro and compact plaques in situ. *J Biol Chem* 281:24566–24574.

Yang, Y., Estrada, E.Y., Thompson, J.F., Liu, W., and Rosenberg, G.A. 2007. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. *J Cereb Blood Flow Metab* 27:697–709.

Yazdani, U. and Terman, J.R. 2006. The semaphorins. *Genome Biol* 7:211.

Yin, K.J., Cirrito, J.R., Yan, P., Hu, X., Xiao, Q., Pan, X., Bateman, R., Song, H., Hsu, F.F., Turk, J., *et al.* 2006. Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism. *J Neurosci* 26:10939–10948.

Yoshisue, H. and Hasegawa, K. 2004. Effect of MMP/ADAM inhibitors on goblet cell hyperplasia in cultured human bronchial epithelial cells. *Biosci Biotechnol Biochem* 68:2024–2031.

Yoshiyama, Y., Sato, H., Seiki, M., Shinagawa, A., Takahashi, M., and Yamada, T. 1998. Expression of the membrane-type 3 matrix metalloproteinase (MT3-MMP) in human brain tissues. *Acta Neuropathol (Berl)*. 96:347–350.

Yu, Q. and Stamenkovic, I. 1999. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev* 13:35–48.

Yu, Q. and Stamenkovic, I. 2000. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 14:163–176.

Yu, W.H. and Woessner, J.F., Jr. 2000. Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7). *J Biol Chem* 275:4183–4191.

Yu, W.H., Woessner, J.F., Jr., McNeish, J.D., and Stamenkovic, I. 2002. 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.

Zou, Z., Chung, B., Nguyen, T., Mentone, S., Thomson, B., and Biemesderfer, D. 2004. Linking receptor-mediated endocytosis and cell signaling: evidence for regulated intramembrane proteolysis of megalin in proximal tubule. *J Biol Chem* 279:34302–34310.

Zucker, S., Hymowitz, M., Rollo, E.E., Mann, R., Conner, C.E., Cao, J., Foda, H.D., Tompkins, D.C., and Toole, B.P. 2001. Tumorigenic potential of extracellular matrix metalloproteinase inducer. *Am J Pathol* 158:1921–1928.

Zwick, E., Hackel, P.O., Prenzel, N., and Ullrich, A. 1999. The EGF receptor as central transducer of heterologous signalling systems. *Trends Pharmacol Sci* 20:408–412.

Editor: Michael M. Cox