

# Matrix metalloproteinases in multiple myeloma

## Review Article

**Els Van Valckenborgh, Kewal Asosingh, Ivan Van Riet, Ben Van Camp and Karin Vanderkerken\***

Department of Hematology and Immunology, Vrije Universiteit Brussel (VUB), Brussels, Belgium

**\*Correspondence:** Dr. Karin Vanderkerken, Vrije Universiteit Brussel, Department HEIM, Laarbeeklaan 103, B-1090 Brussels, Belgium; Phone: 0032 2 477 44 18; Fax: 0032 2 477 44 05; E-mail: Karin.Vanderkerken@vub.ac.be

**Key Words:** matrix metalloproteinases, multiple myeloma, angiogenesis, homing, osteolytic bone disease

**Abbreviations:** bone marrow (BM); bone marrow stromal cells (BMSCs); 1.25-dihydroxyvitamin D<sub>3</sub>, ([1.25(OH)<sub>2</sub>VitD<sub>3</sub>]); extracellular matrix (ECM); glycosylphosphatidylinositol (GPI); hepatocyte growth factor (HGF); human umbilical vein endothelial cells (HUVECs); insulin-like growth factor-1 (IGF-1); Interleukin-6 (IL-6); matrix metalloproteinases (MMPs); monoclonal gammopathy of unknown significance, (MGUS); Multiple myeloma (MM); Oncostatin M (OSM); tissue inhibitors of matrix metalloproteinases (TIMPs); transforming growth factor- $\beta$ , (TGF- $\beta$ ); tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )

Received: 30 March 2004; Accepted: 5 April 2004; electronically published: April 2004

## Summary

**Multiple myeloma is a B-cell malignancy characterized by the monoclonal proliferation of plasma cells in the bone marrow, the presence of monoclonal immunoglobulins in the serum, the development of osteolytic lesions and the induction of angiogenesis. Matrix metalloproteinases are described as endopeptidases and are known to be involved in cancer development. Formerly, it was believed that the enzymes were only important in the degradation of extracellular matrix components. However, new substrates have been discovered making the functions of matrix metalloproteinases extended and complex. Here, an overview has been given about the expression and regulation of matrix metalloproteinases in multiple myeloma. With the literature we demonstrate that the enzymes are involved in tumor growth, angiogenesis, homing and the development of osteolytic lesions, all important events in the progression of multiple myeloma.**

## I. Introduction

Multiple myeloma (MM) is a B-cell malignancy with several specific characteristics. Our group has demonstrated the postgerminal origin of MM cells (Bakkus et al, 1992). These cells migrate from the intravascular to the extravascular compartment of the bone marrow (BM), a process called "homing". In the BM, the myeloma cells receive signals from the microenvironment essential for survival and growth leading to the accumulation of the tumor cells in the BM. The malignant plasma cells produce a monoclonal immunoglobulin that can be detected in the serum of patients and can be used to follow the development of the disease. Osteoclast-activating factors and angiogenic factors, produced by MM cells and the BM environment, result in the induction of osteolytic lesions and the formation of new blood vessels (angiogenesis). In advanced stages of the disease, tumor cells can be observed in the peripheral blood and at extramedullary sites. Symptoms of MM are kidney problems, bone pain especially in the back or ribs, fatigue and recurrent infections. Despite a lot of research and progress in treatment, the disease remains incurable. More understanding of the biology of MM can lead to new

approaches to therapy and better treatments of patients. An interesting target are the matrix metalloproteinases (MMPs). It has been suggested that matrix metalloproteinases are involved in a number of events underlying MM progression. This review focuses on the expression, regulation and the role of MMPs in MM disease.

## II. Matrix metalloproteinases

Matrix metalloproteinases are a family of zinc-dependent endopeptidases involved in physiological (embryogenesis and wound healing) (Matrisian, 1990) and pathological (multiple sclerosis, rheumatoid arthritis and cancer) tissue degradation (Jackson et al, 2001; Lindberg et al, 2001; Vihinen and Kähäri, 2002). More than 20 members of the human MMP family are known. They are able to degrade structural components of the extracellular matrix (ECM) (reviewed by Sternlicht and Werb, 2001 and Vihinen and Kähäri, 2002). New substrates, like growth factors (GF), GF binding proteins, GF receptors, adhesion molecules, chemokines and inhibitors, have been discovered, making the functions of MMPs diverse and complex. They cannot only regulate migration and

invasion, but also cell growth, differentiation, angiogenesis and metastasis (Chang and Werb, 2001; Egeblad and Werb, 2002). Formerly, the members of the family were divided into subgroups depending on their substrate specificity (collagenases, gelatinases,

stromelysines and membrane-type MMPs). Because of the growing list of substrates, all MMPs are given a number and can be classified according to their structure (Egeblad and Werb, 2002).

**Table 1:** The human MMP family and their new substrates

Structural class	Enzyme names	New substrates
Minimal domain	MMP-7 (matrilysin)	1-PI, 1-AT, 4 integrin, FasL, TNF-, plasminogen, TFPI, E-cadherin, OPN, IgG, CTGF, syndecan-1, fibrinogen
	MMP-26 (matrilysin-2)	IGFBP-1, 1-PI, fibrinogen
Simple hemopexin domain	MMP-1 (collagenase-1)	1-AT, TFPI, CTGF, MCP-1, -2, -3 and -4, SAA, IGFBP-3, IL-1, AFP, SDF-1, MBL, 1-AC, 2-M, 1-PI, C1q, fibrinogen, TNF-
	MMP-3 (stromelysin-1)	1-AT, OPN, E-cadherin, IgG, CTGF, MCP-1, -2, -3 and -4, SAA3, IGFBP-3, IL-1, SDF-1, HB-EGF, FasL, MBL, uPA, plasminogen, PAI-1, (2)-antiplasmin, fibrinogen, 1-AC, 2-M, 1-PI, C1q, TNF-
	MMP-8 (collagenase-2)	1-AT, TFPI, MBL, CXCL-6, CXCL-9, CXCL-10, fibrinogen, 2-M, 1-PI, C1q
	MMP-10 (stromelysin-2)	Fibrinogen
	MMP-12 (metalloelastase)	Fibrinogen, factor XII, plasminogen, apolipoprotein, uPAR, MBP, 1-AT, pro-TNF, TFPI, 2-M, 1-PI
	MMP-13 (collagenase-3)	CTGF, MCP-3, SDF-1, factor XII
	MMP-19	IGFBP-3
	MMP-20 (enamelysin)	Amelogenin
Gelatin-binding	MMP-2 (gelatinase A)	MCP-3, IGFBP-3, IL-1, SAA, AFP, FGFR1, plasminogen, big endothelin-1, SDF-1, LTBP1, MBL, KiSS-1, 1-AC, 1-PI, C1q, fibrinogen, proTGF-, proTNF-
	MMP-9 (gelatinase B)	1-AT, plasminogen, TFPI, IL-1, SDF-1, LTBP1, IL-8, CXCL-6, CXCL-5, MBP, substance P, IGFBP-3, MBL, KiSS-1, B-crystallin, CXCL-9, CXCL-10, 2-M, 1-PI, C1q, fibrinogen, proTGF-, proTNF-
Furin-activated secreted	MMP-11 (stromelysin-3)	1-PI, IGFBP, 2-M
	MMP-28 (epilysin)	
Vitronectin-like insert	MMP-21	1-AT
Transmembrane	MMP-14 (MT1-MMP)	2-M, 1-PI, SDF-1, MCP-3, KiSS, factor XII, MBL, pro- v integrin, gC1qR, syndecan-1, CD44, tTG, fibrinogen, proTNF-
	MMP-15 (MT2-MMP)	tTG
	MMP-16 (MT3-MMP)	KiSS-1, syndecan-1, tTG
	MMP-24 (MT5-MMP)	KiSS-1
GPI-linked	MMP-17 (MT4-MMP)	pro-TNF-
	MMP-25 (MT6-MMP)	
Type II transmembrane	MMP-23	

Based on Sternlicht and Werb, 2001; Egeblad and Werb, 2002 and additional references: Winyard et al, 1991; Michaelis et al, 1992; Mitchell et al, 1993; Proost et al, 1993; Fowlkes et al, 1994; Sires et al, 1994; Chandler et al, 1996; Levi et al, 1996; Llano et al, 1997; Suzuki et al, 1997; von Bredow et al, 1997; Ugwu et al, 1998; Edelstein et al, 1999; Mañes et al, 1999; Fernandez-Patron et al, 1999; Powell et al, 1999; Belaouaj et al, 2000; English et al, 2000; Lijnen et al, 2000, 2001; McQuibban et al, 2000, 2001, 2002; Van Den Steen et al, 2000, 2003a, 2003b; Agnihotri et al, 2001; Belkin et al, 2001; Matsuno et al, 2001; Stix et al, 2001; Andolfo et al, 2002; Butler et al, 2002; Cunningham et al, 2002; Dallas et al, 2002; Deryugina et al, 2002; Gearing et al, 2002; Hashimoto et al, 2002; Li et al, 2002; Park et al, 2002; Rozanov et al, 2002; Endo et al, 2003; Marchenko et al, 2003; Sadowski et al, 2003; Starckx et al, 2003; Takino et al, 2003; Nakamura et al, 2004.

**Abbreviations:**

1-PI: 1-protease inhibitor; 1-AT: 1-antitrypsin; TNF: tumor necrosis factor; TFPI: tissue factor pathway inhibitor; OPN: osteopontin; CTGF: connective tissue growth factor; IGFBP: insulin-like growth factor-binding protein; MCP: monocyte chemoattractant protein; SAA: serum amyloid A; IL: interleukin; AFP: amyloid fibril protein; SDF: stromal cell-derived factor; MBL: mannose-binding lectin; 1-AC: 1-antichymotrypsin; 2-M: 2-macroglobulin; HB-EGF: heparin-binding epidermal growth factor-like growth factor; uPA: urokinase-type plasminogen activator; PAI: plasminogen activator inhibitor; uPAR: urokinase plasminogen activator receptor; MBP: myelin basic protein; FGFR: fibroblast growth factor receptor; LTBP: latent TGF-beta-binding protein; TGF: transforming growth factor; tTG: tissue transglutaminase.

The MMPs can be divided into 8 structural groups: minimal-domain MMPs, simple hemopexin-domain-containing MMPs, gelatin-binding MMPs, furin-activated secreted MMPs, vitronectin-like insert MMPs, transmembrane MMPs, glycosylphosphatidylinositol (GPI)-anchored MMPs and type II transmembrane MMPs. Enzymes belonging to the first 5 groups are secreted, the others are membrane-type MMPs. **Table 1** gives an overview of the human MMP family and their new substrates.

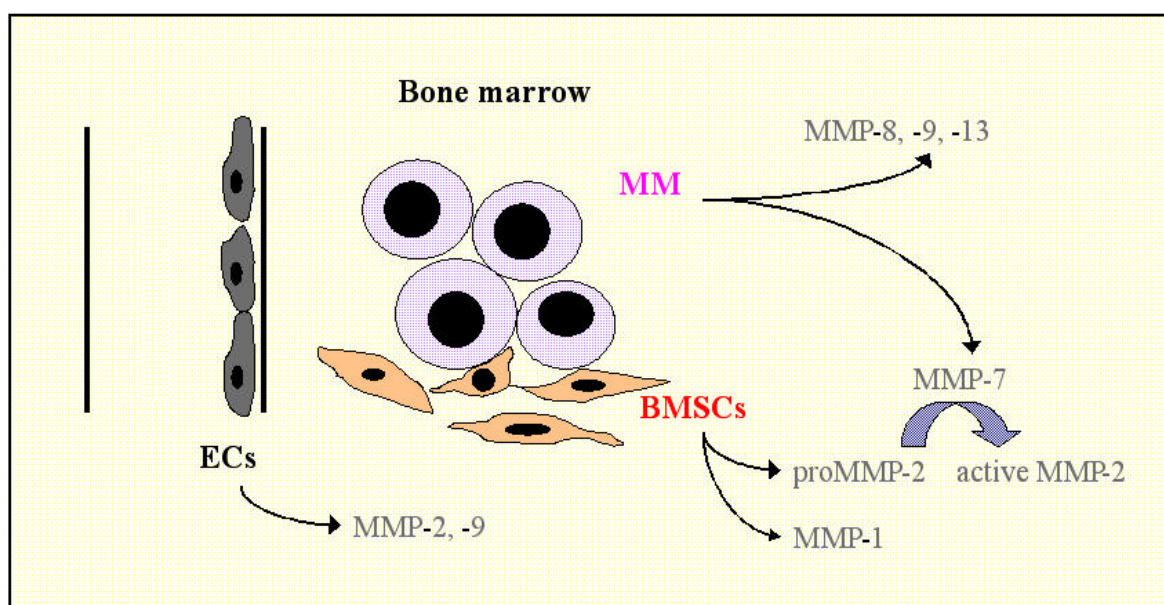
The production of MMPs can be regulated at different levels. The transcription is under control of several cytokines, growth factors and tumor promoters. The enzymes are synthesized as inactive proenzymes and are activated by proteolytic cleavage of the propeptide domain, where the cysteine residue in the conserved sequence interacts with the zinc ion in the catalytic domain. Activation of MMPs can be achieved by interaction with other active MMPs or proteinases from the plasminogen/plasmin system. The activity of MMPs can be inhibited by endogenous inhibitors with the most important tissue inhibitors of metalloproteinases (TIMPs). At this moment, four TIMPs have been described. The balance between active MMPs and TIMPs determines the net proteolytic activity of MMPs. This equilibrium is highly regulated in normal tissue remodeling, but is disturbed in pathological conditions.

### III. Matrix metalloproteinases in multiple myeloma: expression, regulation and activation

#### A. Expression of MMPs in MM

Several groups reported the expression of MMPs in MM cells. The production of MMP-9 has been demonstrated in purified myeloma cells isolated from MM patients (Barillé et al, 1997) and 5T33MM cells isolated

from the 5T33MM mouse model (Van Valckenborgh et al, 2002a). MMP-2 was not secreted by these cells. On the contrary, Vacca et al. were also able to detect MMP-2 in the human MM cell line U266 (1998) and bone marrow plasma cells from MM patients (1999). MMP-2 and -9 are gelatinases and belong to the gelatin-binding MMPs. MMP-7, a minimal domain MMP, has a large number of substrates and is produced by human MM cell lines and MM cells from patients (Barillé et al, 1999). Interestingly, MMP-2, -7 and -9 are involved in several processes in cancer, like tumor growth, angiogenesis, invasion and metastasis (Powell et al, 1993; Watanabe et al, 1993; Hua and Muschel, 1996; Deryugina et al, 1997; Wilson et al, 1997; Hasegawa et al, 1998; Itoh et al, 1998; Itoh et al, 1999; Nishizuka et al, 2001; Huang et al, 2002). The expression of MMP-8 and -13 has also been investigated and detected in the human MM cell line RPMI 8226 and malignant plasma cells from plasmacytomas (Wahlgren et al, 2001). Our group was able to detect MMP-8 and -13 by RT-PCR in 5T2MM-diseased bone marrow cells (Van Valckenborgh et al, 2003). MMP-8 and -13 are collagenases belonging to the structural group of the simple hemopexin-domain containing MMPs. The enzymes are expressed in several cancers and it is suggested that they are involved in invasion (Pendás et al, 2000; Kim et al, 2001; Ala-Aho et al, 2002; Moilanen et al, 2002). However, their possible role in the different processes in tumor progression is not yet defined. Since the bone marrow stromal microenvironment is involved in the development of MM, it appears important to investigate the production of MMPs in bone marrow stromal cells (BMSCs). BMSCs secrete MMP-2 and MMP-1 (Barillé et al, 1997). Endothelial cells (ECs) isolated from MM patients were compared with human umbilical vein endothelial cells (HUVECs). MMECs secreted more (3-4 times higher) active MMP-2 and -9 than HUVECs (Vacca et al, 2003). **Figure 1** gives an overview of the expression of MMPs in MM.



**Figure 1.** The secretion of MMPs by multiple myeloma (MM) cells, bone marrow stromal cells (BMSCs) and endothelial cells (ECs) in multiple myeloma-diseased bone marrow.

Expression of MMPs has also been investigated in other hematological malignancies. MMP-2 and -9 are the most studied and one or both enzymes seems to be produced by leukemia and lymphoma cells (Van Ranst et al, 1991; Ries et al, 1996, 1999; Devy et al, 1997; Kossakowska et al, 1998; Vacca et al, 1998).

## B. Regulation of MMPs in MM

The expression of MMPs can be regulated by cytokines, hormones, growth factors, cell-matrix and cell-cell interactions.

### 1. Cytokines and hormones

Several cytokines are involved in the pathogenesis of MM. Therefore, it is interesting to investigate the role of these cytokines in the regulation of MMPs. Interleukin-6 (IL-6), Oncostatin M (OSM), IL-1, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), transforming growth factor- $\beta$  (TGF- $\beta$ ) and IL-10 were not able to regulate MMP-2 and MMP-9 production in respectively BMSCs and MM cells (Barillé et al, 1997). Dexamethasone and 1.25(OH) $_2$ VitD $_3$ , which can inhibit myeloma cell growth, did not regulate MMP-2 and -9. MMP-1 on the other hand is upregulated by OSM, IL-1 and TNF- $\alpha$  and downregulated by dexamethasone (Barillé et al, 1997). The receptor for IL-6 consists of a signal-transducing molecule IL-6R $\alpha$  and a specific ligand-binding protein IL-6R $\beta$ . This molecule can be found on the membrane, but also exists in a soluble form, sIL-6R $\beta$ . The latter molecule (sIL-6R $\beta$ ) is able to significantly increase MMP-1 and MMP-2 production by BMSCs (Barillé et al, 2000).

### 2. Bone marrow microenvironment

MM cells are in contact with the bone marrow microenvironment. Cocultures of MM cells with BMSCs is a way to investigate the role of the BM microenvironment in the regulation of MMPs. MMP-1 production by BMSCs is upregulated in response to MM cells and also MMP-9 production is slightly increased in cocultures (Barillé et al, 1997). The BM microenvironment is a complex structure of various extracellular components and many cell types. BM endothelial cells are the first cells encountered by the MM cells upon entry into the BM environment from the blood circulation. Interaction of BMECs with MM cells induces MMP-9 expression in MM cells. This was demonstrated in the 5T33MM mouse model (Van Valckenborgh et al, 2002a) and in human MM cells (Vande Broek et al, 2004). This is similar in T lymphoma where MMP-9 secretion was enhanced following coculture of lymphoma cells and ECs and where the role of ICAM-1/LFA-1 was evidenced in this upregulation (Aoudjit et al, 1998). However, in MM, it was demonstrated that hepatocyte growth factor (HGF) was involved in the induction of MMP-9 (Vande Broek et al, 2004).

MM cells express the integrin  $\alpha$ 3 which can bind to ECM proteins present in the BM, like vitronectin and fibronectin. MM cells incubated with VN and FN resulted in an increased release of MMP-2 and MMP-9. This

upregulation can be inhibited by a neutralizing anti- $\alpha$ 3 antibody (Ria et al, 2002).

## 3. Syndecan-1

It has been described that syndecan-1 is involved in the regulation of MMP-9. Syndecan-1 is a transmembrane heparan sulfate proteoglycan able to inhibit cell invasion, mediate cell-cell adhesion and regulate cell growth. Expression of syndecan-1 on the surface of MM cells downregulates MMP-9 production (Kaushal et al, 1999). Interestingly, syndecan-1 is shed from the surface of myeloma cells and it has been suggested that a non-matrix-type metalloproteinase, like ADAM (a disintegrin and metalloproteinase) is responsible for this process (Holen et al, 2001). A recent report demonstrated that soluble syndecan-1 promotes MM growth *in vivo* and enhances invasion (Yang et al, 2002). Inhibition of the shedding of syndecan-1 might decrease MMP-9 production by MM cells and might decrease MM progression.

## C. Activation of MMPs in MM

Most of the MMPs are secreted as inactive proenzymes and are activated extracellularly by proteolytic cleavage. Interaction of MMPs with each other can lead to their activation. MMP-7, secreted by MM cells, is responsible for the activation of MMP-2 produced by BMSCs (Barillé et al, 1999). The uPA/plasmin system is also involved in MMP activation (Werb et al, 1977). This was demonstrated with leukemia cells which produce significant amounts of proMMP-9. Activation was achieved by adding plasminogen to the leukemia cells (Devy et al, 1997). uPA converts plasminogen to plasmin which in turn can activate MMPs. Recent results indicate that uPA is expressed by myeloma cells (Hjertner et al, 2000; Asosingh et al, 2002). Addition of plasminogen to proMMP-9 secreting 5T33MM *in vivo* cells resulted in the activation of proMMP-9 (unpublished observations).

## IV. The role of matrix metalloproteinases in multiple myeloma

### A. MMPs and tumor growth

Several reports demonstrated that treatment with MMP inhibitors resulted in a significant decrease of tumor growth (Koivunen et al, 1999; Matsushita et al, 2001; Winding et al, 2002). This suggests that MMPs can generate growth-promoting signals. Two important growth factors in MM are IL-6 and insulin-like growth factor-1 (IGF-1). It has been described that the specific ligand-binding protein of the receptor for IL-6, IL-6R $\beta$ , is released from MM cells by proteolytic cleavage (Thabard et al, 1999). Soluble IL-6R $\beta$  binds to IL-6 leading subsequently to an increased proliferation of MM cells. IGFBPs regulate the bioavailability of IGF by binding the growth factor and are described, especially IGFBP-3, as one of the new substrates of MMPs (see **Table 1**). Serum levels of IGFBP-3 are decreased in MM patients, suggesting that the protein is cleaved (Standal et al, 2002).

Shedding of IGFBPs might increase the amount of bioavailable IGF-1 resulting in increased tumor growth. It is not yet known which enzyme is responsible for the shedding of IL-6R and IGFBP-3 in MM. It has been suggested that members of the ADAM family might be responsible for the cleavage of growth factors (Hargreaves et al, 1998; Standal et al, 2002). Interesting to investigate is whether inhibiting the process of shedding might result in the inhibition of MM progression. Treatment of 5T2MM-diseased mice with the broadspectrum MMP inhibitor SC-964 resulted in a decreased number of tumor cells in the BM compared to vehicle treated animals (Van Valckenborgh et al, 2003). A minor effect of SC-964 on the proliferation of tumor cells has been demonstrated by <sup>3</sup>H-thymidine incorporation (unpublished observations).

### B. MMPs and angiogenesis

Angiogenesis is the formation of new blood vessels and in solid tumors it has been demonstrated that it is required for tumor growth. Like in solid tumors, it has been demonstrated that neovascularization is enhanced in MM (Vacca et al, 1994; Van Valckenborgh et al, 2002b). MMPs are involved in the different processes of angiogenesis, like proteolysis of the ECM, migration of ECs and the release of angiogenic factors from the ECM (Moses, 1997). Vacca et al (1999) demonstrated a larger microvessel area and a higher secretion of MMP-2 and -9 in patients with active MM than in those with nonactive MM, MGUS (monoclonal gammopathy of unknown significance) or control subjects. ECs isolated from the bone marrow of MM patients produce a higher level of MMP-2 and -9 compared to HUVECs (Vacca et al, 2003). Treatment of MM-diseased mice with the broadspectrum MMP inhibitor SC-964 resulted in an almost complete inhibition of angiogenesis (Van Valckenborgh et al, 2003). This was confirmed in the rat aortic ring assay where the outgrowth of blood vessels was significantly decreased with the MMP inhibitor SC-964 (unpublished observations). It has to be elucidated whether selective targeting of the enhanced neovascularisation in MM results in a protective effect against MM disease.

### C. MMPs and homing of MM cells

MM cells home from the intravascular to the extravascular compartment of the bone marrow. This is a multistep process consisting of adhesion of myeloma cells to the ECs followed by chemoattraction and migration through the endothelium and invasion through the basement membrane into the BM. In a recent report, the differential homing capacity of CD45- and CD45+ MM cells was investigated in the 5TMM mouse model (Asosingh et al, 2002). CD45- MM cells have a decreased homing capacity compared to CD45+ MM cells. This could be due to the higher MMP-9 secretion by CD45+ compared to CD45- cells which secrete little or no MMP-9. Further experiments revealed a significant lower invasive capacity of CD45- MM cells compared to CD45+ MM cells. Treatment of the 5TMM cells with the gelatinase inhibitor EGCG resulted in the inhibition of invasion and thus demonstrated the involvement of MMP-

9 in invasion, the last step of homing. The upregulation of MMP-9 after interaction of MM cells with BMECs also indicates that MMP-9 might play a role in the homing process (Van Valckenborgh et al, 2002a; Vande Broek et al, 2004).

### D. MMPs and osteolytic bone disease in MM

An important characteristic of MM is the development of osteolytic lesions. MMPs play a role in normal bone remodeling. MMPs are involved in osteoclast recruitment to sites of bone remodeling (Sato et al, 1998) and the enzymes can degrade mineralized bone matrix (Holliday et al, 1997; Everts et al, 1998). In several cancers, the use of MMP inhibitors have clearly evidenced a role of MMPs in osteolytic bone disease. In the SCID-human model of prostate cancer metastasis, treatment with a broadspectrum MMP inhibitor batimastat prevented mineralized trabeculae degradation *in vivo* and reduced the number of osteoclasts on trabecular surfaces (Nemeth et al, 2002). Also in breast cancer, MMP inhibitors inhibited the development of osteolytic lesions in mice (Lee et al, 2001; Winding et al, 2002). Collagen I is a major constituent of the bone and can be degraded by collagenases like MMP-1, -8 and -13. The denatured collagen I becomes a substrate for MMP-2 and -9. Our group performed a study to investigate the role of MMPs in the development of osteolytic bone disease in MM. Treatment of 5T2MM-diseased mice with the MMP inhibitor SC-964 resulted in a significant decrease in the number of osteolytic lesions and the prevention of cancellous bone loss induced by the presence of 5T2MM cells (Van Valckenborgh et al, 2003). Other evidence suggesting the role of MMPs in osteolytic bone disease is the inhibition of MMPs by biphosphonates. These are used as therapy in MM for preventing bone resorption. Zoledronate significantly inhibits MMP-1 secretion by BMSCs, but strongly upregulates MMP-2 production (Derenne et al, 1999). Clodronate can inhibit *in vitro* the activities of several MMPs, like MMP-2, -9, -13 and MT1-MMP (Teronen et al, 2000).

### V. Natural inhibitors in multiple myeloma

TIMPs are the natural inhibitors of MMPs, but it has been suggested that they are multifunctional. There have been 4 TIMPs described and they are called TIMP-1, -2, -3 and -4. There is some controversy on the functions of TIMPs in cancer development. Because they are able to inhibit MMPs, it was believed that they could inhibit tumor growth, invasion, angiogenesis and metastasis. Several studies confirm this hypothesis (Ahonen et al, 1998; Hajitou et al, 2001; Bloomston et al, 2002; Spurbeck et al, 2002; Ikenaka et al, 2003). However, it has also been demonstrated that high TIMP levels in certain types of malignant tumors in humans are associated with poor outcome (Curran and Murray, 1999; McCarthy et al, 1999). This could be due to the multifunctional role of TIMPs. TIMP-1 and -2 are able to stimulate the growth of

several cells (Docherty et al, 1985; Hayakawa et al, 1992, 1994; Gomez et al, 1997) and TIMP-2 has been described to be involved in the activation of proMMP-2 (Hernandez-Barrantes et al, 2000). TIMP-3 possesses pro-apoptotic capacity (Ahonen et al, 1998; Baker et al, 1999), whereas TIMP-1 have anti-apoptotic effects on certain cell types (Guedez et al, 1998; Li et al, 1999). Recently, DNA array demonstrated a higher level of TIMP-1 and the same level of TIMP-2 in the MMECs compared to the HUVECs (Vacca et al, 2003). This is the only report where TIMPs were investigated in MM. More research is necessary to find out more about the expression and role of TIMPs in MM disease.

Neovastat is an orally bioavailable extract from shark cartilage able to inhibit the activity of MMP-2, -9, -12 and -13 and has also been described to be anti-angiogenic. A phase II clinical trial is going on to evaluate the efficacy of neovastat as monotherapy treatment for patients with MM not responding to standard therapies (Vihinen and Kähäri, 2002).

## VI. Conclusion

Research on MMPs in MM demonstrated that certain enzymes are expressed in the tumor cells and the BM microenvironment and that they are involved in certain processes important for the development of MM. Formerly, it was believed that MMPs were only necessary for the degradation of several components of the ECM. Recently, it has been described that the enzymes are also able to cleave growth factors, cytokines and adhesion molecules resulting in a more complex role of MMPs. The multifunctional role of MMPs suggests further investigations for the recently discovered MMPs in their expression and role in MM. Although clinical trials with MMP inhibitors have not been promising, MMPs are still interesting targets for therapy. More knowledge about the function of the specific MMPs is needed for the beginning of new clinical trials. Recently, it has been demonstrated in a T-cell lymphoma model that an inhibitor with greater selectivity/specificity for MMP-9 in vitro showed greater efficacy against liver metastasis in vivo (Arlt et al, 2002). The development of specific inhibitors for the different MMPs makes it possible to investigate the role of each MMP in MM disease. TIMPs, the natural inhibitors of MMPs and also described as multifunctional molecules, have not yet been described in MM. It is interesting to know whether they are expressed in MM cells and what impact the molecules will have on MM development when they are overexpressed.

## Acknowledgements

This work was financially supported by the Onderzoeksraad-Vrije Universiteit Brussel (OZR-VUB), Fonds voor Wetenschappelijk Onderzoek-Vlaanderen, Belgische Federatie tegen Kanker, Fortis. Karin Vanderkerken and Kewal Asosingh are postdoctoral fellows of the "Fonds voor Wetenschappelijk Onderzoek-Vlaanderen" (FWO-VI).

## References

- Agnihotri R, Crawford HC, Haro H, Matrisian LM, Havrda MC, Liaw L (2001) Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). **J Biol Chem.** 276, 28261-7
- Ahonen M, Baker AH, Kähäri VM (1998) Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. **Cancer Res.** 58, 2310-5
- Ala-Aho R, Johansson N, Baker AH, Kähäri VM (2002) Expression of collagenase-3 (MMP-13) enhances invasion of human fibrosarcoma HT-1080 cells. **Int J Cancer** 97, 283-9
- Andolfo A, English WR, Resnati M, Murphy G, Blasi F, 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
- Aoudjit F, Potworowski EF, St-Pierre Y (1998) Bi-directional induction of matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 during T lymphoma/endothelial cell contact: implication of ICAM-1. **J Immunol.** 160,2967-73
- Arlt M, Kopitz C, Pennington C, Watson KL, Krell HW, Bode W, Gansbacher B, Khokha R, Edwards DR, Krüger A (2002) Increase in gelatinase-specificity of matrix metalloproteinase inhibitors correlates with antimetastatic efficacy in a T-cell lymphoma model. **Cancer Res.** 62, 5543-50
- Asosingh K, Menu E, Van Valckenborgh E, Vande Broek I, Van Riet I, Van Camp B, Vanderkerken K (2002) Mechanisms involved in the differential bone marrow homing of CD45 subsets in 5T murine models of myeloma. **Clin Exp Metastasis** 19, 583-91
- Baker AH, George SJ, Zaltsman AB, Murphy G, Newby AC (1999) Inhibition of invasion and induction of apoptotic cell death of cancer cell lines by overexpression of TIMP-3. **Br J Cancer** 79, 1347-55
- Bakkus MHC, Heirman C, Van Riet I, Van Camp B, Thielemans K (1992) Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intraclonal variation. **Blood** 80, 2326-35
- Barillé S, Akhouni C, Collette M, Mellier MP, Rapp MJ, Harousseau JL, Bataille R, Amiot M (1997) Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. **Blood** 90, 1649-55
- Barillé S, Bataille R, Rapp MJ, Harousseau JL, Amiot M (1999) Production of metalloproteinase-7 (matrilysin) by human myeloma cells and its potential involvement in metalloproteinase-2 activation. **J Immunol.** 163, 5723-8
- Barillé S, Collette M, Thabard W, Bleunven C, Bataille R, Amiot M (2000) Soluble IL-6R alpha upregulated IL-6, MMP-1 and MMP-2 secretion in bone marrow stromal cells. **Cytokine** 12, 1426-9
- Belaouaj AA, Li A, Wun TC, Welgus HG, Shapiro SD (2000) Matrix metalloproteinases cleave tissue factor pathway inhibitor. Effects on coagulation. **J Biol Chem.** 275, 27123-8
- Belkin AM, Akimov SS, Zaritskaya LS, Ratnikov BI, Deryugina EI, Strongin AY (2001) Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion. **J Biol Chem.** 276, 18415-22
- Bloomston M, Shafii A, Zervos EE, Rosemurgy AS (2002) TIMP-1 overexpression in pancreatic cancer attenuates tumor growth, decreases implantation and metastasis, and inhibits angiogenesis. **J Surg Res.** 102, 39-44
- Butler GS, Sim D, Tam E, Devine D, Overall CM (2002) Mannose-binding lectin (MBL) mutants are susceptible to

- matrix metalloproteinase proteolysis: potential role in human MBL deficiency. **J Biol Chem.** 277, 17511-9
- Chandler S, Cossins J, Lury J, Wells G (1996) Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. **Biochem Biophys Res Commun.** 228, 421-9
- Chang C, Werb Z (2001) The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. **Trends Cell Biol.** 11, S37-43
- Cunningham AC, Hastly KA, Enghild JJ, Mast AE (2002) Structural and functional characterization of tissue factor pathway inhibitor following degradation by matrix metalloproteinase-8. **Biochem J.** 367, 451-8
- Curran S, Murray GI (1999) Matrix metalloproteinases in tumour invasion and metastasis. **J Pathol.** 189, 300-8
- Dallas SL, Rosser JL, Mundy GR, Bonewald LF (2002) Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. **J Biol Chem.** 277, 21352-60
- Derenne S, Amiot M, Barillé S, Collette M, Robillard N, Berthaud P, Harousseau JL, Bataille R (1999) Zoledronate is a potent inhibitor of myeloma cell growth and secretion of IL-6 and MMP-1 by the tumoral environment. **J Bone Miner Res.** 14, 2048-56
- Deryugina EI, Luo GX, Reisfeld RA, Bourdon MA, Strongin A (1997) Tumor cell invasion through matrigel is regulated by activated matrix metalloproteinase-2. **Anticancer Res.** 17, 3201-10
- Deryugina EI, Ratnikov BI, Postnova TI, Rozanov DV, Strongin AY (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, 9749-56
- Devy L, Noël A, Baramova E, Bajou K, Trentesaux C, Jardillier JC, Foidart JM, Jeannesson P (1997) Production and activation of matrix metalloprotease-9 (MMP-9) by HL-60 promyelocytic leukemia cells. **Biochem Biophys Res Commun.** 238, 842-6
- Docherty AJ, Lyons A, Smith BJ, Wright EM, Stephens PE, Harris TJ, Murphy G, Reynolds JJ. (1985) Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. **Nature** 318, 66-9
- Edelstein C, Shapiro SD, Klezovitch O, Scanu AM (1999) Macrophage metalloelastase, MMP-12, cleaves human apolipoprotein(a) in the linker region between kringle IV-4 and IV-5. Potential relevance to lipoprotein(a) biology. **J Biol Chem.** 274, 10019-23
- Egeblad, M., and Werb, Z (2002) New functions for the matrix metalloproteinases in cancer progression. **Nat Rev Cancer** 2, 161-174
- Endo K, Takino T, Miyamori H, Kinsen H, Yoshizaki T, Furukawa M, Sato H (2003) Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. **J Biol Chem.** 278, 40764-70
- English WR, Puente XS, Freije JM, Knauper V, Amour A, Merryweather A, Lopez-Otin C, 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, 14046-55
- Everts V, Delaisse JM, Korper W, Beertsen W (1998) Cysteine proteinases and matrix metalloproteinases play distinct roles in the subosteoclastic resorption zone. **J Bone Miner Res.** 13, 1420-30
- Fernandez-Patron C, Radomski MW, Davidge ST (1999) Vascular matrix metalloproteinase-2 cleaves big endothelin-1 yielding a novel vasoconstrictor. **Circ Res.** 82,906-11
- Fowlkes JL, Enghild JJ, Suzuki K, Nagase H (1994) Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures. **J Biol Chem.** 269, 25742-6
- Gearing AJ, Thorpe SJ, Miller K, Mangan M, Varley PG, Dudgeon T, Ward G, Turner C, Thorpe R (2002) Selective cleavage of human IgG by the matrix metalloproteinases, matrilysin and stromelysin. **Immunol Lett.** 81, 41-8
- Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP (1997) Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. **Eur J Cell Biol.** 74, 111-22
- Guedez L, Stetler-Stevenson WG, Wolff L, Wang J, Fukushima P, Mansoor A, Stetler-Stevenson M (1998) In vitro suppression of programmed cell death of B cells by tissue inhibitor of metalloproteinases-1. **J Clin Invest.** 102, 2002-10
- Hajitou A, Sounni NE, Devy L, Grignet-Debrus C, Lewalle JM, Li H, Deroanne CF, Lu H, Colige A, Nusgens BV, Franken F, Maron A, Yeh P, Perricaudet M, Chang Y, Soria C, Calberg-Bacq CM, Foidart JM, Noël A (2001) Down-regulation of vascular endothelial growth factor by tissue inhibitor of metalloproteinase-2: effect on in vivo mammary tumor growth and angiogenesis. **Cancer Res.** 61,3450-7
- Hargreaves PG, Wang F, Antcliff J, Murphy G, Lawry J, Russell RG, Croucher PI (1998) Human myeloma cells shed the interleukin-6 receptor: inhibition by tissue inhibitor of metalloproteinase-3 and a hydroxamate-based metalloproteinase inhibitor. **Br J Haematol.** 101, 694-702
- Hasegawa S, Koshikawa N, Momiyama N, Moriyama K, Ichikawa Y, Ishikawa T, Mitsuhashi M, Shimada H, Miyazaki K (1998) Matrilysin-specific antisense oligonucleotide inhibits liver metastasis of human colon cancer cells in a nude mouse model. **Int J Cancer** 76, 812-6
- Hashimoto G, Inoki I, Fujii Y, Aoki T, Ikeda E, Okada Y (2002) Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor 165. **J Biol Chem.** 277, 36288-95
- Hayakawa T, Yamashita K, Tanzawa K, Uchijima E, Iwata K (1992) Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum. **FEBS Lett.** 298, 29-32
- Hayakawa T, Yamashita K, Ohuchi E, Shinagawa A (1994) Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2). **J Cell Sci.** 107, 2373-9
- Hernandez-Barrantes S, Toth M, Bernardo MM, Yurkova M, Gervasi DC, Raz Y, Sang QA, Fridman R (2000) Binding of active (57 kDa) membrane type 1-matrix metalloproteinase (MT1-MMP) to tissue inhibitor of metalloproteinase (TIMP)-2 regulates MT1-MMP processing and pro-MMP-2 activation. **J Biol Chem.** 275, 12080-9
- Hjertner O, Qvigstad G, Hjorth-Hansen H, Seidel C, Woodliff J, Epstein J, Waage A, Sundan A, Børset M (2000) Expression of urokinase plasminogen activator and the urokinase plasminogen activator receptor in myeloma cells. **Br J Haematol.** 109, 815-22
- Holen I, Drury NL, Hargreaves PG, Croucher PI (2001) Evidence of a role for a non-matrix-type metalloproteinase activity in the shedding of syndecan-1 from human myeloma cells. **Br J Haematol.** 114, 414-21
- Holliday LS, Welgus HG, Fliszar CJ, Veith GM, Jeffrey JJ, Gluck SL (1997) Initiation of osteoclast bone resorption by interstitial collagenase. **J Biol Chem.** 272, 22053-8
- Hua J, Muschel RJ (1996) Inhibition of matrix metalloproteinase 9 expression by a ribozyme blocks metastasis in a rat sarcoma model system. **Cancer Res.** 56, 5279-84
- Huang S, Van Arsdall M, Tedjarati S, McCarty M, Wu W, Langley R, Fidler IJ (2002) Contributions of stromal

- metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. **J Natl Cancer Inst.** 94, 1134-42
- Ikenaka Y, Yoshiji H, Kuriyama S, Yoshii J, Noguchi R, Tsujinoue H, Yanase K, Namisaki T, Imazu H, Masaki T, Fukui H (2003) Tissue inhibitor of metalloproteinases-1 (TIMP-1) inhibits tumor growth and angiogenesis in the TIMP-1 transgenic mouse model. **Int J Cancer** 105, 340-6
- Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S (1998) Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. **Cancer Res.** 58, 1048-51
- Itoh T, Tanioka M, Matsuda H, Nishimoto H, Yoshioka T, Suzuki R, Uehira M (1999) Experimental metastasis is suppressed in MMP-9-deficient mice. **Clin Exp Metastasis** 17, 177-81
- Jackson C, Nguyen M, Arkell J, Sambrook P (2001) Selective matrix metalloproteinase (MMP) inhibition in rheumatoid arthritis--targeting gelatinase A activation. **Inflamm Res.** 50, 183-6
- Kaushal GP, Xiong X, Athota AB, Rozypal TL, Sanderson RD, Kelly T (1999) Syndecan-1 expression suppresses the level of myeloma matrix metalloproteinase-9. **Br J Haematol.** 104, 365-73
- Kim MH, Albertsson P, Xue Y, Nannmark U, Kitson RP, Goldfarb RH (2001) Expression of neutrophil collagenase (MMP-8) in Jurkat T leukemia cells and its role in invasion. **Anticancer Res.** 21, 45-50
- Koivunen E, Arap W, Valtanen H, Rainisalo A, Medina OP, Heikkilä P, Kantor C, Gahmberg CG, Salo T, Kontinen YT, Sorsa T, Ruoslahti E, Pasqualini R (1999) Tumor targeting with a selective gelatinase inhibitor. **Nat Biotechnol.** 17, 768-74
- Kossakowska AE, Hinek A, Edwards DR, Lim MS, Zhang CL, Breitman DR, Prusinkiewicz C, Stabbler AL, Urbanski LS, Urbanski SJ (1998) Proteolytic activity of human non-Hodgkin's lymphomas. **Am J Pathol.** 152, 565-76
- Lee J, Weber M, Mejia S, Bone E, Watson P, Orr W (2001) A matrix metalloproteinase inhibitor, batimastat, retards the development of osteolytic bone metastases by MDA-MB-231 human breast cancer cells in Balb C nu/nu mice. **Eur J Cancer** 37, 106-13
- Levi E, Fridman R, Miao HQ, Ma YS, Yayon A, Vlodavsky I (1996) Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1. **Proc Natl Acad Sci U S A.** 93, 7069-74
- Li G, Fridman R, Kim HR (1999) Tissue inhibitor of metalloproteinase-1 inhibits apoptosis of human breast epithelial cells. **Cancer Res.** 59, 6267-75
- Lijnen HR, Arza B, Van Hoef B, Collen D, Declerck PJ. (2000) Inactivation of plasminogen activator inhibitor-1 by specific proteolysis with stromelysin-1 (MMP-3). **J Biol Chem.** 275, 37645-50
- Lijnen HR, Van Hoef B, Collen D (2001) Inactivation of the serpin alpha(2)-antiplasmin by stromelysin-1. **Biochim Biophys Acta** 1547, 206-13
- Lindberg RL, De Groot CJ, Montagne L, Freitag P, van der Valk P, Kappos L, Leppert D (2001) The expression profile of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in lesions and normal appearing white matter of multiple sclerosis. **Brain** 124, 1743-53
- Li Q, Park PW, Wilson CL, Parks WC (2002) Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. **Cell** 111, 635-46
- Llano E, Pendas AM, Knauper V, Sorsa T, Salo T, Salido E, Murphy G, Simmer JP, Bartlett JD, Lopez-Otin C (1997) Identification and structural and functional characterization of human enamelysin (MMP-20). **Biochemistry** 36, 15101-8
- Mañes S, Llorente M, Lacalle RA, Gomez-Mouton C, Kremer L, Mira E, Martinez-A C (1999) The matrix metalloproteinase-9 regulates the insulin-like growth factor-triggered autocrine response in DU-145 carcinoma cells. **J Biol Chem.** 274, 6935-45
- Marchenko GN, Marchenko ND, Strongin AY (2003) The structure and regulation of the human and mouse matrix metalloproteinase-21 gene and protein. **Biochem J.** 372, 503-15
- Matrisian LM (1990) Metalloproteinases and their inhibitors in matrix remodeling. **Trends Genet.** 6, 121-5
- Matsuno H, Yudoh K, Watanabe Y, Nakazawa F, Aono H, 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, 22-8
- Matsushita A, Onda M, Uchida E, Maekawa R, Yoshioka T (2001) Antitumor effect of a new selective matrix metalloproteinase inhibitor, MMI-166, on experimental pancreatic cancer. **Int J Cancer** 92, 434-40
- McCarthy K, Maguire T, McGreal G, McDermott E, O'Higgins N, Duffy MJ (1999) High levels of tissue inhibitor of metalloproteinase-1 predict poor outcome in patients with breast cancer. **Int J Cancer** 84, 44-8
- McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM (2000) Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. **Science** 289, 1202-6
- McQuibban GA, Butler GS, Gong JH, Bendall L, Power C, Clark-Lewis I, Overall CM (2001) Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. **J Biol Chem.** 276, 43503-8
- McQuibban GA, Gong JH, Wong JP, Wallace JL, Clark-Lewis I, Overall CM (2002) Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo. **Blood** 100, 1160-7
- Michaelis J, Vissers MC, Winterbourn CC (1992) Cleavage of alpha 1-antitrypsin by human neutrophil collagenase. **Matrix Suppl.** 1,80-1
- Mitchell TI, Jeffrey JJ, Palmiter RD, Brinckerhoff CE (1993) The acute phase reactant serum amyloid A (SAA3) is a novel substrate for degradation by the metalloproteinases collagenase and stromelysin. **Biochim Biophys Acta** 1156, 245-54
- Moilanen M, Pirila E, Grenman R, Sorsa T, Salo T (2002) Expression and regulation of collagenase-2 (MMP-8) in head and neck squamous cell carcinomas. **J Pathol.** 197, 72-81
- Moses MA (1997) The regulation of neovascularization of matrix metalloproteinases and their inhibitors. **Stem Cells** 15, 180-9
- Nakamura H, Suenaga N, Taniwaki K, Matsuki H, Yonezawa K, Fujii M, Okada Y, Seiki M (2004) Constitutive and induced CD44 shedding by ADAM-like proteases and membrane-type 1 matrix metalloproteinase. **Cancer Res.** 64, 876-82
- Nemeth JA, Yousif R, Herzog M, Che M, Upadhyay J, Shekarriz B, Bhagat S, Mullins C, Fridman R, Cher ML (2002) Matrix metalloproteinase activity, bone matrix turnover, and tumor cell proliferation in prostate cancer bone metastasis. **J Natl Cancer Inst.** 94, 17-25
- Nishizuka I, Ichikawa Y, Ishikawa T, Kamiyama M, Hasegawa S, Momiyama N, Miyazaki K, Shimada H (2001) Matrilysin stimulates DNA synthesis of cultured vascular endothelial cells and induces angiogenesis in vivo. **Cancer Lett.** 173, 175-82
- Park HI, Turk BE, Gerkema FE, Cantley LC, Sang QX (2002) Peptide substrate specificities and protein cleavage sites of human endometase/matrilysin-2/matrix metalloproteinase-26. **J Biol Chem.** 277, 35168-75

- Pendás AM, Uría JA, Jiménez MG, Balbín M, Freije JP, López-Otín C (2000) An overview of collagenase-3 expression in malignant tumors and analysis of its potential value as a target in antitumor therapies. **Clin Chim Acta** 291, 137-55
- Powell WC, Knox JD, Navre M, Grogan TM, Kittelson J, Nagle RB, Bowden GT (1993) Expression of the metalloproteinase matrilysin in DU-145 cells increases their invasive potential in severe combined immunodeficient mice. **Cancer Res.** 53, 417-22
- Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM (1999) The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. **Curr Biol.** 9, 1441-7
- Proost P, Van Damme J, Opendakker G (1993) Leukocyte gelatinase B cleavage releases encephalitogens from human myelin basic protein. **Biochem Biophys Res Commun.** 192, 1175-81
- Ria R, Vacca A, Ribatti D, Di Raimondo F, Merchionne F, Dammacco F (2002) Alpha(v)beta(3) integrin engagement enhances cell invasiveness in human multiple myeloma. **Haematologica** 87, 836-45
- Ries C, Lottspeich F, Dittmann KH, Petrides PE (1996) HL-60 leukemia cells produce an autocatalytically truncated form of matrix metalloproteinase-9 with impaired sensitivity to inhibition by tissue inhibitors of metalloproteinases. **Leukemia** 10,1520-6
- Ries C, Loher F, Zang C, Ismail MG, Petrides PE (1999) Matrix metalloproteinase production by bone marrow mononuclear cells from normal individuals and patients with acute and chronic myeloid leukemia or myelodysplastic syndromes. **Clin Cancer Res.** 5, 1115-24
- Rozanov DV, Ghebrehwet B, Postnova TI, Eichinger A, Deryugina EI, Strongin AY (2002) The hemopexin-like C-terminal domain of membrane type 1 matrix metalloproteinase regulates proteolysis of a multifunctional protein, gC1qR. **J Biol Chem.** 277, 9318-25
- Sadowski T, Dietrich S, Koschinsky F, Sedlacek R (2003) Matrix metalloproteinase 19 regulates insulin-like growth factor-mediated proliferation, migration, and adhesion in human keratinocytes through proteolysis of insulin-like growth factor binding protein-3. **Mol Biol Cell.** 14, 4569-80
- Sato T, Foged NT, Delaissé JM (1998) The migration of purified osteoclasts through collagen is inhibited by matrix metalloproteinase inhibitors. **J Bone Miner Res.** 13, 59-66
- Sires UI, Murphy G, Baragi VM, Fliszar CJ, Welgus HG, Senior RM (1994) Matrilysin is much more efficient than other matrix metalloproteinases in the proteolytic inactivation of alpha 1-antitrypsin. **Biochem Biophys Res Commun.** 204, 613-20
- Spurbeck WW, Ng CY, Strom TS, Vanin EF, Davidoff AM (2002) Enforced expression of tissue inhibitor of matrix metalloproteinase-3 affects functional capillary morphogenesis and inhibits tumor growth in a murine tumor model. **Blood** 100, 3361-8
- Standal T, Borset M, Lenhoff S, Wisloff F, Stordal B, Sundan A, Waage A, Seidel C (2002) Serum insulinlike growth factor is not elevated in patients with multiple myeloma but is still a prognostic factor. **Blood** 100, 3925-9
- Starckx S, Van den Steen PE, Verbeek R, van Noort JM, Opendakker G (2003) A novel rationale for inhibition of gelatinase B in multiple sclerosis: MMP-9 destroys alpha B-crystallin and generates a promiscuous T cell epitope. **J Neuroimmunol.** 141, 47-57
- Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. **Annu Rev Cell Dev Biol.** 17, 463-516
- Stix B, Kahne T, Sletten K, Raynes J, Roessner A, Rocken C (2001) Proteolysis of AA amyloid fibril proteins by matrix metalloproteinases-1, -2, and -3. **Am J Pathol.** 159, 561-70
- Suzuki M, Raab G, Moses MA, Fernandez CA, 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, 31730-7
- Takino T, Koshikawa N, Miyamori H, Tanaka M, Sasaki T, Okada Y, Seiki M, Sato H (2003) Cleavage of metastasis suppressor gene product KiSS-1 protein/metastatin by matrix metalloproteinases. **Oncogene** 22, 4617-26
- Teronen O, Laitinen M, Salo T, Hanemaaijer R, Heikkila P, Kontinen YT, Sorsa T (2000) Inhibition of matrix metalloproteinases by bisphosphonates may in part explain their effects in the treatment of multiple myeloma. **Blood** 96, 4006-7
- Thabard W, Barillé S, Collette M, Harousseau JL, Rapp MJ, Bataille R, Amiot M (1999) Myeloma cells release soluble interleukin-6/Ralpha in relation to disease progression by two distinct mechanisms: alternative splicing and proteolytic cleavage. **Clin Cancer Res.** 5, 2693-7
- Ugwu F, Van Hoef B, Bini A, Collen D, Lijnen HR (1998) Proteolytic cleavage of urokinase-type plasminogen activator by stromelysin-1 (MMP-3). **Biochemistry** 37, 7231-6
- Vacca A, Ribatti D, Roncali L, Ranieri G, Serio G, Silvestris F, Dammacco F (1994) Bone marrow angiogenesis and progression in multiple myeloma. **Br J Haematol.** 87, 503-8
- Vacca A, Ribatti D, Iurlaro M, Albini A, Minischetti M, Bussolino F, Pellegrino A, Ria R, Rusnati M, Presta M, Vincenti V, Persico MG, Dammacco F (1998) Human lymphoblastoid cells produce extracellular matrix-degrading enzymes and induce endothelial cell proliferation, migration, morphogenesis, and angiogenesis. **Int J Clin Lab Res.** 28, 55-68
- Vacca A, Ribatti D, Presta M, Minischetti M, Iurlaro M, Ria R, Albini A, Bussolino F, Dammacco F (1999) Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. **Blood** 93, 3064-73
- Vacca A, Ria R, Semeraro F, Merchionne F, Coluccia M, Boccarelli A, Scavelli C, Nico B, Gernone A, Battelli F, Tabilio A, Guidolin D, Petrucci MT, Ribatti D, Dammacco F (2003) Endothelial cells in the bone marrow of patients with multiple myeloma. **Blood** 102, 3340-8
- Vande Broek I, Asosingh K, Allegaert V, Leleu X, Facon T, Vanderkerken K, Camp BV, Van Riet I (2004) Bone marrow endothelial cells increase the invasiveness of human multiple myeloma cells through upregulation of MMP-9: evidence for a role of hepatocyte growth factor. **Leukemia** 4 [Epub ahead of print]
- Van den Steen PE, Proost P, Wuyts A, Van Damme J, Opendakker G (2000) Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-alpha and leaves RANTES and MCP-2 intact. **Blood** 96, 2673-81
- Van Den Steen PE, Wuyts A, Husson SJ, Proost P, Van Damme J, Opendakker G (2003a) Gelatinase B/MMP-9 and neutrophil collagenase/MMP-8 process the chemokines human GCP-2/CXCL6, ENA-78/CXCL5 and mouse GCP-2/LIX and modulate their physiological activities. **Eur J Biochem.** 270, 3739-49
- Van den Steen PE, Husson SJ, Proost P, Van Damme J, Opendakker G (2003b) Carboxyterminal cleavage of the chemokines MIG and IP-10 by gelatinase B and neutrophil collagenase. **Biochem Biophys Res Commun.** 310, 889-96
- Van Ranst M, Norga K, Masure S, Proost P, Vandekerckhove F, Auwerx J, Van Damme J, Opendakker G. (1991) The cytokine-protease connection: identification of a 96-kD THP-1 gelatinase and regulation by interleukin-1 and cytokine inducers. **Cytokine.** 3, 231-9

- Van Valckenborgh E, Bakkus M, Munaut C, Noel A, St Pierre Y, Asosingh K, Van Riet I, Van Camp B, Vanderkerken K (2002a) Upregulation of matrix metalloproteinase-9 in murine 5T33 multiple myeloma cells by interaction with bone marrow endothelial cells. **Int J Cancer** 101, 512-8
- Van Valckenborgh E, De Raeve H, Devy L, Blacher S, Munaut C, Noel A, Van Marck E, Van Riet I, Van Camp B, Vanderkerken K (2002b) Murine 5T multiple myeloma cells induce angiogenesis in vitro and in vivo. **Br J Cancer** 86, 796-802
- Van Valckenborgh, P. I. Croucher, H. De Raeve, C. Carron, K. Asosingh, I. Van Riet, B. Van Camp and K. Vanderkerken (2003) The effect of the broad\_spectrum matrix metalloproteinase inhibitor SC-964 on tumor growth, development of osteolytic lesions and angiogenesis in multiple myeloma: A study in the 5T2MM model. **Hematol J.** 4 (Suppl. 1), S186
- Vihinen P, Kähäri VM (2002) Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. **Int J Cancer** 99, 157-66
- von Bredow DC, Nagle RB, Bowden GT, Cress AE (1997) Cleavage of beta 4 integrin by matrilysin. **Exp Cell Res.** 236, 341-5
- Wahlgren J, Maisi P, Sorsa T, Sutinen M, Tervahartiala T, Pirilä E, Teronen O, Hietanen J, Tjäderhane L, Salo T (2001) Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. **J Pathol.** 194, 217-24
- Watanabe H, Nakanishi I, Yamashita K, Hayakawa T, Okada Y (1993) Matrix metalloproteinase-9 (92 kDa gelatinase/type IV collagenase) from U937 monoblastoid cells: correlation with cellular invasion. **J Cell Sci.** 104, 991-9
- Werb Z, Mainardi CL, Vater CA, Harris ED Jr. (1977) Endogenous activation of latent collagenase by rheumatoid synovial cells. Evidence for a role of plasminogen activator. **N Engl J Med.** 296, 1017-23
- Wilson CL, Heppner KJ, Labosky PA, Hogan BL, Matrisian LM (1997) Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. **Proc Natl Acad Sci U S A** 94, 1402-7
- Winding B, NicAmhlaoibh R, Misander H, Hoegh-Andersen P, Andersen TL, Holst-Hansen C, Heegaard AM, Foged NT, Brüner N, Delaissé JM (2002) Synthetic matrix metalloproteinase inhibitors inhibit growth of established breast cancer osteolytic lesions and prolong survival in mice. **Clin Cancer Res.** 8, 1932-9
- Winyard PG, Zhang Z, Chidwick K, Blake DR, Carrell RW, Murphy G (1991) Proteolytic inactivation of human alpha 1 antitrypsin by human stromelysin. **FEBS Lett.** 279, 91-4
- Yang Y, Yaccoby S, Liu W, Langford JK, Pumphrey CY, Theus A, Epstein J, Sanderson RD (2002) Soluble syndecan-1 promotes growth of myeloma tumors in vivo. **Blood** 100, 610-7



Els Van Valckenborgh