DDT Vol. 6, No. 9 May 2001

Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis

Hussein D. Foda and Stanley Zucker

Matrix metalloproteinases (MMPs) are a family of proteinases that play an important role in cancer as well as in numerous other diseases. In this article, we summarize the current views on the role of MMPs in cancer with respect to invasion, metastasis and angiogenesis. A positive correlation between tumor progression and the expression of multiple MMP family members in tumor tissues has been demonstrated in numerous human and animal studies. It has been assumed that cancer cells are responsible for producing the MMPs in human tumors. However, recent evidence suggests that tumor cells have docking sites that bind stromal-cell-secreted MMPs. Furthermore, the role of MMPs produced by endothelial cells, especially MMP-2 and MT1-MMP, appear to be crucial for tumor angiogenesis, which is a requirement for cancer growth and dissemination.

***Hussein D. Foda and Stanley Zucker**
Dept of Medicine and Research
VAMC Northport and the Divisions of Pulmonary/Critical Care Medicine and Medical Oncology
State University of New York at Stony Brook
NY 11794, USA
*e-mail:
hfoda@mail.som.sunysb.edu

▼ Matrix metalloproteinases (MMPs) are a family of proteinases that were first described in 1962 when Gross and Lapiere identified an enzyme from a vertebrate source (tadpole tail) with proteolytic action capable of attacking collagen[1,2]. The initial MMP was called interstitial collagenase because it cleaved collagen type I, II and III at a single site in the triple helix of the molecule. More recently, many functions, other than proteolytic action on extracellular matrix proteins, have been described for MMPs. If MMPs had been discovered today, they might have been labeled as angiogenesis proteinases or as growth factor proteinases because of their important role in these biological processes.

MMPs appear to have an important role in cancer as well as in numerous other disease states. Cancer is the second leading cause of death in the USA. In the majority of patients, the growth and metastasis of cancer evolves over several years and many steps are involved. The role of MMPs in many of the complicated aspects of cancer dissemination has been intensively investigated over the past 20 years. In this review we will examine the role of MMPs in cancer invasion, metastasis and angiogenesis.

MMP characteristics

MMPs were initially characterized by their ability to degrade components of the extracellular matrix (ECM). These enzymes require a zinc ion at their active site and are inhibited by zinc- and calcium-chelating agents. MMPs are secreted in a latent form and thus require activation for proteolytic activity. They are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs). The MMP family consists of at least 20 enzymes that share considerable homology (30–50%) within their major domains (signal peptide, propeptide, catalytic, hinge and hemopexin-like domains[3]). MMPs and TIMPs are tightly regulated at the levels of transcription, release and activation[4]. The 72 kDa MMP-2 (gelatinase A) is the most widely distributed of the MMPs and is expressed constitutively by most cells including endothelial and epithelial cells[4]. The 92 kDa MMP-9 (gelatinase B) is produced by inflammatory cells, including blood neutrophils and tissue macrophages, as well as by stimulated connective tissue cells. MMP-2 and MMP-9 have an important role in basement membrane turnover. MMP-2 is secreted as an inactive pro-form; when it is converted to a 62 kDa active form it can degrade collagen types IV and V, laminin and elastin. In intact cells, MMP-2 is activated at the cell surface by a process involving interaction of the C-terminal component of MMP-2 with a plasma membrane activation mechanism[5–7]. Sato and colleagues reported the identification of a membrane-type matrix metalloproteinase (MT1-MMP) that appears to be important in cellular activation of MMP-2 (Ref. 8). Strongin

1359-6446/01/$ – see front matter PII: S1359-6446(01)01752-4

and coworkers[9] and Zucker and colleagues[10] have demonstrated that the plasma-membrane-dependent activation of MMP-2 in endothelial cells, fibroblasts and cancer cells required the formation of a tri-molecular complex composed of proMMP-2, TIMP-2 and MT1-MMP (Ref. 8). MT1-MMP converts the 72 kDa proMMP-2 to a 64 kDa intermediate, and then auto-activation to the 62 kDa activated MMP-2 occurs[9,11].

The role of MMPs in cancer dissemination

A positive correlation between tumor progression and the expression of multiple MMP family members (MMP-1, MMP-2, MMP-7, MMP-9, MMP-11 and MT1-MMP) in tumor tissues has been demonstrated in numerous human and animal studies[12,13]. The ratio of activated to total MMP-2 levels has also been correlated with tumor aggressiveness[14]. On the basis of numerous studies, it was proposed that pharmacologic targeting of MMP activity might provide a mechanism to prevent cancer dissemination[13]. Further support for the role of MMPs in cancer dissemination came from the demonstration that TIMPs can interfere with experimental metastasis[15,16]. However, the role of MMPs and TIMPs in cancer is far more complicated than suggested initially. For example, increased TIMP-1 levels in human cancer tissues have been associated with poor prognoses[17]. It is uncertain whether this reflects the growth potentiating properties of TIMPs or some other undetermined property of TIMPs (Refs 18,19). Other experimental studies with cancer cells transfected with TIMP-1 cDNA demonstrated that MMPs act primarily to alter the extracellular environment to allow sustained cancer cell growth in an ectopic site, as opposed to having a specific role of allowing the cells to extravasate from the blood stream[20–22]. Furthermore, in some experimental tumor systems, increased MMP production did not correlate with increased metastasis[23]. One potential explanation of this finding is that excess proteolysis might degrade matrix signals and receptors, thereby disrupting cell matrix interactions and inhibiting migration[24].

Cleavage of matrix components by MMPs releases polypeptide fragments with new biological properties, as well as releasing signaling components embedded within the matrix. For example, cleavage of laminin-5, a component of the ECM, by MT1-MMP and MMP-2 promotes migration of cells[25,26]. Several soluble growth factors are secreted and stored in an inactive form bound to extracellular matrix molecules. During enhanced proteolysis, these factors are then freed to act on their target receptors. MMP-3 has been shown to cleave the matrix molecule decorin, resulting in the release of transforming growth factor-β (TGF-β) in its more biologically active form[27]. Receptors for growth factors are targeted for proteolysis by MMPs including FGF type I receptor, which mediates the effects of fibroblast growth factor[28]. IGF-binding protein-l has been identified as a potential physiologic substrate for MMP-11. Whereas IGF-binding proteins can confer latency on IGF-I and IGF-II, their degradation can restore the activity of these growth factors, thereby affecting tumor growth[29].

Production of MMPs by tumor stromal cells

As a result of numerous studies of oncogene-transformed cells, cancer cell lines, and experimental tumor models, it had long been assumed that cancer cells were responsible for producing the MMPs in human tumors[30,31]. This concept came under attack in 1990 when Basset and colleagues[32] reported that stromal fibroblasts surrounding tumor cells, and not the tumor cells themselves, were responsible for producing MMP-11 in human breast cancer. Similarly, other investigators have employed *in situ* hybridization to demonstrate the localization of MMP-l, MMP-2, MMP-3 and MTl-MMP mRNA primarily in stromal fibroblasts, especially in proximity to invading cancer cells but not in the carcinoma cells in human breast, colorectal, lung, prostate, and ovarian cancers[22]. MMP-9 has been localized primarily to inflammatory cells (macrophages and neutrophils) rather than fibroblasts or tumor cells in colorectal cancer tissue. Immunolocalization studies that identify MMPs using specific antibodies, rather than mRNA expression, however, have generally identifed MMP-2 and MMP-9 protein in cancer cells. This data reinforces the concept that tumor cells possess docking sites that bind stromal-cell-secreted MMPs (i.e. MMP-9 binding to collagen type IV components and to CD-44 on the cell surface[33,34]); MMP-2 binds to cell surface $\alpha_v\beta_3$ integrin[35]. Hence, tumor cells might function as a receptacle for stromal MMPs. The function of MMPs identified in the cytoplasm of cancer cells remains to be determined. Osteoblasts have a receptor for MMP-13 that leads to catabolism of the internalized proteinase[36].

An explanation for the production of MMPs by reactive stromal cells in a tumor came from the discovery of extracellular matrix metalloproteinase inducer (EMMPRIN), by Biswas[37]. EMMPRIN (originally known as tumor collagenase-stimulating factor) is an intrinsic plasma membrane glycoprotein produced in large amounts by cancer cells, which stimulates local fibroblasts to synthesize MMP-l, MMP-2 and MMP-3. Tumor cell interactions with fibroblasts via EMMPRIN lead to fibroblast-induced local degradation of basement membrane and ECM components, thus facilitating tumor cell invasion. On examination of human lung and breast cancer tissue, EMMPRIN expression in cancer cells far exceeded that of normal epithelial cells[38]. Recent studies have demonstrated that MMP-l binds to EMMPRIN

on the tumor cell surface, thus indicating that following EMMPRIN stimulation of MMP synthesis and secretion by fibroblasts, a surface-localized MMP-l–EMMPRIN complex arms the cancer cell for degradation of the ECM (Ref. 39).

Cell surface protrusions (invadopodia)

Invadopodia (podosomes) are specialized cell-surface structures that have been identified on transformed malignant cells and are composed of a meshwork of microfilaments. Invadopodia are involved in the growth of cells on collagen-like matrices and invasion of the underlying matrix. Invadopodia utilize proteases to degrade a variety of immobilized substrates including fibronectin, laminin, type I and IV collagens and other ECM components. Several integral membrane enzymes of different classes have been identified as important functional components of invadopodia[40]. These include the serine proteases, seprase (surface expressed protease) and dipeptidyl peptidase IV, which must form oligomeric structures for expression of proteolytic activity and also MT-MMP (Ref. 40). Various integrins and EMMPRIN have also been identified in invadopodia. Plasma membranes shed vesicles containing densely clustered MMP-9 and MMP-2 (Ref. 41), which might facilitate directional proteolysis of the ECM during cell migration and especially during cancer invasion.

The role of MMPs in angiogenesis

One of the early events in the transition of a tumor from the pre-neoplastic state to the neoplastic phenotype is the ability of the tumor to promote angiogenesis[42]. Results of numerous experimental studies support the concept that ingrowth of new blood vessels is required for continued tumor growth[43]. Pathological studies of human tumors have demonstrated a correlation between increased numbers of tumor blood vessels and poor prognoses[44]. Whereas initial studies were directed towards identifying and characterizing positive regulators of angiogenesis including the growth factors VEGF, αFGF, βFGF, EGF, TGF-α, TGF-β, PGE2. TNF-α, PD-ECGF, angiopoietin, angiogenin and interleukin 8, the 1990s witnessed the characterization of cell surface receptors for angiogenic factors. Most recently, research is underway to elucidate the signal transduction pathways involved in angiogenesis[45,46].

Tumor angiogenesis is a complex process that requires: (1) degradation of the basement membrane and ECM surrounding blood vessels; (2) chemotaxis of endothelial cells toward an angiogenic stimulus; (3) proliferation of endothelial cells; and (4) remodeling of the basement membrane as the new blood vessel forms. This remodeling is thought to be a result of MMP activity[47,48]. Endothelial cells produce MMP-1, MMP-2, MMP-3 and MT-MMP. The role of MMP-2 and MT-MMP in angiogenesis has been the most studied[49–51].

MMP-2 and MT-MMP have a crucial role in angiogenesis. Treatment of human umbilical vein endothelial cells with phorbol ester (PMA) leads to the activation of MMP-2 and the induction of MT-MMP (Ref. 52); this is accompanied by the formation of multicellular tube structures when cells are cultured within a collagen gel[53]. In an *in vitro* model of microvascular endothelial cell angiogenesis, cells cultured on a collagen matrix constitutively expressed low levels of latent MMP-2. However, when the same cells were cultured in a 3D collagen matrix, formation of an endothelial network ensued that was accompanied by a large increase in MMP-2 production. A large portion of the MMP-2 produced was activated and this increase was accompanied by an increase in MT-MMP. The addition of the MMP-inhibitor marimastat (BB-94) prevented tube formation while blocking the activation of MMP-2 (Ref. 54).

MMPs have been shown to aid endothelial cells in invading and neovascularizing fibrin-rich tissue both *in vitro* and *in vivo*[53]. This ability to invade this fibrin-rich tissue was independent of plasminogen activator (PA) activity. MT1-MMP appears to be the dominant MMP in this effect because cells lacking MT1-MMP had no fibrinolytic activity and, therefore, were unable to invade the fibrin-rich tissue[55]. These observations are relevant in cancer invasion where a fibrin-rich 'provisional matrix' is a prominent component of the tumor ECM (Ref. 56).

Integrins comprise a family of heterodimeric cell surface adhesion molecules that bind to components of the ECM (Ref. 57). The integrin $\alpha_v\beta_3$ was shown to directly bind MMP-2 on the surface of cells, through the MMP-2 hemopexin domain[35]. Angiogenesis depends on specific endothelial cell adhesive events mediated by the integrin $\alpha_v\beta_3$. A fragment of MMP-2 that comprises the C-terminal hemopexin-like domain (termed PEX) prevents MMP-2 binding to $\alpha_v\beta_3$. Recently, it has been shown that the PEX fragment can disrupt angiogenesis and tumor growth[58]. A therapeutic strategy that employs PEX to inhibit tumor angiogenesis has been proposed.

The role of MMP-9 in angiogenesis is less clear than that of MMP-2 and MT1-MMP. Recently, thrombospondin-1, a known angiogenic promoting glycoprotein, has been shown to act via the upregulation of MMP-9 in promoting bovine aortic endothelial cells to form tubes[59]. MMP-9 has also been shown to activate the pro-angiogenic factor TGF β1 (Ref. 34). However, other investigators have found that the upregulation of MMP-9 did not correlate with an increase in migratory or invasiveness of pulmonary artery endothelial cells[60].

Recent evidence suggests that endothelial cells, in response to extracellular matrix alterations, increase their production of the transcriptional factor Egr-1. This increased Egr-1, in turn, leads to the upregulation of MT1-MMP and the increased invasiveness of endothelial cells in culture[61]. Another transcription factor, ETS-1, increases the expression of MMP-1, MMP-3 and MMP-9 and also leads to an increased invasiveness of endothelial cells in an *in vitro* system[62].

In a study of endometrial carcinoma biopsies, increased tumor MMP-2 and MMP-9 mRNA were demonstrated by *in situ* hybridization. Increased MMP-2 and MMP-9 expression correlated with enhanced angiogenesis and invasiveness of tumors[63]. In an *in vivo* model of tumor angiogenesis and invasion that was recently reported, treatment with an MMP-2 antisense oligonucleotide caused suppression of the tumor angiogenic activity and invasiveness[42]. These data strongly implicate MMP-2 as an essential requirement for angiogenesis.

The recently isolated angiogenesis inhibitor, angiostatin, inhibits endothelial cell proliferation, neovascularization and metastatic tumor growth[64]. Angiostatin is a cleavage product of plasminogen, which is an abundant plasma protein. Both MMP-12 and MMP-2 cleave plasminogen and generate angiostatin[65,66]. Recently, O'Reilly and colleagues have reported that Lewis lung carcinoma cells produced MMP-2, which was responsible for cleavage of plasminogen and production of angiostatin by these tumor cells[66].

The fact that MMPs might be responsible for the production of angiostatin is intriguing, given that tumor-derived MMPs have been recognized as tumor promoters by degrading matrix barriers and by enhancing angiogenesis. The regulation of angiogenesis by MMPs suggests that clinical strategies that are directed at decreasing the enzymatic activity of tumor MMPs might also decrease the release of angiogenesis inhibitors. Therefore, the results of clinical trials using such therapeutic strategies need to be re-addressed. Furthermore, MMP inhibitors with more specific action need to be designed to inhibit the tumor-promoting aspects of MMPs without interfering with the angiostatin-promoting activity of MMPs.

Conclusion

MMPs have a complex and key role in cancer growth and metastasis. Neo-angiogenesis by tumors is a crucial step for cancer growth and dissemination. MMPs produced by endothelial cells, especially MMP-2 and MT1-MMP, are crucial for tumor angiogenesis. To determine the role that MMPs have in angiogenesis inhibition, further study is required.

References

1 Nagai, Y. *et al.* (1966) Tadpole collagenase. Preparation and purification. *Biochemistry* 5, 3123–3130
2 Gross, J. and Lapiere, C.M. (1962) Collagenolyticactivity in amphibian tissues: a tissue culture assay. *Proc. Natl. Acad. Sci. U. S. A.* 48, 1014–1022
3 Matrisian, L.M. (1990) Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet.* 6, 121–125
4 Birkedal-Hansen, H. *et al.* (1993) Matrix metalloprotease: a review. *Crit. Rev. Oral Biol. Med.* 4, 197–250
5 Murphy, G. *et al.* (1992) The C-terminal domain of 72 kDa gelatinase A is not required for catalysis, but is essential for membrane activation and modulates interaction with tissue inhibitors of metalloproteinases. *Biochem. J.* 283, 637–641
6 Ward, R.V. *et al.* (1994) Cell surface-mediated activation of progelatinase A: demonstration of the involvement of the C-terminal domain of progelatinase A in cell surface binding and activation of progelatinase A by primary fibroblasts. *Biochem. J.* 304, 263–269
7 Overall, C. M. and Sodek, J. (1990) Concanavalin A produces a matrix-degradative phenotype in human fibroblasts. *J. Biol. Chem.* 265, 21141–21151
8 Sato, H. *et al.* (1994) A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* 370, 61–65
9 Strongin, A.Y. *et al.* (1995) Mechanism of cell surface activation of 72 kDa type IV collagenase. *J. Biol. Chem.* 270, 5331–5338
10 Zucker, S. *et al.* (1998) Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinae 1 (MT1-MMP). *J. Biol. Chem.* 273, 1216–1222
11 Atkinson, S.J. *et al.* (1995) Intermolecular autolytic cleavage can contribute to the activation of proglatinase A by cell membranes. *J. Biol. Chem.* 270, 30479–30485
12 Ellerbroek, S.M. and Stack, M.S. (1999) Membrane associated matrix metalloproteinases in metastasis. *Bioessays* 21, 940–949
13 Stetler-Stevenson, W.G. *et al.* (1996) Matrix metalloproteinases and tumor invasion: from correlation and causality to the clinic. *Semin. Cancer Biol.* 7, 147–154
14 Davies, B. *et al.* (1993) Activity of type IV collagenases in benign and malignant breast disease. *Br. J. Cancer* 67, 1126–1131
15 DeClerck, Y.A. and Imren, S. (1994) Protease inhibitors: role and potential therapeutic use in human cancer. *Eur. J. Cancer* 14, 2170–2180
16 Montgomery, A.M. *et al.* (1994) Effect of tissue inhibitor of the matrix metalloproteinases-2 expression on the growth and spontaneous metastasis of a human melanoma cell line. *Cancer Res.* 54, 5467–5473
17 Lu, X.Q. *et al.* (1991) Immunological quantitation of levels of tissue inhibitor of metalloproteinase-1 in human colon cancer. *Cancer Res.* 51, 6231–6235
18 Guedez, L. *et al.* (1998) *In vitro* suppression of programmed cell death of B cells by tissue inhibitor of metalloproteinases-1. *J. Clin. Invest.* 102, 2002–2010
19 Hayakawa, T. *et al.* (1994) Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2). *J. Cell Sci.* 107, 2373–2379
20 Cameron, M.D. *et al.* (2000) Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. *Cancer Res.* 60, 2541–2546
21 Chambers, A.F. and Matrisian, L.M. (1997) Changing views of the role of matrix metalloproteinases in metastasis. *J. Natl. Cancer Inst.* 89, 1260–1270
22 Nelson, A.R. *et al.* (2000) Matrix metalloproteinases: biologic activity and clinical implications. *J. Clin. Oncol.* 18, 1135–1149
23 Zucker, S. *et al.* (1992) Secretion of gelatinases and tissue inhibitors of metalloproteinases by human lung cancer cell lines and revertant cell lines: not an invariant correlation with metastasis. *Int. J. Cancer* 52, 366–371
24 Parks, W.C. *et al.* (1998) Matrix metalloproteinases in tissue repair. In *Matrix Metalloproteinases* (Parks, W.C. and Mecheam, R.P., eds), pp. 263–300, Academic Press

25 Giannelli, G. *et al.* (1997) Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. *Science* 277, 225–228

26 Koshikawa, N. *et al.* (2000) Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. *J. Cell Biol.* 148, 615–624

27 Imai, K. *et al.* (1997) Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-β1 release. *Biochem. J.* 322, 809–814

28 McCawley, L.J. and Matrisian, L.M. (2000) Matrix metalloproteinases: multifunctional contributors to tumor progression. *Mol. Med. Today* 6, 149–156

29 Noel, A. *et al.* (1997) Emerging roles for proteinases in cancer. *Invasion Metastasis* 17, 221–239

30 Liotta, L.A. (1992) Cancer cell invasion and metastasis. *Sci. Am.* 266, 54–59

31 Nicolson, G.L. (1991) Tumor and host molecules important in the organ preference of metastasis. *Semin. Cancer Biol.* 2, 143–154

32 Basset, P. *et al.* (1990) A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature* 348, 699–704

33 Olson, M.W. *et al.* (1998) High affinity binding of latent matrix metalloproteinase-9 to the alpha2(IV) chain of collagen IV. *J. Biol. Chem.* 273, 10672–10681

34 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

35 Brooks, P.C. *et al.* (1996) Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin $\alpha_v\beta_3$. *Cell* 85, 683–693

36 Barmina, O.Y. *et al.* (1999) Collagenase-3 binds to a specific receptor and requires the low density lipoprotein receptor-related protein for internalization. *J. Biol. Chem.* 274, 30087–30093

37 Biswas, C. *et al.* (1995) The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Res.* 55, 434–439

38 Polette, M. *et al.* (1997) Tumor collagenase stimulatory factor (TCSF) expression and localization in human lung and breast cancers. *J. Histochem. Cytochem.* 45, 703–709

39 Guo, H. *et al.* (2000) EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface. *Cancer Res.* 60, 888–891

40 Nakahara, H. *et al.* (1997) Transmembrane/cytoplasmic domain-mediated membrane type 1-matrix metalloprotease docking to invadopodia is required for cell invasion. *Proc. Natl. Acad. Sci. U. S. A.* 94, 7959–7964

41 Nguyen, M. *et al.* (1998) Active and tissue inhibitor of matrix metalloproteinase-free gelatinase B accumulates within human microvascular endothelial vesicles. *J. Biol. Chem.* 273, 5400–5404

42 Fang, J. *et al.* (2000) Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3884–3889

43 Folkman, J. (1992) The role of angiogenesis in tumor growth. *Semin. Cancer Biol.* 3, 65–71

44 Weidner, N. *et al.* (1991) Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *New Engl. J. Med.* 324, 1–8

45 Ferrara, N. *et al.* (1995) The regulation of blood vessel growth by vascular endothelial growth factor. *Ann. N. Y. Acad. Sci.* 752, 246–256

46 Dvorak, H.F. *et al.* (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am. J. Pathol.* 146, 1029–1039

47 Fisher, C. *et al.* (1994) Interstitial collagenase is required for angiogenesis *in vitro*. *Dev. Biol.* 162, 499–510

48 Moscatelli, D. and Rifkin, D.B. (1988) Membrane and matrix localization of proteinases: a common theme in tumor cell invasion and angiogenesis. *Biochim. Biophys. Acta* 948, 67–85

49 Nagase, H. and Woessner, J.F., Jr (1999) Matrix metalloproteinases. *J. Biol. Chem.* 274, 21491–21494

50 Galis, Z.C. (1998) Metalloproteases in remodeling of vascular extracellular matrix. *Fibrinolysis & Proteolysis* 13, 54–63

51 Stetler-Stevenson, W.G. (1999) Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. *J. Clin. Invest.* 103, 1237–1241

52 Foda, H.D. *et al.* (1996) Activation of human umbilical vein endothelial cell progelatinase A by phorbol myristate acetate: a protein kinase C-dependent mechanism involving a membrane-type matrix metalloproteinase. *Lab. Invest.* 74, 538–545

53 Haas, T.L. and Madri, J.A. (1999) Extracellular matrix-driven matrix metalloproteinase production in endothelial cells: implications for angiogenesis. *Trends Cardiovasc. Med.* 9, 70–77

54 Haas, T.L. *et al.* (1998) Three-dimensional type I collagen lattices induce coordinate expression of matrix metalloproteinases MT1-MMP and MMP-2 in microvascular endothelial cells. *J. Biol. Chem.* 273, 3604–3610

55 Hiraoka, N. *et al.* (1998) Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. *Cell* 95, 365–377

56 Dvorak, H.F. (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *New Engl. J. Med.* 315, 1650–1659

57 Silletti, S. and Cheresh, C.A. (1999) A link between integrin and MMPs in angiogensis. *Fibrinol. Proteol.* 13, 226–238

58 Brooks, P.C. *et al.* (1998) Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. *Cell* 92, 391–400

59 Qian, X. *et al.* (1997) Thrombospondin-1 modulates angiogenesis *in vitro* by up-regulation of matrix metalloproteinase-9 in endothelial cells. *Exp. Cell Res.* 235, 403–412

60 Puyraimond, A. *et al.* (1999) Examining the relationship between the gelatinolytic balance and the invasive capacity of endothelial cells. *J. Cell Sci.* 112, 1283–1290

61 Haas, T.L. *et al.* (1999) Egr-1 mediates extracellular matrix-driven transcription of membrane type 1 matrix metalloproteinase in endothelium. *J. Biol. Chem.* 274, 22679–22685

62 Oda, N. *et al.* (1999) ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin β3. *J. Cell Physiol.* 178, 121–132

63 Iurlaro, M. *et al.* (1999) Angiogenesis extent and expression of matrix metalloproteinase-2 and -9 correlate with upgrading and myometrial invasion in endometrial carcinoma. *Eur. J. Clin. Invest.* 29, 793–801

64 O'Reilly, M.S. *et al.* (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 79, 315–328

65 Cornelius, L.A. *et al.* (1998) Matrix metalloproteinases generate angiostatin: effects on neovascularization. *J. Immunol.* 161, 6845–6852

66 O'Reilly, M.S. *et al.* (1999) Regulation of angiostatin production by matrix metalloproteinase-2 in a model of concomitant resistance. *J. Biol. Chem.* 274, 29568–29571