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Matrix metalloproteinases in tumor invasion

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Abstract. Controlled degradation of extracellular matrix (ECM) is essential for the growth, invasion, and metastasis of malignant tumors, and for tumor-induced angiogenesis. Matrix metalloproteinases (MMPs) are a family of zinc-dependent neutral endopeptidases collectively capable of degrading essentially all ECM components and they apparently play an important role in all these aspects of tumor development. Furthermore, recent evidence suggests that MMPs also play a role in tumor cell survival. In this review, we discuss the current concept concerning the role of MMPs and their inhibitors in tumor invasion, as a basis for prognosis and targeted therapeutic intervention in cancer.

Key words. Squamous cell carcinoma; invasion; matrix; metalloproteinase.

Introduction

Degradation of extracellular matrix (ECM) is an important feature in many physiological and pathological conditions. In general, ECM and basement membranes can be degraded by four classes of proteolytic enzymes: cysteine proteinases, aspartic proteinases, serine proteinases, and metalloproteinases [see ref. 1]. There is much evidence that matrix metalloproteinases (MMPs) play an important role in ECM remodeling in physiologic situations, such as fetal tissue development and postnatal tissue repair, and that excessive breakdown of ECM by MMPs occurs in many pathologic conditions including periodontitis, autoimmune blistering disorders of the skin, dermal photoaging, rheumatoid arthritis, osteoarthritis, and chronic ulcerations. In addition, controlled degradation of ECM by MMPs is thought to play an important role in tumor invasion and metastasis [for reviews see refs 2–5].

Matrix metalloproteinases

To date, 18 mammalian MMPs have been identified and according to structure and substrate specificity they can be divided into subgroups of collagenases, stromelysins, gelatinases, membrane-type MMPs (MT-MMPs) and other MMPs (fig. 1). MMPs have a characteristic multidomain structure (fig. 1) consisting of (i) a *signal peptide*, (ii) a *propeptide*, which is essential for maintaining the proMMP in a latent form, (iii) a *catalytic domain* containing the highly conserved Zn^{2+}-binding site (HExGHxxGxxHS/T), (iv) a proline-rich *hinge region* that links the catalytic domain to (v) the *hemopexin-like* domain, which determines the substrate specificity of the MMP. In addition, the catalytic domain of gelatinases contains three repeats of the *fibronectin-type II domain*, involved in binding of these enzymes to gelatin. MT-MMPs contain a *transmembrane domain* of 20 hydrophobic amino acids in the C-terminal end of the hemopexin domain followed by a 24 amino-acid intracellular domain [5].

Collagenases

Collagenase-1 (MMP-1), collagenase-2 (MMP-8), and collagenase-3 (MMP-13) are the principal secreted neutral proteinases capable of degrading native fibrillar collagens of types I, II, III, V, and XI in the extracellular space. Collagenases cleave fibrillar collagens at a specific site between Gly775 and Leu/Ile776 of the α

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chains, generating N-terminal 3/4 and C-terminal 1/4 fragments, which rapidly denature to gelatin at body temperature and become susceptible to degradation by other MMPs, e.g., gelatinases. MMP-1 preferentially degrades type III collagen and MMP-8 prefers type I collagen. MMP-13 cleaves type II collagen more effectively than type I and III collagens and displays stronger gelatinolytic activity than MMP-1 and MMP-8 [6, 7]. Interestingly, both human and mouse MMP-13 cleave type I collagen in the N-terminal nonhelical telopeptide [8]. This N-telopeptidase activity is required for resorption of type I collagen during fetal development and early postnatal life of mice, whereas triple helicase activity appears to be required during intense collagen resorption, e.g., involution of postpartum uterus, as well as in normal turnover of collagen in murine skin [9].

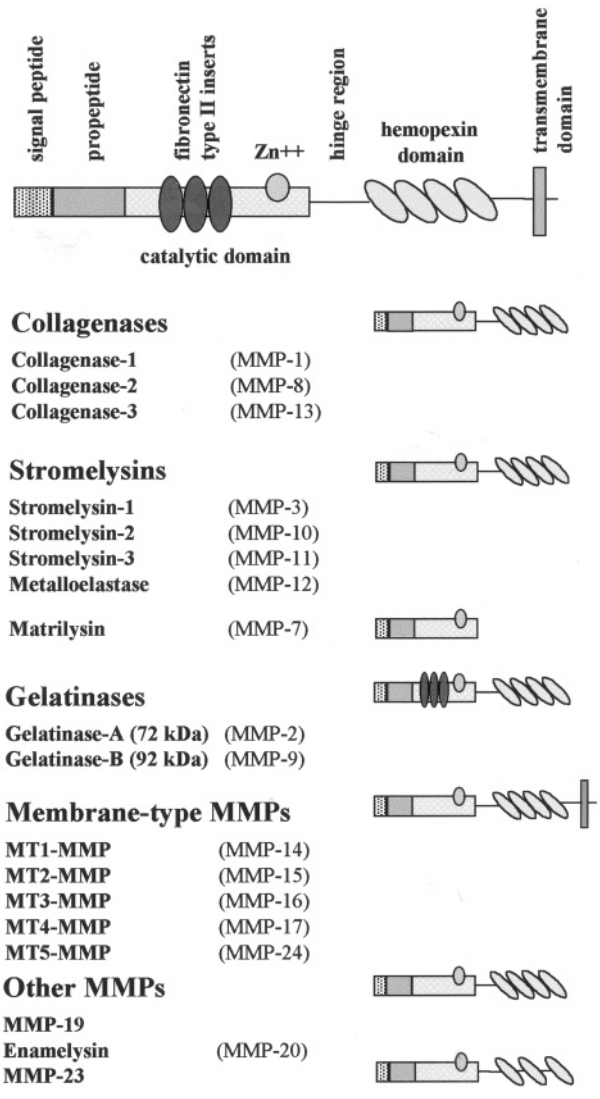

Figure 1. Structure of mammalian matrix metalloproteinases.

MMP-1 is produced by various types of cells, e.g., fibroblasts, keratinocytes, endothelial cells, macrophages, hepatocytes, chondrocytes, osteoblasts, and tumor cells [1]. MMP-8 is synthesized by polymorphonuclear leukocytes during their maturation in bone marrow, stored in the intracellular granules, and secreted in response to external stimuli [10, 11]. MMP-8 is also expressed by chondrocytes, rheumatoid synovial fibroblasts, endothelial cells, in osteoarthritic cartilage, and melanoma cells [12–15]. Interestingly, MMP-8 is expressed abundantly in postpartum uterus of mouse [16].

Human MMP-13 is highly homologous (86% at the amino acid level) to rat and mouse interstitial collagenases with only 50% homology to human MMP-1 indicating that these rodent collagenases are counterparts to human MMP-13 instead of MMP-1 [17]. Furthermore, no rat or murine homologues for human MMP-1 have been found. MMP-13 is characterized by wide substrate specificity and limited expression compared to other MMPs. In addition to fibrillar collagens and gelatin, MMP-13 degrades type IV, IX, X, and XIV collagens, the large tenascin C isoform, fibronectin, laminin, aggrecan core protein, fibrillin-1, and serine proteinase inhibitors [6, 7, 18, 19]. Latent MMP-13 is activated by stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), 72-kDa gelatinase (MMP-2), MT1-MMP (MMP-14), MT2-MMP (MMP-15), trypsin, and plasmin [6, 20, 21] and its activity is inhibited by tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-3 and less potently by TIMP-2 [6, 16]. MMP-13 activates latent MMP-2 and 92-kDa gelatinase (MMP-9) [20].

Apparently due to its wide substrate specificity, the physiologic expression of human MMP-13 is restricted, as so far it has only been detected in developing fetal bone [22, 23]. In contrast, human MMP-13 is expressed in pathologic conditions characterized by excessive degradation of collagenous ECM, i.e., osteoarthritis and rheumatoid arthritis [7, 14, 23–26], chronic cutaneous ulcers [27], intestinal ulcerations [28], chronically inflamed periodontal tissue [29], and in atherosclerotic plaques and aortic aneurysms [30, 31]. MMP-13 expression has also been detected in invasive malignant tumors, i.e., breast carcinomas [17, 32, 33], squamous cell carcinomas (SCCs) of the head and neck [34–36] and vulva [37], chondrosarcomas [38], and malignant melanomas [39].

Stromelysins

Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) are closely related with respect to structure and substrate specificity. MMP-3 and MMP-10 are expressed by fibroblastic cells and by normal and transformed

epithelial cells in culture and in vivo [2–5]. MMP-3 and MMP-10 degrade a wide range of ECM proteins, e.g., type IV, V, IX, and X collagens, proteoglycans, gelatin, fibronectin, and laminin fibrillin-1 [1, 19, 40]. MMP-3 also cleaves α1-proteinase inhibitor, tumor necrosis factor (TNF)-α precursor, and myelin basic protein [40], and degrades and inactivates interleukin (IL)-1β [41].

Stromelysin-3 (MMP-11), matrilysin (MMP-7), and metalloelastase (MMP-12) are often included in the stromelysin subgroup, although they are structurally less closely related to MMP-3 and MMP-10 [1]. MMP-11 is expressed in breast cancer, uterus, placenta, and involuting mammary gland [42]. To date, human MMP-11 has not been shown to degrade any ECM component, but it degrades serine proteinase inhibitors, α1-proteinase inhibitor and α1-antitrypsin [43].

Matrilysin (MMP-7) is the smallest MMP, as it lacks the hemopexin domain. MMP-7 is expressed by normal glandular epithelial cells in endometrium, small intestinal crypts, skin, and airways [44, 45]. MMP-7 is also expressed by malignant epithelial cells in tumors of the gastrointestinal tract, prostate, and breast [46, 47]. In addition to a wide range of ECM components, including fibronectin, laminin, nidogen, type IV collagen, and proteoglycans, MMP-7 cleaves β4 integrin [48].

Macrophage metalloelastase (MMP-12) is expressed in placenta, by alveolar macrophages in pulmonary emphysema and by fibroblasts in granulomatous diseases of the intestine and skin [28, 49, 50]. MMP-12 degrades elastin, type IV collagen, type I gelatin, fibronectin, laminin, vitronectin, proteoglycans, myelin basic protein, and α1-antitrypsin [51].

Gelatinases

Gelatinase-A (72-kDa gelatinase, MMP-2) is expressed by various cell types, including fibroblasts, keratinocytes, endothelial cells, chondrocytes, osteoblasts, and monocytes, and by different types of transformed cells [1]. Gelatinase-B (92-kDa gelatinase, MMP-9) is produced by normal alveolar macrophages, polymorphonuclear leukocytes, osteoclasts, keratinocytes, invading trophoblasts, and by several types of transformed cells [1]. Gelatinases degrade type IV, V, VII, X, XI, and XIV collagens, gelatin, elastin, proteoglycan core proteins, myelin basic protein, fibronectin, fibrillin-1, and precursors of TNF-α and IL-1β [1, 2, 19, 40, 41]. MMP-2 also degrades native type I collagen [52] and MMP-9 cleaves N-terminal telopeptide of type I collagen in an acidic environment [53], suggesting that MMP-2 and MMP-9 also play a role in the remodeling of collagenous ECM.

Membrane-type MMPs

At present, five MT-MMPs are known: MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT4-MMP (MMP-17), and MT5-MMP (MMP-24) (fig. 1). MT-MMPs contain a cleavage site for furin proteinases between propeptide and catalytic domain providing the basis for furin-dependent activation of latent MT-MMPs prior to secretion. MT1-MMP activates latent MMP-2 at the cell membrane by a mechanism involving interaction of the C terminus of proMMP-2 with MT1-MMP/TIMP-2 complex [54–56], but it also cleaves type I, II, and III collagen, gelatin, fibronectin, laminin-1, vitronectin, cartilage proteoglycans, and fibrillin-1 [19, 21, 57]. MT1-MMP is expressed by dermal fibroblasts, malignant epithelial cells, and osteoclasts [56, 58, 59]. MT2-MMP is expressed in human placenta, brain, and heart. MT2-MMP also activates proMMP-2 and proMMP-13 and degrades laminin, fibronectin, and tenascin [21, 60]. MT3-MMP is expressed in lung, placenta, kidney, ovary, intestine, prostate, spleen, heart, and skeletal muscle [61]. MT3-MMP activates proMMP-2 and also hydrolyzes gelatin, casein, type III collagen, and fibronectin, and can be detected both in membrane-bound and soluble form [62, 63]. MT4-MMP is expressed in the brain, leukocytes, colon, ovary, testis, breast carcinomas, and breast cancer cell lines [64]. The substrate specificity of MT4-MMP is not known. MT5-MMP activates latent MMP-2 and is predominantly expressed in brain, kidney, pancreas, and lung, and at a high level in brain tumors [65]. Interestingly, MT5-MMP is also shed from the cell membrane, suggesting that it may function both as membrane-bound and soluble proteinase [66].

Other MMPs

Three recently cloned human MMPs do not fit well into any subgroup mentioned above, based on structure and substrate specificity. MMP-19 was cloned from human mammary gland and liver cDNA and is expressed in a wide variety of human tissues [67, 68]. The ability of MMP-19 to degrade native ECM components is not known. Enamelysin (MMP-20) was cloned from odontoblasts, has a restricted expression in dental tissues and degrades amelogenin [69]. Human MMP-23 was cloned from an ovary cDNA library [70]. Interestingly, MMP-23 contains no signal peptide or hemopexin-like domain, and its ability to degrade native ECM components is not known [70].

Regulation of MMP activity

Most MMPs are not constitutively expressed by cells in vivo, but their expression is induced by exogenous sig-

nals, e.g., cytokines, growth factors, or altered cell-matrix and cell-cell contacts [see refs 1, 2]. However, collagenase-2 (MMP-8) and 92-kDa gelatinase (MMP-9) are stored in secretory granules of neutrophils and eosinophils [71] and matrilysin (MMP-7) in secretory epithelial cells in exocrine glands of e.g., skin, gastrointestinal tract, and airways [44, 45]. Expression of MMPs is regulated at the level of transcription, although modulation of MMP mRNA half-life by growth factors and cytokines has also been documented. The proteolytic activity of MMPs is regulated by zymogen activation and inhibition by specific inhibitors, i.e., TIMPs, and by nonspecific proteinase inhibitors, e.g., α1-proteinase inhibitor and α2-macroglobulin.

Transcriptional regulation

Expression of most MMPs (MMP-1, -3, -7, -9, -10, -12, -13, and -19) is induced at the transcriptional level, e.g., by growth factors and cytokines, oncogenes, hormones, and contact to the ECM [for a review see ref. 1]. The promoters of these inducible MMPs contain a conserved AP-1 cis-element between -65 and -79 with respect to the transcription start site [see ref. 72]. The extracellular stimuli activate the AP-1 transcription factor complexes (dimers composed of members of Fos and Jun families) [73], which bind to the AP-1-binding site in the promoter and stimulate transcription of the MMP genes. The promoter regions of the AP-1-responsive MMPs also contain one or multiple polyomavirus enhancer A-binding protein-3 (PEA3) elements, which bind transcription factors of the ETS family and cooperate with the AP-1 element for maximal activation of MMP-1, MMP-3, and MMP-9 promoters [74, 75]. Expression of ETS-1 has been demonstrated in stromal fibroblasts adjacent to invading tumor cells and in endothelial cells during tumor vascularization [76, 77]. Transcription factor NF-κB plays a pivotal role in expression of MMP-9 in fibroblasts and vascular smooth muscle cells [78].

The promoter of the MMP-2 gene is relatively unresponsive to stimulation in cultured cells and it lacks not only the adjacent AP-1 and PEA3 elements, but also the classical TATA box [see ref. 72]. MT1-MMP is also constitutively expressed by different types of cells in culture [79].

The induction of expression of the components of the classical AP-1 dimer, c-Jun, and c-Fos is mediated by three distinct classes of mitogen-activated protein kinases (MAPKs), i.e., extracellular signal-regulated kinase (ERK), stress-activated protein kinase/Jun N-terminal kinases (SAPK/JNKs), and p38 [see ref. 72]. In general, the ERK1,2 cascade is activated by mitogenic signals, resulting in phosphorylation of various substrates, including Elk-1, and in subsequent activation of c-*fos* transcription. SAPK/JNKs and p38 are activated by cytokines (TNF, IL-1) and cellular stress, such as UV light, resulting in phosphorylation of c-Jun and ATF-2, which then induce c-*jun* transcription [see ref. 73]. The balance between distinct MAPK pathways is thought to regulate cell growth, differentiation, survival, and death. Constitutively active mutants of Raf-1 and MEK1 transform fibroblasts in vitro [80, 81] and in vivo activation of the ERK1/2 pathway has been observed in renal and breast carcinomas [82, 83]. Our recent findings together with observations by others indicate that distinct MAPKs play an important role in the regulation of MMP expression [84–88]. Specifically, blocking the p38 MAPK pathway by a specific chemical inhibitor potently inhibits expression of MMP-13, MMP-1, and MMP-9 by transformed keratinocytes and SCC cells, and inhibits their invasion through Matrigel and collagen [86, 89]. In addition, blocking the ERK1,2 pathway by a specific chemical inhibitor PD98059 inhibits expression of MMP-1 and MMP-9 by SCC cells and inhibits their invasion in vitro [89].

Activation of latent MMPs

Most MMPs are secreted as latent precursors (zymogens), which are proteolytically activated in the extracellular space [for a review see ref. 90]. Latent MMPs are retained in the proform by a 'cysteine switch' formed by covalent interaction of the conserved cysteine in the propeptide with the catalytic zinc [91]. Various compounds, e.g., organomercurials (APMA) can react with cysteine, converting it to a nonbinding form, exposing the catalytic site, and resulting in autocatalytic cleavage of the propeptide. The propeptide of most MMPs can also be cleaved by a number of other extracellular proteinases, e.g., plasmin and other MMPs [1–4]. All MT-MMPs and MMP-11 contain a potential cleavage site for the prohormone convertases (e.g., furin) which occur in the Golgi complex and pericellular space, suggesting a role for these proteinases in their proteolytic processing [92, 93]. Activation of latent MMP-2 and MMP-13 at the cell membrane by MT1-MMP provides a potent way of directing their activity to the pericellular environment.

Inhibition of MMP activity

The activity of MMPs in the pericellular space is strictly controlled by nonspecific inhibitors, e.g., α2-macroglobulin and by the specific inhibitors, TIMPs.

Tissue inhibitors of metalloproteinases (TIMPs)

At present, four members of the TIMP gene family are known: TIMP-1, -2, -3, and -4. All TIMPs share struc-

tural features, especially 12 conserved cysteine residues, which form six disulfide bonds [for a review see refs 94, 95]. TIMPs bind to the zinc-binding catalytic site of the MMPs with a 1:1 molar ratio. In addition, TIMP-2 and TIMP-1 can bind to the hemopexin domain of latent MMP-2 and MMP-9, respectively. TIMP-1 inhibits the activity of most MMPs, with the exception of MT1-MMP and MMP-2. TIMP-2 also inhibits the activity of most MMPs, except MMP-9. TIMP-3 inhibits the activity of MMP-1, -2, -3, -9, and -13 [18, 96], and human TIMP-4 that of MMP-2, -9, and -7 [94, 97].

TIMPs are expressed by a variety of cell types and they obviously play an important role e.g., in tissue development, angiogenesis, cancer cell invasion, and metastasis, by regulating MMP activity and stimulating cell growth [94, 95]. TIMP-1, TIMP-2, and TIMP-4 are secreted in soluble form, whereas TIMP-3 is associated with the ECM [98]. Expression of TIMP-1 in cultured cells is stimulated e.g., by growth factors, cytokines, and phorbol ester, whereas the expression of TIMP-2 is constitutive [95]. Expression of TIMP-3 is induced in response to mitogenic stimulation and during normal cell cycle progression [see ref. 95] and it is inhibited by TNF-α in fibroblasts [99]. Expression of TIMP-4 in vivo is especially abundant in the heart [97], but it is also expressed at the sites of tissue injury, i.e., dermal wounds and vascular injury [100, 101].

Proteolysis of ECM during tumor invasion

Tumor growth involves alterations in the stromal ECM, and malignant tumors often induce a fibroproliferative response in the adjacent stroma, characterized by increased expression of type I and III procollagens [102]. The formation of tumor stroma is often viewed as a nonspecific host attempt to wall off the tumor, and it is thought to have a negative influence on tumor progression. During the process of metastasis formation, malignant cells detach from the primary tumor, invade the stromal tissue, enter the circulation, arrest at the peripheral vascular bed, extravasate, invade the target organ interstitium and parenchyma, and form a metastatic colony [4, 103]. Tumor cells must escape the host immune surveillance and therefore only a fraction of circulating tumor cells successfully initiate metastatic colonies [103]. Tumor-induced angiogenesis is essential for growth of the primary tumor and metastases, and new blood vessels are also frequent sites for tumor cell entry into the circulation. It is conceivable that proteolytic degradation of ECM plays a crucial role in all the above-mentioned aspects of tumor development.

MMPs in tumor invasion

A considerable body of evidence has accumulated implicating MMPs in cancer spread. In fact, several MMPs were first purified and cloned from tumor cell lines or tissues. A number of studies have demonstrated a positive correlation between MMP expression and invasive and metastatic potential of malignant tumors, including colon, lung, head and neck, basal cell, breast, thyroid, prostate, ovarian, and gastric carcinomas [see refs 3, 4]. For example, expression of MMP-1 is associated with poor prognosis in colorectal cancer and esophageal cancer [104, 105] and expression of MMP-2 and MMP-3 is closely related to lymph node metastasis and vascular invasion in SCC of the esophagus [106]. Similarly, abundant expression of MMP-13 in SCCs of the head and neck and vulva is associated with their metastasis capacity [34, 37]. MMP-11 expression correlates with increased local invasiveness in head and neck SCCs [107], and the level of MMP-2 expression with poor prognosis of cervical SCCs [108]. It should be noted that all MMPs whose expression has been documented in malignant tumors can also be expressed by nonneoplastic cells. However, MMP-13, MMP-7, and MT1-MMP can be regarded as transformation-specific MMPs at least in keratinocytes, as they are expressed by malignantly transformed keratinocytes in SCCs, but not in normal keratinocytes, indicating that their expression serves as a marker for transformation [34, 37, 109]. In addition, the expression of MMP-2 appears to serve as a marker for malignant transformation of cervical epithelial cells [108, 110].

Direct evidence for the role of distinct MMPs in tumor growth and invasion has recently been provided by mice with targeted disruption of a specific MMP gene [3]. Mice lacking MMP-7 show decreased intestinal tumorigenesis [111], and MMP-11-deficient mice show impaired tumor formation in response to chemical mutagenesis [112]. MMP-2 knockout mice show reduced tumor growth and formation of metastases by Lewis lung carcinomas and B16-BL6 melanoma cells [113]. In addition, studies with MMP inhibitors have provided supporting evidence for the role of MMPs in tumor progression and metastasis [see refs 4, 114].

In malignant tumors, most MMPs are produced by nonmalignant stromal cells rather than tumor cells [115]. Tumor cells can also secrete factors, such as extracellular MMP inducer (EMMPRIN), which enhances the expression of MMP-1, MMP-2, and MMP-3 by fibroblasts [116]. In addition, many growth factors and cytokines secreted by tumor-infiltrating inflammatory cells as well as by tumor or stromal cells are capable of modulating MMP expression. Invasion of malignant tumors involves an interplay between tumor cells, stromal cells, and inflammatory cells and it is likely that all these cells express distinct, although some-

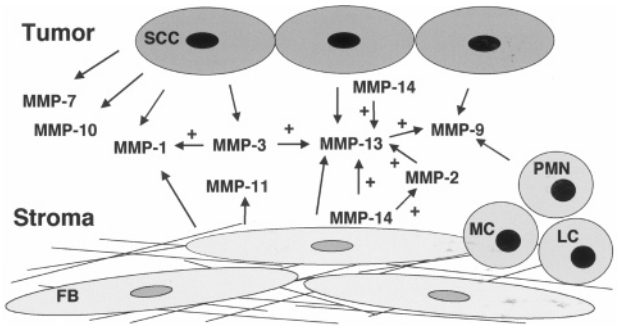

Figure 2. Expression of distinct MMPs by tumor cells, stromal fibroblasts (FB), and inflammatory cells in squamous cell carcinomas (PMN, polymorphonuclear leukocyte; MC, monocyte; LC, lymphocyte). Activation of latent MMPs is indicated by +.

what overlapping patterns of MMP [see refs 4, 115]. For example, in SCC invading tumor cells, infiltrating inflammatory cells and stromal fibroblasts express distinct MMPs, which may complement each other's substrate specificity and form a network of MMP cascades, in which a single MMP cleaves a particular native or partially degraded ECM component and activates other latent MMPs (fig. 2). For example, in SCCs, MMP-13 is mainly expressed by tumor cells, whereas MMP-1 is expressed mainly by stromal fibroblasts, matrilysin (MMP-7) is only expressed by tumor cells, MMP-2 by stromal fibroblasts, MT1-MMP by tumor and stromal cells, and MMP-9 by tumor cells and inflammatory cells [34, 35, 37, 71]. In SCCs, colocalization of the cells expressing MT1-MMP and MMP-2 with tumor cells producing MMP-13 likely creates optimal conditions for pericellular activation of tumor cell-derived latent MMP-13 and stromal cell-derived MMP-2. In addition, expression of MMP-3 and MMP-7 by some SCC tumor cells provides further activity for a tumor-driven proteolytic cascade, in which latent MMP-13 can be activated by MT1-MMP or MMP-3, both expressed by tumor cells [37, 117]. Furthermore, expression of MMP-9 by invading tumor cells and adjacent tumor infiltrating inflammatory cells adds a further link to this MMP cascade, as MMP-13 can activate latent MMP-9. As the substrate specificity of the MMPs present in the peritumoral environment of SCCs is different, each MMP can be proposed to play a distinct role at different stages of SCC growth and invasion. In this context it is interesting, that MMP-3, MMP-7, MMP-9, and MMP-12 have recently been shown to generate angiostatin from plasminogen, indicating that their expression in the peritumoral area may in fact serve to limit tumor-induced angiogenesis [118–120].

TIMPs in tumor growth and invasion

A number of studies have demonstrated the expression of TIMPs in tumor stroma and tumor tissue, but the prognostic value of TIMP expression is not well established. For example, in breast cancer, TIMP-2 expression correlates with tumor recurrence [121], and in cervical carcinomas, TIMP-2 expression correlates with a poor prognosis [109, 122]. Similarly, in malignant breast cancer, TIMP-1 expression is enhanced compared to nonmalignant breast tumor [122]. However, the MMP:TIMP ratio is elevated in cervival carcinomas with poor prognosis, indicating that evaluation of either MMP or TIMP expression alone is not always sufficient for prognostication of malignancies [123]. In general, there is convincing evidence that overexpression of TIMPs by cancer cells or by the host reduces the invasive and metastatic capacity of tumor cells. In cutaneous and oral SCCs, expression of TIMP-1, TIMP-2, and TIMP-3 is detected in stromal cells adjacent to the tumor [124–126], suggesting that their expression represents a host attempt to limit tumor invasion and tumor-induced angiogenesis. This notion is supported by the observations of Polette et al. [127] indicating that the presence of TIMP-1 and TIMP-2 in SCCs correlates with less aggressive growth.

Inhibition of cancer cell invasion has been achieved in vitro and in vivo by recombinant TIMPs or by overexpression of TIMPs using a variety of gene delivery vehicles. Recombinant TIMP-2 inhibited the invasion of HT-1080 fibrosarcoma cells in vitro [128, 129], but had no effect on tumor cell growth. Overexpression of TIMP-1 in gastric cancer cells reduced metastasis of clones expressing high levels of TIMP-1 [130]. TIMP-1 also reduced the growth rate and inhibited invasion of astrocytoma cells [131], prevented metastasis of gastric cancer cells [132] and inhibited invasion and growth of mammary carcinoma cells [133]. The ability of TIMP-1 to alter tumor development at different stages has been highlighted by the use of transgenic mice. Mice with constitutive overexpression of TIMP-1 in the liver showed suppressed tumor initiation, growth, and angiogenesis when crossed with transgenic mice expressing the SV40 T antigen, which develop hepatocellular carcinomas [134]. The effects on tumor initiation apparently represent a protective effect of TIMP-1 on the liver. These observations were also supported by a recent study in which TIMP-1 overexpression in the brain prevented tumor formation [135].

Overexpression of TIMP-2 in melanoma cells reduced their MMP activity and suppressed tumor growth in the skin of immunodeficient mice [136], but did not affect the rate of spontaneous metastasis. Conversely, Oku et al. [137] demonstrated that melanoma cells overexpressing TIMP-2 had a reduced metastatic activity in vivo.

Using a different melanoma cell line, Koop et al. [138] showed that melanoma cells overexpressing TIMP-1 had a reduced metastasis capacity due to inhibition of tumor growth following extravasation. TIMP-4 overexpression in breast carcinoma cells also inhibits invasion in vitro and tumor growth in vivo, and results in a reduction in lymph node and lung metastasis [139]. Together these studies highlight both similar and diverse effects of overexpression of individual TIMPs on tumor cell phenotype in vivo.

Further studies have demonstrated distinct effects of individual TIMPs on cell survival. Valente et al. [140] reported that overexpression of TIMP-2 reduced invasion and angiogenesis, but also protected the melanoma cells from apoptosis although it increased necrosis. Furthermore, TIMP-1 promotes the survival of B cells through modulation of CD40 levels [141]. However, we and others have recently shown that adenoviral-mediated gene delivery of TIMP-3 promotes apoptosis of a number of cell types including human melanoma, HeLa, MCF-7 breast carcinoma, and HT-1080 fibrosarcoma cells, while TIMP-1 and -2 have no effect on the viability of these cells [142–144]. This apoptotic effect was associated with a reduced capacity of TIMP-3-transduced cells to bind to ECM components suggesting disruption of the cell-matrix survival pathway. TIMP-3-overexpressing stable colon carcinoma cell lines show reduced tumor growth in vivo [145] and in this model, TIMP-3 overexpression resulted in apoptosis of tumor cells through stabilization of TNF-α receptors on the cell surface [146]. This suggests that individual TIMPs may modulate the levels of death proteins at the cell surface, as demonstrated by findings that shedding of FAS from the cell surface is mediated by MMP-induced cleavage and is inhibited by synthetic MMP inhibitors [147]. Furthermore, TIMP-3 inhibits the activity of TNF-α convertase (TACE or ADAM-17) providing further evidence for complex regulation of death ligands and receptors by MMPs and TIMPs [148], which may play an important role in the survival, growth, and invasion of malignant cells.

At present, several synthetic MMP inhibitors are in clinical trials to evaluate their ability to inhibit the growth and invasion of malignant tumors in vivo [3, 114]. Gene delivery of TIMP-1, -2, and -3 into malignant cells may also be a potent way of inhibiting tumor invasion and survival [142]. Furthermore, an effective way of inhibiting the expression of MMPs may be by blocking signaling pathways mediating the activation of MMP transcription. The on-going clinical trials are expected to show whether the concept of MMP inhibition has a place in the therapeutic arsenal aimed at inhibiting the growth, invasion, and metastasis of malignant tumors arising from different histogenetic backgrounds.

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