Matrix metalloproteinases and the development of cancer

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Proteolytic remodeling of the extracellular matrix is an important aspect of the creation and progression of cancer. Matrix metalloproteinases are important at several points during multistage neoplastic progression in tumor cells and responding blood vessels, inflammatory cells and stroma.

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Introduction

Invasion of cells from one tissue into a neighboring tissue occurs during many physiological processes, both normal and pathological. These include the invasion of blood vessels into sites of tissue growth and inflammation, cell migration during wound healing, embryo implantation, ovulation, involution of the mammary gland during lactation, and the dissemination of tumors. In all of these processes the invading cells must breach barriers opposing their movement. These barriers include basement membranes, the stromal matrix, and cell–cell junctions. A common mechanism is believed to facilitate breaching of all of these barriers during invasion, namely release of proteolytic enzymes from either the invading cells, the opposing and responding cells, or both. The types of proteinases involved, the types of cells expressing them, and their precise roles are likely to be different for different types of tissues and circumstances. Nevertheless, there are three classes of proteinases (matrix metalloproteinases, serine proteases and cysteine proteases) that have altered distribution, increased expression and/or increased activity, during these processes, and are therefore believed to be involved in the matrix remodeling that facilitates invasion.

The concept that tumorigenesis is a multistep process has been well documented and is widely accepted. Historically, this has been thought to be exclusively a process of the progressive acquisition of mutations in key growth control genes, (oncogenes or tumor suppressor genes). Such mutations bestow upon cells traits associated with malignancy, for example, enhanced proliferation, invasive capability and the ability to grow in ectopic tissue environments. During tumor development, changing relationships between the premalignant and malignant cells and their microenvironment characterize all stages of the tumorigenic process. Although intrinsic factors are necessary for cellular transformation, extrinsic factors affecting the distribution, composition, and function of the extracellular matrix (ECM) into which a tumor expands, clearly make just as important a contribution to neoplastic progression and malignant conversion. Proteolytic enzymes are some of these extrinsic factors. It is significant that expression of the genes encoding these extrinsic factors does not result directly from mutation. Instead, altered expression of the normal genes is part of the response of the tumor and host to the neoplastic process. Studies of proteolysis in tumorigenesis have previously focused on basement membrane destruction during tumor invasion and metastasis. However, recent evidence suggests that proteinases, specifically matrix metalloproteinases (MMPs), are also involved in the earlier stages of tumor progression.

Table 1

The matrix metalloproteinase multigene family.

Matrix metalloproteinase	EC name	Substrates
Minimal domain MMP		
Matrilysin (Pump-1)	MMP-7	Proteoglycans, laminin, fibronectin, gelatins, collagen IV, elastin, entactin, tenascin
Hemopexin/vitronectin domain MMPs		
Simple-type		
Collagenase-1 (interstitial)	MMP-1	Collagens, I, II, III, VII, X, gelatins
Collagenase-2 (neutrophil)	MMP-8	Collagens I, II, III
Collagenase-3	MMP-13	Aggrecan, collagen I, II, III, gelatins
Collagenase-4	MMP-18	Collagen I
Metalloelastase	MMP-12	Elastin, fibrinogen, fibronectin
Stromelysin-1 (transin)	MMP-3	Proteoglycans, laminin, gelatins, collagens III, IV, V, IX, Fibronectin, gelatins, entactin, SPARC, collagenase-1
Stromelysin-2	MMP-10	Proteoglycans, laminin, gelatins, collagen III, IV, V, IX, Fibronectin
Furin activated-type		
Stromelysin-3	MMP-11	Laminin, fibronectin, α1-proteinase inhibitor
Membrane-type MMP-1	MMP-14	Collagen I, II, III, fibronectin, vitronectin, proteoglycans, Progelatinase A, procollagenase-3
Membrane-type MMP-2	MMP-15	Progelatinase A
Membrane-type MMP-3	MMP-16	Unknown
Membrane-type MMP-4	MMP-17	Unknown
Fibronectin-type		
Gelatinase A (72 KDa type IV collagenase)	MMP-2	Gelatins, collagens I, IV, V, VII, X, fibronectin, elastin, Procollagenase-3
Gelatinase B (92 KDa type IV collagenase)	MMP-9	Gelatins, collagens IV, V, elastin

EC name, enzyme commission nomenclature. Data from [1,4,5,47–50].

Here we review the data suggesting that MMPs are involved in neoplasia, the tissue-specific expression patterns observed for MMPs during tumorigenesis and the mechanisms regulating their proteolytic activities, and the evidence suggesting that MMPs participate in neoplastic progression at multiple points.

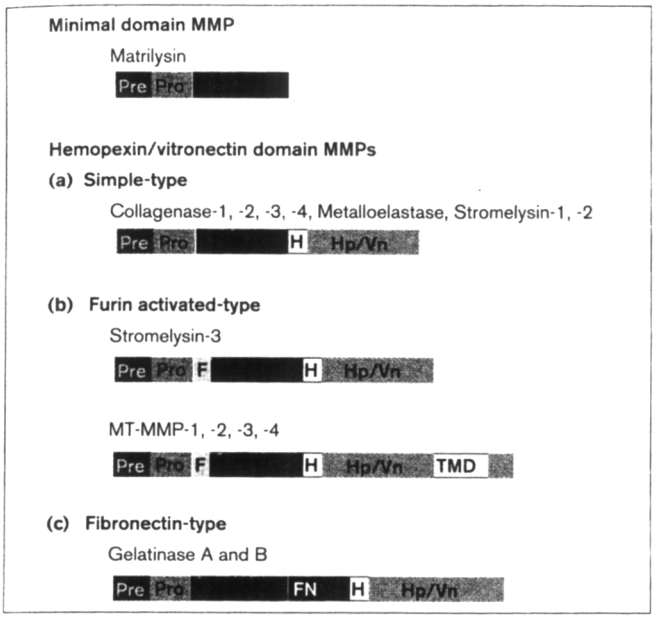
The MMP multigene family

The MMP multigene family currently has 15 members (Table 1). MMPs are named for their catalytically active chelated zinc and calcium dependence, and are therefore characterized as metal-binding proteinases. There are MMP family members with enzymatic activity against virtually all components of the ECM and basement membranes. Most importantly, the MMP family includes the only enzymes capable of cleaving and denaturing fibrillar collagens. The compositions of the ECM and basement membrane vary depending on location, but generally include various forms of collagens, glycoproteins (laminin, fibronectin, entactin, nidogen), proteoglycans, and glycosaminoglycans. Penetration and/or degradation of basement membranes or ECM requires several different proteolytic activities because of their diversity in macromolecular composition. Thus, during matrix remodeling, a complex cascade of proteolytic events occurs involving multiple MMP family members.

MMP family members share many common features in addition to their zinc and calcium dependence. At the transcriptional level, MMPs are responsive to cytokines, growth factors, and hormones [1]. Furthermore, changes in the pericellular environment that modify cell–ECM interactions and/or composition of the ECM can confer differential expression of MMP mRNAs [2,3].

MMPs share extensive sequence homology. The MMP family can be grouped and subdivided based on the number and characteristics of specific functional protein domains, as shown in Figure 1 ([4]; Table 1). We now have some information about the functions of these domains. With the exception of matrilysin, all MMP family members contain a carboxy-terminal hemopexin/vitronectin-like domain. This domain may have different functions in different MMP family members. In progelatinase A and B it is thought to mediate interactions with specific proteinase inhibitors (discussed below); in collagenase-1 and -2, however, it is associated with inhibitor and substrate binding [5]. The hinge region, which links the hemopexin and catalytic domains, may be important in determining substrate specificity. Generally, the hinge region is variable in length and composition among family members, but MMPs that can degrade fibrillar collagens all contain a hinge of distinct size and composition [6].

Figure 1



Domain structure of MMP family members. Classification of MMP family members based on functional domain structure. Catalytic, domain containing the catalytic active site and metal binding sites; F, furin-recognition domain; FN, domain containing homology to the collagen binding region of fibronectin; H, 'hinge' region linking catalytic and hemopexin domains; Hp/Vn, carboxy-terminal domain containing homology to hemopexin and vitronectin; Pre, domain containing signal sequence; Pro, a propeptide domain that is cleaved during activation; TMD, transmembrane spanning domain. Modified from [4].

Structure–function studies have confirmed that the substrate specificity is dictated by this region [7]. In all MMP family members the catalytic domain contains three conserved histidines. The structure of the full length active collagenase [8] confirms that the zinc contained in the catalytic site is coordinated by these conserved histidines. Gelatinase A and B do contain these conserved histidine amino acid residues, but also contain a 182-amino-acid insertion in this domain that is homologous to the collagen-binding region of fibronectin, and is required for collagen binding of gelatinase A as well as cleavage [9].

All MMPs are synthesized as inactive zymogens. Following activation (discussed below) MMPs are subject to further regulation by a family of naturally occurring inhibitor proteins termed tissue inhibitors of metalloproteinases, or TIMPs. To date, three human TIMPs (TIMP-1, TIMP-2, and TIMP-3) have been cloned [10], and the existence of three additional MMP inhibitor proteins (imp-a, imp-b, and TIMP-4) has been reported ([11]; Table 2). TIMPs bind either proMMPs or active MMPs with 1:1 stoichiometry, thereby inhibiting the autocatalytic activation of latent enzymes as well as the proteolytic capacity of active proteinases. TIMPs do, however, show some differences in their abilities to form complexes with MMPs. For example, TIMP-1 forms a tight complex with progelatinase B, whereas TIMP-2 instead forms a tight complex with progelatinase A. TIMPs can be proteolytically inactivated by diverse proteinases, for example neutrophil elastase (a serine proteinase) trypsin and stromelysin-3 [12]. MMPs can also be inhibited by noncovalent association with α2-macroglobulin [13].

Activation of MMPs

The zymogen forms of MMPs are inactive. Crystallographic studies have confirmed that this is due to coordinate bonding between the active site zinc atom and an unpaired cysteine thiol group located near the carboxyl end of MMP propeptides [13]. The primary mechanism for MMP activation is interruption of the cysteine–zinc interaction, referred to as the cysteine switch ([14]; Fig. 2). In cell-free systems, the cysteine–zinc atom interaction can be interrupted by organomercurials and chaotropic agents. Limited proteolysis of the propeptide also destabilizes the cysteine–zinc bond. Interruption of the cysteine–zinc bond, by any means, results in conformational changes, opening the switch (Fig. 2). Following opening, autocatalytic or proteolytic cleavage of the remainder of the propeptide yields a truncated and catalytically competent enzyme.

MMPs containing a furin-like recognition domain in their propeptides (stromelysin-3 and membrane-type MMPs (MT-MMPs)) are activated intracellularly by a group of calcium-dependent transmembrane serine proteinases of

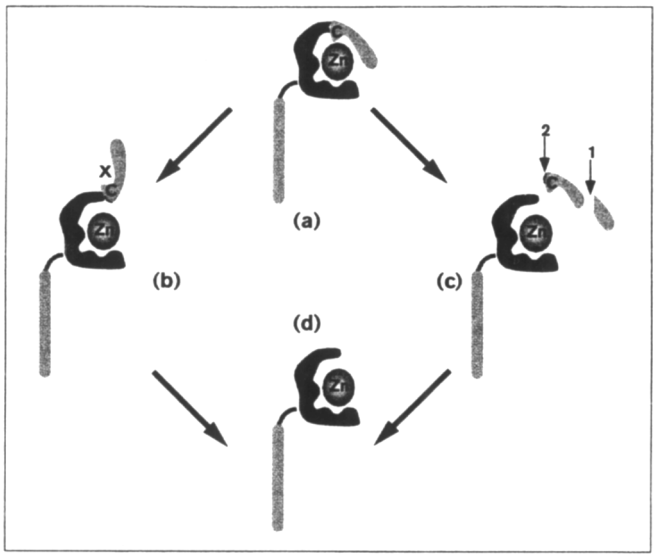
Table 2

The TIMP multigene famiy

MMP inhibitor	Molecular weight (x 10^3)	Target MMP
TIMP-1	28.5	Collagenase-1, gelatinase B > gelatinase A >>> MT-MMP-1
TIMP-2	21	Gelatinase A > stromelysin-1, gelatinase B, MT-MMP-1
TIMP-3	24–25	Gelatinase A = gelatinase B>MT-MMP-1
TIMP-4	nr	unknown
imp-a	29	unknown
imp-b	30	unknown

Nr, molecular weight has not been reported; >, activity towards protein to the left of symbol is greater than that towards protein to the right of symbol; =, equivalent activity towards both proteins. Data from [1,4,5,47–50].

Figure 2



Multistep activation of MMPs. **(a)** In the zymogen, the zinc ion is ligated by three histidines (H) in the catalytic domain and a cysteine in the pro-peptide. The cysteine switch mechanism of activation can be triggered in one of two ways. **(b)** Interaction of the cysteine with a thiol reagent (X), such as an organomercurial or certain chaotropic agents, produces a conformational change making the catalytic site accessible. This conformation may have enzymatic activity. Subsequently, the pro-peptide is removed by autocatalytic cleavage (see (d)). **(c)** A prote-olytic enzyme such as trypsin can also cleave the propeptide (1) trigger-ing a confor-mational change and activation of the cysteine switch. **(d)** An additional proteolytic step(s) which may be autocatalytic (2), cleaves the remainder of the propeptide from either (b) or (c) making the MMP fully active.

the subtilisin group termed furin/PACE/kex-2-like proteinases (Fig. 3). MMPs without this recognition sequence are secreted in latent form. Serine proteinases, such as plasmin or urokinase-type plasminogen activator, elastase and trypsin, cleave propeptide domains of secreted proMMPs and consequently induce autocatalytic activation of collagenase-1, stromelysin-1 and gelatinase B; progelatinase A is resistant to activation by serine proteinases. Some of the activated MMPs can further activate other proMMPs. For example, stromelysin-1 activates pro-collagenase-1 and pro-gelatinase B [15], whereas pro-gelatinase A is resistant to activation by other MMPs. Thus, serine proteinases are believed to be initiators for a complex array of activation cascades of proMMPs *in vivo*.

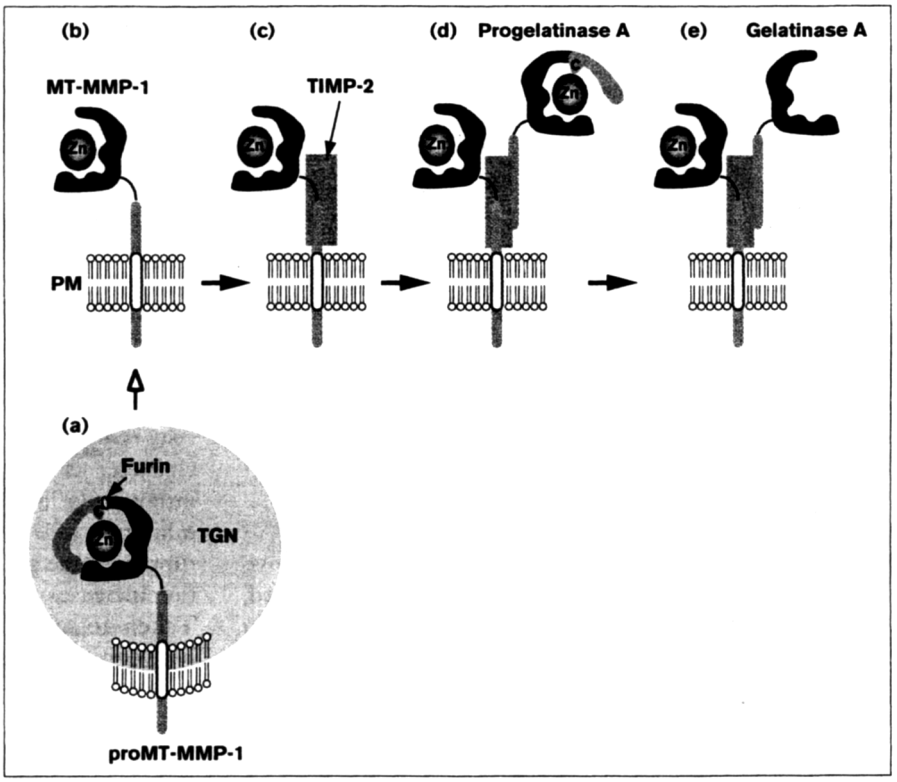
Cell-mediated activation mechanisms are also possible, the model being activation of gelatinase A. This mechanism remained elusive until recently when several MT-MMPs, proteinases that span the plasma membrane, were discovered [5]. MT-MMPs are located in plasma membrane-associated ternary complexes. The prototype is the complex that contains progelatinase A, TIMP-2 and activated MT-MMP-1 (Fig. 3). Activated MT-MMP-1 acts as a cell-surface receptor for TIMP-2. (MT-MMP-1 and TIMP-2 can also form a secreted binary complex [16]). The cell-surface form of the activated binary complex in turn acts as a receptor for progelatinase A, which binds via its carboxy-terminal hemopexin domain [5,17]. MT-MMP-1 activity is sensitive to inhibition by TIMP-2 and TIMP-3, but is not inhibited by TIMP-1 [18,19], the inhibitor that is most readily activated by inflammation [20]. Thus MT-MMP-1 could function as an activator of progelatinase and as a broad spectrum proteinase in the presence of high concentrations of TIMP-1. The facts that MT-MMP-1 is present in high concentrations in tumors and that it is insensitive to TIMP-1 mean that widespread proteolysis of the ECM might be possible at tumor sites. A recent report suggests that gelatinase A may also be localized at the cell surface through interactions with an adhesion receptor [21]. Moscatelli and Rifkin [22] have proposed four possible advantages for having degradative enzymes in a bound state at the cell surface. First, bound proenzymes may be more readily activated and the bound enzymes generated may be more active than the same enzymes found in the soluble phase. Second, bound enzymes may be protected from activation by inhibitors. Third, binding enzymes to the cell surface may concentrate the components of a multistep pathway, thereby increasing the rate of reactions. Fourth, immobilizing enzymes on the surface of a cell or in the matrix may provide a way to restrict the activity of the enzyme, so that only substrates in the vicinity of the cell or only adjacent matrix components are degraded. Localizing activation to the cell surface thus links MMP expression with proteolysis and invasion, and this may actually provide the most significant control point for MMP activity.

MMP expression and neoplasia

The association of MMPs with malignant transformation is well documented both *in vitro* and *in vivo* [1]. Several MMPs were first cloned from tumor cell lines (gelatinase A, gelatinase B, collagenase-3, matrilysin, stromelysin-1, stromelysin-2, MT-MMP-1 and MT-MMP-4), or as metastasis-specific genes from metastatic tumors (stromelysin-3) [1,4,5]. In fact, expression of all members of the MMP family, whether constitutive or inducible, has been documented in cultured neoplastic cells from diverse developmental lineages ([1,23]; Table 3). MMPs have also been associated with the malignant phenotype in a wide variety of human tissues, including lung, prostate, stomach, colon, breast, squamous carcinoma of the head and neck, melanoma and osteosarcoma. It is notable that two MMP family members are associated with poor clinical outcome: collagenase-1 expression in colorectal carcinoma [24] and stromelysin-3 expression in metastatic breast cancer [4].

There is increasing evidence that the expression profiles for MMPs observed in cultured neoplastic cells are not representative of *in vivo* situations. Neoplastic cells in culture express most members of the MMP family, either constitutively or following induction by oncogenes,

Figure 3



Cell-surface activation of progelatinase A by MT-MMP. **(a)** The newly synthesized proMT-MMP-1 is activated by a furin-like enzyme intracellularly in the trans-Golgi network (TGN) to produce the active MT-MMP **(b)**. **(c)** Active MT-MMP-1 then interacts with TIMP-2 and progelatinase A to form a trimolecular complex **(d)**. **(e)** The MT-MMP-1 then cleaves the progelatinase A tethered to the cell surface through MT-MMP and TIMP-2.

growth factors or cytokines (Table 3). These expression profiles have led investigators to speculate that expression of proteolytic enzymes by tumor cells was a critical step in the transformation process. *In situ* hybridization studies have revealed however, that expression of MMPs in tumors *in vivo* is not limited to neoplastic cells, but frequently originates from tumor-associated stromal cells. For example, in carcinomas collagenase-1, gelatinase A, gelatinase B, metalloelastase, stromelysin-2, and stromelysin-3 are expressed by various stromal cells, (activated fibroblasts, macrophages, neutrophils, endothelial cells) (Table 4). On the other hand, expression of collagenase-3 has only been observed in breast carcinoma cells; this enzyme was initially identified from a breast-tumor-derived cDNA library [25]. The 'normal' expression profile for collagenase-3 in humans is still undefined; in rodents it is widely expressed, particularly in bone. In carcinomas of the colon, prostate, and lung, matrilysin mRNA is also expressed only in tumor cells. In breast carcinomas, however, matrilysin expression has been observed in both tumor and tumor-associated fibroblasts [25]. There has been a recent report of matrilysin expression in an osteosarcoma, confirming that its expression is not limited to cells of epithelial origin [26]. Stromelysin-1 expression has also been observed in both epithelial and mesenchymal cells, the expression being dependent on the stage and/or grade of the tumor. Stromelysin-1 is expressed in stromal fibroblasts in squamous cell carcinomas, although in the later stages of the disease, spindle cell carcinomas, its expression shifts to tumor cells [27]. As these cells are typically vimentin-positive and cytokeratin-negative, it is

Table 3

MMP and TIMP expression in cultured neoplastic cells.

MMP/TIMP	Epithelial	Fibroblast	Other mesenchymal
Matrilysin	+	nr	nr
Collagenase-1	+	+	+
Collagenase-2	+	+	nr
Collagenase-3	+	+	nr
Metalloelastase	nr	nr	+
Stromelysin-1	+	+	+
Stromelysin-2	+	+	nr
Stromelysin-3	+	+	+
MT-MMP-1	+	+	+
MT-MMP-2	+	nr	+
MT-MMP-3	nr	nr	nr
MT-MMP-4	+	nr	nr
Gelatinase A	+	+	+
Gelatinase B	+	+	+
TIMP-1	+	+	+
TIMP-2	+	+	+
TIMP-3	+	nr	nr

(+), Expression was detected by either northern analysis, immunocytochemistry, or zymography; nr, expression has not been reported in these cell types. Data from [1,4,26,46].

likely that expression in the tumor cells reflects the 'de-differentiated' nature of the epithelial cells that have undergone epithelial/mesenchymal conversion [27].

These studies have several implications. Most notably, increased expression of MMP family members does correlate with ECM remodeling during tumorigenesis and with the malignant phenotype. With the exception of matrilysin, most MMPs appear to maintain spatial restriction *in vitro* and are expressed either by stromal cells or by tumor cells but typically not by both. Thus, the mechanisms regulating cell-type specificity in various types of tumors do not appear to be impaired as a consequence of neoplastic transformation. The observation that the tumor-associated expression of MMPs in cultured cells is independent of lineage may result from the fact that culture environments fail to recapitulate the microenvironmental complexities present *in vivo*.

Mechanism of induction of stromal MMPs

The tissue specificity of MMP expression described above may have a role in determining where a MMP is expressed, but does not explain why MMPs are often expressed in normal tissue that is adjacent to a tumor. Aside from soluble growth factors and inflammatory cytokines that induce MMP gene expression, are there other mechanisms regulating stromal expression of MMPs adjacent to tumor cells? There are several reported examples, where direct cell–cell contact between neoplastic and stromal cells in culture has resulted in MMP expression in either or both populations of cells [28]. Furthermore, alterations in cell shape, actin cytoskeleton and cellular interactions with integrins induces the production of certain MMPs in mesenchymal cells [29]. Clearly the tumor stromal cells have different properties from quiescent fibroblasts [30]. Recently, Biswas and colleagues [31] demonstrated the existence of a soluble protein that is not a growth factor or a cytokine, named 'collagenase stimulatory factor', that is secreted by lung carcinoma cells. This novel protein coordinately induces the expression of collagenase-1, gelatinase A and stromelysin-1 in tumor-associated fibroblasts [30]. The idea that a tumor-cell-derived soluble factor might coordinately stimulate stromal MMP expression is supported by the observation that intensity of hybridization for MMP mRNAs *in situ* decreases in stromal cells furthest away from tumor cells. The significance of this upregulation of MMP gene expression in the stromal compartment during tumor development and metastasis is underscored by the recent obser vation indicating that ultraviolet light, which induces basal cell carcinomas in the skin, is a potent inducer of collagenase expression in the dermis *in vivo* [32]. Thus, proteinases appear to be part of the 'crosstalk' between epithelial cells and adjacent stromal cells.

Tumor growth, invasion and metastasis

The functional evidence linking MMPs to primary tumor growth, invasion, and metastasis historically comes from four main sources. There is correlative evidence showing that MMPs are expressed after transformation *in vitro* and in advanced stage tumors *in vivo*. They appear important both in *in vitro* models of invasion and in *in vivo* models of invasion and/or metastasis after injection into syngeneic or immunodeficient animals. And finally, transgenic mice ectopically overexpressing an MMP and/or a TIMP are altered in their ability to develop tumors.

Transfection of specific MMPs into immortalized cells increases the tumorigenicity of the recipient cells [4], but overexpression of TIMPs in neoplastic cells decreases their tumorigenicity, suggesting that these natural inhibitors may function as natural suppressors of cellular invasion. Overexpression of TIMP-2 in Ras-transformed rat embryo fibroblasts reduces their *in vivo* growth rate and locally invasive character as well as reducing lung colony formation after intravenous injection in nude mice [33,34]. TIMP-1 also inhibits *in vivo* metastasis in animal models [1,15], and disruption of the gene for TIMP-1 by homologous recombination increases invasive behavior and metastasis *in vivo* [35]. Taken together, these studies suggest that the balance between ECM-degrading proteinases and their inhibitors is a key factor regulating growth, invasion, and metastasis of neoplastic cells *in vivo*.

TIMPs also possess anti-angiogenic activities, suppressing vascularization of tumors and limiting primary tumor growth [3]. Nanomolar amounts of TIMP-2 block the angiogenic response to basic fibroblast growth factor (bFGF), an important angiogenic cytokine produced by vascularized human tumors. Its ability to do this appears to be independent of its MMP inhibition activity. TIMP-1 and TIMP-2 also inhibit endothelial cell invasion of human amniotic membranes *in vivo* [3]. Apparently, TIMP-2 can inhibit the proliferation of human microvascular endothelial cells, whereas TIMP-1 can block invasion, by regulating proteolysis. The effects of TIMP-1 on endothelial cell invasion can be minimized by the addition of antibodies that neutralize gelatinases [3].

Ectopic expression of MMPs in transgenic animal models suggests that MMPs and TIMPs are involved in neoplastic progression prior to malignant conversion as well as invasion and metastasis. The observation that MMPs are important in stromal–epithelia interactions in mammary development [36] suggested that a transgenic model in which an auto-activating stromelysin-1 (ST-1) gene was targeted to mammary epithelia would be informative. These animals show mammary hyperplasia in virgin animals, decreased mammary differentiation during pregnancy and lactation, an enhanced stromal reaction in the involuting gland, and induction of hyperplasia, angiogenesis, carcinoma *in situ*, and adenocarcinoma with age [37]. A remarkable feature of this model is that when ST-1 overexpressing mice are intercrossed with transgenic mice

Table 4

MMP and TIMP expression in human carcinomas.

MMP/TIMP	Neoplasia	Localization
Matrilysin	Basal cell carcinoma	Tumor epithelium
	Breast	Tumor epithelium; stromal fibroblasts (focal)
	Colorectal	Tumor epithelium
	Gastric	Tumor epithelium
	Prostate	Tumor epithelium
Collagenase-1	Breast	Tumor epithelium (focal), Stromal fibroblasts (tumor associated)
	Colorectal	Stroma
	Gastric	Stromal fibroblasts
	Head and neck	Stromal fibroblasts (tumor associated)
Collagenase-2	Breast	Not detected
Collagenase-3	Breast	Tumor epithelium (focal)
Metalloelastase	Breast	Macrophages (isolated) and necrotic areas
Stromelysin-1	Basal cell carcinoma	Stromal fibroblasts
	Breast	Stromal fibroblasts (tumor associated)
	Colorectal	Stromal cells
	Head and neck	Stromal fibroblasts (tumor associated)
Stromelysin-2	Breast	Not detected
	Colorectal	Not detected
	Head and neck	Stromal fibroblasts (tumor associated)
Stromelysin-3	Basal cell carcinoma	Stromal fibroblasts (tumor associated)
	Breast	Stromal fibroblasts (tumor associated)
	Colorectal	Stromal cells
	Head and neck	Stromal fibroblasts (tumor associated)
MT-MMP-1	Breast	Stromal fibroblasts (diffuse)
	Colorectal	Stromal cells
	Gastric	Tumor epithelium
	Head and neck	Stromal cells
MT-MMP-4	Breast	Positive expression by northern
Gelatinase A	Breast	Stromal fibroblasts (focal)
	Colorectal	Stromal fibroblasts (tumor associated)
	Head and neck	Stromal cells
Gelatinase B	Breast	Endothelial, macrophage, and inflammatory cells
	Colorectal	Macrophages and inflammatory cells
TIMP-1	Breast	Tumor epithelium and stromal fibroblasts (tumor associated)
	Colorectal	Tumor epithelium and stromal fibroblasts (tumor associated)
	Head and neck	Tumor epithelium and tumor endothelium
TIMP-2	Breast	Stromal cells
	Head and neck	Stromal cells (focal) and tumor endothelium
TIMP-3	Breast	Stromal fibroblasts (tumor associated)

Except for MT-MMP-4 all data in table reflects mRNA expression as demonstrated by *in situ* hybridization. Data from [1,4,24,25,51–56].

constitutively or temporally overexpressing human TIMP-1, the ST-1 phenotype is quenched [37,38]. Other animal models also suggest a role for MMPs in the early stages of tumor progression. For example, in mice in which tissue collagenase is expressed in squamous epithelium [39], skin is hyperproliferative, developing hyperkeratosis and acanthosis. Although the animals do not develop malignant disease *de novo*, they display an increased incidence of malignant conversion when treated with carcinogens compared to untreated littermates [39]. Following the observation that the expression of matrilysin is increased in the early stages of colorectal tumorigenesis, Matrisian and colleagues [40,41] intercrossed matrilysin-deficient mice with Min (multiple intestinal neoplasia) mice that develop *de novo* intestinal carcinomas as a direct result of a mutant APC tumor suppressor gene. The resultant Min/matrilysin-deficient mice develop 60% fewer intestinal tumors, suggesting that matrilysin is important in the development of lesions in this model [41]. Furthermore, in a transgenic model that develops metastatic squamous carcinomas of the skin due to expression of human papillomavirus type 16 in basal keratinocytes, expression and activity of several MMPs and serine proteinases have been observed in 100% of animals at the dysplastic stage concomitant with the onset of angiogenesis ([42], and our unpublished observations). Although only 20% of these animals go on to develop fully malignant disease, activation of proteolytic enzymes at a pre-malignant stage suggests that they are involved in the onset of angiogenesis as well as malignant conversion.

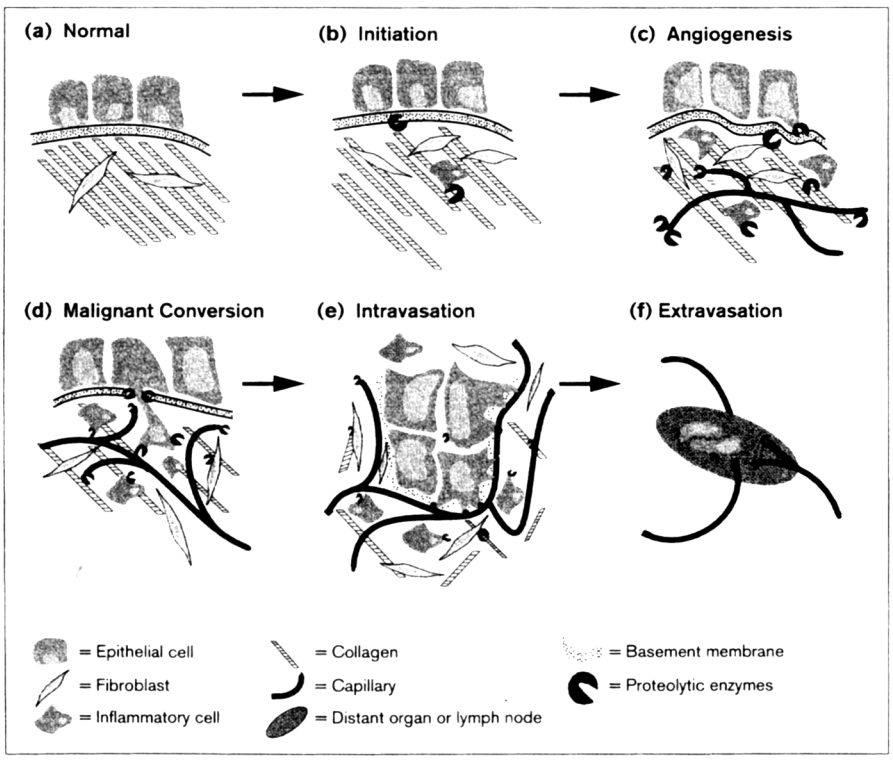
The mechanism by which MMPs enhance metastatic competence of tumor cells appears to be straightforward; dissolution of basement membranes and interstitial collagens facilitates the intravasation and extravasation of tumor cells, thus promoting growth in ectopic tissue environments. The mechanism underlying the growth-promoting properties of MMPs remains unclear, but growth promotion may be an indirect effect of focal dissolution of the basement membrane. Loss of the basement membrane has been shown to diminish the frequency of apoptotic cell death [36], alter cell polarity [43] and increase the ability of cells to migrate [2]. Furthermore, basement membranes are vast reservoirs of latent growth factors, such as bFGF or vascular endothelial growth factor (VEGF), that are probably released from it upon proteolysis. Constitutively increasing the local concentration of growth factors would undoubtedly promote proliferation and subsequent invasion through a compromised basement membrane. Furthermore, our laboratory, among others (see [29] for review), has reported that bioactive fragments of ECM molecules produced through proteolysis can lead to upregulation of MMP gene expression, thus allowing a vicious cycle of ECM degradation, neoplastic growth and tumor invasion to be initiated; these interactions may also stimulate cellular proliferation, alter apoptotic processes, and enhance viability of tumor cells.

The inhibitory activities of the TIMPs may be most important when considering that proteolysis indirectly stimulates proliferation. By maintaining the integrity of the ECM, proteinase inhibitors preserve the delicate balance that exists between tumor cells, matrix-bound growth factors and cytokines, and matrix components. Consequently proteinase inhibitors could also have a marked cytostatic effect on tumors. It is important to remember that MMPs and TIMPs are also synthesized and secreted by normal cells in normal tissue remodeling. In tumors MMP activity may be enhanced as a result of constitutive overexpression, constitutive activation, or loss of inhibition. Loss or impairment of regulation at any level in MMP biosynthesis would compromise cellular homeostasis and contribute to neoplastic progression.

Are MMPs targets for cancer therapy?

It is now evident that MMPs are broadly expressed during multistep initiation and progression of solid tumors (Fig. 4). Several key steps in the progression to malignancy may directly involve MMPs. First, after tumor initiation, MMPs expressed by both epithelial and stromal cells induce subtle modifications in the ECM. The resulting release of ECM-bound growth factors stimulates proliferation, a stromal response, and recruitment of inflammatory cells. As incipient neoplasia progresses, in addition to the ECM remodeling required for growth and expansion of the neoplastic mass, new blood vessels must be recruited to facilitate further growth [44]. Angiogenesis requires MMPs for capillary invasion and remodeling of connective tissue and

Figure 4



Involvement of MMPs at several steps in malignant progression. **(a)** Under normal circumstances, ECM turnover is a dynamic process where the net ratio between proteinases and inhibitors is balanced. **(b)** Following tumorigenic initiation MMPs expressed by neoplastic or responding stromal cells induce subtle alterations to ECM composition and turnover, stimulating release of ECM-bound growth or angiogenic factors, and recruitment of inflammatory cells. **(c)** Angiogenesis requires MMP action for capillary invasion and remodeling of connective tissue and for further release of latent angiogenic factors from ECM reservoirs. **(d)** As cells undergo malignant conversion, MMPs facilitate breaching of basement membranes and stromal invasion. **(e)** Intravasation and **(f)** extravasation further require MMP-stimulated proteolysis as tumor cells enter and exit the vasculature.

for release of the angiogenic factors that are also bound in a latent state to ECM and basement membrane reservoirs. As premalignant lesions undergo malignant conversion, MMPs are involved in breaching of basement membrane barriers. During metastasis, MMPs are involved in the invasive activity, intravasation and distant colonization. Any and all of these steps represent possible targets for therapeutic intervention.

The ability of TIMPs, from tumor or stromal sources, to inhibit tumor growth in transgenic models has provided a test of the concept that MMPs are potential therapeutic targets. Indeed, several low molecular weight MMP inhibitors are already in clinical trials [34].

There are several possible ways to intervene in MMP action in cancer. The most obvious approach is to directly inhibit active MMPs. This approach would work for some tumors by limiting net proteolysis, but in some cases inhibition of MMPs may also promote tumor growth. Some tumor cells grow better, *in vivo* and in culture, in the presence of TIMPs [1,35]. Such tumors may be particularly sensitive to ECM composition [45]. Moreover, inhibition of MMPs may also inhibit the beneficial effects of tumor-infiltrating lymphocytes and macrophages, which use MMPs for extravasation and invasion. Perhaps a more promising approach is to inhibit the activators of MMPs instead. These activators include other MMPs and cysteine and serine proteinases [46]. By inhibiting the beginning of the activation cascade, one can more effectively reduce the total MMP activity generated. Another advantage is that the activation reactions appear to be localized at or near cell surfaces, where enzyme reactions (and their inhibition) become diffusion limited. In such zones, effective MMP concentrations are very high and it is therefore difficult to inhibit the enzymatic activity. If the MMP activators are directly inhibited, however, the effective MMP concentration can be significantly decreased inhibiting the vicious cycle described above. Thus, inhibition of an MMP at an early stage in tumor evolution may have profound effects on overall MMP expression. It remains to be determined whether carcinomas, which primarily activate the stroma as the source of MMPs, or melanoma and fibrosarcomas, which actively produce MMPs, will represent better targets for therapy.

Conclusion and perspective

Solid tumors, unlike tumor cells in culture, are not homogeneous clusters of single cell types. Instead, they are a mixture of neoplastic cells and host-derived non-tumor stromal cells all interconnected and embedded in a dynamic ECM. As we are now beginning to understand, this amalgamation of cell types, each of which can express different proteinases, results in a complex pericellular environment containing a mixture of proteinases. In human tumors, MMP mRNAs have been found in stromal fibroblasts and vascular cells adjacent to clusters of malignant tumor cells, as well as in some tumor cells, depending on the enzyme in question and the type of tumor being examined. On the other hand, MMP proteins are often associated with tumor cells, suggesting that proteinases made by stromal cells near the invasive front localize to the tumor cells due to cell-surface receptors or local activation. Thus, activation at the cell surface may link MMP expression by stromal cells with invasion of tumor cells, and may actually provide the most significant control point in MMP activity. Hence, MMPs may be important in any one of multiple critical events in tumor evolution including neoplastic growth (by facilitating ECM degradation), proliferation (by releasing sequestered growth factors, such as bFGF, from ECM reservoirs), angiogenesis (by stimulating release of factors such as VEGF from the ECM), generation of a reactive stroma, and promotion of tumor cell invasion of basement membranes as tumor cells enter and exit the vasculature during metastasis.

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