**Review**

**Interstitial Collagenases as Markers of Tumor Progression**

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Abstract

Degradation of the extracellular matrix is the *sine qua non* of tumor invasion and metastasis. Most of this degradation is mediated by matrix metalloproteinases (MMPs), a family of enzymes that, collectively, degrades the extracellular matrix. Although the basement membrane-degrading enzymes, MMP-2 and MMP-9, have been given considerable attention for their roles in invasion and metastasis, the interstitial collagenases, a subfamily of MMPs that cleaves the stromal collagens types I and III, have received relatively little recognition for their part in these processes. This subfamily is comprised of collagenase 1 (MMP-1), collagenase 3 (MMP-13), and the MT-MMPs, membrane-bound MMPs, and numerous reports over the last several years document the expression of these MMPs in a wide variety of advancing tumors. Of particular interest is a single nucleotide polymorphism in the MMP-1 promoter that increases the transcription of this gene and that is associated with melanoma and with ovarian and endometrial cancers. The collagenases can mediate tumor invasion through several mechanisms, which include constitutive production of enzyme by the tumor cells, induction of collagenase production in the neighboring stromal cells, and interactions between tumor/stromal cells to induce collagenase production by one or both cell types. Thus, evidence indicates that elevated expression of the interstitial collagenases is associated with a poor prognosis in a variety of cancers, and therefore, these MMPs can serve as a marker of tumor progression.

Background

Malignant tumors have the ability to invade normal tissue and spread to distant sites, giving rise to metastases, the major factors in the morbidity and mortality of cancer. Invasion and metastasis involve attachment of tumor cells to the basement membrane, degradation of the local connective tissue, followed by penetration and migration through proteolyzed stroma (1–4). Matrix degradation is mediated by the concerted action of several proteinases, including members of the serine, cysteine, aspartate, and MMP families (1–5). However, the majority of connective tissue destruction is carried out by the MMPs, a family of zinc-dependent enzymes that degrades all components of connective tissues (1–5). Most MMPs are secreted as zymogens, requiring proteolytic cleavage of the “pro” portion to be catalytically active (2, 3, 5, 6). Although this process can be facilitated by other MMPs, it is often effected by serine proteinases such as plasmin or urokinase (1–5, 7–9). Thus, although MMPs may be the direct mediators of connective tissue destruction, serine proteinases have a role in a proteolytic cascade that culminates in the activation of MMPs and the initiation of tumor invasion (Refs. 7–9; Fig. 1).

Currently, 26 human MMPs have been identified, and these enzymes are classified according to their substrate specificity and structural similarities (2, 3, 5, 10–12). There are four major subgroups: (a) interstitial collagenases; (b) gelatinases; (c) stromelysins; and (d) MT-MMPs. The interstitial collagenases degrade the structural collagen types I, II, and III, with the characteristic cleavage site for type I collagen at Gln^729/Lys^730 (2, 3, 5, 13). The gelatinases are effective primarily against type IV collagen, although a limited ability to degrade stromal collagens has been noted (2, 3, 5, 13). The stromelysins have broad substrate specificity, degrading non-collagen matrix molecules, such as proteoglycans, laminin, and fibronectin, but they indirectly mediate collagen degradation by contributing to the activation of other latent MMP family members (2, 3, 5). The MT-MMPs represent membrane-bound forms of the enzymes, which activate latent MMP-2 and which can cleave collagen at the classic site (2, 3, 5, 10–12, 14). Thus, we will consider MT-MMPs as interstitial collagenases, along with the more traditional enzymes, MMP-1 (collagenase-1) and MMP-13 (collagenase-3). Although neutrophil collagenase (MMP-8; collagenase-2) degrades stromal collagens, it contributes more to connective tissue degradation in arthritic disease than to tumor invasion and metastasis (1–5, 15).

Numerous studies have documented the important role of the gelatinases/type IV collagenases (MMP-2 and MMP-9) in tumor invasion, and several recent reviews suggest new functional roles for MMPs in supporting tumor growth, modulating the extracellular matrix, regulating the availability of growth factors, and facilitating angiogenesis (16–19). However, relatively little attention has been devoted to the specific role of the interstitial collagenases in malignancy. Within the last several years, an increasing number of reports has documented the

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4 The abbreviations used are: MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; SNP, single nucleotide polymorphism; LOH, loss of heterozygosity.

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presence of these enzymes in aggressive tumors, suggesting a definitive correlation between this subfamily of MMPs and tumor prognosis. Thus, this review focuses on expression of the interstitial collagenases in cancer. A consistent theme is that the collagenases are probably not major players in the initial stages of tumor formation and invasion, but rather, that they contribute substantially to the later stages of tumor dissemination. Thus, their expression may serve as a marker of tumor progression.

MT1-MMP (MMP-14)

To date, five membrane-bound MMPs (MT-MMPs) have been identified (5, 10–12, 14). Similar to the other members of the MMP family, the MT-MMPs are synthesized in a latent form, but unlike the secreted enzymes, they are activated intracellularly by a furin-dependent mechanism (5, 10–12, 14, 20). The activated enzymes are then embedded in the plasma membrane, where their surface localization suggests that they may

Fig. 1 Proteolytic cascades for activation of collagenases in tumor cell invasion. A, activation of procollagenase-3 (proMMP-13) secreted by tumor cells and collagenolytic abilities of MT-MMP. Collagenase-3 can be activated by MMP-3 secreted by fibroblasts. Additionally, active cell surface MT1-MMP activates procollagenase-3, either directly or indirectly through the activation of proMMP-2 (5). MMP-13 then proceeds to degrade collagen, whereas the membrane-bound MT1-MMP, which is already active, directly cleaves collagen, thereby facilitating tumor invasion. B, expression and activation of procollagenase 1 (proMMP-1) by tumor cells and fibroblasts. Similar to the activation cascade of collagenase-3, procollagenase-1 can be activated by MMP-3, secreted by the fibroblasts. Activation of proMMP-1 is initiated by serine proteinases secreted by either fibroblast and tumor cells. Full activation of MMP-1 is obtained by stromelysin (MMP-3) secreted by fibroblasts (5). MMP-1 subsequently degrades collagen, leading to tumor invasion. In both A and B, constitutive expression of proMMP-1 and proMMP-13 by tumor cells may be enhanced by stimuli secreted by surrounding fibroblasts, which express low basal levels of MMPs.
modulate a number of important cell-matrix interactions. They activate latent MMPs, i.e., MMP-2 and MMP-13, but they are not universal activators of all MMPs (5, 20, 21). Because MMP-2 has been immunolocalized to the cell membrane of the tumor cells, the model is that MT-MMPs function as a receptor molecule to capture proMMP-2 on the cell surface before activating it (5, 20). In addition, the transmembrane/cytoskeletal domain of the MT-MMPs has been shown to mediate the spatial organization of these enzymes to the invadopodia and subsequent degradation of the extracellular matrix (5, 10, 14, 20). Finally, they can degrade interstitial collagens (5, 10, 14), although the importance of this collagenolytic activity in mediating tumor progression may not yet be fully appreciated or understood. However, a recent report describes the unique ability of the MT-MMPs to regulate cell invasion and branching morphogenesis in three-dimensional collagen matrix of type I collagen (14). Under the experimental conditions used, none of the secreted interstitial collagens (MMP-1 and MMP-13) or the gelatinases (MMP-2 and MMP-9) was able to mediate invasion, whereas in contrast, two of the three membrane-bound MMPs, MT1-MMP and MT2-MMP, enabled cells to penetrate type I collagen and to initiate tubulogenesis. Of particular interest is the fact that soluble forms of these enzymes were ineffective, indicating that they must be confined to the pericellular space/compartment where they are concentrated at the cell-matrix interface and are protected from circulating proteinase inhibitors.

The MT-MMPs have often been detected in the stromal cells adjacent to the invading tumor, indicating that a host/tumor cell interaction may mediate matrix degradation and tumor invasion (20–25). In squamous cell carcinoma (24), for example, cell-cell contact between the tumor cells and normal fibroblasts increased levels of MT1-MMP, which was followed by activation of proMMP-2 on the surface of the tumor cells. This scenario clearly implicates both MT1-MMP and stromal cells as important mediators of tumor invasion and stresses the role of host/tumor cell interactions in facilitating tumor invasion (Fig. 1A). However, evidence suggests that as the tumors progress, MT-MMP expression is increasingly linked to the tumor cells (24–30), and this expression has been documented in several types of carcinomas where these enzymes appear to contribute to the metastatic phenotype (24–35). Furthermore, in genitourinary tumors, MT-MMP expression seems to be a common finding (33–35). Many of these tissues express MT-MMPs in the regulated process of normal development (36–38), suggesting a link between the expression of MT-MMPs during regulated development and their re-expression during the dysregulated process of carcinogenesis.

Several interesting reports describe MT1-MMP expression in brain tumors: gliomas (39, 40) and advanced pediatric neuroblastoma (41). A study of 46 gliomas revealed MT1-MMP transcripts in both normal neuronal tissue and malignant cells. Also interesting is the fact that there was no close association between MT1-MMP expression and MMP-2. This finding suggests that this membrane-bound MMP may be a specific marker for advancing brain malignancies, and that the ability of this enzyme to directly degrade matrix components may contribute to disease progression. Taken together, all of these reports indicate that expression of MT-MMPs by the tumor cells represents an unfavorable prognostic marker.

Collagenase-3 (MMP-13)

Human collagenase-3 (MMP-13) was first identified in breast carcinoma (42–44). At that time, it was suggested that this MMP might be associated exclusively with malignancy, and that it was produced by the tumor cells (42–44). Although this is an attractive hypothesis, MMP-13 expression has since been documented in stromal cells immediately adjacent to the tumor (43, 44) and in nonmalignant conditions, such as chronic ulcers (45, 46) and arthritis (47–50). Because MMP-13 expression in fibroblasts is rare (47–49), perhaps the expression in stromal cells occurs in response to a particular factor(s) produced by the tumor cells (Fig. 1A).

Increasingly, expression of MMP-13 has been documented in certain cancers that are aggressive and invasive (43, 44). Squamous cell carcinomas of the head and neck, in particular, are noted for their propensity to rapid progression and poor clinical outcome (43, 44). In these tumors, MMP-13 has been seen mostly in tumor cells at the invading front, with only a subset of the intermingled stromal cells expressing this enzyme (44, 45, 51–53). Only malignant squamous cell tumors, but not premalignant and benign lesions, express MMP-13, supporting the concept that MMP-13 can serve as a prognostic marker in these carcinomas with characteristically poor outcomes.

MMP-13 expression has also been noted in chondrosarcoma, a malignancy of mesenchymal origin (54, 55). A study of 29 patients revealed universal expression of this enzyme by the tumor cells, leading the authors to conclude that MMP-13 gene expression signified those patients at risk for recurrence (55). Another example of the correlation between MMP-13 expression and tumor progression is seen with malignant melanoma (56). In this study, MMP-1 expression was also up-regulated in these tumors, implying a dual role for the interstitial collagenases in this disease (See below). Premalignant and grade I tumors were consistently negative for MMP-13 (and MMP-1), but these enzymes were seen in Clark’s grades III and IV, which represent advanced cancers, again strengthening the link between expression of the interstitial collagenases and progressing tumors.

However, expression of MMP-13 appears to be relatively restricted, confined to a few normal tissues and perhaps to particular types of tumors. Thus, it appears to serve as a marker of tumor progression in a specific subset of cancers.

Collagenase-1 (MMP-1)

Of the interstitial collagenases, MMP-1 (collagenase-1) is the most ubiquitously expressed (15, 57–59). It is produced by a wide variety of normal cells, e.g., stromal fibroblasts, macrophages, endothelial cells, and epithelial cells, as well as by numerous tumors, suggesting a broad-based role for this collagenase in biology. Normally, expression of MMP-1 by most cells is low but is readily induced by phorbol esters, growth factors, and inflammatory cytokines. In contrast, some tumors display constitutively high levels of MMP-1 expression, even in the absence of apparent external stimuli (9).
Recently, MMP-1 has been described in a wide variety of advanced cancers (56, 60–68), and in nearly all instances, there was a significant negative correlation between expression of MMP-1 and survival. Some reports document MMP-1 production by tumor cells and correlate this with the invasive potential of the tumors, whereas other investigations support the belief that MMP-1 is predominantly expressed by the surrounding stromal fibroblasts. Still other reports indicate that in certain tumors both tumor cells and stromal cells express MMP-1, emphasizing stromal/tumor cell interactions in the regulation of MMP-1 production (Refs. 9 and 69; Fig. 1B). The important point is not which cells, i.e., stromal, tumor, or both, are producing MMP-1, but rather, that this enzyme is expressed at the site of the progressing tumor.

The level of MMP-1 expression, and hence its potential to mediate connective tissue degradation and tumor progression, can be influenced by a genetic variation in the MMP-1 promoter (70). This variation is a SNP located at −1607 bp, where an insertion of a guanine base (G) creates the sequence, 5′-GGAT-3′, the core binding site for members of the Ets family of transcription factors (71). This SNP is not a rare mutation or genetic variation found in a few tumor cells (70). Genotyping of 100 normal individuals indicated that the distribution of this SNP in the normal population is approximately: 30% = 1G homozygous; 30% = 2G homozygous; and 40% = 1G/2G heterozygous. However, in tumor cells cultured in vitro, the incidence of the 2G allele rises to 62% (P ≤ 0.001), supporting the hypothesis that it correlates with aggressive tumors. This in vitro correlation has been upheld in vivo in studies of ovarian and endometrial carcinomas. In both studies, the patients had a significantly higher incidence of the 2G allele, compared with noncancer controls. Furthermore, patients with the 2G allele expressed higher levels of MMP-1 protein (65, 68). Thus, this SNP may provide a mechanism for elevating MMP-1 gene expression and for facilitating tumor progression by mediating enhanced degradation of the interstitial matrix. Because the 2G SNP results in increased transcription of the MMP-1 gene in normal fibroblasts and in tumor cells (70), increased MMP-1 production by either the tumor cells or the surrounding stromal cells could enhance invasion (Fig. 1B).

A recent report further supports a functional role for the 2G SNP in progressing tumors. This study describes the LOH at the MMP-1 locus of chromosome 11q.22 and links this to the development of metastatic melanoma (72). LOH is usually associated with the loss of a tumor suppressor gene, and several putative tumor suppressors have been assigned to this locus (73). However, LOH in these metastatic tumors is significantly (P = 0.04) associated with retention of the 2G allele, i.e., the allele expressing higher levels of MMP-1. Although LOH is a random event with an equal probability of losing either allele, the hypothesis is that heterozygotic tumors retaining the 2G allele have a selective aggressive and invasive advantage, which is manifested by an increased number of metastases.

Each member of the Ets family contains a highly homologous DNA-binding domain, which recognizes the core sequence motif 5′-GGA(A/T)-3′ (71). Certain Ets family members, especially E1AF, Ets-1, Ets-2, and Erg proteins, have been associated with an aggressive phenotype, tumor progression, and elevated MMP expression (74–79). The high constitutive levels of MMP-1 seen in some aggressive tumors (9, 70) may result from the presence of the 2G allele and from elevated expression of the transcription factors that bind to this site. The hypothesis is, therefore, that heightened MMP-1 expression results from the presence of: (a) the 2G allele; and (b) the appropriate transcription factors that bind to this site (Fig. 2). In the absence of these factors, MMP-1 expression from the 2G allele is not necessarily increased compared with the 1G allele. Although the precise identity of the Ets family member(s) binding to this 2G SNP is not known, perhaps several Ets proteins can function to drive transcription (74–79). In any case, given the strong link between increased MMP-1 expression, the presence of the 2G allele, and a poor clinical outcome, a simple genetic analysis of this polymorphism may provide a useful and potentially important mechanism for predicting prognosis in certain cancers.
Conclusions and Challenges for the Future

Given the well-documented increases in the collagenases as tumors progress, the question arises as to how these increases are mediated. Tumor cells are constantly changing both their genotype and their phenotype as they respond to genetic, physiological, and pharmacological pressures. As a result of this genetic instability, errors in gene regulation accrue as the tumor continues to metastasize and progress (73, 80–87). How do these changes affect levels of the collagenases (and other MMPs) and the invasive behavior of the tumors? Tumor cells may display high constitutive expression of the collagenases, even in the absence of apparent external stimuli. What mechanisms drive this expression? One possibility is that it results from an accumulation of errors/mutations in the regulation of mechanisms drive this expression? One possibility is that it results from an accumulation of errors/mutations in the regulation of signal transduction pathways targeted to the collagenase/MMP promoters (50, 84–87). However, other changes and mutations are also occurring within the cells, i.e., the transition to a more mesenchymal phenotype with a loss in keratin and E-cadherin and an increase in vimentin and MMP-1 (76). These changes suggest that a cohort of genes, including MMP-1, is expressed as tumors progress. Still another change associated with an increased MMP gene expression and aggressive behavior of the tumor is resistance to chemotherapeutic drugs. The mechanistic link between the development of drug resistance and an increase in invasive behavior is unclear but may involve increases in the expression of growth factor receptors and heightened tyrosine kinase activity, both of which can be linked to MMP expression (83–87). Thus, one unwanted but not uncommon result of conventional chemotherapy may be a metastatic tumor that is more invasive and aggressive than the primary one (80–86).

There is, therefore, considerable appeal in selecting MMPs as targets for therapeutic strategies that will block either enzyme activity or enzyme synthesis at all stages of tumor invasion and metastasis (88–97). The vitamin A analogues, retinoids, have been successful therapeutic agents in some cancers (92–101), perhaps because they block transcription of several MMP genes (15, 92, 95–97). Retinoids, such as all-trans- or 13-cis-retinoic acid, engage all three retinoic acid receptors, α, β, and γ, and act, at least in part, through the activator protein-1 site in the promoters of most MMP genes (92). The emergence of new retinoids, which are targeted at specific retinoic acid receptors/retinoid X receptors (93–95) or the delivery of traditional retinoids via novel routes (102), may provide a new approach to blocking tumor invasion.

Similarly, the concept of inhibiting MMP activity is attractive, although difficulties associated with enzyme specificity, drug delivery, drug stability, rates of clearance, and achievement of clinically effective concentrations remain to be resolved (88–90). The introduction of new “second generation” inhibitors, which are directed to a particular MMP and which make use of our knowledge of the crystal structures of the different MMPs (91), may circumvent some of these difficulties, and indeed, several promising specific MMP inhibitors are being tested (90). Another intriguing possibility is the use of two or more drugs with totally different targets (30). The effectiveness of this new therapeutic strategy has been described with the MMP inhibitor AG3340, which when used in combination with standard chemotherapeutic agents achieves both antiproliferative and anti-invasive effects on tumors (30).

Our increasing knowledge of the signal/transduction pathways participating in MMP gene expression has led to new therapeutic strategies that are designed to block specific pathways, with subsequent inhibition of MMP synthesis (50, 85–87). For instance, Ras and mitogen-activated protein kinase signaling are important for MMP expression, and Ras function depends on posttranslational modifications such as farnesylation and geranylgeranylation for plasma membrane localization. Farnesylation inhibitors or geranylgeranyltransferase inhibitors can prevent Ras localization, thus preventing the ability of Ras to transduce signals through the various pathways that regulate the transcription of oncogenes and MMPs. In addition, downstream targets, such as mitogen-activated protein kinase kinase/extracellular signal-regulated kinase, can be inhibited by compounds such as PD98059, and its p.o. available counterpart, PD-184352, thereby potentially increasing the specificity of targets and decreasing more generalized toxicities. Some of these therapies are in clinical trials, where their efficacy may result from their ability to block MMP gene expression, along with inhibiting cell growth.

Thus, the use of standard chemotherapeutic agents together with newer compounds with novel modes of action is emerging as a treatment modality, as is the development of therapies that totally abandon the traditional treatments in favor of a more targeted gene-specific approach. By understanding the roles of the collagenases in tumor invasion, we may develop drugs that are targeted to specific enzymes and that are effective at various stages of tumor growth and progression. As our knowledge of the molecular mechanisms regulating expression of the MMPs continues to increase, this concept comes closer to becoming a reality.

References


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