REVIEW

Changing Views of the Role of Matrix Metalloproteinases in Metastasis

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Metastatic spread of cancer continues to be the greatest barrier to cancer cure. Understanding the molecular mechanisms of metastasis is crucial for the design and effective use of novel therapeutic strategies to combat metastases. One class of molecules that has been repeatedly implicated in metastasis is the matrix metalloproteinases (MMPs). In this review, we re-examine the evidence that MMPs are associated with metastasis and that they make a functional contribution to the process. Initially, it was believed that the major role of MMPs in metastasis was to facilitate the breakdown of physical barriers to metastasis, thus promoting invasion and entry into and out of blood or lymphatic vessels (intravasation, extravasation). However, recent evidence suggests that MMPs may have a more complex role in metastasis and that they may make important contributions at other steps in the metastatic process. Studies using intravital videomicroscopy, as well as experiments in which levels of MMPs or their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]) are manipulated genetically or pharmacologically, suggest that MMPs are key regulators of growth of tumors, at both primary and metastatic sites. On the basis of this evidence, a new view of the functional role of MMPs in metastasis is presented, which suggests that MMPs are important in creating and maintaining an environment that supports the initiation and maintenance of growth of primary and metastatic tumors. Further clarification of the mechanisms by which MMPs regulate growth of primary and metastatic tumors will be important in the development of novel therapeutic strategies against metastases. [J Natl Cancer Inst 1997;89:1260–70]

Considerable research has been directed toward understanding both the steps involved in metastatic spread of cancer cells and the underlying molecular mechanisms. Understanding the molecular basis of metastasis is crucial for the development and appropriate clinical use of novel therapeutics directed at prevention of metastasis and its consequences to the patient. Here we will discuss one class of molecules, the matrix metalloproteinases (MMPs), enzymes that have been repeatedly implicated in metastasis. Our goals in this review are to re-examine the evidence that MMPs are associated with the metastatic phenotype, that they contribute functionally to metastasis, and how they do so. We will not attempt to review the full literature on these topics, since it is extensive and many recent reviews have summarized and critically evaluated much of this literature *(1–7)*. Instead, we will focus conceptually on changing ideas about the nature of the roles of MMPs in the metastatic process, based primarily on recent studies that suggest that their major contribution may be somewhat different and more complex than previously assumed. Clarification of the molecular nature and timing of the contributions of MMPs to metastasis are important in part because MMPs are viewed as an appropriate target for antimetastasis therapies, and use of this therapeutic strategy will be maximized if the roles of MMPs in metastasis, both temporally and spatially, are well understood.

Overview of the Metastatic Process

Metastasis is the spread of cancer from a primary tumor to distant sites of the body and is a defining feature of cancer *(8,9)*. Metastasis is defined by end points, i.e., metastatic lesions detected in specific organs distant from a primary tumor, while steps by which metastases form have often been inferred rather than directly observed. Some experimental and clinical evidence supports some of these steps, but the internal nature of the process has prevented it from being fully understood. Sequential steps in the process (Fig. 1) [reviewed in *(8–12)*] are believed to include the following: escape of cells from the primary tumor, intravasation (entry of cells into the lymphatic or blood circulation), survival and transport in the circulation, arrest in distant organs, extravasation (escape of cells from the circulation), and growth of cells to form secondary tumors in the new organ environment. Angiogenesis, the recruitment of new blood vessels, is required for the primary and metastatic tumors to grow beyond minimal size, and evasion of immune destruction is necessary at various steps throughout the process. The end point, formation of detectable metastatic lesions, thus can be prevented by interruption at any one or more of these steps.

Metastasis is known to be an inefficient process, from both clinical observations and experimental studies [*(13–17);* re-

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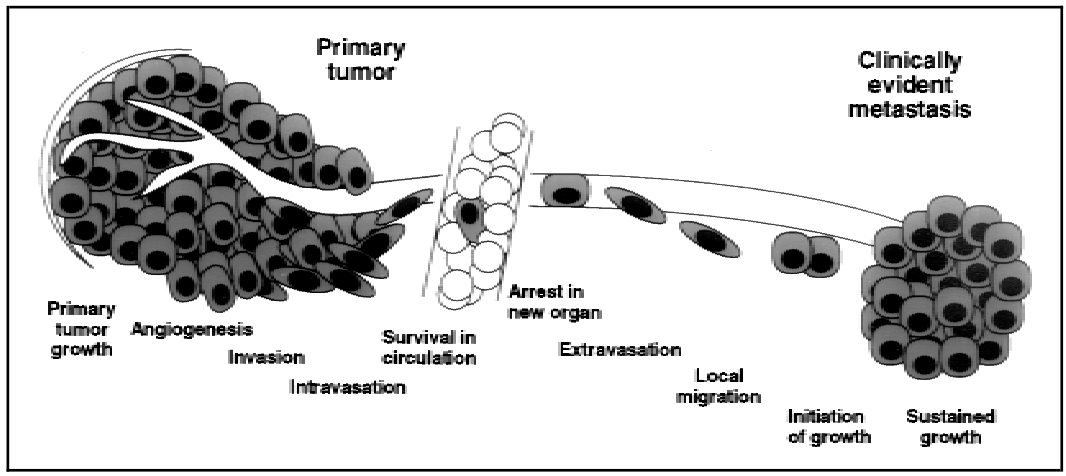

Fig. 1. Metastatic process. Tumor cells are believed to proceed through the sequential steps indicated to form clinically detectable metastases.

viewed in *(18)*]. Large numbers of cells can be shed into the circulation from a primary tumor, and yet not all of these cells will form metastases. When cells are injected into the circulation of experimental animals, only a small fraction of the cells will succeed in forming metastases. Furthermore, experimental studies *(19–21)* indicate that individual metastases are likely clonal in origin. Thus, the majority of cells that successfully escape from a primary tumor will not complete all of the steps necessary to give rise to metastatic tumors. Steps that have been considered to be major contributors to this inefficiency, and thus rate limiting for metastasis, include cell survival in, and escape from, the circulation. Relatively few cells arriving in a target organ were believed to survive initial arrest, due to hemodynamic destruction, and of those that did survive, few were believed to succeed in extravasating. These views of the major rate-limiting steps in metastasis have been questioned by recent studies using *in vivo* videomicroscopy to directly monitor the fate of cells during the metastatic process, as discussed below.

Metastasis is the final stage in tumor progression from a normal cell to a fully malignant cell. Considerable progress has been made in identifying molecular changes that accompany, and may be responsible for, the clinical, pathologic, and cytogenetic changes that occur during the progression of specific cancers *(22,23).* The best-developed example is the characterization of molecular progression in colon cancer, in which specific changes (e.g., loss of tumor-suppressor genes and mutation of oncogenes) are preferentially associated with specific stages of progression *(24,25).* However, the final stage in tumor progression to a metastatic phenotype has eluded characterized at a molecular genetic level, in colon or other cancers. Initially, there was hope that a single metastasis-specific gene could be identified to be responsible for conversion to a metastatic phenotype. Early DNA transfection studies indicated that some cells could be converted to a metastatic phenotype by this strategy, suggesting that metastatic ability could have a genetic basis [reviewed in *(26)*]. In addition, transfection with a variety of oncogenes (e.g., ras and src) could produce metastatic cells [reviewed in *(27)*]. These studies indicated that a variety of downstream, oncogene-regulated genes could functionally contribute to metastatic behavior of the cells. For example, metastatic H-ras-transfected NIH 3T3 cells had increased levels of a variety of gene products, including proteinases and adhesive proteins, accompanied by decreases in other gene products, including proteinase inhibitors *(28).* Loss of tumor-suppressor gene function also has been implicated in the conversion to metastatic ability in specific tumor types, although none is likely to be universally implicated in all tumor types [e.g., nm23 *(29);* KAI1 *(30),* KiSS-1 *(31);* reviewed in *(32)*].

Thus, phenotypically there appear to be cellular abilities necessary for metastasis in many tumor types, while genotypically there does not seem to be a single master ''metastasis gene'' that regulates these properties in all tumors. It appears more likely that regulation of expression of genes that contribute functionally to metastasis can occur in a tissue-specific manner, with different regulatory genes (e.g., oncogenes and tumor-suppressor genes) inducing the multiple aspects of the metastatic phenotype in specific tumors. Included among the required traits is sufficient proteolytic capacity to complete all the steps in metastasis. MMPs and their inhibitors have been repeatedly implicated in this context. We next will summarize this family of enzymes and their inhibitors and then will consider their relationship to the process of metastasis.

Metalloproteinases and Their Inhibitors

MMPs are a family of secreted or transmembrane proteins that are capable of digesting extracellular matrix and basement membrane components under physiologic conditions. Currently, 16 family members have been identified (Fig. 2). They share a catalytic domain with the HEXGH motif responsible for ligating zinc, which is essential for catalytic function. MMPs are also characterized by a distinctive PRCGVPD sequence in the pro domain that is responsible for maintaining latency in the zymogens. MMP family members differ from each other structurally by the presence or absence of additional domains that contribute to activities, such as substrate specificity, inhibitor binding, matrix binding, and cell-surface localization [reviewed in *(6,33)*]. There are three major subgroups of MMPs, identified by their substrate preferences: collagenases degrade fibrillar collagen, stromelysins prefer proteoglycans and glycoproteins as substrates, and gelatinases are particularly potent in degradation of nonfibrillar and denatured collagens (gelatin).

MMP activity is highly regulated at many levels. The messenger RNA (mRNA) for most family members is transcriptionally regulated by biologically active agents, such as growth fac-

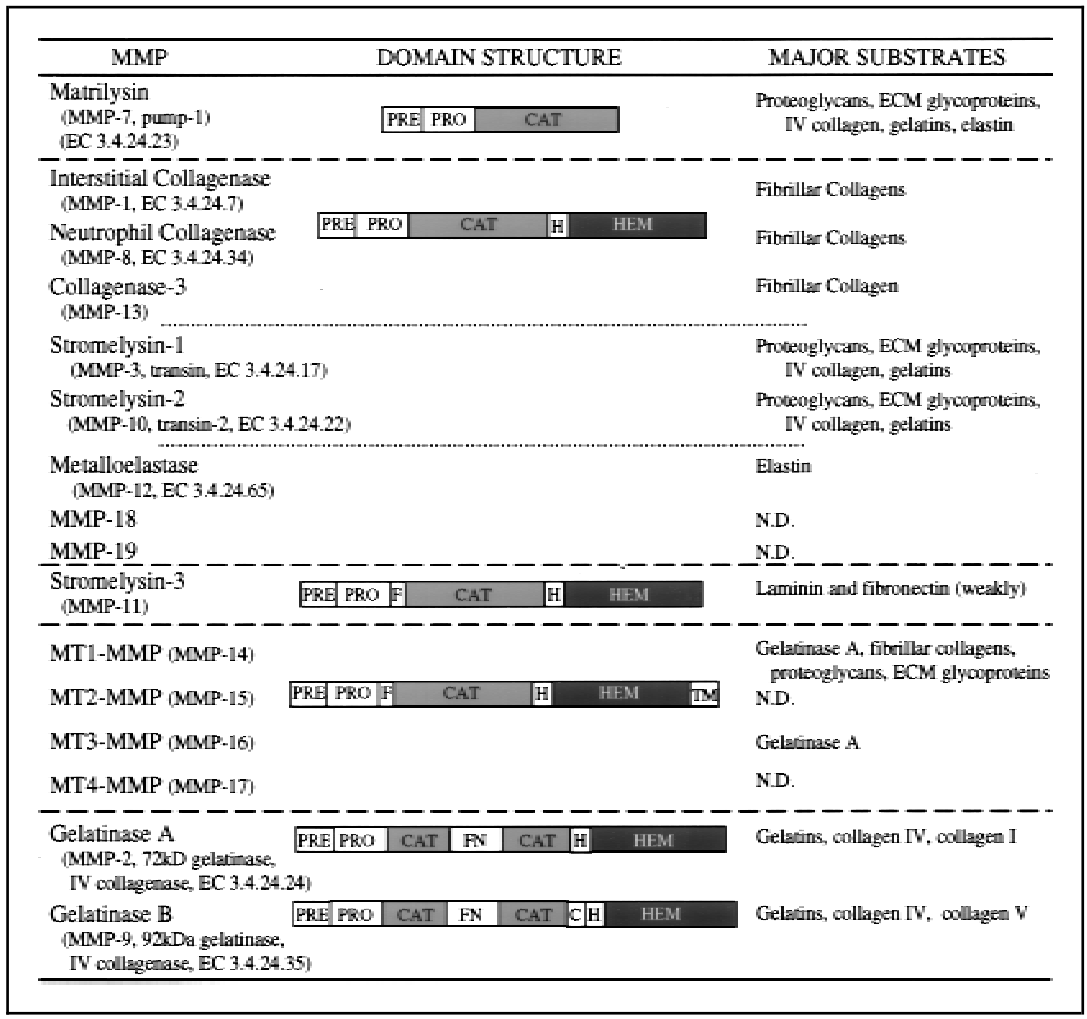

Fig. 2. Matrix metalloproteinase (MMP) family. Subgroups are arranged by domain structure and separated by a dashed line. Within the largest, hemopexin-domain subgroup, family members with some distinct but subtle structural features are separated by a dotted line. This table is adapted from Powell and Matrisian *(6)* with the addition of information from references *(123–127).* MT = membrane type; PRE = leader sequence; PRO = prodomain; CAT = catalytic domain; H = hinge domain; HEM = hemopexin-like domain; F = furin consensus site; FN = fibronectin-like domain; C = collagen-like domain; TM = transmembrane domain; and ND = not determined.

tors, hormones, oncogenes, and tumor promoters. There is evidence for regulation at the level of mRNA stability, translational control, and storage in secretory granules for specific MMPs in specific cell types. In general, however, the protein is rapidly secreted in a latent form and requires extracellular activation. Proteinase cascades involving other MMPs as well as other enzyme classes have been implicated in MMP activation. Once the enzymes are active, they are susceptible to inhibition by the general serum proteinase inhibitor α2-macroglobulin and by a family of specific tissue inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). There are currently four members of the TIMP family that have in common their MMP inhibitory activity but differ in properties, such as expression patterns and association with latent MMPs (Table 1). TIMPs act to inhibit metalloproteinase activity by forming a complex with active MMPs and are believed to be specific for enzymes of this family, although they do not distinguish effectively between individual family members.

MMPs were originally described as enzymes responsible for dissolution of the tadpole tail *(34).* Subsequent work focused on the association of these activities with systems characterized by dramatic connective tissue remodeling, such as uterine involution, wound healing, and joint destruction in arthritic conditions. The notion that MMPs are the major class of enzymes responsible for matrix degradation is supported not only by the ''smoking gun'' nature of their association with these processes but also by the observation that members of this family are the only enzymes known to denature and digest fibrillar collagens. More recently, experiments involving genetic manipulation of MMPs or their inhibitors and specific synthetic inhibitors provide additional support for the essential role of these enzymes in normal and pathologic matrix destruction.

Table 1. TIMP family*

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Molecular mass	28 kd	21 kd	24 kd	22 kd
Messenger RNA	0.9 kb	1.1/3.5 kb	4.5–5.0 kb	1.4 kb
Associated proteins	proGELB	proGELA	ECM	Not determined
Major sites of expression	Ovary, bone	Placenta	Kidney, brain	Heart

*Information presented in this table is reviewed in *(33)* and presented in *(117–122).*

Association of MMPs With Cancer

During metastasis, there are a series of collagen-containing structural barriers that cells must pass (*see* Fig. 1). Extracellular matrix and basement membrane barriers must be breached for cells to intravasate and extravasate. The basement membrane underlying endothelial cells presents, in many organs, a continuous collagen-containing structural barrier to completion of the metastatic process. Within tissue, at either primary or secondary tumor sites, extracellular matrices appear to require degradation to permit tumor cell invasion and spread. By logical inference, metastatic cells require sufficient degradative enzymatic capacity to break down these proteinaceous structural barriers. Alternatively, some of the required proteolytic activity may be derived from tumor-associated host tissues, including adjacent stromal tissue and tumor-infiltrating immune cells. Support for a requirement for enhanced proteolytic function associated with cancer comes from pathologic studies of tumors, in which defects in basement membranes adjacent to tumors are commonly associated with malignant but not benign tumors *(35).*

MMPs have been associated with the malignant phenotype for several decades [early reviews in *(36–40)*]. Several studies *(41–43)* presented evidence that malignant tumors contained proteolytic activity capable of degrading collagen *in vitro.* With the advent of more sophisticated biochemical and molecular biologic techniques, it became possible to identify individual proteases responsible for the activities detected in tumor cells. Proteases of all five major classes (i.e., serine, aspartic, cysteine, threonine, and metalloproteinases) have been linked with the malignant phenotype *(44,45).* From early work of Liotta et al. *(41,42)* and Tryggvason et al. *(43),* interest was focused on type IV collagenase, the enzyme responsible for degradation of type IV collagen, a major structural protein in basement membrane. The enzymes responsible for this activity are now recognized to be either gelatinase A (72 kd type IV collagenase) or gelatinase B (92 kd type IV collagenase). The first member of the MMP family to be cloned was transin, the rat homologue of stromelysin-1, which was identified as an oncogene and growth factor-inducible gene *(46).* Subsequent work identified the product of this complementary DNA (cDNA) as a protease that was overexpressed in malignant mouse skin tumors *(47)* and was related to the prototypic member of the MMP family, interstitial collagenase *(48,49).* Since that time, extensive literature demonstrating the association of MMP family members and tumor progression has developed [reviewed in *(5,50)*]. Several generalizations can be made: 1) The number of different MMP family members that can be detected tends to increase with progression of the tumor, 2) the relative levels of any individual MMP family members tend to increase with increasing tumor stage, and 3) MMPs can be made by either tumor cells themselves or, quite commonly, as a host response to the tumor. The expression pattern of MMPs, therefore, supports a role for these enzymes in later stages of tumor progression. MMPs are found most abundantly in tumors in which the basement membrane is breached and there is evidence for local invasion and distant metastases. In this review, we are considering the role of MMPs in tumor progression, but it should be remembered that they are not the only proteolytic contributors to this process, and interactions between members of other classes of proteolytic enzymes provide additional levels of complexity and regulation *(4,40,45).*

Members of the TIMP family have also been associated with cancer. The literature on expression of TIMPs and MMPs in tumors has been thoroughly reviewed recently by Denhardt *(7)* and will not be considered further in detail here. An important point that must be made, however, is that the simplistic expectation that malignant tumors would have increased MMP expression accompanied by decreased TIMP expression is often not met. In several cases, malignant tumors have been shown to have increased rather than decreased TIMP levels [e.g., *(51)*]. Furthermore, the tissue localization of both specific MMPs and TIMPs in and around a tumor can be complex, with variable expression within the tumor versus adjacent stromal cells [reviewed in *(7)*]. Tumor localization studies can give only a snapshot at one point in time, and there are difficulties in interpreting these studies: for example, is overexpression of a particular enzyme or inhibitor an indication of a functional role for it in the malignant process or a sign of (effective or ineffective?) host response? One promising approach to address this question involves the use of transgenic or knockout mice to address the effects of altered host levels of specific MMPs or TIMPs [reviewed in *(52)*]. The localization and interplay between proteases and their inhibitors *in vivo* is complex and as yet poorly understood.

Evidence for a Functional Role for MMPs and Their Inhibitors in Metastasis

Evidence that MMPs play a functional role in metastasis came originally from experiments with recombinant or genetically manipulated levels of TIMP-1. Schultz et al. *(53)* first showed that an intraperitoneal injection of recombinant TIMP-1 reduced lung colonization of intravenously injected B16F10 melanoma cells. A reduction in TIMP-1 levels by antisense RNA in mouse fibroblasts resulted in formation of metastatic tumors in nude mice *(54).* Subsequent studies *(55–58)* using recombinant or transfected TIMP-1 or TIMP-2 in experimental and spontaneous metastasis assays further suggested that MMPs could play a causal role in metastasis. Assuming that the primary activity of TIMP in these assays is inhibition of MMP activity, these results provide strong support for a role for MMPs in the establishment of metastatic lesions.

Studies with synthetic MMP inhibitors further support a requirement for MMP activity in the establishment of metastatic foci. These low-molecular-weight compounds are unlikely to have complicating activities distinct from inhibition of metalloproteinase activity, and their specificity appears to be restricted to enzymes closely related to matrix metalloproteinases. The British Biotech inhibitor batimastat (BB-94) was shown to reduce metastasis of melanoma, mammary carcinoma, and colorectal tumor cells in experimental metastasis assays *(59–61)* and of human colon *(62)* and breast *(63)* tumor cells injected orthotopically in nude mice. Other broad-spectrum MMP inhibitors have shown similar results in lung colonization assays *(64,65).* In addition, combination therapy with a gelatinase A-specific inhibitor and cytotoxic agents reduced invasion and metastasis of subcutaneously injected Lewis lung carcinomas *(66).*

Finally, there is evidence for a role for specific MMP family

members in tumor cell invasion and metastasis. Bernhard et al. *(67)* have demonstrated that gelatinase B expression is strongly associated with the metastatic ability of rat embryo fibroblasts and that its overexpression results in increased metastatic potential following injection into nude mice *(68),* while ribozyme inhibition of this enzyme decreases lung colonization *(69).* Transfection of a gelatinase A cDNA in a bladder cancer cell line increased the area of lung metastases *(70),* and MT1–MMP overexpression enhanced the survival of mouse lung carcinoma cells in the lungs of intravenously injected mice *(71).* A role for the MMP matrilysin in tumor invasion was demonstrated by transfection into human prostate cells and measuring invasion into the diaphragm of immunodeficient mice *(72).*

The effect of MMP activity in spontaneous and experimental metastasis assays has been associated with the ability to degrade basement membrane and extracellular matrix components, thus facilitating invasion through connective tissue and blood vessel walls. This view was supported by *in vitro* studies measuring invasion through amnion basement membrane, smooth muscle cell-generated basement membrane, or reconstituted basement membrane (Matrigel; Collaborative Research, Inc., Waltham, MA). An inhibition of *in vitro* invasion has been observed following the addition of recombinant or transfected TIMP-1 or TIMP-2 *(40,53,73–76),* and targeted disruption of the TIMP-1 gene resulted in an increase in *in vitro* invasion *(77).* Other studies *(64,78)* using synthetic inhibitors of metalloproteinases also support an effect of MMPs on the penetration of basement membranes. It should be noted, however, that following transfection of various MMP family members, positive effects on *in vitro* invasion have been documented *(79),* but there are also examples of a lack of a consistent effect in these assays *(80,81).* In addition, no change in *in vitro* invasion was detected in loss-of-function studies in which expression of stromelysin-3 *(81)* and matrilysin *(80)* were ablated by use of antisense technology. These results raise the possibility that at least some MMPs may affect steps in metastasis other than extravasation.

Evidence Suggesting That MMPs Play a Role in the Growth of Primary and Secondary Tumors

As outlined above, MMPs and their inhibitors have been strongly linked with the process of metastasis, both by the association of increased proteolytic capacity with the metastatic phenotype and functionally as contributors to the process. How MMPs and their inhibitors contributed functionally to metastasis has been more difficult to address experimentally. Many initial conclusions about the mechanistic role of MMPs in metastasis have been derived by inference rather than direct experimentation. Metastasis is an *in vivo* process and is hard to observe directly. Most metastasis assays are end point assays, in which input (numbers, type of cells injected, etc.) and output (numbers of metastases counted at the end of the experiment) are known, while mechanisms by which the input resulted in the output are based on inference. In logically considering steps believed to be required for successful metastasis (*see* Fig. 1), extravasation from blood vessels in target organs has been assumed to be a difficult, rate-limiting process. Because MMPs are able to degrade proteins that make up blood vessel basement membrane, and because the basement membrane underlying vascular endothelial cells appears to be a clear physical barrier to metastasis, the assumption has been made that the major contribution of MMPs to metastasis is in facilitating extravasation. Mechanistic conclusions have also been based on *in vitro* assays that were thought to be appropriate models for *in vivo* processes. For example, *in vitro* invasion assays through basement membrane proteins (Matrigel) have been used to model extravasation. However, as discussed below, logical inferences about rate-limiting steps *in vivo* do not necessarily hold up to experimental scrutiny, and *in vitro* assays may not model the assumed steps *in vivo.*

A procedure for direct *in vivo* observation of early steps in metastasis has been developed *(82).* This procedure, intravital videomicroscopy, has provided evidence that suggests that some of our assumptions about mechanisms of metastasis need to be revised, based on evidence obtained from direct observation of the process. From results using this procedure, it can be concluded that the role of MMPs and their inhibitors in metastasis may be different, and more complex, than previously assumed.

Intravital videomicroscopy (IVVM) permits direct observation of the microcirculation *in vivo.* It allows observations to be made on steps in metastasis and the steps in the process that are affected by molecules, such as MMPs, implicated in metastasis [reviewed in *(83,84)*]. The microvasculature of living experimental animals is observed in real time, and interactions of tumor cells with host tissue can be observed and quantified. Results from a series of experiments by use of this procedure suggest that early steps in metastasis, including destruction of cells in the circulation and extravasation, contribute less to metastatic inefficiency than previously assumed. Rather, the regulation of growth of individual extravasated cells in target tissue appears to be rate limiting. Here, we will review some of the evidence from IVVM, as well as recent findings using other approaches, that suggest that the primary functional contribution of MMPs and their inhibitors in metastasis may be at steps after the extravasation stage.

Previously, it was believed that the majority of cells that escape from a tumor into the circulation were destroyed by hemodynamic forces. However, this belief was not supported by direct observation of intravenously injected cancer cells in chick embryos or mice *(85,86).* Evidence from IVVM *(86)* suggests that the majority of injected cells not only survive injection and arrest in a target organ but succeed in extravasating. Metastatic inefficiency thus arises from failure of the majority of extravasated cells to successfully grow in the target organ. Moreover, it also had been assumed that highly metastatic cells are better able to extravasate than are poorly metastatic cells. However, studies with IVVM have not supported this idea. In mouse liver, the timing and steps in extravasation were identical for mammary carcinoma cells of high and low metastatic ability, and the difference between the cell lines manifested itself at the postextravasation growth stage *(87).* In addition, the ability to extravasate was identical for malignant, ras-transformed NIH 3T3 cells and control fibroblasts (NIH 3T3 and primary mouse embryo fibroblasts), whereas the postextravasation growth behavior of these cells reflected their transformed versus normal phenotypes *(88).* Together, these studies suggest that extravasation may be a relatively easy process, while rate-limiting steps in metastasis occur after the cells have extravasated.

The nature of the contribution of MMPs to the metastatic process was examined directly by IVVM, by using B16F10 mouse melanoma cells engineered to overexpress TIMP-1. The TIMP-1 overexpressing cells had been shown to have markedly reduced metastatic ability *(57,89),* as measured by end point assays [intravenous injection into mouse or chick embryos and counting of tumors that formed in mouse lung or chick chorioallantoic membrane, a structure that is structurally and functionally similar to lung, with complete endothelial lining and basement membrane *(90)*]. Similarly, the TIMP-expressing cells showed reduced *in vitro* invasive ability *(76).* When these cells were assessed using IVVM, the expectation was that the reduced metastatic ability of the overexpression of TIMP-1 cells would manifest itself in defective extravasation. However, both cell lines were found to extravasate with identical kinetics, with nearly all cells having successfully extravasated by 36 hours after injection *(86,91).* The reduced metastatic ability of the TIMP-1-expressing cells was manifested by 3 days after injection, when the morphology of micrometastatic colonies was strikingly different from that of control cells (Fig. 3); instead of forming tight, growing colonies in contact with the outer surface of arterioles, the TIMP-1-expressing cells lacked adhesive contacts to other tumor cells and to vessels (where IVVM has shown micrometastases to form) and had abundant stroma between the cells *(91).* Thus, in this model, overexpression of TIMP-1 had a clear end point effect (i.e., the cells formed fewer, and smaller, metastases) but had no inhibitory effect on extravasation. These findings pointed to a role for MMPs in the regulation of post-extravasation growth. Similar conclusions, which will not be summarized here, can be drawn from other IVVM studies [reviewed in *(83,84)*].

In light of results from IVVM studies, it is necessary to re-evaluate earlier literature from the perspective of potential effects of MMPs on growth of metastatic lesions as opposed to an effect on extravasation. As discussed previously, experimental and spontaneous metastasis assays that rely on the presence of detectable secondary tumors cannot readily distinguish between these possibilities. However, effects on the growth of the primary tumor can often be detected, and in some studies an analysis of the relative size of secondary tumors has been informative. In early studies by Khokha et al. *(54),* antisense reduction of TIMP-1 levels in murine fibroblasts allowed these cells to grow into tumors when injected subcutaneously into nude mice; these tumor cells were then capable of metastatic colonization of the lungs. Recently, the effect of TIMP-1 on the initiation and growth of liver tumors was documented in transgenic mice expressing either sense or antisense TIMP-1 constructs *(92).* TIMP-1 overexpression inhibited SV40 T-antigen-induced tumor initiation, growth, and angiogenesis, while TIMP-1 reduction resulted in more rapid tumor initiation and progression. TIMP-1 transfection in B16F10 melanoma cells resulted in a decline in primary tumor growth following a subcutaneous injection as well as a reduction in lung colonization following an intravenous injection *(57).* These results are contrasted with an early study by Schultz et al. *(53)* also using B16F10 cells, in which an intraperitoneal injection of recombinant TIMP-1 reduced the number of lung colonies but did not alter the size of lung nodules nor did the growth of subcutaneously injected tumors. These authors suggested that the primary effect of TIMP was to inhibit extravasation, a conclusion that was supported by effects on invasion of an amniotic membrane *in vitro.* These apparently contradictory results might be explained by a difference in the experimental protocol or by effects of tumor versus host expression of TIMP-1, since systemic TIMP-1 was elevated following injection of recombinant protein, while in the transfection studies, tumor cell TIMP-1 levels were specifically altered. This possibility is supported by the recent, elegant studies of Soloway et al. *(93).* Using co-isogenic cells and genetically manipulated mice varying in expression of TIMP-1, these authors demonstrated that lung colonization is influenced by the TIMP-1 genotype of the tumor but not that of the host. Although systemic TIMP-1 may influence extravasation, the initiation and growth of primary tumor cells can be markedly affected by alterations in tumor TIMP-1 levels.

TIMP-2 has also been demonstrated to reduce tumor cell growth as well as metastasis. Transfection or retroviral introduction of TIMP-2 into transformed rat embryo fibroblasts reduced primary tumor growth as well as hematogenous metastasis *(75,94).* TIMP-2 overexpression reduced the growth of metastatic human melanoma cells injected subcutaneously in immunocompromised mice, although it did not prevent metastasis in this study *(95).* The growth-inhibitory effect of TIMP-2 was shown to require a three-dimensional collagen matrix and was not observed in gelatin-coated dishes; in the presence of matrix, TIMP-2 expressing melanoma cells demonstrated a reduction in growth rate and assumed a differentiated morphology. Thus, it appears that both TIMP-1 and TIMP-2 can have growth inhibitory effects, and this effect can be dependent on the cel-

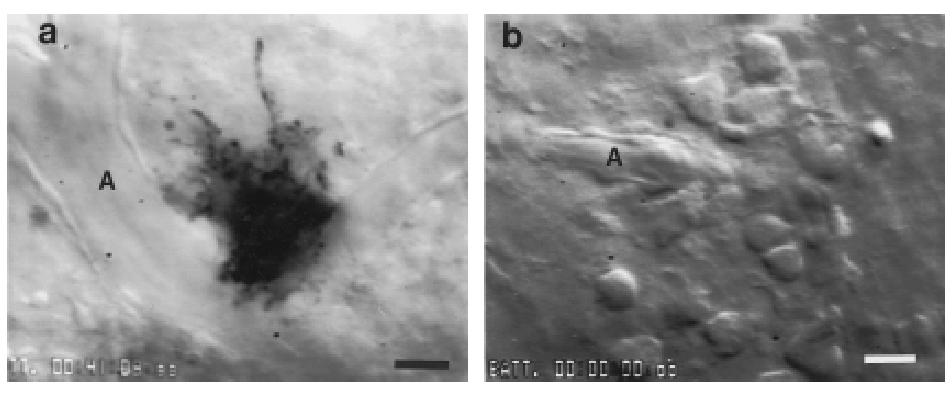

Fig. 3. Morphology of nascent micrometastases, 3 days after injection of **(a)** control B16F10 and **(b)** tissue inhibitor of metalloproteinases-1 (TIMP-1) overexpressor cells. B16F10 cells formed melanotic, tight perivascular cuffs around arterioles, visible in intravital videomicroscopy by focusing up and down through the lesion, and had a compact tumor morphology. In contrast, TIMP-1 overexpressor cells formed loosely dispersed groups of amelanotic cells, near but not attached to arterioles, lacking homotypic contacts between cells. A = arteriole; bars (**a** and **b**) = 20 μm. Reprinted with permission from Koop et al. *(91).*

lular environment and on the tumor cells themselves producing the inhibitor.

The effect of TIMPs on the growth of primary tumors and metastatic lesions is further complicated by the observation that TIMP-1 and TIMP-2 also display growth-promoting activity for a variety of cell types *(96–100).* In fact, TIMP-1 was originally identified as erythroid-potentiating activity, a growth factor for hematopoietic cells of the erythrocyte lineage *(101,102).* A recent study *(103)* has dissociated the erythroid-potentiating effect of TIMP-1 from its MMP-inhibitory activity, demonstrating that TIMPs are bifunctional molecules. There are several recent examples of systems in which TIMPs either have no effect or enhance tumor growth and/or metastasis, effects contrary to that expected from its antimetalloproteinase activity. Soloway et al. *(93)* demonstrated that, although the lack of TIMP-1 expression enhanced lung colonization in two pairs of isogenic cells with wild-type and mutant TIMP-1, in a third pair, lung colonization was reproducibly decreased in the absence of functional TIMP-1. Overexpression of TIMP-1 in the gastrointestinal tract also enhanced development of benign adenomas in a line of transgenic mice carrying a germline mutation in the adenomatous polyposis coli (APC) gene (Heppner KJ, Brown PD, Matrisian LM: manuscript submitted for publication). TIMP-3 overexpression in mouse epidermal cells had no effect on growth, tumorigenicity, or invasion *(104).* It is possible that the growth-promoting effects of TIMPs are cell type specific, manifest only in cells which, for example, have an appropriate receptor for the domain of TIMP containing the growth-promoting activity. Alternatively, factors such as the relative concentrations of specific TIMPs and/or MMPs and the extracellular environment may all affect how a tumor cell responds to alterations in TIMP expression.

Although the role of MMPs in tumor establishment and growth is difficult to decipher from experiments using multifunctional TIMPs, additional support for such a role comes from studies with the synthetic MMP inhibitors. The first published study *(105)* with batimastat demonstrated that this compound dramatically reduced tumor burden in an ovarian ascites xenograft. Batimastat caused a delay in growth of the primary tumor and a reduction in the weight of metastases in B16-BL6 melanoma cells *(59),* and an effect on the regrowth of resected breast tumors was observed following orthotopic injection of human breast cancer cells in nude mice *(63).* Batimastat also inhibited primary tumor growth in an orthotopic model of colon cancer *(62)* and of a hemangioma *(78).* In systems in which the relative size of metastatic nodules was noted, batimastat reduced the size of lung or liver colonies following injection of rat mammary carcinoma or human colorectal cancer cells *(60,61).* Other MMP inhibitors have also been reported to alter the growth of primary tumors and their metastases *in vivo,* either alone *(65,106)* or in combination with standard chemotherapeutic agents *(66).* Batimastat does not alter the growth of tumor cells in plastic culture dishes *(105).* It is not clear, however, if MMP inhibitors may suppress growth in three-dimensional collagen, as has been observed for TIMP-2 *(95).* In some cases, the effects of MMP inhibitors on growth *in vivo* may be related to their effects on tumor angiogenesis. MMP inhibitors block angiogenesis as assayed in chick and rodent models of neovascularization *(78,107–109).* The mechanism of inhibition of tumor growth may reflect both indirect effects on angiogenesis as well as more direct effects on the growth of tumor cells themselves.

The most definitive evidence for a role for MMPs in tumor cell establishment and growth comes from studies in which levels of a specific MMP were manipulated. Stromelysin-3 was originally isolated from the stromal surrounding malignant breast carcinomas *(110).* This MMP was overexpressed in human breast cancer cells or removed by antisense RNA from murine fibroblasts, which were then assayed for subcutaneous tumor development in nude mice. Manipulation of stromelysin-3 levels altered the tumorigenicity of the cells but did not alter the growth of established tumors, their invasion, or their metastatic capability *(81).* Collagenase expression in the skin of transgenic mice resulted in earlier onset and increased numbers of papillomas arising after chemical initiation and promotion *(111).* Expression of stromelysin-1 in mammary glands of transgenic mice resulted in the development of aggressive malignant mammary tumors *(112).* In contrast, chemically initiated mammary tumors were actually reduced in other stromelysin-1 transgenic mice *(113).* However, this effect was determined to be related to an increase in both proliferation and apoptosis in target mammary epithelial cells, a result that is conceptually consistent with a tumor-promoting effect of stromelysin-1 on spontaneous-derived tumors. Taken together, these results suggest that stromelysin expression can promote tumor take and suggest that metalloproteinases may favor cancer cell survival in a tissue environment initially not permissive for tumor growth.

Matrilysin is distinct from other MMPs in that it is expressed in epithelial-derived rather than mesenchymal-derived cells and is expressed in the epithelial component of both benign and malignant stages of many common adult adenocarcinomas *(114,115).* Genetic manipulation of matrilysin levels in human colon tumor cell lines resulted in an effect on the tumorigenicity of the cells following orthotopic injection into the cecum of nude mice, with little detectable effect on invasive or metastatic ability *(80).* With the use of matrilysin-deficient mice, the role of matrilysin in the development of benign intestinal adenomas was determined in mice carrying a germline mutation of the APC gene. A significant reduction in both number and size of intestinal adenomas was observed in matrilysin-null mice compared with wild-type control mice *(116).* Interestingly, the tumors arising in the matrilysin-deficient mice induced gelatinase A in the surrounding stroma, suggesting that MMP activity provided a selective advantage for initiated cells to grow into detectable tumors. In gain-of-function experiments, matrilysin expression in the mammary epithelium of transgenic mice significantly accelerated development of MMTV-neu-induced tumors (Rudolph-Owen LA, Matrisian LM: unpublished results). These results support a role for MMPs, and matrilysin in particular, in the development and growth of early stage tumors.

New View of the Contributions of MMPs to Metastasis

MMPs have long been associated with metastasis, and there is no doubt that they are major functional contributors to the metastatic process. The nature of their contribution originally was assumed to be primarily facilitation of the breakdown of

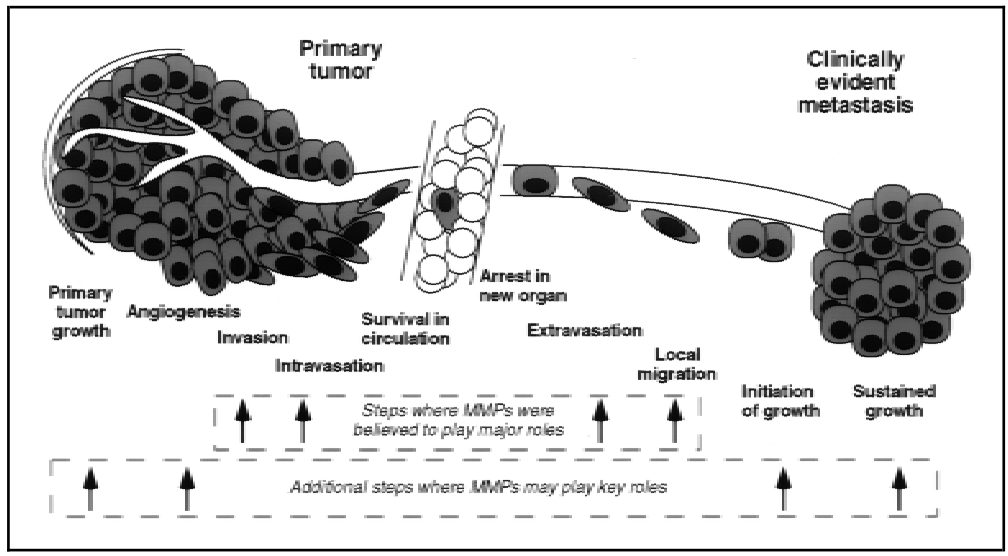

Fig. 4. Role of metalloproteinases in the metastatic process. Matrix metalloproteinases (MMPs) have classically been associated with steps in the metastatic cascade that involve matrix degradation, including invasion, intravasation, extravasation, and local migration. Evidence discussed in this review expands the role of MMPs to steps involving the growth of the primary tumor, angiogenesis, the initiation of growth at an ectopic site, and the sustained growth of metastatic foci to become clinically detectable tumors.

physical barriers between a primary tumor and distant sites for metastasis. As shown in Fig. 4, these steps include local invasion and intravasation of cancer cells, facilitating their departure from the primary tumor and their access to the lymphatic or blood circulations, and extravasation and local invasion of cancer cells in distant organs, as a first step toward the establishment of secondary tumors. Recent evidence, summarized in this review, suggests that MMPs play a much broader role in metastasis than previously believed, and that action of MMPs at steps both before and after the breakdown of the apparent physical barriers to metastasis may in fact be of greater importance. MMPs and their inhibitors appear to be important regulators of the growth of tumors, both at the primary site and as metastases (Fig. 4). The mechanisms of this growth regulation are not yet fully characterized, but a number of mechanisms are possible. First, MMPs appear to contribute to the initiation of growth, at both primary and secondary sites. One can speculate that this may involve regulation of the growth environment by, for example, regulating access to growth factors from the extracellular matrix surrounding the growing tumor, either directly or via a proteolytic cascade. Similarly, MMPs and their inhibitors appear to regulate the sustained growth of tumors. Beyond the maintenance of an appropriate growth environment, the role of MMPs in angiogenesis is likely important at this stage. Angiogenesis is required for growth of tumors, primary and metastases, beyond small size, and MMPs play a contributory role in regulation of angiogenesis. Details of the mechanisms by which MMPs and their inhibitors contribute to creating an environment that favors the initiation and continued growth of primary and metastatic tumors remain to be elucidated, but are of key importance in cancer therapy. An understanding of the molecular role of MMPs at each of the sequential steps required to produce clinically evident metastases will be important in the design and appropriate use of novel therapeutics designed to combat metastasis.

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Notes

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