

Review

Collagen – A Necessary Accomplice in the Metastatic Process

PAUL S. NERENBERG, RAMON SALSAS-ESCAT and COLLIN M. STULTZ

Harvard-MIT Division of Health Sciences and Technology and Department of Electrical Engineering and Computer Science Massachusetts Institute of Technology, U.S.A.

Abstract. *The prognosis for cancer patients with metastatic disease remains poor. For cancer to metastasize from a primary tumor to distinct sites in the body, both the extracellular matrix and basement membrane – physiological barriers whose primary structural constituent is collagen – must be degraded to allow the passage of tumor cells. Collagen has long been assumed to be a passive background upon which the biochemical events of metastasis take place, but recent experimental developments instead point to a novel active role for collagen in the immune response to metastasis. Along with a new hypothesis for the mechanism of collagen degradation, these data suggest innovative approaches to prevent the spread of cancer from the primary tumor site.*

In 2006 there were an estimated 1.4 million new cases of cancer diagnosed in the United States, along with an estimated 560,000 deaths due to cancer (1). Worldwide, 6.7 million people died from cancer in 2002, and as of 2006, this annual figure was expected to rise to 10.3 million by 2020. While there has been significant progress in treating cancer – the 5-year relative survival rate increased from 50% in 1974-76 to 65% in 1995-2001 – the prognosis for patients whose cancer has metastasized remains relatively unchanged (2).

Metastases are responsible for approximately 90% of cancer deaths (3) and are found during the initial diagnosis in a significant fraction (~30%) of cancer cases (4, 5). Moreover, metastases are likely to be present in an additional 20% of cases at the time of diagnosis (5). As existing treatments for metastatic disease are relatively

ineffective, new approaches aimed at inhibiting the processes responsible for the spread of cancer from initial tumor sites are needed.

What is Metastasis?

Malignant cancer typically spreads by one of three pathways: (i) hematogenous spread (ii), lymphatic spread, and/or, (iii) seeding within body cavities (5). For both hematogenous and lymphatic spread, tumor cells propagate from their initial site to vascular or lymphatic structures where they are then be transported to distant sites. This is the preferred pathway for cancers arising from mesenchymal tissues (connective tissues, as well as blood and lymphatic vessels) and tumors of epithelial origin.

In this review we elucidate the roles of collagen in the extracellular matrix (ECM), and matrix metalloproteinases (MMPs), the enzymes that degrade collagen, in the metastatic process. Collagen, a major component of the ECM and the basement membrane (BM), provides a protective barrier that must be compromised before tumor cells can metastasize to distant sites. In fact, numerous experiments support a positive correlation between the metastatic ability of cancer and MMP expression (6).

A number of steps are involved in the metastatic process (7). Without augmenting its own blood supply, a tumor is usually limited to a maximum size of 1-2 mm in diameter (8), which is presumed to be the diffusion distance for oxygen and nutrients from blood vessels. Sustained angiogenesis is the tumor's solution to the blood supply problem. A common mutation in malignant cancer cells, the inactivation of both alleles the *TP53* gene, results in a decrease in levels of the antiangiogenic molecule thrombospondin-1, which in turn allows angiogenic factors to dominate the local environment of the tumor (5).

While tumor hypoxia leads to the release of hypoxia-inducible factor-1 (HIF-1) – a protein that promotes angiogenesis by stimulating the production of vascular endothelial growth factor (VEGF) – MMPs have also been implicated in the release of both pro- and antiangiogenic

Correspondence to: Collin M. Stultz, Harvard-MIT Division of Health Sciences and Technology and Department of Electrical Engineering and Computer Science Massachusetts Institute of Technology, MIT, Building 36-796, 77 Massachusetts Ave. Cambridge Ma. 02139, U.S.A. Tel: +617 253 4961, Fax: +617 324 3644, e-mail: cmstultz@mit.edu

Key Words: Collagen, cancer, MMPs, metastasis, collagenolysis, review.

factors. MMPs can release basic fibroblast growth factor (bFGF) stored in the ECM, which may aid in the vascularization of the tumor (9). Conversely, MMPs can also degrade plasminogen in the ECM into angiostatin-like fragments, which are angiogenesis inhibitors (10, 11).

Only certain subclones can invade the local tissue of the associated organ on their way to reaching the vascular system. Invasion begins when (invasive) subclone cells separate from one another and then adhere to and penetrate the surrounding basement membrane. This separation is thought to be caused by a loss or reduction in E-cadherin expression in these cells (12), which has been correlated with increased aggressiveness in many carcinomas (13).

Metastasis and the Basement Membrane

Basement membrane is composed primarily of type IV collagen and glycoproteins, the most prevalent of which is laminin (14). Many tumor cells of mesenchymal origin contain laminin receptors that recognize laminin in the BM. Once a tumor cell "realizes" that it is attached to the BM, it begins to express MMPs that degrade the BM and gain internal access to the tissue being invaded. Recent experiments suggest that BM degradation is dominated by the membrane-type MMPs (MT-MMPs) (15), despite earlier evidence that type IV collagenase (MMP-2) was primarily responsible for this process (16-18).

After the BM is sufficiently degraded, tumor cell migration is mediated by fibronectin receptors on tumor cell membranes, as well as a variety of motility factors. As the surrounding ECM is degraded by MMPs released by these invasive cells, soluble matrix proteins are cleaved, which can stimulate further migration (19). Some of the major proteins known to promote tumor cell motility (both chemotaxis and haptotaxis) include types I and IV collagen, laminin, fibronectin, and thrombospondin (20).

Once invasive cells reach the basement membrane surrounding a blood vessel (not necessarily one created by the tumor's angiogenesis), they again degrade it, thus beginning the process of intravasation – an invasion of the blood vessel through the basement membrane and endothelium. The BM is again degraded – a process mediated by MMPs – and eventually the invaded tumor cells only need traverse the thin endothelial lining of the blood vessel wall (21). After entering the vasculature, tumor cells must survive until they arrest in new (and often distant) tissue.

To start life anew in distant places, tumor cells employ a variety of methods including surrounding themselves in fibrin and platelets, mechanical wedging, or the use of targeted endothelial cell receptors found on the surface of some of these cells (5, 22). Once attached to the

endothelium, tumor cells begin the process of extravasation. Extravasation again requires the degradation of basement membrane using MMPs. Upon entry to the ECM, tumor cells may migrate before initiating growth of a new tumor.

It has been implied that the low probability of successful metastasis (<0.1%) to remote tissues reflects the difficulty inherent in the circulation, arrest, or extravasation stages of the metastatic process (23, 24), but more recent data instead points to tumor cells' difficulty in achieving sustained growth in new tissue (25, 26). Current research suggests that extravasation may in fact not be necessary for such growth – tumor cells can arrest in blood vessels and proliferate to form endothelial cell-like structures in what is termed "vasculogenic mimicry" (27). Regardless of methodology, if cell proliferation is able to proceed, the secondary tumor also has the potential to metastasize to additional sites within the body.

As the degradation of the BM plays an essential role in several stages of the metastatic sequence, a thorough understanding of the molecular mechanism underlying collagenolysis is of particular interest. Such data may lead to a deeper understanding of the metastatic process and potentially lead to new therapies for this devastating complication of tumorigenesis.

Collagen

Structural characteristics. About one quarter of all of proteins in the human body belong to the class of structural proteins known as collagens, which form fibers that add significant tensile strength to tendons, bone, and structural constituents of skin and virtually all internal organs. Collagen provides integrity to these structures, protecting and supporting the softer tissues and connecting them to the skeleton (28).

Collagen is composed of three polypeptide chains form a triple helical structure. A repeating sequence of three amino acids (XYG) forms this structure, in which every third amino acid is glycine (29). The tight internal packing of the triple-helix only allows residues without a side-chain to occupy every third position. Many of the remaining positions in the chain are occupied by proline (Pro or P) and a post-translational modification of proline, hydroxyproline (Pro-OH, Hyp, or O) (30).

Regions in the collagen sequence that are rich in POG repeats (imino rich regions) are essential for the overall triple helical structure of collagen, but, for example, represent only 10% of the total type I collagen sequence (30). Indeed, not only imino acids are found in the X and Y positions of the XYG triplets, but other amino acids can be present. There are several stretches within different collagen sequences that are relatively imino poor in that few or no imino acids are present. These imino poor regions, rather

than playing a structural role, are thought to carry biological information in cell recognition (30). Most importantly, the regions downstream of the cleavage sites in several types of collagen have a low content of imino acids and may be an important factor in collagen degradation.

Host-guest studies have been carried out using collagen-like model peptides to dissect the contribution to stability of different XYG triplets (31-34). These studies found greater stability for triplets containing O and/or P and lower melting temperatures for imino poor sequences. An algorithm to predict the stability of collagen-like peptides from their sequence is available (35) and compiles the knowledge acquired since these early experiments. Collagen-like peptides have also been studied using other experimental techniques to gain insights into the dynamics and thermodynamics of collagen. NMR spectroscopy has been used to determine the dynamics of collagen-like peptides (36). Calorimetric techniques have been employed to follow the unfolding of such peptides (37). Detailed molecular dynamics simulations have also described the conformational landscape of collagen-like peptides (38), as well as their folding mechanisms (39). All these studies have contributed to the notion that the collagen triple helix does not adopt a uniform structure throughout its entire length, but rather it adopts a variety of local structures (with unique dynamical characteristics) that are determined by the underlying amino acid sequence.

Matrix Metalloproteinases

General structural and sequence motifs. Matrix metalloproteinases are a family of Zn^{2+} dependent endopeptidases that are capable of cleaving components of the extracellular matrix, including collagen, fibronectin and laminin (40). For example, a number of members of the MMP family such as fibroblast collagenase (MMP-1), gelatinase A (MMP-2), neutrophil collagenase (MMP-8), collagenase-3 (MMP-13), membrane-type I MMP (MMP-14), collagenase-4 (MMP-18), and MMP-22 are the only enzymes that can cleave native triple helical interstitial collagens (types I and III) (41, 44). There are at least 25 members of the MMP family, and all but two (MMP-7 and MMP-26) of them share the archetypal structure shown in Figure 2. This structure consists of two domains – a catalytic domain and a hemopexin-like domain – connected by a linker of variable length. Some members of the MMP family contain additional domains, *e.g.* the transmembrane domain of MT1-MMPs or the fibronectin like domains of MMP-2 (42-44).

MMPs are expressed as zymogens and can be activated intra or extracellularly (41). These zymogens include a propeptide that prevents access to the catalytic site. This propeptide can be cleaved by membrane-type MMPs or other proteases, including activated MMPs (40). The

catalytic domain contains a conserved sequence motif (HEXXHXXGXXH), with glutamate being the catalytic residue and the histidine residues responsible for coordinating the Zn^{2+} ion. The hemopexin or binding domain is of little known function (43). It is considered to be necessary for collagen binding. Some MMPs retain catalytic activity for cleaving gelatin (unfolded collagen) in the absence of the binding domain, but lose the ability to cleave triple helical collagen. The structures of full MMPs or of the different domains of several MMPs have been previously reported (45).

The Collagen Degradation Cycle in Metastasis

Overview and significance. Collagen serves as an effective barrier that tumor cells must break through to metastasize to distinct sites throughout the body (46). However, collagen is not just a passive player in the metastatic sequence. Recent experimental developments point to a far more complex role for this structural protein. It is known that collagen degradation products serve as chemotactic stimuli for monocytes (47). As collagen is degraded during multiple steps of metastasis, the resulting collagen fragments may play a role in recruiting tumor associated macrophages (TAMs). These TAMs are found in abundance in most solid tumors (48), and clinical studies have demonstrated a correlation between poor prognosis and high concentration of TAMs (49).

The presence of additional monocytes/macrophages in and around tumor sites can, in principle, either promote or inhibit the metastatic process (50). The principle factors behind this transformation appear to be interleukin-6 (IL-6) and colony-stimulating factor 1 (CSF-1) (51). When soluble CSF-1 is released by tumor cells, the differentiation of monocytes into pro-tumor macrophages – macrophages that will promote tumor growth and metastasis – is promoted. Similarly, the presence of IL-6 will inhibit monocyte differentiation to mature dendritic cells, which direct the immune response against tumor cells. Tumor cells can also express CSF-1 on the surface of their cell membranes; in such cases, monocytes will differentiate into anti-tumor macrophages, *i.e.* macrophages that attack tumor cells and inhibit metastasis. Likewise, tumor cells can express factors such as IL-4, IL-12, and IL-13, all of which act to promote the differentiation of monocytes to mature dendritic cells. Given this, it is possible that aggressive tumors may be distinguished from less aggressive ones by their relative expression of factors that promote pro-tumor macrophage differentiation and inhibit dendritic cell maturation *versus* the production of factors that promote dendritic cell maturation and promote anti-tumor macrophage differentiation.

Pro-tumor macrophages themselves express factors responsible for several stages of the metastatic process,

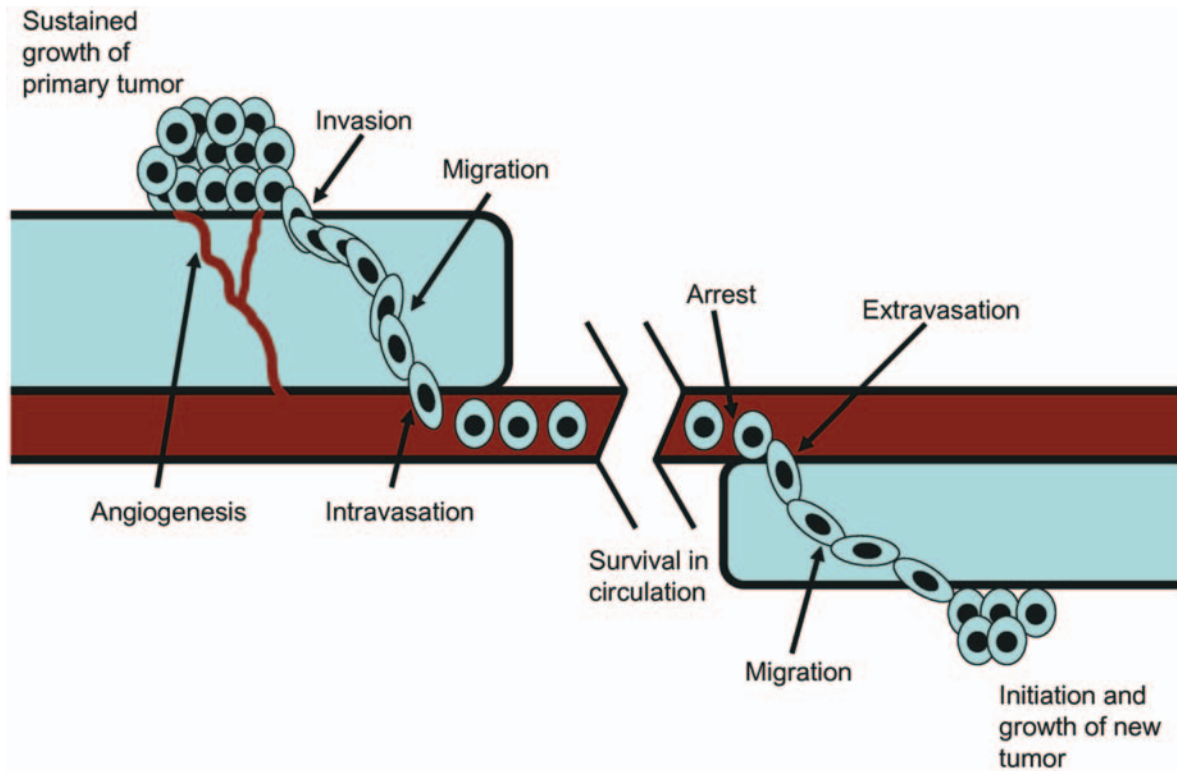


Figure 1. Basic progression of metastasis from a primary tumor to distinct sites. In addition to the mechanism presented in this figure, invasive cells may also enter the vascular system through tumor-associated blood vessels created by angiogenesis, thus omitting the invasion and migration phases of metastasis.

including angiogenesis, invasion, and intra- and extravasation. It has been observed that macrophages release soluble forms of VEGF, angiopoietins, IL-1, IL-8, tumor necrosis factor- α (TNF- α), and MMPs (including MMP-2) that are known contributors to angiogenesis (52, 53). IL-1, a pro-inflammatory cytokine, is of particular interest because it has been observed that melanoma cells require its presence for invasion and angiogenesis, specifically in the form of IL-1 β (54). IL-1 β stimulates expression of VEGF and TNF- α , which play important roles in angiogenesis. Similarly, by stimulating expression of adhesion molecules on endothelial and tumor cells, IL-1 β enhances the invasive ability of malignancies (55, 56).

As mentioned above, macrophages also express MMPs, which in turn lead to further degradation of ECM and BM. Additionally, there is a complex relationship between MMPs and IL-1 β . It is known that MMP-1 deactivates IL-1 β through proteolysis, while the gelatinases (MMP-2, 3, and 9) activate IL-1 β by cleaving its precursor protein (57, 58). New observations demonstrate the ability of collagen degradation products to not only act as chemotactic agents for monocytes, but also to directly stimulate the expression of IL-1 β by monocytes (59). This research has uncovered an important positive feedback element in the immune response to cancer relating to collagen degradation – not

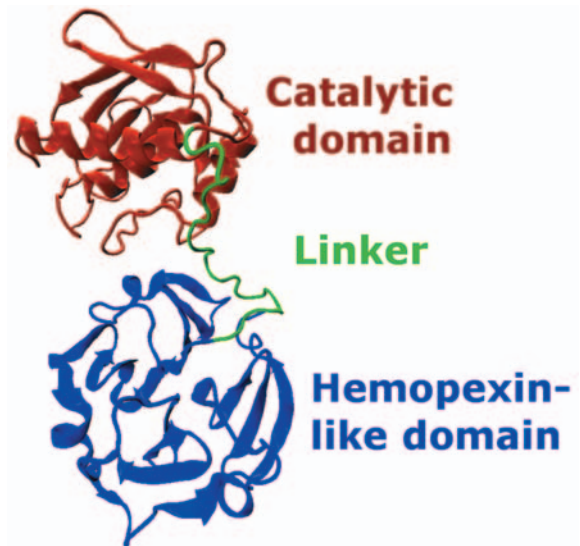


Figure 2. MMP-1 (1FBL) – archetypal MMP structure showing the catalytic and hemopexin-like domains connected by a linker.

only is collagen degraded by tumor cells, but these degradation products attract additional monocytes, which then differentiate to become macrophages that carry out

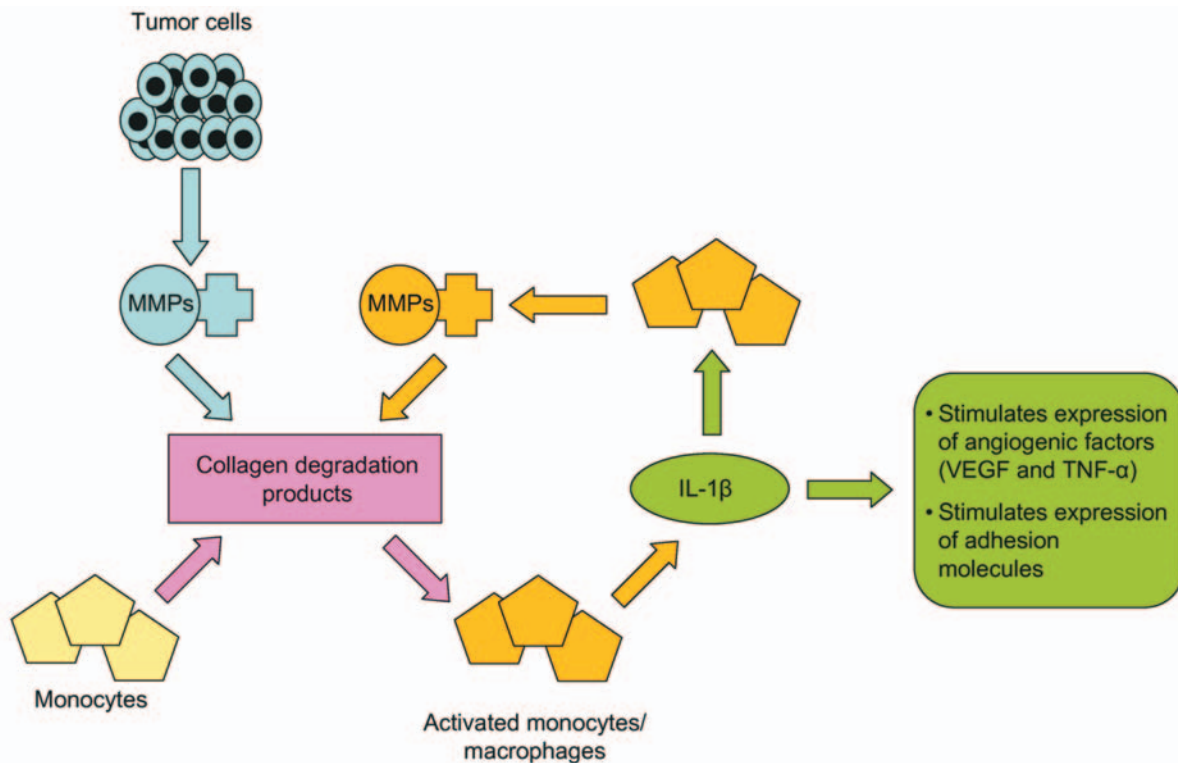


Figure 3. MMPs expressed by both invasive tumor cells and activated macrophages degrade the ECM or BM thus producing fragments of collagen. These collagen degradation products serve as both chemotactic attractants for monocytes and stimulators of IL-1 β production in activated monocytes/macrophages. In turn, these activated macrophages express MMPs which lead to further collagen degradation near the site of metastasis.

further degradation of collagen. Furthermore, collagen degradation products result in the production of IL-1 β , which is responsible for multiple factors in the progression of metastasis (Figure 3).

These observations are corroborated by independent experiments that link degraded type IV collagen to angiogenesis and tumor growth (60, 61). In these experiments, type IV collagen found in BM of angiogenic blood vessels is degraded by MMP-2. A so-called cryptic site, or site that is hidden in non-degraded collagen, can then be bound by an antibody known as HUIV26. Moreover, when HUIV26 is administered systemically, angiogenesis and tumor growth are both strongly inhibited *in vivo*. Other research using the D93 antibody, which is derived from another HUIV antibody, shows similar results (62). Together with earlier data regarding their chemotactic aspects, these data imply a pivotal role for collagen degradation products in the metastatic process.

Future Research and Treatment Possibilities

Known approaches to MMP inhibition. MMPs are regulated at different levels *in vivo*, including gene transcription, proenzyme activation, and enzymatic inhibition (63). These

three areas divide the efforts directed to clinical MMP inhibition and are reported in more detail elsewhere (64).

Efforts to regulate MMP gene transcription have been directed towards avoiding the action of extracellular factors that bind to receptors and trigger MMP expression. Other approaches attempt to block the signal transduction pathways that carry the ligand binding information from the cell surface to the nucleus. Finally, the transcription factors that regulate MMP expression have been also targeted (64).

The *in vivo* activation of MMP has been also identified as a possible avenue of inhibition of MMP activity. In particular, the ability of MT1-MMP to activate other MMPs by cleaving their propeptides has made it a candidate for inhibition (65). Monoclonal antibodies against MT1-MMP have been shown to block its enzymatic activity and inhibit cell migration and invasion. Another method of inhibiting activation has been to use small peptides that contain a highly conserved sequence (PRCGXPDV) found in the propeptides of MMPs (66, 67). These small peptides bind competitively to several MMPs, including MMP-2 and MMP-3, thus preventing them from activating other MMPs. As a byproduct of this competitive binding, enzymatic activity on ECM and BM may also be reduced.

The classical approach to regulate MMP activity has been the inhibition of its enzymatic activity, and it has been the approach used in the majority of clinical trials to date (64). Small synthetic inhibitors have constituted the principal avenue of research. Unfortunately, their *in vitro* effectiveness has not translated into wide success in clinical trials, even though some compounds appear to be promising (64). The naturally occurring tissue inhibitors of metalloproteinases (TIMPs) have been considered as an alternative, but there are many variables to consider, such as the fact that only 4 TIMPs inhibit a wide range of MMPs, therefore specificity is hard to achieve.

Alternative approaches to MMP inhibition. Collagen monomers adopt a left-handed triple-helical structure with a diameter of approximately 15Å. However, structures of the catalytic domain of MMPs reveal a catalytic site that is small relative to the structure of the collagen triple-helix (approximately 5-8Å wide) (68). It becomes apparent that collagen in its triple helical folded structure cannot fit into the active site of the enzyme. A number of different hypotheses have been proposed to explain the structural changes that must precede collagenolysis. In particular, it has been proposed that collagen unfolding prior to collagen degradation is facilitated by MMPs in an active process known as "molecular tectonics" (42, 69). It has been suggested that the different MMP domains (catalytic, binding and/or linker) actively force the native triple-helical structure of collagen to unfold by binding to collagen at distinct regions near the collagenase cleavage site and executing a set of coordinated motions (42, 69, 70).

Our approach to collagen degradation emphasizes the role of the substrate structure and stability. We do not underestimate the interaction between collagen and MMPs, but we do not support the concept of active unfolding by the latter. Detailed molecular simulations have shown that the imino poor region of type III collagen downstream of the scissile bond exists in equilibrium between folded and partially unfolded structures that would make the scissile bond accessible to the active site of MMPs (38). This finding suggested that imino poor regions of collagen preexist, as a function of temperature, in a partially unfolded structure that could be recognized, bound, and cleaved by MMPs. These results, together with NMR studies of the backbone dynamics of the model peptide T3-785 (36), are consistent with other data which imply that the structural stability of collagen is a function of its amino acid content; *i.e.*, imino poor regions have lower melting temperatures than imino rich regions (71).

Therefore, an alternative view of collagen degradation is one in which collagen exists as a distribution of well folded imino rich regions together with imino poor regions that are in equilibrium between the two folded (native) and partially unfolded (vulnerable) states (72). It is the vulnerable states

that would be recognized and bound by MMPs *via* their collagen binding domains, both directing the catalytic domain to the correct site for cleavage, and displacing the conformational equilibrium towards vulnerable states.

This alternative scenario of collagen degradation presented in this review suggests new approaches to MMP inhibition. One alternative would be the design of small molecules or peptides that bind to the partially unfolded imino poor regions of collagen and therefore compete with MMPs. Such binding partners could range from small peptides to antibodies designed against particular collagen sequences. In the latter case, if the sequence were hidden in the triple helical structure of collagen, the antibodies would not recognize or bind to the native folded structure, but binding could occur with the partially unfolded structure.

Another approach would involve preferentially stabilizing the folded ensemble of key imino poor regions of collagen *versus* the partially unfolded ensemble of structures, *i.e.* stabilizing the triple helix of collagen. Designing a molecule or peptide that preferentially binds to an imino poor triple helix *versus* an imino rich triple helix may be difficult though.

One last approach is centered in mapping the interaction between the collagen binding domains (CBD) of MMPs (in general the hemopexin domain) and the partially unfolded imino poor regions. Such mapping could be carried out *via* X-ray crystallography and would reveal which residues within the CBD are involved in binding. This information could then lead to the development of small molecules or peptides than would compete and bind to the key amino acids of the CBD.

Controlling the immune response to metastasis. Further research into the immune response to cancer may yield information about additional factors involved in the differentiation and education of monocytes in the tumor environment. The current view of this response, however, presently offers many opportunities for halting the metastatic process that merit further investigation.

Therapies that inhibit or otherwise neutralize IL-6 and soluble CSF-1 would appear to be the most direct route to preventing pro-macrophage differentiation and encouraging dendritic cell maturation. Indeed, recent trials using targeted antibodies against IL-6 have shown promise in terms of both efficacy and tolerance (73, 74). Such therapies would prevent tumor cells from enlisting the support of the body's monocytes and halt the metastatic process before invasion.

Additionally inhibiting "second string" chemokines and cytokines, *e.g.* TNF- α and IL-8, may have a similar effect in halting the metastatic process after the arrival of monocytes to the site of the tumor. There are currently several

therapies in development targeted against TNF- α – some are antibodies, while others aim to either prevent its synthesis or its signaling properties (75). While an antibody to IL-8 has been developed and shown to inhibit many, if not all, of IL-8's downstream effects, it has not yet been tested in human patients (76, 77).

Therapies directed toward IL-1 β have not yet been developed, as research into IL-1 β knock-out models is relatively new. Similarly, targeted antibodies for degraded type IV collagen have been found only within the last few years. Nevertheless, this research implies that antibodies to either IL-1 β or collagen degradation products may be effective in preventing angiogenesis and reducing tumor invasiveness, much as antibodies are for other chemokines and cytokines. Antibodies to degraded collagen are additionally interesting because they may reduce the initial recruitment of monocytes to the tumor site, as well as the expression of IL-1 β in monocytes already near the tumor.

Based on current data, there are several therapeutic avenues for halting the metastatic process. If used together, these therapies may potentially block the majority of the biochemical signals that trigger the early steps of the process, which would significantly slow the progression of metastatic disease. Future research to find molecules or antibodies that inhibit multiple chemokines and cytokines would help to simplify therapies and potentially avoid unwanted interactions and side effects, but currently known antibodies and drug molecules may already be satisfactory in this regard.

New Views of Collagen Degradation in Metastasis

Research carried out in the last few years has offered new views of collagen's roles in its own degradation and in metastasis. Collagen can no longer be considered a static and passive background upon which metastasis takes places. Rather, collagen is an active participant in the metastatic process through its interactions with MMPs and its role in the immune response to cancer.

One such new view is the mechanism by which collagen is degraded. While there is a significant body of work that seemingly verifies the hypothesis of molecular tectonics, *i.e.* active unwinding of collagen by MMPs, these same data also support the alternative hypothesis of native and vulnerable states of collagen. Other experiments are unable to be reconciled with molecular tectonics, but are plausibly explained by this two state hypothesis. Future experiments regarding the kinetics of collagen-MMP binding and the structural dynamics of collagen-like peptides, in concert with crystallographic structures of MMP complexed with collagen-like peptides, will expose the mechanism by which MMPs degrade collagen. Once this mechanism is known, more effective therapies may be

created to reduce the degradation of ECM and BM inherent in the metastatic process.

Secondly, it is now recognized that collagen degradation products are a critical part of the immune response to metastasis. These degradation products not only recruit macrophages to the tumor site, where they may in turn be "hijacked" to express a wide variety of pro-tumor factors, but they also stimulate the expression of the pro-inflammatory cytokine IL-1 β from such macrophages. As IL-1 β has been shown to enable both angiogenesis and invasion, halting its expression by preventing macrophage recognition of collagen degradation products may significantly slow the progression of metastatic disease.

References

- 1 American Cancer Society Facts and Figures 2006.
- 2 Sporn MB: The war on cancer: a review. *Ann NY Acad Sci* 833: 137-146, 1997.
- 3 Sporn MB: The war on cancer. *Lancet* 347: 1377-1381, 1996.
- 4 Greenlee RT *et al*: Cancer statistics, 2001. *CA Cancer J Clin* 51: 15-36, 2001.
- 5 Kumar V, Cotran RS and Robbins SL: Neoplasia. *In*: Basic Pathology. Philadelphia, Saunders, pp. 165-210, 2003.
- 6 Ray JM and Stetler-Stevenson WG: The role of matrix metalloproteases and their inhibitors in tumour invasion, metastasis and angiogenesis. *Eur Respir J* 7: 2062-2072, 1994.
- 7 Chambers AF and Matrisian LM: Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 89: 1260-1270, 1997.
- 8 Folkman J: Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285: 1182-1186, 1971.
- 9 Folkman J and Klagsbrun M: Angiogenic factors. *Science* 235: 442-447, 1987.
- 10 Lijnen HR *et al*: Generation of an angiostatin-like fragment from plasminogen by stromelysin-1 (MMP-3). *Biochemistry* 37: 4699-4702, 1998.
- 11 Patterson BC and Sang QA: Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). *J Biol Chem* 272: 28823-28825, 1997.
- 12 Li G and Herlyn M: Dynamics of intercellular communication during melanoma development. *Mol Med Today* 6: 163-169, 2000.
- 13 Beavon IR: The E-cadherin-catenin complex in tumour metastasis: structure, function and regulation. *Eur J Cancer* 36: 1607-1620, 2000.
- 14 Kalluri R: Basement membranes: structure, assembly, and role in tumour angiogenesis. *Nat Rev Cancer* 3: 422-433, 2003.
- 15 Hotary K *et al*: A cancer cell metalloprotease triad regulates the basement membrane transmigration program. *Genes Dev* 20: 2673-2686, 2006.
- 16 Liotta LA *et al*: Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 284: 67-69, 1980.
- 17 Salo T, Liotta LA and Tryggvason K: Purification and characterization of a murine basement membrane collagen-degrading enzyme secreted by metastatic tumor cells. *J Biol Chem* 258: 3058-3063, 1983.

- 18 Albini A *et al*: Tumor cell invasion inhibited by TIMP-2. *J Natl Cancer Inst* 83: 775-779, 1991.
- 19 Egeblad M and Werb Z: New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2: 161-174, 2002.
- 20 Woodhouse EC, Chuaqui RF and Liotta LA: General mechanisms of metastasis. *Cancer* 80: 1529-1537, 1997.
- 21 Roos E and Dingemans KP: Mechanisms of metastasis. *Biochim Biophys Acta* 560: 135-166, 1979.
- 22 Orr FW, Wang HH, Lafrenie RM, Scherbarth S and Nance DM: Interactions between cancer cells and the endothelium in metastasis. *J Pathol* 190: 310-329, 2000.
- 23 Liotta LA, Kleinerman J and Saidel GM: Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. *Cancer Res* 34: 997-1003, 1974.
- 24 Fidler IJ: The relationship of embolic homogeneity, number, size and viability to the incidence of experimental metastasis. *Eur J Cancer* 9: 223-227, 1973.
- 25 Morris VL *et al*: Early interactions of cancer cells with the microvasculature in mouse liver and muscle during hematogenous metastasis: videomicroscopic analysis. *Clin Exp Metastasis* 11: 377-390, 1993.
- 26 Koop S *et al*: Fate of melanoma cells entering the microcirculation: over 80% survive and extravasate. *Cancer Res* 55: 2520-2523, 1995.
- 27 Maniotis AJ *et al*: Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am J Pathol* 155: 739-752, 1999.
- 28 Stryer L: *Biochemistry*. New York, W.H. Freeman and Company, 1089 p, 1988.
- 29 Rich A and Crick FHC: The molecular structure of collagen. *J Mol Biol* 3: 482-506, 1961.
- 30 Brodsky B and Ramshaw JAM: The Collagen Triple-Helix Structure. *Matrix Biology* 15: 545-554, 1997.
- 31 Bella J, Brodsky B and Verman HM: Crystal and molecular structure of a collagen like peptide at 1.9 Å resolution. *Science* 266: 75-81, 1994.
- 32 Shah NK, Ramshaw JAM, Kirkpatrick A, Shah C and Brodsky B: A host-guest set of triple helical peptides: Stability of Gly-X-Y triplets containing common non-polar residues. *Biochemistry* 35: 5510-5517, 1996.
- 33 Kramer RZ, Bella J, Mayville P, Brodsky B and Berman HM: Sequence dependent conformational variations of collagen triple-helical structure. *Nat Struct Biol* 6: 454-457, 2001.
- 34 Kramer RZ, Bella J and Brodsky B: The crystal structure of a collagen-like peptide with a biologically relevant sequence. *J Mol Biol* 311: 131-147, 2001.
- 35 Persikov AV, Ramshaw JAM and Brodsky B: Prediction of Collagen Stability from Amino Acid Sequence. *J Biol Chem* 280: 19343-19349, 2005.
- 36 Li MH, Fan P, Brodsky B and Baum J: Two-dimensional NMR assignments and conformation of (Pro-Hyp-Gly)₁₀ and a designed collagen triple-helical peptide. *Biochemistry* 32: 7377-7387, 1993.
- 37 Persikov AV, Xu Y and Brodsky B: Equilibrium thermal transitions of collagen model peptides. *Protein Sci* 13: 893-902, 2004.
- 38 Stultz CM: Localized Unfolding of Collagen Explains Collagenase Cleavage at Imino-Poor sites. *J Mol Biol* 319: 997-1003, 2002.
- 39 Stultz CM: The folding mechanism of collagen-like model peptides explored through detailed molecular simulations. *Protein Sci* 15: 2166-2177, 2006.
- 40 Jones CB, Sane DC and Herrington DM: Matrix metalloproteinases: A review of their structure and role in acute coronary syndrome. *Cardiovasc Res* 59: 812-823, 2003.
- 41 Chakraborti S, Mandal M, Das A, Mandal A and Chakraborti T: Regulation of matrix metalloproteinases: An overview. *Mol Cell Biochem* 253: 269-285, 2003.
- 42 Overall CM: Molecular determinants of metalloproteinase substrate specificity. *Mol Biotechnol* 22: 51-86, 2002.
- 43 Bode W *et al*: Structural properties of matrix metalloproteinases. *Cell Mol Life Sci* 55: 639-652, 1999.
- 44 Lauer-Fields JL, Juska D and Fields GB: Matrix metalloproteinases and collagen metabolism. *Biopolymers* 66: 19-32, 2002.
- 45 Maskos K: Crystal structures of MMPs in complex with physiological and pharmacological inhibitors. *Biochimie* 87: 249-263, 2005.
- 46 Fields GB: A Model for Interstitial Collagen Catabolism by Mammalian Collagenases. *J Theor Biol* 153: 585-602, 1991.
- 47 Postlethwaite AE and Kang AH: Collagen-and collagen peptide-induced chemotaxis of human blood monocytes. *J Exp Med* 143: 1299-1307, 1976.
- 48 Normann SJ: Macrophage infiltration and tumor progression. *Cancer Met Rev* 4: 277-291, 1985.
- 49 Bingle L, Brown NJ and Lewis CE: The role of tumour-associated macrophages in tumor progression: implications for new anticancer therapies. *J Pathol* 196: 846-852, 2002.
- 50 Pollard JW: Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 4: 71-78, 2004.
- 51 Menetrier-Caux C *et al*: Inhibition of the differentiation of dendritic cells from CD34(+) progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. *Blood* 92: 4778-4791, 1998.
- 52 Carmeliet P and Jain RK: Angiogenesis in cancer and other diseases. *Nature* 407: 249-257, 2000.
- 53 Boudreau N and Myers C: Breast cancer-induced angiogenesis: multiple mechanisms and the role of the microenvironment. *Breast Cancer Res* 5: 140-146, 2003.
- 54 Voronov E *et al*: IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci* 100: 2645-2650, 2003.
- 55 Chirivi RG *et al*: IL-1α gene-transfected human melanoma cells increase tumor-cell adhesion to endothelial cells and their retention in the lung of nude mice. *Int J Cancer* 67: 856-863, 1996.
- 56 Vidal-Vanalocho F *et al*: Interleukin 1 (IL-1)-dependent melanoma hepatic metastasis *in vivo*; increased endothelial adherence by IL-1-induced mannose receptors and growth factor production *in vitro*. *J Natl Cancer Inst* 88: 198-205, 1996.
- 57 Ito A *et al*: Degradation of interleukin 1β by matrix metalloproteinases. *J Biol Chem* 271: 14657-14660, 1996.
- 58 Parks WC, Wilson CL and Lopez-Boado YS: Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 4: 617-629, 2004.
- 59 Thomas AH, Edelman ER and Stultz CM: Collagen fragments modulate innate immunity. *Exp Biol Med* 232: 406-411, 2007.
- 60 Xu J *et al*: Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth *in vivo*. *J Cell Biol* 154: 1069-1079, 2001.

- 61 Roth JM *et al*: Inhibition of experimental metastasis by targeting the HUIV26 cryptic epitope in collagen. *Am J Pathol* 168: 1576-1586, 2006.
- 62 Pernasetti F *et al*: Novel anti-denatured collagen humanized antibody D93 inhibits angiogenesis and tumor growth: An extracellular matrix-based therapeutic approach. *Int J Oncol* 29: 1371-1379, 2006.
- 63 Folgueras AR, Pendas AM, Sanchez LM and Lopez-Otin C: Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int J Dev Bio* 48: 411-424, 2004.
- 64 Westermarck J and Kahari VM: Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* 13: 781-792, 1999.
- 65 Galvez BG, Matias-Roman S, Albar JP, Sanchez-Madrid F and Arroyo AG: Membrane type 1-matrix metalloproteinase is activated during migration of human endothelial cells and modulates endothelial motility and matrix remodelling. *J Biol Chem* 276: 37491-37500, 2001.
- 66 Melchiori A, Albini A, Ray JM and Stetler-Stevenson WG: Inhibition of tumor cell invasion by a highly conserved peptide sequence from the matrix metalloproteinase enzyme prosegment. *Cancer Res* 52: 2353-2356, 1992.
- 67 Fotouhi N *et al*: Potent peptide inhibitors of stromelysin based on the prodomain region of matrix metalloproteinases. *J Biol Chem* 269: 30227-30231, 1994.
- 68 Lang R *et al*: Crystal structure of the catalytic domain of Mmp-16/Mt3-Mmp: characterization of Mt-Mmp specific features. *J Mol Biol* 336: 213-225, 2004.
- 69 Bode W and Maskos K: Structural basis of the matrix metalloproteinases and their physiological inhibitors, the tissue inhibitors of metalloproteinases. *Biol Chem* 384: 863-872, 2003.
- 70 Murphy G and Knauper V: Relating Matrix Metalloproteinase Structure to Function: Why the "hemopexin" domain? *Matrix Biology* 15: 511-518, 1997.
- 71 Long G, Braswell E, Zhu D, Baum J and Brodsky B: Characterization of collagen-like peptides containing interruptions in the repeating Gly-X-Y sequence. *Biochemistry* 32: 11688-11695, 1993.
- 72 Nerenberg PS, Salas-Escat R and Stultz CM: Do collagenases unwind triple-helical collagen prior to peptide bond hydrolysis? Reinterpreting experimental observations with mathematical models. *Proteins* in press, 2007.
- 73 Trikha M *et al*: Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. *Clin Cancer Res* 9: 4653-4665, 2003.
- 74 Jang H *et al*: Proceedings of ASCO Annual Meeting New Orleans, 2004.
- 75 Yan L, Anderson GM, DeWitte M and Nakada MT: Therapeutic potential of cytokine and chemokine antagonists in cancer therapy. *Eur J Cancer* 42: 793-802, 2006.
- 76 Yang XD, Corvalan JR, Wang P, Roy CM and Davis CG: Fully human anti-interleukin-8 monoclonal antibodies: potential therapeutics for the treatment of inflammatory disease states. *J Leukoc Biol* 66: 401-410, 1999.
- 77 Huang S *et al*: Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. *Am J Pathol* 161: 125-134, 2002.

Received July 9, 2007

Accepted July 30, 2007