Matrix Metalloproteinases

Changing Roles in Tumor Progression and Metastasis

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Articles on tumor invasion, metastasis, and angiogenesis in normal and disease states have been well represented among the pages of The American Journal of Pathology. In addition to exciting interest in a variety of disease processes, these studies have been central in defining the emerging field in cancer research known as the tumor microenvironment. Early studies in this field established the importance of the extracellular matrix on tumor cell growth and differentiation. With time, the role of the extracellular matrix and matrix metalloproteinases in the regulation of tumor invasion, metastasis, and angiogenesis was recognized, and AJP has published seminal articles in this field. Moreover, recent studies show evidence for a role of matrix metallopeptinases in the regulation of inflammation within tumor lesions, making the targeting of matrix metalloproteinases in cancer therapy even more complex. This review attempts to summarize the contribution of AJP to some of the key changes that have led to the evolution of this field. (Am J Pathol 2012, 181:1895–1899; http://dx.doi.org/10.1016/j.ajpath.2012.08.044)

It is now well established that the tumor microenvironment has a major influence on the development, invasion, and metastasis of cancer. Stephen Paget, who noted the propensity for some types of cancer to metastasize to specific organs, suggesting that the metastatic site is not simply a matter of chance, was probably the first to recognize the importance of the microenvironment. This concept of nonrandom metastasis is embodied in Paget’s 1889 seed-soil hypothesis, which proposes that metastatic cancer cells (seeds) interact with specific organ microenvironments (soil) to result in metastasis formation. We now know that the metastatic potential of a tumor cell is dependent on genetic alterations within cells of the primary tumor and also results from a dynamic series of interactions between structural, soluble, and changing cellular elements of the extracellular matrix and stromal tissue compartment. This commentary will briefly summarize The American Journal of Pathology contributions to the evolution of this field.

During the past 40 years, there has been increasing recognition that metastatic disease is responsible for the demise of most patients with cancer, resulting in a concurrent exponential increase in studies on the metastatic process. The seminal observations by Paget challenged the prevailing viewpoint of his time that cancer metastasis was a random process. However, James Ewing, who proposed that metastatic dissemination of cancer was purely dependent on the anatomical distribution of the vascular system, later challenged Paget’s seed-soil hypothesis in 1928. This controversy was finally resolved with the work of Fidler and colleagues, who studied experimental metastasis in syngeneic mice to show that subsequent metastatic growth at a distant organ site was site specific, consistent with Paget’s original hypothesis. Critical to this work was the in vivo selection and characterization of invasive and metastatic mouse tumor models. For example, tumors s.c. implanted in mice showed a different pattern of gene expression and metastasis formation than tumors implanted in the tissue of origin (orthotopic implantation), once again demonstrating the influence of the local microenvironment on tumor growth, selection, and metastasis.

During the past 30 years, the scope of metastasis research has continued to expand, generating new roles for proteases and an increased understanding of the molecular mechanisms driving tumor angiogenesis. Moreover, transcriptome profiling of metastatic versus nonmetastatic tumors has revealed crucial information...
pertaining to the immunological characteristics of cancer progression and metastasis and metabolic profiling of the tumor microenvironment. The aim of this commentary is to review the changing role of matrix metalloproteinases (MMPs) in cancer progression and metastasis from past years to current studies, with an emphasis on the protumorigenic and antitumorigenic activities of these enzymes.

**Tumor Invasion and MMPs: Early Concepts**

The invasive nature of malignant tumors has long been associated with the ability to degrade collagens, just as the resistance of cartilage to tumor invasion is associated with the presence of collagenase inhibitors. However, it was not until Liotta recognized the importance of basement membrane (type IV collagen) degradation in delineating the invasive and metastatic potential of carcinoma that the enzymatic activities associated with cancer cells became better defined. These investigators identified and purified the type IV collagenase that became the second member of the MMP family (MMP-2). During the next 15 years, the MMP family expanded to include 23 zinc-dependent endopeptidases, many of which were first identified by their overexpression in tumor cells. Their structure and substrates have been reviewed elsewhere. Early immunohistochemical studies revealed an association of MMPs with tumor invasion and progression, demonstrating enhanced expression in tumor cells. Interestingly, MMPs were also observed in stromal cells at the invasive front of lung and colorectal tumors. The physiological inhibitors of MMPs include the tissue inhibitors of metalloproteinases (TIMPs), some of which are ubiquitously expressed in normal and tumor tissue.

The expression of MMPs and TIMPs in the tumor microenvironment is diverse. Additional studies identified the expression of MMP-1, MMP-3, MMP-7, MMP-9, MMP-12, and MMP-14 in a variety of tumor types, including non-Hodgkin’s lymphomas. Preliminary in vivo studies suggested great promise using natural (TIMP) and synthetic MMP inhibitors to inhibit tumor invasion and metastasis in mouse tumor models. These and other such studies (many published in *AJP*) generated great interest in the development and clinical testing of a variety of MMP inhibitors for the treatment of patients with cancer. Unfortunately, these studies showed significant adverse effects with no therapeutic benefit. The failure of these drugs was attributed to several causes, including the design of clinical trials in which primarily patients with late-stage cancer were studied.

**New Roles for MMPs in Tumor Progression and Angiogenesis**

During the past 15 years, the accumulation of evidence supporting the role of MMPs and TIMPs in the tumor microenvironment has become increasingly complex. For example, overexpression of MMPs, such as MMP-3, in normal breast epithelium has resulted in invasive tumor formation via induction of an epithelial-to-mesenchymal transition and increased genomic instability. MMPs have also altered cell-cell adhesion through cleavage of E-cadherin, leading to enhanced cell motility, another feature of the epithelial-to-mesenchymal transition. Moreover, MMPs can also activate growth factor signaling by increasing the bioavailability of factors, such as transforming growth factor-β, fibroblast growth factor-2, and vascular endothelial growth factor (VEGF)-A, leading to tumor progression through stimulation of tumor fibroblasts and angiogenesis.

Tumor angiogenesis has long been a focus of studies reported in *AJP*. During the past two decades, nearly 20% of the top 25 cited articles in *AJP* pertained to the subject of physiological and/or pathological angiogenesis, with the most highly cited articles of both decades dealing directly with this topic. The process of tumor angiogenesis begins with blood vessel recruitment in response to the release of angiogenic growth factors and cytokines from tumor cells and the release of proteases, such as MMP-9, into the surrounding extracellular matrix. Indeed, we know that tumor blood vessels are formed by several processes, including the sprouting of endothelial cells from local capillaries and small blood vessels in response to angiogenic growth factors (angiogenesis) and the recruitment of endothelial progenitors from bone marrow (vasculogenesis). Tumors may also co-opt existing blood vessels, illustrated by the formation of localized tumor cell growth around pre-existing tissue blood vessels. Furthermore, tumor cells have also formed vascular channels and expressed endothelial markers, a process known as vasculogenic mimicry, first described by Hendrix and colleagues in *AJP*. Notably, all of these mechanisms of tumor vascularization have some requirement for MMP activity, primarily MMP-1, MMP-2, MMP-9, and MMP-14. In addition to their pro-angiogenic function, it is appreciated that MMPs can also promote anti-angiogenic activity. Indeed, a variety of endogenous angiogenesis inhibitors are derived from MMP-mediated degradation of extracellular matrix macromolecules. These inhibitors include angiostatin, a 38-kDa fragment of plasminogen; endostatin, a fragment of type XVIII collagen; and tumstatin, which is derived from type IV collagen. Therefore, the timing of MMP-directed cancer therapy is critical in regulating the balance of endogenous angiogenesis inhibitors with pro-angiogenic agents, which in turn is a critical determinant controlling the angiogenic switch and initiation of tumor angiogenesis.

Normal blood vasculature consists of two interacting cell types, endothelial cells and surrounding pericytes, that share a common basement membrane and communicate via physical contact and paracrine signaling. In fact, these pericyte–endothelial cell interactions are required for vessel survival, maturation, and stabilization. Conversely, tumor-associated blood vessels demonstrate functional and anatomical heterogeneity, and often become leaky in the sense that they allow the escape of circulating plasma macromolecules. This occurs in response to the production of vascular permeability factor (alias VEGF) by tumor cells. This hyperpermeability of tumor blood vessels is recognized as an initial step of
both pathological and physiological angiogenesis, similar to that observed in wound healing and inflammation. This results in an investment of the tumor vessels with a provisional matrix composed principally of fibrin that stimulates endothelial cell growth and migration. The structural basis for blood vessel leakiness in tumors is associated with defective endothelial cells that form gaps between cells and transcellular holes. In addition to the defective endothelial lining composed of branched, disorganized, and loosely connected endothelial cells, the pericyte covering of tumor vessels is also abnormal. Although ubiquitously present, tumor vessel-associated pericytes show morphological abnormalities, including an abnormally loose association with endothelia and long cytoplasmic projections that extend deep into the tumor.

It is well documented that tumor progression and metastasis depend on the formation and recruitment of new blood vessels in response to the release of pro-angiogenic factors, such as basic fibroblast growth factor-2, VEGF, and/or IL-8. The concept that a dominance of anti-angiogenic factors, including endogenous angiogenesis inhibitors (eg, thrombospondin and TIMPs), is superseded by an overabundance of pro-angiogenic factors before the initiation of tumor vascularization is referred to as the angiogenic switch. This idea resulted in attempts to target new cancer therapies aimed at inhibiting tumor angiogenesis and Food and Drug Administration approval of the first angiogenesis-targeted therapeutic agent, bevacizumab, in 2004. In addition to the potential therapeutic value in understanding tumor vascularization, investigators also realized the prognostic value in studying tumor angiogenesis. Although first demonstrated for melanoma, Weidner and colleagues used sections from 49 patients with primary invasive breast cancer to demonstrate a statistically significant correlation between the density of microvessels and the incidence of metastasis. Highly cited studies from AJP reported this strong correlation in prostate and ovarian cancers. Similar studies confirmed this correlation in non-small-cell lung cancer, and later studies in early-stage breast cancer showed that microvessel density predicted relapse-free and overall survival.

**Cancer Immunity and MMPs**

Research into MMP regulation of immune responses is accelerating. Given the close association between cancer progression and inflammation, the role of MMPs in regulating the immune response has become an area of increasing importance. One mechanism of interest suggests that the cleavage of pro-tumor necrosis factor-α from the cell surface promotes NF-κB signaling in tumor cells. The activation of NF-κB signaling, and Stat-3 signaling, leads to the activation of a feed-forward loop, resulting in production of chemokines and recruitment of tumor-infiltrating immune cells. The activation of NF-κB signaling can also stimulate MMP production in tumor and surrounding stromal cells. In addition, tumor-infiltrating immune cells (eg, neutrophils, macrophages, and T cells) can release cytokines that further stimulate tumor growth. Interestingly, MMPs may also exert tumor-suppressor activity through proteolytic modification of chemokines, such as CXC stromal-derived factors and members of the monocyte chemoattractant protein family, resulting in loss of activity and suppression of the tumor immune response. The MMPs most closely associated with modulating the immune response include MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-12. Thus, such as tumor angiogenesis, modulation of tumor-associated inflammation using MMP-directed therapies will depend on identification of the specific MMP target and an understanding of the correct therapeutic window.

**New Roles for MMPs: The Premetastatic Niche**

MMPs also play a role in establishing the premetastatic niche, or environment conducive to tumor cell growth at a secondary site in the body. The premetastatic niche is generated before the arrival of disseminated primary tumor cells and initiated by soluble factors, including VEGF-A, transforming growth factor-β, and tumor necrosis factor-α, secreted by tumor cells at the primary site. Premetastatic niche formation is associated with increased fibronectin production that can facilitate attachment of VEGF receptor 1–positive bone marrow–derived progenitor cells to perpetuate a microenvironment favorable to metastasis. Progenitor cells in the premetastatic niche have contributed to the release of soluble kit-ligand and VEGF into the microenvironment, aiding in stem and progenitor cell recruitment from the bone marrow and angiogenesis, respectively. In addition, Hiratsuka and colleagues reported that the S100 chemokines, S100A8 and S100A9, are up-regulated in the lung by primary tumor cells and function synergistically to disrupt vascular integrity to facilitate lung metastasis. MMPs also play a role in establishing the premetastatic niche, modulating the immune response. MMP activation also increases the production of MMPs, further promoting extracellular matrix degradation and release of growth factors. Furthermore, MMP-3 and MMP-10, along with angiopoietin 2, are upregulated in the lung by primary tumor cells and function synergistically to disrupt vascular integrity to facilitate lung metastasis. In support of these findings, spontaneous lung metastasis of MDA-MB-231-Luc-D3H1 cells was significantly inhibited when expression of all three factors was knocked down. Although the activation of MMPs in distant sites has aided premetastatic niche formation and metastasis, further studies are needed to identify specific mechanisms in MMP-mediated metastatic niche formation and therapeutic development and delivery to prevent the formation of a premetastatic niche.
Concluding Remarks

This survey of the past 25 plus years summarizes some of the major developments regarding the association between MMPs and immunoregulatory factors within the tumor microenvironment. In doing so, it is evident that AJP has played a major role in furthering our understanding of tumor progression, metastasis, and angiogenesis, as well as the role of MMPs in these processes. The original concept of MMPs directing tumor and endothelial cell invasion has expanded to an investigation of their role in the generation of endogenous angiogenesis inhibitors, regulation of tumor immune responses, and generation of the premetastatic niche. However, MMPs are not confined to the pathological characteristics of tumor invasion, metastasis, and angiogenesis. AJP contains numerous reports on the role of MMPs in a variety of other pathological conditions, such as arthritis, idiopathic pulmonary fibrosis, biliary fibrosis, dissecting aortic aneurysms, myocardial infarction, pre eclampsia, and macular degeneration. With its broad focus, AJP continues to foster the integration between various disciplines that rapidly advances our understanding of basic and translational knowledge of the pathogenesis, classification, diagnosis, and mechanisms of disease.

References