**The Biology Behind**

**Matrix Metalloproteinases, Angiogenesis, and Cancer**


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**Introduction**

The MMPs are a family of zinc-containing endopeptidases that degrade various components of the ECM. A number of MMPs have been strongly implicated in multiple stages of cancer progression including the acquisition of invasive and metastatic properties. Thus, efforts have been made for the past 20 years to develop MMPIs that can be used to halt the spread of cancer, which is what ultimately kills the person. However, initial clinical trials using first generation MMPIs proved to be disappointing (1, 2). In the ensuing years, much has been learned about the roles of specific MMPs in the different processes of carcinogenesis and more specific MMPIs are being developed and brought to clinical trials. However, because the MMPIs do not directly kill cancer cells, but instead target such processes as angiogenesis (the development of new blood vessels), invasion, and metastatic spread, the dosing and scheduling for optimal efficacy is not the same as required for conventional cytotoxic drugs. Thus, alternate methods that do not use the maximum tolerated dose for determining dosing in Phase I trials are needed. In this issue, Lockhart et al. (3) describe the use of a wound angiogenesis assay to measure the biological activity of an MMPI, BMS-275291, during its Phase I clinical trial. This assay, then, is a new biomarker for measuring the activity of certain nontoxic chemotherapeutic drugs. To evaluate a new bioassay for MMPIs, an understanding of the MMPs, how they are involved in the end point being measured (wound angiogenesis in this case), and how they are involved in cancer progression is needed.

**Overview of MMPs**

MMPs are a diverse family of enzymes capable of degrading various components of the ECM. Common properties of the MMPs include the requirement of zinc in their catalytic site for activity and their synthesis as inactive zymogens that generally need to be proteolytically cleaved to be active. Normally the MMPs are expressed only when and where needed for tissue remodeling that accompanies various processes such as during embryonic development, wound healing, uterine and mammary involution, cartilage-to-bone transition during ossification, and trophoblast invasion into the endometrial stoma during placenta development (reviewed in Ref. 4). However, aberrant expression of various MMPs has been correlated with pathological conditions, such as periodontitis, rheumatoid arthritis, and tumor cell invasion and metastasis (4).

There are now over 20 members of the MMP family, and they can be subgrouped based on their structures (2, 5, 6). The minimal domain structure consists of a signal peptide, prodomain, and catalytic domain. The propeptide domain contains a conserved cysteine residue (the “cysteine switch”) that coordinates to the catalytic zinc to maintain inactivity. MMPs with only the minimal domain are referred to as matrilysins (MMP-7 and -26). The most common structures for secreted MMPs, including collagenases and stromelysins, have an additional hemopexin-like domain connected by a hinge region to the catalytic domain (MMP-1, -3, -8, -10, -12, -13, -19, and -20). The gelatinases (MMP-2 and -9) contain inserts that resemble collagen-binding type II repeats of fibronectin within their catalytic domains in addition to the simple hemopexin domain structure. The addition of a furin-recognized cleavage site on the carboxyl side of the cysteine switch is found in MMP-11 and -28. Both of these MMPs are activated by furin in the trans-Golgi network and are secreted in their active form. The MT-MMPs are membrane bound rather than secreted, and all have furin-cleavage recognition sites between their pro- and catalytic domains. Four MT-MMPs (MT1-, MT2-, MT3-, and MT5-MMP) have a transmembrane domain and short cytoplasmic tail, whereas MT4- and MT6-MMP are bound to the membrane via a glycosylphosphatidylinositol moiety at their COOH termini, and MMP-23 has an NH2-terminal signal anchor and a cysteine-rich, proline-rich, interleukin-1-like domain rather than the hemopexin domain.

MMP expression and activity are regulated at several levels. In most cases, MMPs are not synthesized until needed. Transcription can be induced by various signals including cytokines, growth factors, and mechanical stress (2). In certain cases, regulation of mRNA stability and translational efficiency have been reported. Because most MMPs are secreted as inactive zymogens, they need to be activated, usually by proteolytic cleavage of their NH2-terminal prodomains. Some MMPs are activated by other serine proteases such as plasmin and furin, whereas some of the MMPs can activate other members of their family. The most well characterized is the activation of pro-MMP-2 by MT1-MMP. In some cases, initial cleavage in the prodomain allows partial unfolding, exposing additional sites for cleavage including autocatalysis (2). Additional regulation of MMP activity is accomplished by the presence of endogenous
MMP inhibitors such as α₂-macroglobulin and the TIMPs (7). There are four human TIMPs, all of which are secreted, low-molecular-weight proteins that noncovalently bind to the active site of MMPs in a 1:1 ratio (7, 8). Besides inhibiting MMP activity, it has become apparent that the TIMPs have other activities and in fact, TIMP-2 is required for the activation of pro-MMP-2 by MT1-MMP (9). By these various methods, MMP activity is tightly regulated temporally and spatially in normal physiological processes. However, in pathological situations such as cancer, one or more of these regulatory controls has been bypassed.

Overview of Angiogenesis

Angiogenesis is a complex process by which new blood vessels are formed from existing vessels; it involves multiple interactions between endothelial cells, surrounding pericytes, and smooth muscle cells, ECM, and angiogenic cytokines/growth factors (see Fig. 1). The multiple steps include degradation of the basement membrane surrounding an existing vessel, migration and proliferation of endothelial cells into the new space, maturation, differentiation, and adherence of the endothelial cells to each other, and lumen formation. Angiogenesis can be initiated by the release of proangiogenic factors (e.g., VEGF, bFGF, and tumor necrosis factor-α) from inflammatory cells, mast cells, macrophages, or tumor cells (10). These factors bind to their respective cell-surface receptors (Y-shaped receptors in Fig. 1) on endothelial cells, leading to their activation, which includes the induction of cell proliferation, increased expression of cell adhesion molecules (e.g., integrins α1β1, α2β1, α5β1, and αvβ3; T-shaped receptors in Fig. 1), secretion of MMPs, and increased migration and invasion (10–12). bFGF is mitogenic for many cell types including endothelial cells, and, although it does not have a conventional signal peptide for secretion, it can be found sequestered in the ECM bound to heparan-sulfate-containing proteoglycans, where it is released by ECM-degrading enzymes (i.e., MMPs; Refs. 13, 14). bFGF induces cell proliferation, protease production, chemotaxis, and modulates integrin expression in endothelial cells (14). VEGF is a potent mitogen and chemoattractant for endothelial cells and induces the release of MMP-2, MMP-9, and MT1-MMP by endothelial cells (15, 16). In addition, VEGF, also known as vascular permeability factor, induces vascular permeability factor, which allows leakage of plasma proteins, such as fibronectin and other clotting proteins (17). Activation of the clotting system results in deposition of fibrin in the provisional stroma (17). Fibronectin and fibrin then bind and activate their integrin receptors (α5β1 and αvβ3) on activated endothelial cells. The integrin αvβ3 localizes to the tips of endothelial cells in sprouting vessels, and its expression is reduced on mature vessels (16). Interestingly, αvβ3 integrin has also been shown to bind MMP-2, which may participate in its activation and serve to localize it to the surface of invading endothelial cells (11, 12, 18). More recent reports suggest αvβ3 integrin may actually have antiangiogenic effects as a receptor for endogenous angiogenesis inhibitors, thrombospondins, tumstatin, and the isolated hemopexin domain of MMP-2, and by down-regulating expression of the VEGF receptor, VEGFR-2 (flk-1; Ref. 11). Ligand binding to integrins initiates intracellular signaling, which promotes cell survival of activated endothelial cells and potentiates signaling by ligand-activated growth factor receptors (12). TGFβ, released from bound ECM and activated by MMPs, is a potent chemoattractant for monocytes and macrophages, and stimulates their production of proangiogenic factors, bFGF, tumor necrosis factor-α, and interleukin-1α (19, 20). TGFβ also induces the expression of MMP-2 and -9, and PDGF-A and -B by endothelial cells and down-regulates TIMP expression (19, 20). Mechanical/shear stress on extruding endothelial cells also induces expression and secretion of PDGF-B (21). PDGF-BB homodimer acts as an autocrine growth factor for endothelial cells as well as stimulating chemotaxis, proliferation, and the differentiation of pericytes and smooth muscle cells required for vessel maturation (16, 21). Contact between endothelial cells and pericytes further induces TGFβ expression (21). TGFβ also contributes to vessel maturation/stabilization by stimulating the secretion of chondroitin sulfate, the principle ECM component between endothelial and smooth muscle cells, by smooth muscle cells (19).

Role of MMPs in Angiogenesis

MMPs contribute to angiogenesis not only by degrading basement membrane and other ECM components, allowing en-
dothelial cells to detach and migrate into new tissue, but also by releasing ECM-bound proangiogenic factors (bFGF, VEGF, and TGFβ). In addition, MMP degradation of ECM components generates fragments with now-accessible integrin binding sites, triggering integrin intracellular signaling. By directly binding to αvβ3, MMP-2 may itself initiate integrin signaling and thereby contribute to endothelial cell survival and proliferation (18). However, MMPs also are able to generate endogenous angiogenesis inhibitors from larger precursors: cleavage of plasminogen by MMPs releases angiostatin; endostatin is the COOH-terminal fragment of the basement membrane collagen XVIII, which can be generated by cleavage by cathepsins and MMPs; and generation of the hemopexin domain of MMP-2 from MMP-2 may be through autocatalysis (12, 18). Thus, the MMPs have both pro- and antiangiogenic functions. On the whole, however, MMPs are required for angiogenesis, and MMPIs have been shown to inhibit angiogenesis in animal models (22).

Role of MMPs in Cancer

Expression of various MMPs has been found to be up-regulated in virtually every type of human cancer and correlates with advanced stage, invasive and metastatic properties and, in general, poor prognosis (1, 6). Early expression of MMPs, either by the tumor cells themselves or by surrounding stromal cells, helps to remodel the ECM and release ECM- and/or membrane-bound growth factors, which provides a favorable microenvironment for the establishment of the primary tumor (see Fig. 2). As the tumor grows, an angiogenic switch occurs (possibly in part because of hypoxia) in which the balance of proangiogenic factors (e.g., bFGF and VEGF) overcomes the expression of angiogenic inhibitors (e.g., thrombospondins, angiostatin, and IFNs; Ref. 23). Both MMP-2 and MMP-9 have been implicated in the induction of the angiogenic switch in different model systems (6). In other tumor models, activating mutations of protooncogenes K-ras or H-ras in tumor cells up-regulate expression of VEGF and down-regulate expression of thrombospondin, whereas oncogenic erbB2 signaling up-regulates expression of other proangiogenic factors and down-regulates thrombospondin (24). The angiogenic switch can occur very early in some cancers, even before malignant progression, with increased vessel density seen in precancerous lesions (23). Further up-regulation of MMP expression, in particular the gelatinases, which can degrade basement membrane components, allows the tumor cells to invade into the adjacent stroma and to break down the basement membranes associated with capillaries and lymphatic vessels allowing tumor cells to enter the circulation (intravasation; Ref. 5). MMPs are also involved in cell migration by removing sites of adhesion, exposing new binding sites, cleaving cell-cell or cell-matrix receptors, and releasing chemoattractants from the ECM (25). Similar to intravasation, MMPs are necessary for the circulating tumor cells to be able to exit the blood vessels (extravasation), although this step does not appear to be rate limiting for the establishment of metastases (5). At the distant site, MMPs are required for local migration, establishment of a microenvironment conducive for metastatic growth, and angiogenesis for sustained growth. Thus, MMPs contribute to the carcinogenic process at multiple stages.

Evaluation of MMPIs

The rationale for developing MMPIs for cancer therapy is very strong and has been around for decades. Animal studies with synthetic MMPIs have been compelling, but translation into human clinical trials has not been as promising. Poor bioavailability of first-generation MMPIs led to second-generation MMPIs that were p.o. active but that caused such unwanted side effects as musculoskeletal pain and inflammation with long-term administration (1, 2). These side effects may have been in part attributable to the doses used, based on the maximum tolerated dose, which led to the inhibition of related metalloproteinases, such as the ADAMs (disintegrin and met-
alloproteinase) and ADAMTSs (ADAMs with thrombospondin-type motifs), which have “sheddase” activity (the ability to shed active inflammatory cytokines and growth factors from cell membranes; Refs. 1, 2). The MMPI used in the Phase I clinical trial described by Lockhart et al. (3), BMS-275291, was rationally designed to avoid the inhibition of the sheddases (22). A possible explanation for the failure of MMPIs that did make it to Phase III trials was that they were administered to patients with advanced-stage disease (1). From what is now known about the roles of MMPIs in the angiogenic switch, tumor angiogenesis, and acquisition of the invasive phenotype, which occur often before clinical detection, administration of MMPIs to patients in earlier stages would be expected to have more efficacy. Thus, what is needed for the future use of MMPIs in clinical trials is to take into account the tumor stage and type (with perhaps an MMP expression profile to choose more selective MMPIs) and the development of better biomarkers for determining their efficacy and appropriate dosing (1). To this latter end, Lockhart et al. (3) describe the use of a wound angiogenesis assay during the Phase I trial of BMS-275291.

So, is a wound angiogenesis assay an appropriate biomarker for MMPI activity? Given (a) that expression of MMPs, particularly MMP-2, MMP-9, and MT1-MMP, are essential for angiogenesis in general; (b) that these MMPs are also strongly implicated in several stages of cancer progression; and especially (c) that tumor angiogenesis (which occurs essentially the same as wound angiogenesis, except for the initial source of proangiogenic factors) is absolutely required for the growth and dissemination of cancer, the answer is yes. Although MMPIs can have antiangiogenic activity by generating endogenous angiogenesis inhibitors, they also can have antitumorogenic activity via the same mechanism. Therefore, the balance of these pro- and antiangiogenic MMP activities will determine whether the MMPI will be effective at inhibiting angiogenesis in a skin wound as well as at inhibiting MMP activities contributing to tumorigenesis. That the authors found pretreatment wound angiogenesis to be delayed in the cancer patients in the Phase I trial, compared with initial experiments with healthy volunteers, suggests that, in cancer patients, the balance of these two opposing MMP activities may be altered by the presence of cancer. But if a MMPI can still be shown to inhibit wound angiogenesis in these patients, there would seem to be a strong possibility that it would also be able to inhibit the deleterious effects of MMPs in cancer. Thus, this assay should be useful as a biomarker for the efficacy of MMPIs as well as for angiogenesis inhibitors, which are also under development for cancer therapy (24).

References

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