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Matrix metalloproteinases as breast cancer drivers and therapeutic targets

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Abstract

Members of the matrix metalloproteinase (MMP) family have been identified as poor prognosis markers for breast cancer patients and as drivers of many facets of the tumor phenotype in experimental models. Early enthusiasm for MMPs as therapeutic targets was tempered following disappointing clinical trials that utilized broad spectrum, small molecule catalytic site inhibitors. However, subsequent research has continued to define key roles for MMPs as breast cancer promoters, to elucidate the complex roles that that these proteins play in breast cancer development and progression, and to identify how these roles are linked to specific and unique biochemical features of individual members of the MMP family. Here, we provide an overview of the structural features of the MMPs, then discuss clinical studies identifying which MMP family members are linked with breast cancer development and new experimental studies that reveal how these specific MMPs may play unique roles in the breast cancer microenvironment. We conclude with a discussion of the most promising avenues for development of therapeutic agents capable of targeting the tumor-promoting properties of MMPs.

Keywords

Matrix metalloproteinases; MMPs; tissue inhibitors of metalloproteinases; TIMPs; Breast Cancer; Tumor Progression; Epithelial Mesenchymal Transition; EMT; MMP inhibitors; Cancer Biomarkers; Tumor Microenvironment

2. INTRODUCTION

Breast cancer is the leading cause of cancer death in women worldwide (1). While tumors caught at an early, localized stage are effectively treated by neoadjuvant chemotherapy and surgery with excellent long term prognosis, regional lymph node involvement is associated with more frequent relapse (2), and distant metastasis has much poorer survival (3). Studies of the pathological processes involved in tumor progression and metastasis revealed matrix metalloproteinases (MMPs) as prominent molecules involved in shaping the tumor microenvironment and driving cancer progression and metastasis (4–6). These proteases would seem to offer an obvious therapeutic target, and yet in clinical trials, the broad

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spectrum MMP inhibitor marimastat failed to extend progression-free survival of metastatic breast cancer patients (7). Recent studies illuminating the complexities of this family of proteases, with respect to structure, molecular function, and mechanisms of involvement in tumor progression, offer new insights into context and approaches through which they might be more effectively targeted in cancer.

3. THE MATRIX METALLOPROTEINASE FAMILY

3.1. Domain structure of the MMP family

The MMPs form a large family of multi-domain zinc-dependent endopeptidases distributed throughout all kingdoms except protozoa; in humans, there are 23 MMPs (MEROPS database: http://merops.sanger.ac.uk/) (8). These enzymes each possess a signal peptide to direct trafficking through the secretory pathway, a prodomain that functions as an intramolecular inhibitor to maintain the zymogen form of the enzyme in an inactive state, and a well-conserved compact catalytic domain (Figure 1A). While this most simplified example of domain organization can be found in MMP-7 and -26, other MMPs possess additional accessory domains that can function to localize MMPs to particular membrane structures or multiprotein complexes, and can mediate specificity toward particular protein substrates. While most MMPs are soluble extracellular proteins, MMP-14, -15, -16, and -24 are type I membrane proteins directly tethered through C-terminal transmembrane domains, MMP-17 and -25 are membrane localized via C-terminal glycophosphatidylinositol (GPI) anchors, and MMP-23 via an N-terminal type II transmembrane domain. The hemopexin-like (PEX) domains possessed by many MMPs, connected to the catalytic domain by a flexible linker of variable length, can function indirectly in localizing MMPs to the membrane via protein-protein interactions with cell-surface receptors such as integrins (9–11). The versatile PEX adaptor modules also mediate interactions including dimerization and substrate recognition; as the PEX domains are more divergent in sequence and function than catalytic domains, they contribute to distinct patterns of localization and substrate specificity (9, 12). More specialized domain modules include three fibronectin type II repeats that in MMP-2 and MMP-9 assist in recognition of a subset of extracellular matrix substrates including elastin and denatured collagen (13–16). In MMP-23, a unique cysteine array domain with homology to potassium channel blocking toxins may possess ion channel-modulatory activity (17), while the adjacent immunoglobulin-like domain may mediate protein-protein interactions involved in localization or substrate recognition, similar to the PEX domain of other MMPs (18).

3.2. Structural determinants of MMP activity and specificity

The MMP catalytic domain possesses key features characteristic of the larger metzincin clan of metallopeptidases, including a conserved HExxHxxGxxH motif which coordinates the catalytic zinc ion. The catalytic mechanism of proteolysis involves activation of a water molecule by the catalytic zinc and the Glu residue within the zinc binding motif for nucleophilic attack on the scissile peptide bond (19, 20). In the zymogen form, access to the active site cleft is blocked by the prodomain (Figure 1B), which in all MMPs except MMP-26 is held in place by a thiol-zinc interaction involving Cys of a conserved PRCGxPD “cysteine switch” motif. This coordination is disrupted upon interaction with and cleavage

by an activating protease (20, 21). MMP-26 is also activated proteolytically, although its zymogen latency is not well understood since it possesses a mutated and nonfunctional cysteine switch motif (22). Some of the MMPs, including all of the membrane-type MMPs, are proteolytically activated by furin in the cellular secretory pathway, while others are activated extracellularly by serine proteases or other MMPs (20, 23). Proteolytic activation of an MMP enables dissociation of the prodomain from the catalytic domain, exposing the active site cleft and allowing MMP association with protein substrates.

The catalytic domain of active MMPs features a broad, shallow substrate binding cleft capable of accommodating an extended peptide segment of a substrate, positioning a particular peptide bond for cleavage. Binding subsites within the catalytic cleft confer a degree of substrate sequence specificity, the most important of these being the S1′ subsite, responsible for recognition of the P1′ residue proximal to the cleavage site in the direction of the substrate protein C-terminus (20). However, recent structural and biochemical investigations have revealed additional determinants of specificity located distant from the catalytic site, including exosites on the surface of the catalytic domain itself in addition to those on adjacent accessory domains (16, 24–27). Intriguingly, separate substrate recognition sites on the catalytic and hemopexin domains can work cooperatively to orchestrate proteolysis of specific substrates. This has been illustrated for collagenolysis by MMP-1, where reorientation of the catalytic and PEX domains relative to each other after substrate binding results in a deformation of the collagen helical structure that is required for proteolysis to proceed (28, 29). Extensive interdomain flexibility has been documented for MMP-9 and MMP-12 as well as MMP-1, and is likely to be a general property of the MMP family (30–32). It may be that cooperative motions and reorientation of domains can facilitate MMP proteolysis of other highly structured substrates. Interdomain flexibility may also be important for other MMP-specific functions; for example, the very long and flexible linker of MMP-9 may enable cell surface tethering by the hemopexin domain while allowing the catalytic domain to access complex substrate networks in the pericellular environment (32). Importantly, ligands that bind to exosites, blocking proteolysis of specific substrates or allosterically inhibiting MMP activity, can present novel avenues for pharmacological targeting of MMPs, as will be considered further in section *6. Therapeutic approaches targeting MMPs*.

4. MMPS IN BREAST CANCER AND THEIR CLINICAL SIGNIFICANCE

4.1. MMPs associated with poor prognosis in breast cancer

A subset of the MMPs has been found to be upregulated in breast cancers in association with poor outcome. At the level of transcription, analyses of large microarray patient datasets have identified MMP-1, -9, -12, -14, and -15 as predictive of adverse outcome in one dataset comprised of primary tumors from 295 patients (33, 34), and MMP-9, -11, and -15 as associated with poor survival in another dataset of primary tumors from 1500 patients (35, 36). Neither of these studies identified associations of any MMPs with positive outcomes. In a more focused study examining expression of MMP-2 and -14 by mRNA *in situ* hybridization in 539 breast cancers, high MMP-14 expression alone predicted significantly shorter overall survival when adjusted for tumor size and lymph node involvement (37).

Gene expression in tumors of several MMPs has been incorporated into clinical prognostic tests. MMP-9 is one of 70 genes in the Rosetta poor prognosis signature for breast cancer patients (38), the basis for the clinically implemented Mammaprint prognostic assay (Agendia Inc., Irvine, CA). MMP-11 is included in a 21 gene signature originally developed to predict recurrence of tamoxifen-treated node-negative breast cancer (39), implemented as the Oncotype DX assay (Genomic Health Inc., Redwood City, CA). MMP-11 is also one of 50 genes in the PAM50 gene set used as a predictor of breast cancer intrinsic subtypes and risk of recurrence (40). Interestingly, while many MMPs are most strongly upregulated in association with high grade or advanced invasive cancers, a global gene analysis study identified MMP-1 as a marker predictive of progression to cancer in atypical ductal hyperplasia, a precancerous breast lesion (41). These data suggest that changes in MMP expression can precede and contribute to the development of breast cancer.

4.2. Prognostic implications are linked to the cell type expressing MMPs

One limitation of studies focusing on gene expression is that transcript abundance may not fully reflect levels of the protein that is responsible for biological activity. Staining tumor specimens for MMPs by immunohistochemistry (IHC) gives a more direct readout of protein levels, although this approach may also detect latent zymogen and/or or inhibited enzyme complexes in addition to active MMPs, depending on the antibodies employed. An additional advantage of IHC is that it can yield spatial information to distinguish, for example, among MMPs expressed by stromal versus tumor cells, or at the invasive front versus within the central tumor mass. In a particularly comprehensive study, IHC staining of MMP-1, -2, -7, -9, -11, -13, and -14 along with tissue inhibitors of metalloproteinases (TIMPs) was quantified in 131 invasive ductal breast tumors, and association with 5-year risk of relapse examined (42). Among MMPs, this study found that total immunostaining scores for MMP-9 and -11 were significantly associated with shorter relapse-free survival. Additionally, MMP-9 staining of tumor cells, stromal fibroblasts, and mononuclear inflammatory cells were each individually prognostic of shorter relapse-free survival, as were fibroblast expression of MMP-1, fibroblast or mononuclear inflammatory cell expression of MMP-7, -11, or -13, or mononuclear inflammatory cell expression of MMP-14 (42). Further analyses of this data set have demonstrated that coexpression of multiple MMPs by tumor-associated fibroblasts and by mononuclear inflammatory cells can distinguish groups of patients with increased risk of distant metastasis (43, 44). While other studies have for the most part corroborated these findings, there are some notable exceptions. For example, a study of 125 patients found high MMP-1 expression to be prognostic of poor cancer specific survival; however, in this study it was MMP-1 expression by tumor cells rather than stromal cells that showed significant association with outcome (45). In another study of 263 patients, high MMP-13 expression by tumor cells and stromal fibroblasts were both significantly associated with poorer overall survival (46). One of the most extensively studied MMPs implicated in breast cancer is MMP-9. One study of 421 patients found high MMP-9 expression in stromal cells to be prognostic for poorer recurrence-free survival and breast cancer specific survival, while MMP-9 expression in tumor cells was associated with smaller tumors and better survival outcomes in this cohort (47). A separate study examining MMP-9 and -14 in 175 breast cancers found stromal MMP-9 to be significantly associated with poor relapse-free survival and overall survival

(48). Yet another study of 270 node-negative breast cancers evaluated MMP-2 and -9 staining by IHC, finding both to be expressed primarily by tumor cells, and both to be prognostic for shorter relapse-free survival (49). MMP-9 is most highly expressed in tumors of the basal-like molecular subtype of breast cancer, most of which are triple negative for estrogen receptor, progesterone receptor, and HER2 (50, 51). Notably, high MMP-9 expression (along with MMP-11) was found to be significantly associated with progression to distant metastasis specifically in the subset of basal-like breast cancers (52), and to be significantly associated with shorter progression-free survival as well as overall survival in another cohort of triple-negative breast cancer patients (53). The differences in observed endpoints for the studies examining tumor cell-expressing MMP-9 may be due to use of different antibodies and histological classifications, where the more recent studies may reflect staining improvements, as well as differences in the cohort populations.

4.3. Circulating MMPs as tumor biomarkers

Beyond viewing MMPs as tissue biomarkers, for the secreted MMPs and particularly MMP-2 and -9, many studies have examined enzyme levels in circulation as potential prognostic serum biomarkers in breast cancer. MMP-2 and -9 activity in serum or plasma, measured via quantitative gelatin zymography, has shown potential for discrimination among breast cancer subclassifications of varying risk (54, 55), for prediction of lymph node metastasis (56), and for assessment of response to therapy in breast cancer patients (57). Some studies have shown measurement of MMP-9 protein in serum by ELISA (56, 58–60) or by Luminex multiplexed protein assays (61, 62) to provide an effective alternative measure of similar prognostic value, and high serum MMP-2 measured by ELISA has also been associated with poor prognosis (63, 64). By contrast, one recent large study of 465 breast cancer patients specifically examining the concentration of MMP-9/TIMP-1 complexes in plasma by ELISA and by in-solution proximity ligation assay found no correlation of this complex with disease-free survival (65). It is worth noting that many studies have implicated high levels of TIMPs as well as MMPs, both in tumor tissues and in serum, as associated with poor prognosis. Although this is counterintuitive when considering their function in quenching MMP activity, associations of TIMPs with poor outcome may relate to MMP-independent signaling functions that have been ascribed to TIMPs (66–68).

5. TUMORIGENIC PROCESSES ACTIVATED BY MMPS IN BREAST CANCER

MMPs can directly facilitate cancer progression by degrading the basement membrane, allowing cancer cells to invade into the surrounding stroma, but MMPs can also act directly on the tumor cells, releasing factors that promote growth or suppress apoptosis (69). Imbalances in MMPs activate cellular processes that cause DNA damage and stimulate genomic instability (70). MMPs play critical roles in the tumor microenvironment: providing nutrients and oxygen to the growing tumor as well as avenues for metastasis through MMP-mediated blood and lymph vessel formation, generating tissue disruptive fibrotic stroma through MMP-induced activation of stromal fibroblasts, and stimulation of tumor-promoting metabolic switches by action of MMPs on adipocytes (4). Finally, MMPs can directly induce phenotypic changes associated with the epithelial-mesenchymal transition (EMT), a developmental process that becomes activated during tumor progression

(6, 71). Here, we will describe how MMPs facilitate these activities, emphasizing some of the most recent findings in this area.

5.1. MMPs promote tumor growth by regulating proliferation and apoptosis

It is almost axiomatic that tumor expansion requires a combination of increased proliferation and decreased apoptosis. One of the most well-studied regulators of cellular proliferation and apoptosis is TGFβ, which can inhibit cell cycle progression in nonmalignant normal cells and early malignant tumor cells, but which can also stimulate proliferation through poorly understood processes in more progressed cancer cells; TGFβ can also inhibit apoptosis in a variety of cell types (72). Most TGFβ is produced as an inactive complex; cleavage of this complex by MMPs is an important mechanism for release of the active cytokine (73–75). MMPs (and members of the ADAM protein family of related metalloproteinases) can activate the epidermal growth factor (EGF) receptor through release of cell membrane-associated ligands, such as HB-EGF, TGFβ, and amphiregulin (4, 69). Studies more than a decade ago revealed that MMPs could stimulate resistance to chemotherapeutics and drive tumor progression through proteolytic inactivation of the cell death receptor Fas and consequent inhibition of the intrinsic apoptosis pathway (76, 77); blocking Fas cleavage by MMPs is a potential avenue for therapeutic intervention (78).

5.2. Stromal MMPs create a tumor-promoting microenvironment

MMPs have been implicated in tumor angiogenesis, the penetration of the tumor by new vessels sprouting from existing ones (4, 79). Release of heparan sulphate-sequestered vascular endothelial growth factor (VEGF) by MMP-9 triggers the angiogenic switch in pancreatic and colorectal cancer models (80, 81); in these models, the tumor-promoting MMP-9 was provided by circulating macrophages and neutrophils. MMP-14 and MMP-2 have been implicated in vasculogenic mimicry (82), a process in which blood and nutrients can reach deep into the tumor through channels that link to new vessels closer to the tumor surface (83). Macrophage-derived MMP-9 was also found to be specifically required for induction of vasculogenesis in animal models, the production of new vessels from progenitor cells derived from the bone marrow (84).

MMPs can also affect the tumor microenvironment by stimulating the development of activated stromal cells. Fibrosis, the excess deposition of collagen and fibroblast proliferation that is associated with most types of cancer, is largely the product of myofibroblasts (85). These cells accumulate through activation of stromal fibroblasts or circulating fibrocytes, or directly from epithelial cells by EMT (85). Myofibroblasts are significant sources of breast cancer MMPs (42, 86, 87), and tumor progression and poor prognosis is associated with stromal expression of MMP-1, MMP-7, and MMP-12 (88), and with fibroblast-specific production of MMP-9, MMP-11, and MMP-14 (42, 87). Cancer cells can also directly secrete variant isoforms of collagen that are resistant to cleavage by MMPs and that can thus function as tracks for guiding cancer cell invasion in MMP-rich microenvironments (89, 90). Another key source of MMPs in the breast cancer microenvironment is the tumor-associated adipocyte (91). Tumor cell-produced factors stimulate de-differentiation of the adipocytes, abundant in the tissue surrounding the

developing breast cancer, to a phenotype associated with increased expression of cytokines and MMP-11, driving breast cancer invasion and metastasis in animal models (92).

5.3. MMPs promote invasion and metastasis

MMPs have long been known to facilitate cancer cell invasion through degradation of the ECM, but MMPs can act directly on the tumor cells to induce invasive cellular characteristics, and new players in this process are still being discovered. Recent findings reveal that MMP-14 directs cancer cell invasion and metastasis in part through cleavage of the Wnt/planar cell polarity protein-tyrosine kinase-7 (PTK7), (93–95). MMPs can also directly stimulate an invasive and metastatic phenotype in epithelial cells through activation of the EMT program (96). MMP-induced EMT has been observed in a variety of epithelial cell types, including kidney (97–100), ovary (101), lens (102), lung (103–105), pancreas (50), and prostate (106), although MMP-induced EMT has been best characterized in breast epithelial cells (107). MMP-3 stimulates spontaneous tumor formation in mouse mammary glands (108–110), and dissection of this process revealed that exposure of cultured mouse mammary epithelial cells to MMP-3 directly activates EMT (111, 112). MMP-3 mediates these effects by stimulating increased expression of Rac1b (113, 114), a splice variant of Rac1 with activated characteristics (115), which in turn stimulates EMT by increasing levels of cellular reactive oxygen species (113, 116, 117), through a process that depends upon cell-ECM interactions (118–120). It may be that many studies in which MMPs have been seen to stimulate cancer cell motility and invasion, although not directly investigating these phenomena in the context of EMT, have in fact been observing the cellular consequences of an incomplete or dysregulated activation of the EMT program.

5.4. MMPs as signaling molecules: noncatalytic functions of MMPs

While most studies of processes involving MMPs in tumor progression have focused on their role as catalytic enzymes, recent studies have found that MMPs can also act as signaling molecules independent of their proteolytic activity. Interactions of MMP substrates with noncatalytic domains of the MMPs are well known to affect selectivity for particular substrates and for individual target sites within those substrates (121), as discussed further above in section *3.2. Structural determinants of MMP activity and specificity*. These exosite interactions can also drive signaling functions: interaction of the MMP-2 or MMP-9 hemopexin domains with integrins or CD44 can stimulate cell survival, migration, and angiogenesis (122). Recent studies have identified two novel functions for the MMP-3 hemopexin domain: interaction with extracellular heat shock protein 90-β (HSP90β) can stimulate mammary epithelial cell invasion and morphogenesis (123), while binding of the MMP-3 hemopexin domain to Wnt5b inhibits canonical Wnt signaling and regulates mammary stem cell formation (124). Interactions of cell surface proteins with regions of TIMP-1 distinct from its MMP inhibitory domain have also been implicated in tumor development: MMP-independent association of TIMP-1 with CD63 drives resistance to apoptosis, induction of EMT, and stem cell differentiation (125–127). Effective inhibition of protumorigenic activities of MMPs and TIMPs will likely have to target both catalytic and noncatalytic functions of these molecules.

6. THERAPEUTIC APPROACHES TARGETING MMPS

6.1. Poor performance of broad spectrum MMP inhibitors in clinical trials

Abundant data showing association of MMPs with poor prognosis in breast cancer, as overviewed in section *4. MMPs in breast cancer and their clinical significance*, along with multiple mechanisms by which MMPs are found to drive breast cancer development and progression as overviewed in section *5. Tumorigenic processes activated by MMPs in breast cancer*, suggest that these molecules should offer promising targets for therapy. However, intensive efforts to develop and translate pharmacological MMP inhibitors for cancer treatment culminated a decade ago in disappointing results in multiple clinical trials (128). Of relevance to breast cancer, a phase III trial of the broad spectrum MMP inhibitor marimastat in metastatic breast cancer found no therapeutic benefit (7). Phase II pilot trials of adjuvant marimastat and rebimastat in early stage breast cancer concluded that large scale studies were not feasible in this setting given the high incidence of musculoskeletal toxicity and failure of chronic dose levels to maintain plasma levels within the target range for these drugs (129, 130). While the pharmaceutical industry has been reluctant to invest further in MMP inhibitors in the aftermath of these trials, basic research supports the idea that more selective inhibitors with lower toxicity may succeed where earlier generation drugs failed.

6.2. Improving the selectivity of small molecule MMP inhibitors

Initial efforts toward improving small molecule inhibitor selectivity focused on tailoring drugs to the size and shape of the variable S1′ pocket of the catalytic domain, and exploring alternatives to the strong zinc-binding functionalities of early inhibitors (131–133). For MMP-12 in particular, enhanced understanding of the molecular determinants of drug affinity have been aided by numerous very high resolution crystal structures (134, 135). Novel and selective inhibitors of MMP-12 have resulted from further optimization of S1′ pocket fit for this enzyme (136), as well as from incorporating P2′ glutamate into pseudo-dipeptides, which in the absence of a traditional zinc-chelating group, can take on a non-canonical binding conformation in which it interacts with the catalytic zinc (137). Recently, diverse approaches have emerged that take advantage of increasing knowledge of MMP exosites, allosteric regulatory mechanisms, and domain interactions of the MMPs (12, 138), that may ultimately pave the way to clinically useful agents. To identify hidden allosteric regulatory sites on the surface of MMP catalytic domains that may offer new opportunities for targeted drug development, a recent study used a series of branched amphiphilic polymers to probe MMP-12 and MMP-14 for binding and effects on substrate hydrolysis (139). MMP catalytic domains are known to be highly flexible and dynamic molecules (140), and the identified polymers inhibited MMP-12 and -14 by association with unique surface patches that in the free enzymes are known to have substantial mobility, the damping of which compromised catalytic function. Intriguingly, computational analyses of 13 MMP family members predicted that similar but distinct allosteric regulatory sites exist in each MMP catalytic domain (139); these sites offer new opportunities for selective drug development.

Other efforts to develop selective inhibitors have focused beyond the catalytic domain, targeting exosites of accessory domains (exclusively or in addition to the catalytic domain)

that are important for proteolysis of specific substrates, for receptor recognition, or for other specific biological functions. Many such inhibitors are peptides that mimic natural substrates or that compete with binding epitopes on binding partner proteins. Although as peptides they possess challenges relating to stability that may preclude direct development as drugs, studies using these peptides as probes help to define the relevant MMP exosites, and can provide proof of principle for the concept of selectively inhibiting a subset of MMP biological activities (141). Triple helical peptides (THPs) that mimic the structure of triple helical collagen substrates, along with THP derivatives that mimic transition states in proteolysis, represent one such class of peptide inhibitors (141). THP substrate and transition state analogues targeting exosites on fibronectin domains along with the catalytic domain show high affinity and selectivity for MMP-2 and -9 (142), and can be designed using exosite affinity to inhibit only a subset of proteolytic activities, in one example blocking cleavage of type V collagen but not interstitial collagen (143). THP substrates and inhibitors have also been used in mapping exosites that are important for proteolysis of a subset of substrates to the catalytic domain of MMP-12 (144) and the PEX domain of MMP-1 (145). Another study focusing on PEX domain exosites has identified an epitope on MMP-9 PEX blade IV that is critical for homodimerization, and another epitope on MMP-9 PEX blade I that is critical for CD44 binding; both epitopes are essential for cell migration (146). Short peptides that mimic these linear epitopes were shown to interrupt the molecular association and to interfere with cellular migration (146). Using similar approaches, MMP-14 PEX domain epitopes on blade IV and blade I were found to be necessary for homodimerization and for heterodimerization with CD44, respectively; peptide mimics were able to block cellular migration and to interfere with metastasis in an orthotopic xenograft model of breast cancer (147). Given increasing structural, biochemical and biological information about PEX domains and their role in cancer-driving processes, it has been possible in some instances to employ a structure-based *in silico* approach to identify small molecule inhibitors that target PEX domain exosites. One such study identified a small molecule inhibitor that selectively blocked MMP-9 dimerization by targeting the PEX domain without affecting catalytic activity; the inhibitor was found to block MMP-9-mediated cellular migration and mammary tumor growth and metastasis in an orthotopic xenograft model (148). Another study identified a small molecule inhibitor that targets MMP-14 PEX; this molecule was shown to block MMP-14 dimerization and to repress tumor growth and collagen degradation in an orthotopic mammary tumor model (149).

6.3. MMP-targeting antibodies as therapeutic inhibitors

MMP-targeting opportunities are not limited to small molecule drugs, and macromolecular function-blocking inhibitors may offer advantages in terms of superior potential for selectivity and reduced toxicity. One option is therapeutic antibodies, a well-established approach to development and translation of highly selective macromolecular drugs. A function blocking monoclonal antibody has been reported that selectively binds human MMP-9 with a K_d of 2.1. nM (150), recognizing an epitope on the surface of the catalytic domain (151). A fully human monoclonal antibody against MMP-14, developed using a phage display platform, was able to inhibit tumor growth and metastasis in an orthotopic xenograft model of breast cancer (152). Another monoclonal antibody was shown to inhibit MMP-14 activation of proMMP-2, and consequently to inhibit lymphangiogenesis, while

other catalytic functions were unaffected (153). Mutagenesis and modeling studies revealed that the antibody targets a loop on the MMP-14 catalytic domain surface far from the active site, blocking an interaction with TIMP-2 that is required for formation of the trimolecular MMP-14·TIMP-2·MMP-14 complex that is responsible for proMMP-2 activation (154). Finally, in a novel approach to antibody generation, mice immunized with a synthetic mimic of the zinc-centered MMP active site structure produced antibodies capable of inhibiting MMP-2 and -9 with a binding mechanism reminiscent of the TIMP mechanism (155). The therapeutic potential of these antibodies was demonstrated in mouse models of inflammatory bowel disease.

6.4. Therapeutic potential of TIMPs and variants

An alternative to therapeutic antibodies is presented by the natural human TIMPs, a family of four protein inhibitors of MMPs and other metalloproteinases, and by recombinant engineered proteins based on the TIMPs. TIMPs are essential anticancer molecules: a recent study found that simultaneous knockout of all four TIMPs conferred powerful cancer-promoting properties on fibroblasts (156). TIMPs bind to MMP catalytic domains using a central core epitope comprised of the N-terminal strand of the TIMP, which coordinates to the active site zinc, and adjacent loops of the TIMP connected to the N-terminal strand by disulfide bonds (157, 158). Additional flanking loops further removed from the core epitope can form additional adventitious interactions with exosites on the surface of the MMP catalytic domain (157, 159). While the core epitope is highly conserved and structural differences in core interactions among different MMP/TIMP complexes are very subtle, the peripheral exosite interactions involve protein regions that are less conserved among both TIMPs and MMPs, and likely account for much of the broad spectrum of affinities among MMP/TIMP complexes, which range from sub-picomolar to high nanomolar (157, 159, 160). The broad interface involving in total more than 20 residues of the TIMP protein further offers opportunities to optimize selectivity toward individual MMPs through mutagenesis (161, 162). Many previous studies have identified sites of mutation capable of modulating TIMP selectivity (163–169), and a recent computational and experimental analysis of TIMP-2 has demonstrated that as a protein evolved for broad inhibition of many MMPs, its sequence lies far from the fitness maximum for optimal affinity toward any individual MMP, and therefore specificity enhancing mutations are common (170). While the simplest approaches to such optimization may involve designed improvements in steric and charge complementarity, a more sophisticated approach taking into account the extensive flexibility of the TIMP molecule may be required. For example, a point mutation of TIMP-1 that improved affinity toward MMP-14 by more than an order of magnitude (166) was subsequently found to enhance affinity solely by reducing flexibility of the binding interface, apparently leading to a lower entropy cost upon formation of the complex (171). Demonstrating potential for engineered TIMPs in therapeutic applications, a designer TIMP developed for selective MMP-14 inhibition was shown to block collagenase activity and CD44 shedding in cell culture models of breast cancer and fibrosarcoma (172). Importantly, as MMP-independent activities of TIMPs have also been described (66–68), it will be important to define the sequence and structural epitopes responsible for these activities, to ascertain the feasibility of developing designer TIMPs selectively targeting individual MMPs yet devoid of unwanted off-target activities. Another challenge will be

developing formulations suitable for therapeutic use; early steps in this direction have recently been taken, exploring the potential of PEGylation, fusion to serum albumin, or nanoparticle delivery to enhance recombinant TIMP availability and efficacy *in vivo* (173–178).

7. SUMMARY AND PERSPECTIVES

MMP family members have been extensively identified in animal models and in human cohort studies as key mediators of tumor progression. However, MMPs are a large and diverse family composed of complex macromolecular proteins, with correspondingly complex functions in cancer development. It would be very unfortunate if the prior disappointing performance of broad spectrum, small molecule catalytic site inhibitors in clinical trials precluded future attempts to implement MMP inhibition as a therapeutic strategy for breast cancer, particularly when considering the exciting new developments in small molecule and macromolecular MMP inhibitors. Ongoing research should focus on fully dissecting the intricate pathways and processes affected by MMPs in breast cancer development, particularly with regard to how these processes may differ between tumor development in model systems and breast cancer progression in humans.

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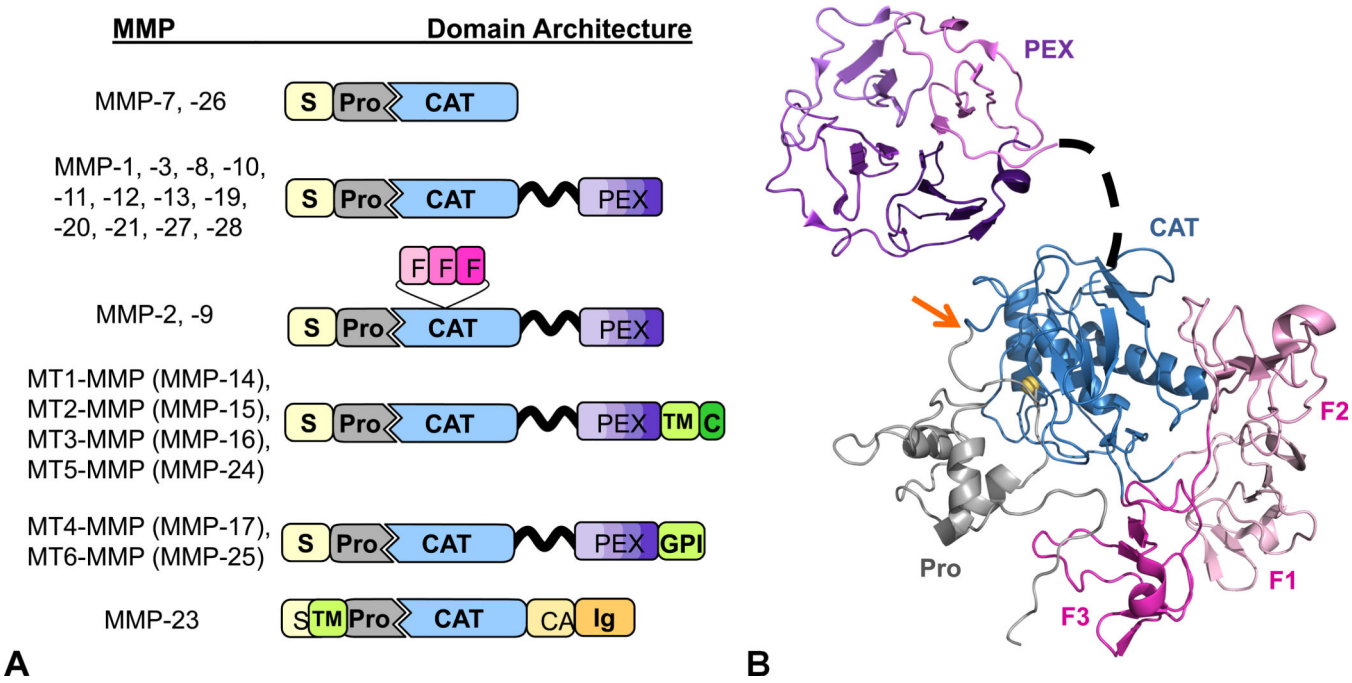

Figure 1. MMP domain structure and protein fold

(A) The various domain organizations of human MMPs are illustrated; S, signal peptide; Pro, propeptide; CAT, catalytic domain; F, fibronectin type II repeats; PEX, hemopexin domain; TM, transmembrane domain; GPI, glycophosphatidylinositol membrane anchor; C, cytoplasmic domain; CA, cysteine array; Ig, immunoglobulin-like domain. The flexible, variable length linker or hinge region is depicted as a wavy black ribbon. (B) The protein structure of the domains of a representative proMMP (proMMP-2) is shown, with individual domains colored as in the cartoon in panel A. The PEX domain has been separated from other domains for visual clarity; the linker connecting CAT and PEX domains, represented by a black dashed line, is flexible, of variable length in different MMPs, and allows for multiple orientations of the PEX domain relative to other domains. The prodomain (gray) blocks the active site by coordination to the catalytic zinc (yellow sphere); activation involves proteolysis near the site indicated by the orange arrow, allowing removal of the prodomain. Figure was generated with PyMOL (Schrodinger, LLC), using coordinates from Protein Databank entry 1GXD (179).

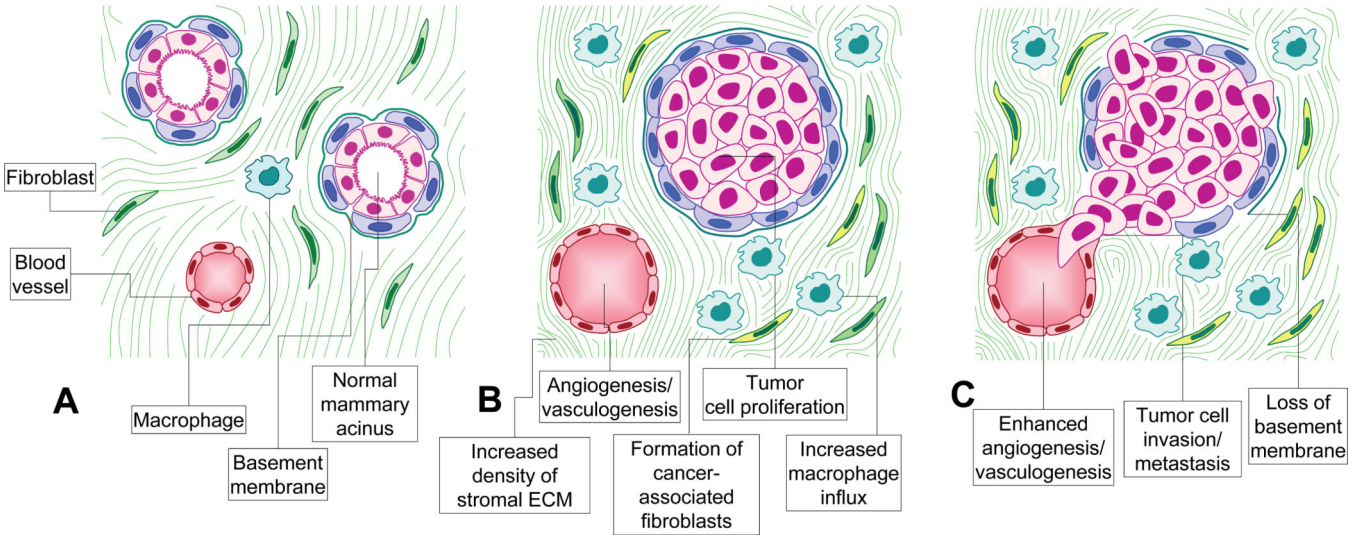

Figure 2. Role of MMPs in breast cancer progression

A. In the normal/premalignant state, lobular acini are embedded in stromal collagen, with rare immune cell infiltrates. Not shown: adipocytes, which in normal human breast tissue are usually separated from the lobular structures by stromal ECM. B. In ductal carcinoma *in situ*, MMP production by activated fibroblasts, infiltrating macrophages and other immune cells, and the tumor cells themselves (as well as by adipocytes) promote epithelial cell proliferation and suppression of apoptosis. C. Progression to invasive breast cancer is associated with increasing abundance of stromal collagen, degradation of the basement membrane, and invasion of cancer cells into the surrounding stromal ECM and the vasculature to begin the process of metastatic dissemination.