

## Matrix metalloproteinases as breast cancer drivers and therapeutic targets

Evette S. Radisky<sup>1</sup>, Derek C. Radisky<sup>1</sup>

<sup>1</sup>Department of Cancer Biology, Mayo Clinic Comprehensive Cancer Center, Jacksonville, Florida 32224

### TABLE OF CONTENTS

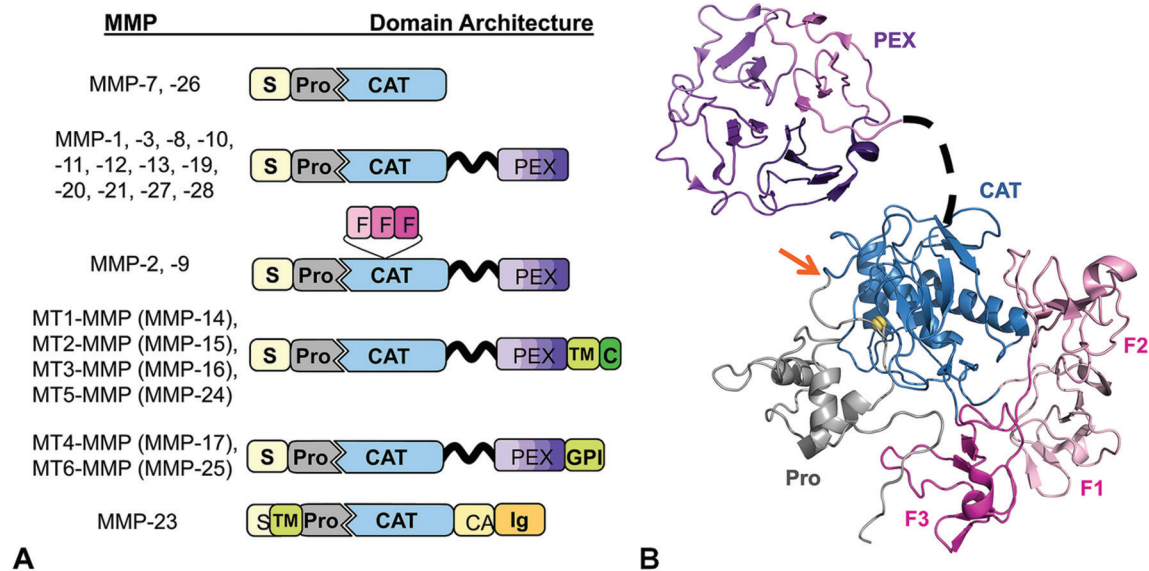
1. Abstract
2. Introduction
3. The matrix metalloproteinase family
  - 3.1. Domain structure of the MMP family
  - 3.2. Structural determinants of MMP activity and specificity
4. MMPs in breast cancer and their clinical significance
  - 4.1. MMPs associated with poor prognosis in breast cancer
  - 4.2. Prognostic implications are linked to the cell type expressing MMPs
  - 4.3. Circulating MMPs as tumor biomarkers
5. Tumorigenic processes activated by MMPs in breast cancer
  - 5.1. MMPs promote tumor growth by regulating proliferation and apoptosis
  - 5.2. Stromal MMPs create a tumor-promoting microenvironment
  - 5.3. MMPs promote invasion and metastasis
  - 5.4. MMPs as signaling molecules: noncatalytic functions of MMPs
6. Therapeutic approaches targeting MMPs
  - 6.1. Poor performance of broad spectrum MMP inhibitors in clinical trials
  - 6.2. Improving the selectivity of small molecule MMP inhibitors
  - 6.3. MMP-targeting antibodies as therapeutic inhibitors
  - 6.4. Therapeutic potential of TIMPs and variants
7. Summary and perspectives
8. Acknowledgements
9. References

### 1. 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 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.

### 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 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.



**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, glycosylphosphatidylinositol 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 1GXN (179).

### 3. THE MATRIX METALLOPROTEINASE FAMILY

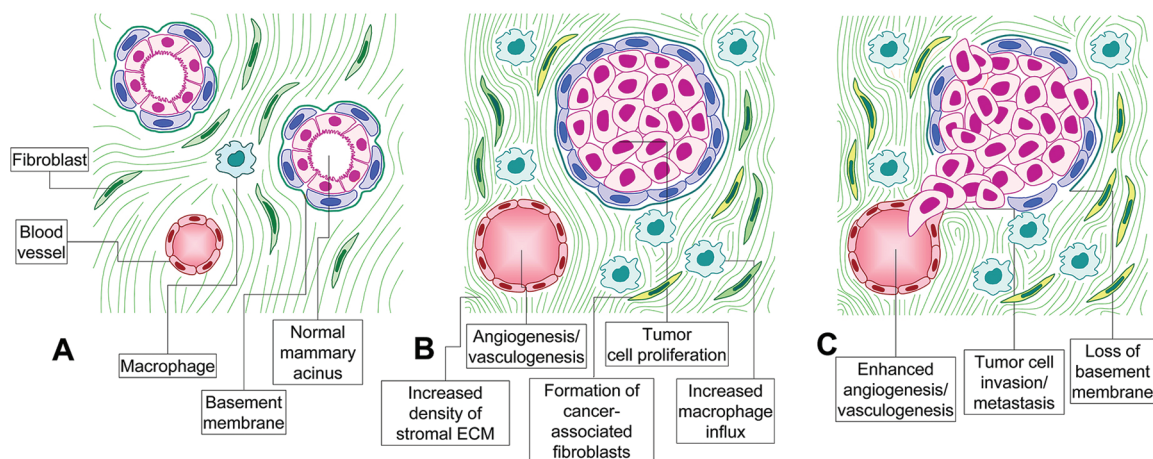
#### 3.1. Domain structure of the MMP family

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: DOI: [merops.sanger.ac.uk](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 glycosylphosphatidylinositol (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 metalloproteases, 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



**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.

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 PRGxPD “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 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 TGF $\beta$ , 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; TGF $\beta$  can also inhibit apoptosis in a variety of cell types (72). Most TGF $\beta$  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, TGF $\beta$ , 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- $\beta$  (HSP90 $\beta$ ) 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.

## 8. ACKNOWLEDGEMENTS

This research was supported by NCI grants R01CA154387 (to E.S.R.), the Susan B. Komen foundation grant KG110542 (to D.C.R.), the Jimmy V Foundation (to D.C.R.), and the Mayo Clinic SPORE in Breast Cancer grant P50CA116201 (P.I. James Ingle).

## 9. REFERENCES

1. A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward and D. Forman: Global cancer statistics. *CA Cancer J Clin*, 61(2), 69-90 (2011)  
DOI: 10.3322/caac.20107
2. L. A. Carey, R. Metzger, E. C. Dees, F. Collichio, C. I. Sartor, D. W. Ollila, N. Klauber-DeMore, J. Halle, L. Sawyer, D. T. Moore and M. L. Graham: American Joint Committee on Cancer tumor-node-metastasis stage after neoadjuvant chemotherapy and breast cancer outcome. *J Natl Cancer Inst*, 97(15), 1137-42 (2005)  
DOI: 10.1093/jnci/dji206
3. S. Dawood, K. Broglio, J. Ensor, G. N. Hortobagyi and S. H. Giordano: Survival differences among women with de novo stage IV and relapsed breast cancer. *Ann Oncol*, 21(11), 2169-74 (2010)  
DOI: 10.1093/annonc/mdq220
4. K. Kessenbrock, V. Plaks and Z. Werb: Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*, 141(1), 52-67 (2010)  
DOI: 10.1016/j.cell.2010.03.015
5. E. S. Radisky and D. C. Radisky: Stromal induction of breast cancer: inflammation and invasion. *Rev Endocr Metab Disord*, 8(3), 279-87 (2007)  
DOI: 10.1007/s11154-007-9037-1
6. E. S. Radisky and D. C. Radisky: Matrix metalloproteinase-induced epithelial-mesenchymal transition in breast cancer. *J*



- Mammary Gland Biol Neoplasia*, 15(2), 201-12 (2010)  
DOI: 10.1007/s10911-010-9177-x
7. J. A. Sparano, P. Bernardo, P. Stephenson, W. J. Gradishar, J. N. Ingle, S. Zucker and N. E. Davidson: Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy: Eastern Cooperative Oncology Group trial E2196. *J Clin Oncol*, 22(23), 4683-90 (2004)  
DOI: 10.1200/JCO.2004.08.054
  8. N. D. Rawlings, A. J. Barrett and A. Bateman: MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res*, 40(Database issue), D343-50 (2012)  
DOI: 10.1093/nar/gkr987
  9. H. Piccard, P. E. Van den Steen and G. Opdenakker: Hemopexin domains as multifunctional liganding modules in matrix metalloproteinases and other proteins. *J Leukoc Biol*, 81(4), 870-92 (2007)  
DOI: 10.1189/jlb.1006629
  10. B. Bauvois: New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: outside-in signaling and relationship to tumor progression. *Biochim Biophys Acta*, 1825(1), 29-36 (2012)  
DOI: 10.1016/j.bbcan.2011.10.001
  11. G. Murphy and H. Nagase: Localizing matrix metalloproteinase activities in the pericellular environment. *FEBS J*, 278(1), 2-15 (2011)  
DOI: 10.1111/j.1742-4658.2010.07918.x
  12. N. Sela-Passwell, G. Rosenblum, T. Shoham and I. Sagi: Structural and functional bases for allosteric control of MMP activities: can it pave the path for selective inhibition? *Biochim Biophys Acta*, 1803(1), 29-38 (2010)  
DOI: 10.1016/j.bbamcr.2009.04.010
  13. G. Murphy, Q. Nguyen, M. I. Cockett, S. J. Atkinson, J. A. Allan, C. G. Knight, F. Willenbrock and A. J. Docherty: Assessment of the role of the fibronectin-like domain of gelatinase A by analysis of a deletion mutant. *J Biol Chem*, 269(9), 6632-6 (1994)
  14. J. M. Shipley, G. A. Doyle, C. J. Fliszar, Q. Z. Ye, L. L. Johnson, S. D. Shapiro, H. G. Welgus and R. M. Senior: The structural basis for the elastolytic activity of the 92-kDa and 72-kDa gelatinases. Role of the fibronectin type II-like repeats. *J Biol Chem*, 271(8), 4335-41 (1996)  
DOI: 10.1074/jbc.271.8.4335
  15. B. Steffensen, U. M. Wallon and C. M. Overall: Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. *J Biol Chem*, 270(19), 11555-66 (1995)  
DOI: 10.1074/jbc.270.19.11555
  16. M. Mikhailova, X. Xu, T. K. Robichaud, S. Pal, G. B. Fields and B. Steffensen: Identification of collagen binding domain residues that govern catalytic activities of matrix metalloproteinase-2 (MMP-2). *Matrix Biol*, 31(7-8), 380-8 (2012)  
DOI: 10.1016/j.matbio.2012.10.001
  17. S. Rangaraju, K. K. Khoo, Z. P. Feng, G. Crossley, D. Nugent, I. Khaytin, V. Chi, C. Pham, P. Calabresi, M. W. Pennington, R. S. Norton and K. G. Chandy: Potassium channel modulation by a toxin domain in matrix metalloprotease 23. *J Biol Chem*, 285(12), 9124-36 (2010)  
DOI: 10.1074/jbc.M109.071266
  18. C. A. Galea, H. M. Nguyen, K. George Chandy, B. J. Smith and R. S. Norton: Domain structure and function of matrix metalloprotease 23 (MMP23): role in potassium channel trafficking. *Cell Mol Life Sci*, 71(7), 1191-210 (2014)  
DOI: 10.1007/s00018-013-1431-0
  19. N. Cerda-Costa and F. X. Gomis-Ruth: Architecture and function of metalloprotease catalytic domains. *Protein Sci*, 23(2), 123-44 (2014)  
DOI: 10.1002/pro.2400
  20. C. Tallant, A. Marrero and F. X. Gomis-Ruth: Matrix metalloproteinases: fold and function of their catalytic domains. *Biochim Biophys Acta*, 1803(1), 20-8 (2010)  
DOI: 10.1016/j.bbamcr.2009.04.003
  21. G. Rosenblum, S. Meroueh, M. Toth, J. F. Fisher, R. Fridman, S. Mobashery and I. Sagi: Molecular structures and dynamics of the stepwise activation mechanism of a matrix metalloproteinase zymogen: challenging the cysteine switch dogma. *J Am Chem Soc*, 129(44), 13566-74 (2007)  
DOI: 10.1021/ja073941l

22. N. D. Marchenko, G. N. Marchenko and A. Y. Strongin: Unconventional activation mechanisms of MMP-26, a human matrix metalloproteinase with a unique PHCGXXD cysteine-switch motif. *J Biol Chem*, 277(21), 18967-72 (2002)  
DOI: 10.1074/jbc.M201197200
23. H. J. Ra and W. C. Parks: Control of matrix metalloproteinase catalytic activity. *Matrix Biol*, 26(8), 587-96 (2007)  
DOI: 10.1016/j.matbio.2007.07.001  
DOI: 10.1074/jbc.M111.273391
24. T. K. Robichaud, B. Steffensen and G. B. Fields: Exosite interactions impact matrix metalloproteinase collagen specificities. *J Biol Chem*, 286(43), 37535-42 (2011)  
DOI: 10.1074/jbc.M111.273391
25. M. O. Palmier, Y. G. Fulcher, R. Bhaskaran, V. Q. Duong, G. B. Fields and S. R. Van Doren: NMR and bioinformatics discovery of exosites that tune metalloelastase specificity for solubilized elastin and collagen triple helices. *J Biol Chem*, 285(40), 30918-30 (2010)  
DOI: 10.1074/jbc.M110.136903
26. E. A. Stura, R. Visse, P. Cuniasse, V. Dive and H. Nagase: Crystal structure of full-length human collagenase 3 (MMP-13) with peptides in the active site defines exosites in the catalytic domain. *FASEB J*, 27(11), 4395-405 (2013)  
DOI: 10.1096/fj.13-233601
27. L. H. Arnold, L. E. Butt, S. H. Prior, C. M. Read, G. B. Fields and A. R. Pickford: The interface between catalytic and hemopexin domains in matrix metalloproteinase-1 conceals a collagen binding exosite. *J Biol Chem*, 286(52), 45073-82 (2011)  
DOI: 10.1074/jbc.M111.285213
28. S. W. Manka, F. Carafoli, R. Visse, D. Bihan, N. Raynal, R. W. Farndale, G. Murphy, J. J. Enghild, E. Hohenester and H. Nagase: Structural insights into triple-helical collagen cleavage by matrix metalloproteinase 1. *Proc Natl Acad Sci U S A*, 109(31), 12461-6 (2012)  
DOI: 10.1073/pnas.1204991109
29. I. Bertini, M. Fragai, C. Luchinat, M. Melikian, M. Toccafondi, J. L. Lauer and G. B. Fields: Structural Basis for Matrix Metalloproteinase 1-Catalyzed Collagenolysis. *J Am Chem Soc*, 134(4), 2100-2110 (2011)  
DOI: 10.1021/ja208338j
30. I. Bertini, V. Calderone, M. Fragai, R. Jaiswal, C. Luchinat, M. Melikian, E. Mylonas and D. I. Svergun: Evidence of reciprocal reorientation of the catalytic and hemopexin-like domains of full-length MMP-12. *J Am Chem Soc*, 130(22), 7011-21 (2008)  
DOI: 10.1021/ja710491y
31. I. Bertini, M. Fragai, C. Luchinat, M. Melikian, E. Mylonas, N. Sarti and D. I. Svergun: Interdomain flexibility in full-length matrix metalloproteinase-1 (MMP-1). *J Biol Chem*, 284(19), 12821-8 (2009)  
DOI: 10.1074/jbc.M809627200
32. G. Rosenblum, P. E. Van den Steen, S. R. Cohen, J. G. Grossmann, J. Frenkel, R. Sertchook, N. Slack, R. W. Strange, G. Opdenakker and I. Sagi: Insights into the structure and domain flexibility of full-length pro-matrix metalloproteinase-9/gelatinase B. *Structure*, 15(10), 1227-36 (2007)  
DOI: 10.1016/j.str.2007.07.019
33. P. M. McGowan and M. J. Duffy: Matrix metalloproteinase expression and outcome in patients with breast cancer: analysis of a published database. *Ann Oncol*, 19(9), 1566-1572 (2008)  
DOI: 10.1093/annonc/mdn180
34. M. J. van de Vijver, Y. D. He, L. J. van't Veer, H. Dai, A. A. Hart, D. W. Voskuil, G. J. Schreiber, J. L. Peterse, C. Roberts, M. J. Marton, M. Parrish, D. Atsma, A. Witteveen, A. Glas, L. Delahaye, T. van der Velde, H. Bartelink, S. Rodenhuis, E. T. Rutgers, S. H. Friend and R. Bernards: A gene-expression signature as a predictor of survival in breast cancer. *New Engl J Med*, 347(25), 1999-2009 (2002)  
DOI: 10.1056/NEJMoa021967
35. D. M. Roy and L. A. Walsh: Candidate prognostic markers in breast cancer: focus on extracellular proteases and their inhibitors. *Breast cancer*, 6, 81-91 (2014)  
DOI: 10.2147/BCTT.S46020
36. C. Curtis, S. P. Shah, S. F. Chin, G. Turashvili, O. M. Rueda, M. J. Dunning, D. Speed, A. G. Lynch, S. Samarajiwa, Y. Yuan, S. Graf, G. Ha, G. Haffari, A. Bashashati, R. Russell, S. McKinney, A. Langerod, A. Green, E. Provenzano, G. Wishart, S. Pinder, P. Watson, F. Markowitz, L. Murphy, I. Ellis, A. Purushotham, A. L. Borresen-Dale, J. D. Brenton, S. Tavare, C. Caldas and S.

- Aparicio: The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*, 486(7403), 346-52 (2012)  
DOI: 10.1038/nature10983
37. B. Tetu, J. Brisson, C. S. Wang, H. Lapointe, G. Beaudry, C. Blanchette and D. Trudel: The influence of MMP-14, TIMP-2 and MMP-2 expression on breast cancer prognosis. *Breast Cancer Res*, 8(3), R28 (2006)  
DOI: 10.1186/bcr1503
38. L. J. van 't Veer, H. Dai, M. J. van de Vijver, Y. D. He, A. A. Hart, M. Mao, H. L. Peterse, K. van der Kooy, M. J. Marton, A. T. Witteveen, G. J. Schreiber, R. M. Kerkhoven, C. Roberts, P. S. Linsley, R. Bernards and S. H. Friend: Gene expression profiling predicts clinical outcome of breast cancer. *Nature*, 415(6871), 530-6 (2002)  
DOI: 10.1038/415530a
39. S. Paik, S. Shak, G. Tang, C. Kim, J. Baker, M. Cronin, F. L. Baehner, M. G. Walker, D. Watson, T. Park, W. Hiller, E. R. Fisher, D. L. Wickerham, J. Bryant and N. Wolmark: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *New Engl J Med*, 351(27), 2817-26 (2004)  
DOI: 10.1056/NEJMoa041588
40. J. S. Parker, M. Mullins, M. C. Cheang, S. Leung, D. Voduc, T. Vickery, S. Davies, C. Fauron, X. He, Z. Hu, J. F. Quackenbush, I. J. Stijleman, J. Palazzo, J. S. Marron, A. B. Nobel, E. Mardis, T. O. Nielsen, M. J. Ellis, C. M. Perou and P. S. Bernard: Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*, 27(8), 1160-7 (2009)  
DOI: 10.1200/JCO.2008.18.1370
41. I. Poola, R. L. DeWitty, J. J. Marshalleck, R. Bhatnagar, J. Abraham and L. D. Leffall: Identification of MMP-1 as a putative breast cancer predictive marker by global gene expression analysis. *Nat Med*, 11(5), 481-3 (2005)  
DOI: 10.1038/nm1243
42. F. J. Vizoso, L. O. Gonzalez, M. D. Corte, J. C. Rodriguez, J. Vazquez, M. L. Lamelas, S. Junquera, A. M. Merino and J. L. Garcia-Muniz: Study of matrix metalloproteinases and their inhibitors in breast cancer. *Br J Cancer*, 96(6), 903-11 (2007)  
DOI: 10.1038/sj.bjc.6603666
43. L. O. Gonzalez, I. Pidal, S. Junquera, M. D. Corte, J. Vazquez, J. C. Rodriguez, M. L. Lamelas, A. M. Merino, J. L. Garcia-Muniz and F. J. Vizoso: Overexpression of matrix metalloproteinases and their inhibitors in mononuclear inflammatory cells in breast cancer correlates with metastasis-relapse. *Br J Cancer*, 97(7), 957-63 (2007)
44. J. M. Del Casar, L. O. Gonzalez, E. Alvarez, S. Junquera, L. Marin, L. Gonzalez, M. Bongera, J. Vazquez and F. J. Vizoso: Comparative analysis and clinical value of the expression of metalloproteases and their inhibitors by intratumor stromal fibroblasts and those at the invasive front of breast carcinomas. *Breast Cancer Res Treat*, 116(1), 39-52 (2009)  
DOI: 10.1007/s10549-009-0351-z
45. P. Bostrom, M. Soderstrom, T. Vahlberg, K. O. Soderstrom, P. J. Roberts, O. Carpen and P. Hirsimaki: MMP-1 expression has an independent prognostic value in breast cancer. *BMC Cancer*, 11, 348 (2011)  
DOI: 10.1186/1471-2407-11-348
46. B. Zhang, X. Cao, Y. Liu, W. Cao, F. Zhang, S. Zhang, H. Li, L. Ning, L. Fu, Y. Niu, R. Niu, B. Sun and X. Hao: Tumor-derived matrix metalloproteinase-13 (MMP-13) correlates with poor prognoses of invasive breast cancer. *BMC Cancer*, 8, 83 (2008)  
DOI: 10.1186/1471-2407-8-83
47. J. M. Pellikainen, K. M. Ropponen, V. V. Kataja, J. K. Kellokoski, M. J. Eskelinen and V. M. Kosma: Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. *Clin Cancer Res*, 10(22), 7621-8 (2004)  
DOI: 10.1158/1078-0432.CCR-04-1061
48. E. Mylona, A. Nomikos, C. Magkou, M. Kamberou, I. Papassideri, A. Keramopoulos and L. Nakopoulou: The clinicopathological and prognostic significance of membrane type 1 matrix metalloproteinase (MT1-MMP) and MMP-9 according to their localization in invasive breast carcinoma. *Histopathology*, 50(3), 338-47 (2007)  
DOI: 10.1111/j.1365-2559.2007.02615.x
49. H. C. Li, D. C. Cao, Y. Liu, Y. F. Hou, J. Wu, J. S. Lu, G. H. Di, G. Liu, F. M. Li, Z. L. Ou, C. Jie, Z. Z. Shen and Z. M. Shao: Prognostic value of matrix metalloproteinases (MMP-2



- and MMP-9) in patients with lymph node-negative breast carcinoma. *Breast Cancer Res Treat*, 88(1), 75-85 (2004)  
DOI: 10.1007/s10549-004-1200-8
50. C. Mehner, A. Hockla, E. Miller, S. Ran, D. C. Radisky and E. S. Radisky: Tumor cell-produced matrix metalloproteinase 9 (MMP-9) drives malignant progression and metastasis of basal-like triple negative breast cancer. *Oncotarget*, 5(9), 2736-2749 (2014)
  51. E. M. Yousef, M. R. Tahir, Y. St-Pierre and L. A. Gaboury: MMP-9 expression varies according to molecular subtypes of breast cancer. *BMC Cancer*, 14(1), 609 (2014)  
DOI: 10.1186/1471-2407-14-609
  52. L. O. Gonzalez, M. D. Corte, S. Junquera, R. Gonzalez-Fernandez, J. M. del Casar, C. Garcia, A. Andicoechea, J. Vazquez, R. Perez-Fernandez and F. J. Vizoso: Expression and prognostic significance of metalloproteases and their inhibitors in luminal A and basal-like phenotypes of breast carcinoma. *Hum Pathol*, 40(9), 1224-33 (2009)  
DOI: 10.1016/j.humpath.2008.12.022
  53. S. Zhao, W. Ma, M. Zhang, D. Tang, Q. Shi, S. Xu, X. Zhang, Y. Liu, Y. Song, L. Liu and Q. Zhang: High expression of CD147 and MMP-9 is correlated with poor prognosis of triple-negative breast cancer (TNBC) patients. *Med Oncol*, 30(1), 335 (2013)  
DOI: 10.1007/s12032-012-0335-4
  54. G. La Rocca, I. Pucci-Minafra, A. Marrazzo, P. Taormina and S. Minafra: Zymographic detection and clinical correlations of MMP-2 and MMP-9 in breast cancer sera. *Br J Cancer*, 90(7), 1414-21 (2004)  
DOI: 10.1038/sj.bjc.6601725
  55. S. B. Somiari, R. I. Somiari, C. M. Heckman, C. H. Olsen, R. M. Jordan, S. J. Russell and C. D. Shriver: Circulating MMP2 and MMP9 in breast cancer -- potential role in classification of patients into low risk, high risk, benign disease and breast cancer categories. *Int J Cancer*, 119(6), 1403-11 (2006)  
DOI: 10.1002/ijc.21989
  56. D. S. Heo, H. Choi, M. Y. Yeom, B. J. Song and S. J. Oh: Serum levels of matrix metalloproteinase-9 predict lymph node metastasis in breast cancer patients. *Oncol Rep*, 31(4), 1567-72 (2014)  
DOI: 10.3892/or.2014.3001
  57. S. M. Ranuncolo, E. Armanasco, C. Cresta, E. Bal De Kier Joffe and L. Puricelli: Plasma MMP-9 (92 kDa-MMP) activity is useful in the follow-up and in the assessment of prognosis in breast cancer patients. *Int J Cancer*, 106(5), 745-51 (2003)  
DOI: 10.1002/ijc.11288
  58. Z. S. Wu, Q. Wu, J. H. Yang, H. Q. Wang, X. D. Ding, F. Yang and X. C. Xu: Prognostic significance of MMP-9 and TIMP-1 serum and tissue expression in breast cancer. *Int J Cancer*, 122(9), 2050-6 (2008)  
DOI: 10.1002/ijc.23337
  59. S. Patel, G. Sumitra, B. C. Koner and A. Saxena: Role of serum matrix metalloproteinase-2 and -9 to predict breast cancer progression. *Clin Biochem*, 44(10-11), 869-72 (2011)  
DOI: 10.1016/j.clinbiochem.2011.04.019
  60. D. Tang, Y. Piao, S. Zhao, X. Mu, S. Li, W. Ma, Y. Song, J. Wang, W. Zhao and Q. Zhang: Expression and correlation of matrix metalloproteinase-9 and heparanase in patients with breast cancer. *Med Oncol*, 31(7), 26 (2014)  
DOI: 10.1007/s12032-014-0026-4
  61. X. Provatopoulou, A. Gounaris, E. Kalogera, F. Zagouri, I. Flessas, E. Goussetis, A. Nonni, I. Papassotiriou and G. Zografos: Circulating levels of matrix metalloproteinase-9 (MMP-9), neutrophil gelatinase-associated lipocalin (NGAL) and their complex MMP-9/NGAL in breast cancer disease. *BMC Cancer*, 9, 390 (2009)  
DOI: 10.1186/1471-2407-9-390
  62. J. Zhang, L. Yin, J. Wu, Y. Zhang, T. Xu, R. Ma, H. Cao and J. Tang: Detection of serum VEGF and MMP-9 levels by Luminex multiplexed assays in patients with breast infiltrative ductal carcinoma. *Exp Ther Med*, 8(1), 175-180 (2014)
  63. S. Leppa, T. Saarto, L. Vehmanen, C. Blomqvist and I. Elomaa: A high serum matrix metalloproteinase-2 level is associated with an adverse prognosis in node-positive breast carcinoma. *Clin Cancer Res*, 10(3), 1057-63 (2004)  
DOI: 10.1158/1078-0432.CCR-03-0047
  64. S. M. Sheen-Chen, H. S. Chen, H. L. Eng, C. C. Sheen and W. J. Chen: Serum levels of matrix metalloproteinase 2 in patients with breast cancer. *Cancer Lett*, 173(1), 79-82 (2001)  
DOI: 10.1016/S0304-3835(01)00657-7

65. S. B. Thorsen, S. L. Christensen, S. O. Wurtz, M. Lundberg, B. S. Nielsen, L. Vinther, M. Knowles, N. Gee, S. Fredriksson, S. Moller, N. Brunner, A. S. Schrohl and J. Stenvang: Plasma levels of the MMP-9:TIMP-1 complex as prognostic biomarker in breast cancer: a retrospective study. *BMC Cancer*, 13, 598 (2013)  
DOI: 10.1186/1471-2407-13-598
66. R. Chirco, X. W. Liu, K. K. Jung and H. R. Kim: Novel functions of TIMPs in cell signaling. *Cancer Metast Rev*, 25(1), 99-113 (2006)  
DOI: 10.1007/s10555-006-7893-x
67. W. G. Stetler-Stevenson: Tissue Inhibitors of Metalloproteinases in Cell Signaling: Metalloproteinase-Independent Biological Activities. *Sci Signal*, 1(27), re6 (2008)  
DOI: 10.1126/scisignal.127re6
68. K. Brew and H. Nagase: The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim Biophys Acta*, 1803(1), 55-71 (2010)  
DOI: 10.1016/j.bbamcr.2010.01.003
69. C. Gialeli, A. D. Theocharis and N. K. Karamanos: Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J*, 278(1), 16-27 (2011)  
DOI: 10.1111/j.1742-4658.2010.07919.x
70. D. C. Radisky and M. J. Bissell: Matrix metalloproteinase-induced genomic instability. *Curr Opin Genet Dev*, 16(1), 45-50 (2006)  
DOI: 10.1016/j.gde.2005.12.011
71. P. Nistico, M. J. Bissell and D. C. Radisky: Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. *Cold Spring Harb Perspect Biol*, 4(2) (2012)  
DOI: 10.1101/cshperspect.a011908
72. J. Massague: TGFbeta signalling in context. *Nat Rev Mol Cell Bio*, 13(10), 616-30 (2012)  
DOI: 10.1038/nrm3434
73. J. P. Annes, J. S. Munger and D. B. Rifkin: Making sense of latent TGFbeta activation. *J Cell Sci*, 116(Pt 2), 217-24 (2003)  
DOI: 10.1242/jcs.00229
74. S. L. Dallas, J. L. Rosser, G. R. Mundy and L. F. Bonewald: Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. *J Biol Chem*, 277(24), 21352-60 (2002)  
DOI: 10.1074/jbc.M111663200
75. O. Tatti, P. Vehvilainen, K. Lehti and J. Keski-Oja: MT1-MMP releases latent TGF-beta1 from endothelial cell extracellular matrix via proteolytic processing of LTBP-1. *Exp Cell Res*, 314(13), 2501-14 (2008)  
DOI: 10.1016/j.yexcr.2008.05.018
76. H. C. Crawford, C. R. Scoggins, M. K. Washington, L. M. Matrisian and S. D. Leach: Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. *J Clin Invest*, 109(11), 1437-44 (2002)  
DOI: 10.1172/JCI0215051
77. N. Mitsiades, W. H. Yu, V. Poulaki, M. Tsokos and I. Stamenkovic: Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res*, 61(2), 577-81 (2001)
78. M. Villa-Morales and J. Fernandez-Piqueras: Targeting the Fas/FasL signaling pathway in cancer therapy. *Expert Opin Ther Targets*, 16(1), 85-101 (2012)  
DOI: 10.1517/14728222.2011.628937
79. S. M. Weis and D. A. Cheresh: Tumor angiogenesis: molecular pathways and therapeutic targets. *Nat Med*, 17(11), 1359-70 (2011)  
DOI: 10.1038/nm.2537
80. G. Bergers, R. Brekken, G. McMahon, T. H. Vu, T. Itoh, K. Tamaki, K. Tanzawa, P. Thorpe, S. Itohara, Z. Werb and D. Hanahan: Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol*, 2(10), 737-44 (2000)  
DOI: 10.1038/35036374
81. L. J. Hawinkels, K. Zuidwijk, H. W. Verspaget, E. S. de Jonge-Muller, W. van Duijn, V. Ferreira, R. D. Fontijn, G. David, D. W. Hommes, C. B. Lamers and C. F. Sier: VEGF release by MMP-9 mediated heparan sulphate cleavage induces colorectal cancer angiogenesis. *Eur J Cancer*, 44(13), 1904-13 (2008)  
DOI: 10.1016/j.ejca.2008.06.031
82. A. R. Hess, E. A. Seftor, R. E. Seftor and M. J.

- Hendrix: Phosphoinositide 3-kinase regulates membrane Type 1-matrix metalloproteinase (MMP) and MMP-2 activity during melanoma cell vasculogenic mimicry. *Cancer Res*, 63(16), 4757-62 (2003)
83. R. E. Seftor, A. R. Hess, E. A. Seftor, D. A. Kirschmann, K. M. Hardy, N. V. Margaryan and M. J. Hendrix: Tumor cell vasculogenic mimicry: from controversy to therapeutic promise. *Am J Pathol*, 181(4), 1115-25 (2012)  
DOI: 10.1016/j.ajpath.2012.07.013
  84. G. O. Ahn and J. M. Brown: Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. *Cancer cell*, 13(3), 193-205 (2008)  
DOI: 10.1016/j.ccr.2007.11.032
  85. C. Mehner and D. C. Radisky: Triggering the landslide: The tumor-promotional effects of myofibroblasts. *Exp Cell Res* (2013)  
DOI: 10.1016/j.yexcr.2013.03.015
  86. K. J. Heppner, L. M. Matrisian, R. A. Jensen and W. H. Rodgers: Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. *Am J Pathol*, 149(1), 273-82 (1996)
  87. J. M. Del Casar, L. O. Gonzalez, E. Alvarez, S. Junquera, L. Marin, L. Gonzalez, M. Bongera, J. Vazquez and F. J. Vizoso: Comparative analysis and clinical value of the expression of metalloproteases and their inhibitors by intratumor stromal fibroblasts and those at the invasive front of breast carcinomas. *Breast cancer research and treatment*, 116(1), 39-52 (2009)  
DOI: 10.1007/s10549-009-0351-z
  88. G. Finak, N. Bertos, F. Pepin, S. Sadekova, M. Souleimanova, H. Zhao, H. Chen, G. Omeroglu, S. Meterissian, A. Omeroglu, M. Hallett and M. Park: Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med*, 14(5), 518-27 (2008)  
DOI: 10.1038/nm1764
  89. S. Han, E. Makareeva, N. V. Kuznetsova, A. M. DeRidder, M. B. Sutter, W. Losert, C. L. Phillips, R. Visse, H. Nagase and S. Leikin: Molecular mechanism of type I collagen homotrimer resistance to mammalian collagenases. *J Biol Chem*, 285(29), 22276-81 (2010)  
DOI: 10.1074/jbc.M110.102079
  90. E. Makareeva, S. Han, J. C. Vera, D. L. Sackett, K. Holmbeck, C. L. Phillips, R. Visse, H. Nagase and S. Leikin: Carcinomas contain a matrix metalloproteinase-resistant isoform of type I collagen exerting selective support to invasion. *Cancer Res*, 70(11), 4366-74 (2010)  
DOI: 10.1158/0008-5472.CAN-09-4057
  91. K. M. Nieman, I. L. Romero, B. Van Houten and E. Lengyel: Adipose tissue and adipocytes support tumorigenesis and metastasis. *Biochimica et biophysica acta*, 1831(10), 1533-41 (2013)  
DOI: 10.1016/j.bbailip.2013.02.010
  92. B. Dirat, L. Bochet, M. Dabek, D. Daviaud, S. Dauvillier, B. Majed, Y. Y. Wang, A. Meulle, B. Salles, S. Le Gonidec, I. Garrido, G. Escourrou, P. Valet and C. Muller: Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. *Cancer Res*, 71(7), 2455-65 (2011)  
DOI: 10.1158/0008-5472.CAN-10-3323
  93. V. S. Golubkov, A. V. Chekanov, P. Cieplak, A. E. Aleshin, A. V. Chernov, W. Zhu, I. A. Radichev, D. Zhang, P. D. Dong and A. Y. Strongin: The Wnt/planar cell polarity protein-tyrosine kinase-7 (PTK7) is a highly efficient proteolytic target of membrane type-1 matrix metalloproteinase: implications in cancer and embryogenesis. *J Biol Chem*, 285(46), 35740-9 (2010)  
DOI: 10.1074/jbc.M110.165159
  94. V. S. Golubkov, N. L. Prigozhina, Y. Zhang, K. Stoletov, J. D. Lewis, P. E. Schwartz, R. M. Hoffman and A. Y. Strongin: Protein-tyrosine pseudokinase 7 (PTK7) directs cancer cell motility and metastasis. *J Biol Chem* (2014)  
DOI: 10.1074/jbc.M114.574459
  95. V. S. Golubkov and A. Y. Strongin: Insights into ectodomain shedding and processing of protein-tyrosine pseudokinase 7 (PTK7). *J Biol Chem*, 287(50), 42009-18 (2012)  
DOI: 10.1074/jbc.M112.371153
  96. S. Lamouille, J. Xu and R. Derynck: Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Bio*, 15(3), 178-96 (2014)  
DOI: 10.1038/nrm3758
  97. S. Cheng and D. H. Lovett: Gelatinase A



- (MMP-2) is necessary and sufficient for renal tubular cell epithelial-mesenchymal transformation. *Am J Pathol*, 162(6), 1937-49 (2003)  
DOI: 10.1016/S0002-9440(10) 64327-1
98. S.Cheng, A. S. Pollock, R. Mahimkar, J. L. Olson and D. H. Lovett: Matrix metalloproteinase 2 and basement membrane integrity: a unifying mechanism for progressive renal injury. *FASEB J*, 20(11), 1898-900 (2006)  
DOI: 10.1096/fj.06-5898fje
  99. T. K. Tan, G. Zheng, T. T. Hsu, Y. Wang, V. W. Lee, X. Tian, Y. Wang, Q. Cao, Y. Wang and D. C. Harris: Macrophage Matrix Metalloproteinase-9 Mediates Epithelial-Mesenchymal Transition *in vitro* in Murine Renal Tubular Cells. *Am J Pathol* (2010)  
DOI: 10.2353/ajpath.2010.090188
  100. G. Zheng, J. G. Lyons, T. K. Tan, Y. Wang, T. T. Hsu, D. Min, L. Succar, G. K. Rangan, M. Hu, B. R. Henderson, S. I. Alexander and D. C. Harris: Disruption of E-cadherin by matrix metalloproteinase directly mediates epithelial-mesenchymal transition downstream of transforming growth factor-beta1 in renal tubular epithelial cells. *Am J Pathol*, 175(2), 580-91 (2009)  
DOI: 10.2353/ajpath.2009.080983
  101. K. D. Cowden Dahl, J. Symowicz, Y. Ning, E. Gutierrez, D. A. Fishman, B. P. Adley, M. S. Stack and L. G. Hudson: Matrix metalloproteinase 9 is a mediator of epidermal growth factor-dependent e-cadherin loss in ovarian carcinoma cells. *Cancer Res*, 68(12), 4606-13 (2008)  
DOI: 10.1158/0008-5472.CAN-07-5046
  102. J. A. West-Mays and G. Pino: Matrix Metalloproteinases as Mediators of Primary and Secondary Cataracts. *Expert Rev Ophthalmol*, 2(6), 931-938 (2007)  
DOI: 10.1586/17469899.2.6.931
  103. S. A. Illman, K. Lehti, J. Keski-Oja and J. Lohi: Epilysin (MMP-28) induces TGF-beta mediated epithelial to mesenchymal transition in lung carcinoma cells. *J Cell Sci*, 119(Pt 18), 3856-65 (2006)  
DOI: 10.1242/jcs.03157
  104. M. L. Stallings-Mann, J. Waldmann, Y. Zhang, E. Miller, M. L. Gauthier, D. W. Visscher, G. P. Downey, E. S. Radisky, A. P. Fields and D. C. Radisky: Matrix metalloproteinase induction of Rac1b, a key effector of lung cancer progression. *Sci Transl Med*, 4(142), 142ra95 (2012)  
DOI: 10.1126/scitranslmed.3004062
  105. C. M. Yamashita, L. Dolgonos, R. L. Zemans, S. K. Young, J. Robertson, N. Briones, T. Suzuki, M. N. Campbell, J. Gaudie, D. C. Radisky, D. W. Riches, G. Yu, N. Kaminski, C. A. McCulloch and G. P. Downey: Matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. *Am J Pathol*, 179(4), 1733-45 (2011)  
DOI: 10.1016/j.ajpath.2011.06.041
  106. J. Cao, C. Chiarelli, O. Richman, K. Zarrabi, P. Kozarekar and S. Zucker: Membrane type 1 matrix metalloproteinase induces epithelial-to-mesenchymal transition in prostate cancer. *J Biol Chem*, 283(10), 6232-40 (2008)  
DOI: 10.1074/jbc.M705759200
  107. C. Foroni, M. Brogini, D. Generali and G. Damia: Epithelial-mesenchymal transition and breast cancer: role, molecular mechanisms and clinical impact. *Cancer Treat Rev*, 38(6), 689-97 (2012)  
DOI: 10.1016/j.ctrv.2011.11.001
  108. M. D. Sternlicht, M. J. Bissell and Z. Werb: The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter. *Oncogene*, 19, 1102-1113 (2000)  
DOI: 10.1038/sj.onc.1203347
  109. M. D. Sternlicht, A. Lochter, C. J. Simpson, B. Huey, J. P. Rougier, J. W. Gray, D. Pinkel, M. J. Bissell and Z. Werb: The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell*, 98(2), 137-46 (1999)  
DOI: 10.1016/S0092-8674(00) 81009-0
  110. C. J. Simpson, M. J. Bissell and Z. Werb: Mammary gland tumor formation in transgenic mice overexpressing stromelysin-1. *Semin Cancer Biol*, 6(3), 159-63 (1995)  
DOI: 10.1006/scbi.1995.0022
  111. A. Lochter, S. Galosy, J. Muschler, N. Freedman, Z. Werb and M. J. Bissell: Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J Cell Biol*, 139(7), 1861-72 (1997)  
DOI: 10.1083/jcb.139.7.1861
  112. A. Lochter, A. Srebrow, C. J. Simpson,

- N. Terracio, Z. Werb and M. J. Bissell: Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties. *J Biol Chem*, 272(8), 5007-15 (1997)  
DOI: 10.1074/jbc.272.8.5007
113. D. C. Radisky, D. D. Levy, L. E. Littlepage, H. Liu, C. M. Nelson, J. E. Fata, D. Leake, E. L. Godden, D. G. Albertson, M. A. Nieto, Z. Werb and M. J. Bissell: Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature*, 436(7047), 123-7 (2005)  
DOI: 10.1038/nature03688
  114. F. Pelisch, D. Khauv, G. Risso, M. Stallings-Mann, M. Blaustein, L. Quadana, D. C. Radisky and A. Srebrow: Involvement of hnRNP A1 in the matrix metalloproteinase-3-dependent regulation of Rac1 pre-mRNA splicing. *J Cell Biochem*, 113(7), 2319-29 (2012)  
DOI: 10.1002/jcb.24103
  115. L. Orlichenko, R. Geyer, M. Yanagisawa, D. Khauv, E. S. Radisky, P. Z. Anastasiadis and D. C. Radisky: The 19-amino acid insertion in the tumor-associated splice isoform Rac1b confers specific binding to p120 catenin. *J Biol Chem*, 285(25), 19153-61 (2010)  
DOI: 10.1074/jbc.M109.099382
  116. C. M. Nelson, D. Khauv, M. J. Bissell and D. C. Radisky: Change in cell shape is required for matrix metalloproteinase-induced epithelial-mesenchymal transition of mammary epithelial cells. *J Cell Biochem* (2008)  
DOI: 10.1002/jcb.21821
  117. M. A. Cichon and D. C. Radisky: ROS-induced epithelial-mesenchymal transition in mammary epithelial cells is mediated by NF- $\kappa$ B-dependent activation of Snail. *Oncotarget*, 5(9), 2827-38 (2014)
  118. Q. K. Chen, K. Lee, D. C. Radisky and C. M. Nelson: Extracellular matrix proteins regulate epithelial-mesenchymal transition in mammary epithelial cells. *Differentiation*, 86(3), 126-32 (2013)  
DOI: 10.1016/j.diff.2013.03.003
  119. K. Lee, Q. K. Chen, C. Lui, M. A. Cichon, D. C. Radisky and C. M. Nelson: Matrix compliance regulates Rac1b localization, NADPH oxidase assembly, and epithelial-mesenchymal transition. *Mol Biol Cell*, 23(20), 4097-108 (2012)  
DOI: 10.1091/mbc.E12-02-0166
  120. D. C. Radisky and C. M. Nelson: Regulation of mechanical stress by mammary epithelial tissue structure controls breast cancer cell invasion. *Oncotarget*, 4(4), 498-9 (2013)
  121. C. M. Overall: Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. *Mol Biotechnol*, 22(1), 51-86 (2002)  
DOI: 10.1385/MB:22:1:051
  122. B. Bauvois: New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: outside-in signaling and relationship to tumor progression. *Biochim Biophys Acta*, 1825(1), 29-36 (2012)  
DOI: 10.1016/j.bbcan.2011.10.001
  123. A. L. Correia, H. Mori, E. I. Chen, F. C. Schmitt and M. J. Bissell: The hemopexin domain of MMP3 is responsible for mammary epithelial invasion and morphogenesis through extracellular interaction with HSP90 $\beta$ . *Genes Dev*, 27(7), 805-17 (2013)  
DOI: 10.1101/gad.211383.112
  124. K. Kessenbrock, G. J. Dijkgraaf, D. A. Lawson, L. E. Littlepage, P. Shahi, U. Pieper and Z. Werb: A role for matrix metalloproteinases in regulating mammary stem cell function via the Wnt signaling pathway. *Cell Stem Cell*, 13(3), 300-13 (2013)  
DOI: 10.1016/j.stem.2013.06.005
  125. K. K. Jung, X. W. Liu, R. Chirco, R. Fridman and H. R. Kim: Identification of CD63 as a tissue inhibitor of metalloproteinase-1 interacting cell surface protein. *EMBO J*, 25(17), 3934-42 (2006)  
DOI: 10.1038/sj.emboj.7601281
  126. R. C. D'Angelo, X. W. Liu, A. J. Najy, Y. S. Jung, J. Won, K. X. Chai, R. Fridman and H. R. Kim: TIMP-1 via TWIST1 Induces EMT Phenotypes in Human Breast Epithelial Cells. *Mol Cancer Res* (2014)  
DOI: 10.1158/1541-7786.MCR-14-0105
  127. V. Egea, S. Zahler, N. Rieth, P. Neth, T. Popp, K. Kehe, M. Jochum and C. Ries: Tissue inhibitor of metalloproteinase-1 (TIMP-1) regulates mesenchymal stem cells through let-7f microRNA and Wnt/ $\beta$ -catenin

- signaling. *Proc Natl Acad Sci U S A*, 109(6), E309-16 (2012)  
DOI: 10.1073/pnas.1115083109
128. L. M. Coussens, B. Fingleton and L. M. Matrisian: Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science*, 295(5564), 2387-92 (2002)  
DOI: 10.1126/science.1067100
  129. K. D. Miller, W. Gradishar, L. Schuchter, J. A. Sparano, M. Cobleigh, N. Robert, H. Rasmussen and G. W. Sledge: A randomized phase II pilot trial of adjuvant marimastat in patients with early-stage breast cancer. *Ann Oncol*, 13(8), 1220-4 (2002)  
DOI: 10.1093/annonc/mdf199
  130. K. D. Miller, T. J. Saphner, D. M. Waterhouse, T. T. Chen, A. Rush-Taylor, J. A. Sparano, A. C. Wolff, M. A. Cobleigh, S. Galbraith and G. W. Sledge: A randomized phase II feasibility trial of BMS-275291 in patients with early stage breast cancer. *Clin Cancer Res*, 10(6), 1971-5 (2004)  
DOI: 10.1158/1078-0432.CCR-03-0968
  131. J. A. Jacobsen, J. L. Major Jourden, M. T. Miller and S. M. Cohen: To bind zinc or not to bind zinc: An examination of innovative approaches to improved metalloproteinase inhibition. *Biochimica et biophysica acta*, 1803(1), 72-94 (2010)  
DOI: 10.1016/j.bbamcr.2009.08.006
  132. C. M. Overall and O. Kleifeld: Towards third generation matrix metalloproteinase inhibitors for cancer therapy. *Br J Cancer*, 94(7), 941-6 (2006)  
DOI: 10.1038/sj.bjc.6603043
  133. L. Devel, B. Czarny, F. Beau, D. Georgiadis, E. Stura and V. Dive: Third generation of matrix metalloprotease inhibitors: Gain in selectivity by targeting the depth of the S1' cavity. *Biochimie*, 92(11), 1501-8 (2010)  
DOI: 10.1016/j.biochi.2010.07.017
  134. B. Czarny, E. A. Stura, L. Devel, L. Vera, E. Cassar-Lajeunesse, F. Beau, V. Calderone, M. Fragai, C. Luchinat and V. Dive: Molecular determinants of a selective matrix metalloprotease-12 inhibitor: insights from crystallography and thermodynamic studies. *J Med Chem*, 56(3), 1149-59 (2013)  
DOI: 10.1021/jm301574d
  135. I. Bertini, V. Calderone, M. Fragai, A. Giachetti, M. Loconte, C. Luchinat, M. Maletta, C. Nativi and K. J. Yeo: Exploring the subtleties of drug-receptor interactions: the case of matrix metalloproteinases. *J Am Chem Soc*, 129(9), 2466-75 (2007)  
DOI: 10.1021/ja065156z
  136. L. Devel, S. Garcia, B. Czarny, F. Beau, E. LaJeunesse, L. Vera, D. Georgiadis, E. Stura and V. Dive: Insights from selective non-phosphinic inhibitors of MMP-12 tailored to fit with an S1' loop canonical conformation. *J Biol Chem*, 285(46), 35900-9 (2010)  
DOI: 10.1074/jbc.M110.139634
  137. L. Devel, F. Beau, M. Amoura, L. Vera, E. Cassar-Lajeunesse, S. Garcia, B. Czarny, E. A. Stura and V. Dive: Simple pseudo-dipeptides with a P2' glutamate: a novel inhibitor family of matrix metalloproteases and other metzincins. *J Biol Chem*, 287(32), 26647-56 (2012)  
DOI: 10.1074/jbc.M112.380782
  138. I. Bertini, M. Fragai and C. Luchinat: Intra- and interdomain flexibility in matrix metalloproteinases: functional aspects and drug design. *Curr Pharm Des*, 15(31), 3592-605 (2009)  
DOI: 10.2174/138161209789271852
  139. Y. Udi, M. Fragai, M. Grossman, S. Mitternacht, R. Arad-Yellin, V. Calderone, M. Melikian, M. Toccafondi, I. N. Berezovsky, C. Luchinat and I. Sagi: Unraveling hidden regulatory sites in structurally homologous metalloproteases. *J Mol Biol*, 425(13), 2330-46 (2013)  
DOI: 10.1016/j.jmb.2013.04.009
  140. I. Bertini, V. Calderone, M. Cosenza, M. Fragai, Y. M. Lee, C. Luchinat, S. Mangani, B. Terni and P. Turano: Conformational variability of matrix metalloproteinases: beyond a single 3D structure. *Proc Natl Acad Sci U S A*, 102(15), 5334-9 (2005)  
DOI: 10.1073/pnas.0407106102
  141. M. W. Ndinguri, M. Bhowmick, D. Tokmina-Roszyk, T. K. Robichaud and G. B. Fields: Peptide-based selective inhibitors of matrix metalloproteinase-mediated activities. *Molecules*, 17(12), 14230-48 (2012)  
DOI: 10.3390/molecules171214230
  142. J. Lauer-Fields, K. Brew, J. K. Whitehead, S. Li, R. P. Hammer and G. B. Fields: Triple-helical transition state analogues: a new class of selective matrix metalloproteinase



- inhibitors. *J Am Chem Soc*, 129(34), 10408-17 (2007)  
DOI: 10.1021/ja0715849
143. J. L. Lauer-Fields, J. K. Whitehead, S. Li, R. P. Hammer, K. Brew and G. B. Fields: Selective modulation of matrix metalloproteinase 9 (MMP-9) functions via exosite inhibitors. *J Biol Chem*, 283(29), 20087-95 (2008)  
DOI: 10.1074/jbc.M801438200
  144. R. Bhaskaran, M. O. Palmier, J. L. Lauer-Fields, G. B. Fields and S. R. Van Doren: MMP-12 catalytic domain recognizes triple helical peptide models of collagen V with exosites and high activity. *J Biol Chem*, 283(31), 21779-88 (2008)  
DOI: 10.1074/jbc.M709966200
  145. J. L. Lauer-Fields, M. J. Chalmers, S. A. Busby, D. Minond, P. R. Griffin and G. B. Fields: Identification of specific hemopexin-like domain residues that facilitate matrix metalloproteinase collagenolytic activity. *J Biol Chem*, 284(36), 24017-24 (2009)  
DOI: 10.1074/jbc.M109.016873
  146. A. Dufour, S. Zucker, N. S. Sampson, C. Kuscus and J. Cao: Role of matrix metalloproteinase-9 dimers in cell migration: design of inhibitory peptides. *J Biol Chem*, 285(46), 35944-56 (2010)  
DOI: 10.1074/jbc.M109.091769
  147. K. Zarrabi, A. Dufour, J. Li, C. Kuscus, A. Pulkoski-Gross, J. Zhi, Y. Hu, N. S. Sampson, S. Zucker and J. Cao: Inhibition of matrix metalloproteinase 14 (MMP-14)-mediated cancer cell migration. *J Biol Chem*, 286(38), 33167-77 (2011)  
DOI: 10.1074/jbc.M111.256644
  148. A. Dufour, N. S. Sampson, J. Li, C. Kuscus, R. C. Rizzo, J. L. Deleon, J. Zhi, N. Jaber, E. Liu, S. Zucker and J. Cao: Small-molecule anticancer compounds selectively target the hemopexin domain of matrix metalloproteinase-9. *Cancer Res*, 71(14), 4977-88 (2011)  
DOI: 10.1158/0008-5472.CAN-10-4552
  149. A. G. Remacle, V. S. Golubkov, S. A. Shiryaev, R. Dahl, J. L. Stebbins, A. V. Chernov, A. V. Cheltsov, M. Pellicchia and A. Y. Strongin: Novel MT1-MMP small-molecule inhibitors based on insights into hemopexin domain function in tumor growth. *Cancer Res*, 72(9), 2339-49 (2012)  
DOI: 10.1158/0008-5472.CAN-11-4149
  150. L. Paemen, E. Martens, S. Masure and G. Opdenakker: Monoclonal antibodies specific for natural human neutrophil gelatinase B used for affinity purification, quantitation by two-site ELISA and inhibition of enzymatic activity. *Eur J Biochem*, 234(3), 759-65 (1995)  
DOI: 10.1111/j.1432-1033.1995.759\_a.x
  151. E. Martens, A. Leyssen, I. Van Aelst, P. Fiten, H. Piccard, J. Hu, F. J. Descamps, P. E. Van den Steen, P. Proost, J. Van Damme, G. M. Liuzzi, P. Riccio, E. Polverini and G. Opdenakker: A monoclonal antibody inhibits gelatinase B/MMP-9 by selective binding to part of the catalytic domain and not to the fibronectin or zinc binding domains. *Biochim Biophys Acta*, 1770(2), 178-86 (2007)  
DOI: 10.1016/j.bbagen.2006.10.012
  152. L. Devy, L. Huang, L. Naa, N. Yanamandra, H. Pieters, N. Frans, E. Chang, Q. Tao, M. Vanhove, A. Lejeune, R. van Gool, D. J. Sexton, G. Kuang, D. Rank, S. Hogan, C. Pazmany, Y. L. Ma, S. Schoonbroodt, A. E. Nixon, R. C. Ladner, R. Hoet, P. Henderikx, C. Tenhoor, S. A. Rabbani, M. L. Valentino, C. R. Wood and D. T. Dransfield: Selective inhibition of matrix metalloproteinase-14 blocks tumor growth, invasion, and angiogenesis. *Cancer Res*, 69(4), 1517-26 (2009)  
DOI: 10.1158/0008-5472.CAN-08-3255
  153. S. Ingvarsen, A. Porse, C. Erpicum, L. Maertens, H. J. Jurgensen, D. H. Madsen, M. C. Melander, H. Gardsvoll, G. Hoyer-Hansen, A. Noel, K. Holmbeck, L. H. Engelholm and N. Behrendt: Targeting a single function of the multifunctional matrix metalloprotease MT1-MMP: impact on lymphangiogenesis. *J Biol Chem*, 288(15), 10195-204 (2013)  
DOI: 10.1074/jbc.M112.447169
  154. S. A. Shiryaev, A. G. Remacle, V. S. Golubkov, S. Ingvarsen, A. Porse, N. Behrendt, P. Cieplak and A. Y. Strongin: A monoclonal antibody interferes with TIMP-2 binding and incapacitates the MMP-2-activating function of multifunctional, pro-tumorigenic MMP-14/MT1-MMP. *Oncogenesis*, 2, e80 (2013)  
DOI: 10.1038/oncsis.2013.44
  155. N. Sela-Passwell, R. Kikkeri, O. Dym, H. Rozenberg, R. Margalit, R. Arad-Yellin, M. Eisenstein, O. Brenner, T. Shoham, T. Danon, A. Shanzer and I. Sagi: Antibodies targeting the catalytic zinc complex of activated

- matrix metalloproteinases show therapeutic potential. *Nat Med*, 18(1), 143-7 (2012)  
DOI: 10.1038/nm.2582
156. M. Shimoda, S. Principe, H. W. Jackson, V. Luga, H. Fang, S. D. Molyneux, Y. W. Shao, A. Aiken, P. D. Waterhouse, C. Karamboulas, F. M. Hess, T. Ohtsuka, Y. Okada, L. Ailles, A. Ludwig, J. L. Wrana, T. Kislinger and R. Khokha: Loss of the Timp gene family is sufficient for the acquisition of the CAF-like cell state. *Nat Cell Biol*, 16(9), 889-901 (2014)  
DOI: 10.1038/ncb3021
  157. J. Batra and E. S. Radisky: Tissue inhibitors of metalloproteinases (TIMPs): inhibition of Zn-dependent metalloproteinases. In: *Encyclopedia of Inorganic and Bioinorganic Chemistry*. Ed R. A. Scott. John Wiley & Sons, Chichester (2014)  
DOI: 10.1002/9781119951438.eibc2168
  158. F. X. Gomis-Ruth, K. Maskos, M. Betz, A. Bergner, R. Huber, K. Suzuki, N. Yoshida, H. Nagase, K. Brew, G. P. Bourenkov, H. Bartunik and W. Bode: Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1. *Nature*, 389(6646), 77-81 (1997)  
DOI: 10.1038/37995
  159. J. Batra, A. S. Soares, C. Mehner and E. S. Radisky: Matrix Metalloproteinase-10/TIMP-2 Structure and Analyses Define Conserved Core Interactions and Diverse Exosite Interactions in MMP/TIMP Complexes. *PLoS One*, 8(9), e75836 (2013)  
DOI: 10.1371/journal.pone.0075836
  160. J. Batra, J. Robinson, A. S. Soares, A. P. Fields, D. C. Radisky and E. S. Radisky: Matrix metalloproteinase-10 (MMP-10) interaction with tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2: binding studies and crystal structure. *J Biol Chem*, 287(19), 15935-46 (2012)  
DOI: 10.1074/jbc.M112.341156
  161. H. Nagase and K. Brew: Engineering of tissue inhibitor of metalloproteinases mutants as potential therapeutics. *Arthritis Res*, 4 Suppl 3, S51-61 (2002)  
DOI: 10.1186/ar573
  162. H. Nagase and K. Brew: Designing TIMP (tissue inhibitor of metalloproteinases) variants that are selective metalloproteinase inhibitors. *Biochem Soc Symp*, (70), 201-12 (2003)
  163. Q. Meng, V. Malinovskii, W. Huang, Y. Hu, L. Chung, H. Nagase, W. Bode, K. Maskos and K. Brew: Residue 2 of TIMP-1 is a major determinant of affinity and specificity for matrix metalloproteinases but effects of substitutions do not correlate with those of the corresponding P1' residue of substrate. *J Biol Chem*, 274(15), 10184-9 (1999)  
DOI: 10.1074/jbc.274.15.10184
  164. S. Wei, Y. Chen, L. Chung, H. Nagase and K. Brew: Protein engineering of the tissue inhibitor of metalloproteinase 1 (TIMP-1) inhibitory domain. In search of selective matrix metalloproteinase inhibitors. *J Biol Chem*, 278(11), 9831-4 (2003)  
DOI: 10.1074/jbc.M211793200
  165. M. H. Lee, M. Rapti, V. Knauper and G. Murphy: Threonine 98, the pivotal residue of tissue inhibitor of metalloproteinases (TIMP)-1 in metalloproteinase recognition. *J Biol Chem*, 279(17), 17562-9 (2004)  
DOI: 10.1074/jbc.M312589200
  166. M. H. Lee, M. Rapti and G. Murphy: Unveiling the surface epitopes that render tissue inhibitor of metalloproteinase-1 inactive against membrane type 1-matrix metalloproteinase. *J Biol Chem*, 278(41), 40224-30 (2003)  
DOI: 10.1074/jbc.M305678200
  167. R. A. Williamson, M. Hutton, G. Vogt, M. Rapti, V. Knauper, M. D. Carr and G. Murphy: Tyrosine 36 plays a critical role in the interaction of the AB loop of tissue inhibitor of metalloproteinases-2 with matrix metalloproteinase-14. *J Biol Chem*, 276(35), 32966-70 (2001)  
DOI: 10.1074/jbc.M101843200
  168. H. Bahudhanapati, Y. Zhang, S. S. Sidhu and K. Brew: Phage display of tissue inhibitor of metalloproteinases-2 (TIMP-2): identification of selective inhibitors of collagenase-1 (metalloproteinase 1 (MMP-1)). *J Biol Chem*, 286(36), 31761-70 (2011)  
DOI: 10.1074/jbc.M111.253328
  169. A. B. Hamze, S. Wei, H. Bahudhanapati, S. Kota, K. R. Acharya and K. Brew: Constraining specificity in the N-domain of tissue inhibitor of metalloproteinases-1; gelatinase-selective inhibitors. *Protein Sci*, 16(9), 1905-13 (2007)  
DOI: 10.1110/ps.072978507
  170. O. Sharabi, J. Shirian, M. Grossman, M. Lebendiker, I. Sagi and J. Shifman: Affinity- and

- specificity-enhancing mutations are frequent in multispecific interactions between TIMP2 and MMPs. *PLoS one*, 9(4), e93712 (2014)  
DOI: 10.1371/journal.pone.0093712
171. M. Grossman, D. Tworowski, O. Dym, M. H. Lee, Y. Levy, G. Murphy and I. Sagi: The intrinsic protein flexibility of endogenous protease inhibitor TIMP-1 controls its binding interface and affects its function. *Biochemistry*, 49(29), 6184-92 (2010)  
DOI: 10.1021/bi902141x
172. M. H. Lee, S. Atkinson, M. Rapti, M. Handsley, V. Curry, D. Edwards and G. Murphy: The activity of a designer tissue inhibitor of metalloproteinases (TIMP)-1 against native membrane type 1 matrix metalloproteinase (MT1-MMP) in a cell-based environment. *Cancer Lett*, 290(1), 114-22 (2010)  
DOI: 10.1016/j.canlet.2009.08.029
173. J. Batra, J. Robinson, C. Mehner, A. Hockla, E. Miller, D. C. Radisky and E. S. Radisky: PEGylation extends circulation half-life while preserving *in vitro* and *in vivo* activity of tissue inhibitor of metalloproteinases-1 (TIMP-1). *PLoS One*, 7(11), e50028 (2012)  
DOI: 10.1371/journal.pone.0050028
174. M. Chaturvedi, I. Figiel, B. Sreedhar and L. Kaczmarek: Neuroprotection from tissue inhibitor of metalloproteinase-1 and its nanoparticles. *Neurochem Int*, 61(7), 1065-71 (2012)  
DOI: 10.1016/j.neuint.2012.07.023
175. M. S. Lee, Y. H. Kim, Y. J. Kim, S. H. Kwon, J. K. Bang, S. M. Lee, Y. S. Song, D. H. Hahm, I. Shim, D. Han and S. Her: Pharmacokinetics and biodistribution of human serum albumin-TIMP-2 fusion protein using near-infrared optical imaging. *J Pharm Pharm Sci*, 14(3), 368-77 (2011)
176. W. K. Kang, E.-K. Park, H. S. Lee, B.-Y. Park, J.-Y. Chang, M.-Y. Kim, H. A. Kang and J.-Y. Kim: A biologically active angiogenesis inhibitor, human serum albumin-TIMP-2 fusion protein, secreted from *Saccharomyces cerevisiae*. *Protein Expr Purif*, 53(2), 331-338 (2007)  
DOI: 10.1016/j.pep.2007.02.001
177. F. Chen, E. S. Radisky, P. Das, J. Batra, T. Hata, T. Hori, A. M. Baine, L. Gardner, M. Y. Yue, G. Bu, G. del Zoppo, T. C. Patel and J. H. Nguyen: TIMP-1 attenuates blood-brain barrier permeability in mice with acute liver failure. *J Cereb Blood Flow Metab*, 33(7), 1041-9 (2013)  
DOI: 10.1038/jcbfm.2013.45
178. M. Chaturvedi, Y. Molino, B. Sreedhar, M. Khrestchatisky and L. Kaczmarek: Tissue inhibitor of matrix metalloproteinases-1 loaded poly (lactic-co-glycolic acid) nanoparticles for delivery across the blood-brain barrier. *Int J Nanomed*, 9, 575 (2014)  
DOI: 10.2147/IJN.S54750
179. E. Morgunova, A. Tuuttila, U. Bergmann and K. Tryggvason: Structural insight into the complex formation of latent matrix metalloproteinase 2 with tissue inhibitor of metalloproteinase 2. *Proc Natl Acad Sci U S A*, 99(11), 7414-9 (2002)]  
DOI: 10.1073/pnas.102185399

**Key words:** Matrix metalloproteinases, MMPs, tissue inhibitors of metalloproteinases, TIMPs, Breast Cancer, Tumor Progression, Epithelial Mesenchymal Transition, EMT, MMP inhibitors, Cancer Biomarkers, Tumor Microenvironment

**Send correspondence to:** Evette S. Radisky, Department of Cancer Biology, Mayo Clinic Comprehensive Cancer Center, Jacksonville, Florida 32224, Tel: 904-953-6372, Fax: 904-953-6372, E-mail: radisky.evette@mayo.edu