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REVIEW

Matrix metalloproteinases involvement in pathologic conditions

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Abstract
Matrix metalloproteinases (MMPs) have a great variability that provides a complex intervention in pathophysiological conditions. MMPs roles in pathology may be grouped into the following main types: (1) tissue destruction, as in cancer invasion and metastasis, rheumatoid arthritis, osteoarthritis, different types of ulcers, periodontal disease, brain injury and neuroinflammatory diseases; (2) fibrosis, as in liver cirrhosis, fibrotic lung disease, otosclerosis, atherosclerosis, and multiple sclerosis; (3) weakening of matrix, as in dilated cardiomyopathy, epidermolysis bullosa, aortic aneurysm and restenotic lesions. Recent data also adds new MMPs functions in angiogenesis and apoptosis. Interesting opposite intervention in escaping mechanisms *vs.* antitumor defensive mechanisms had been also reported. As MMP-7 is expressed by tumor cells of epithelial and mesenchymal origin, it may be used as a biological marker of an aggressive phenotype and as a target of therapeutic intervention. MMPs play a pivotal role in the pathogenesis of arthritis, atherosclerosis, pulmonary emphysema, and endometriosis. Although MMP involvement in pathology is more than simple excessive matrix degradation, or an imbalance between them and their specific tissular inhibitors (TIMPs), MMP inhibition may be of therapeutic benefit, so synthetic MMPs inhibitors had been developed and are currently under clinical testing.

Keywords: matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), extracellular matrix (ECM), carcinogenesis, angiogenesis, apoptosis.

Introduction

Matrix metalloproteinases (MMPs) family consists of more than 26 endopeptidases that share homologous protein sequences, with conserved domain structures and specific domains related to substrate specificity and recognition of other proteins [1]. Their great variability provides a complex intervention in pathophysiological conditions.

MMPs, together with cysteine proteinases, aspartic proteinases, and serine proteinases are proteolytic enzymes involved in extracellular matrix (ECM) and basement membranes (BMs) degradation [2]. ECM remodelling is involved both in physiology, e.g. embryogenesis and in pathologic conditions, such as inflammatory diseases [2] or dermal photoaging [3, 4].

MMPs roles in pathology may be grouped into the following main types:

(1) tissue destruction, as in cancer invasion and metastasis [1], rheumatoid arthritis, osteoarthritis [5, 6], decubitus ulcer, gastric ulcer [7], corneal ulceration, periodontal disease, brain injury [8] and neuroinflammatory diseases [9];

(2) fibrosis, as in liver cirrhosis, fibrotic lung disease, otosclerosis, atherosclerosis, and multiple sclerosis [10];

(3) weakening of matrix, as in dilated cardiomyopathy, epidermolysis bullosa, aortic aneurysm [11] and restenotic lesions [12].

Considering the main action mechanisms, MMPs roles may be discussed in terms of tissue destruction, cancer invasion and metastasis, angiogenesis, apoptosis, escaping mechanisms, and antitumor defensive mechanism, and as a pivotal role in the pathogenesis of arthritis, atherosclerosis, pulmonary emphysema, and endometriosis. Contrabalancing their effects, synthetic MMPs inhibitors were developed and are currently under clinical testing.

MMPs role in tissue destruction

As for most biological processes, matrix degradation is a precise event, attributed to proteinases that are produced and released on demand from activated cells. By means of specific cell surface receptors, the cell recognizes a particular matrix molecule and is instructed to produce the appropriate metalloproteinase, which is then released into the pericellular space where it degrades its specific substrate. The sites of matrix degradation may be isolated by reorganization of cell membrane, analogous to the ruffled border of osteoclasts. As the cell moves beyond the site of matrix degradation, excess proteinase would spill into the open extracellular space. Thus, tissue inhibitors of metalloproteinases (TIMPs) may act in the tissue environment to neutralize used proteinases thereby preventing excessive and unwanted degradation away from the sites of metalloproteinase production.

Collagenases (MMP-1 or collagenase-1, MMP-8 or collagenase-2 and MMP-13 or collagenase-3) degrade native fibrillar collagens of types I, II, III, V, and XI, being involved in various pathologic conditions.

MMP-1 is produced by tumor cells, while MMP-8 is a product of rheumatoid synovial fibroblasts, osteoarthritic cartilage, and melanoma cells [2].

MMP-1 activity is present in the wound environment, being produced by fibroblasts, macrophages, and other cells within the granulation tissue. The expression of collagenase-1 by fibroblasts or macrophages, revealed by *in situ* hybridization and immunohistochemistry in cutaneous wounds, chronic ulcers, necrobiotic lesions, blisters, and carcinomas, is seen in less than half of the cases [13], and collagenase mRNA or protein is not detected in any cell in intact healthy skin. However, MMP-1 is prominently and invariably expressed by basal keratinocytes at the migratory front of cutaneous wounds, including normally healing wounds, burns, various forms of blisters and chronic ulcers, with injury that breached the BM [13–16]. The restricted pattern of collagenase-1expression indicates that its induction is a common response to injury of skin and suggests that re-epithelialization factors in the wound environment may affect collagenase-1 expression and activity.

Although keratinocytes are capable of secreting TIMP-1 *in vitro*, TIMP-1 mRNA seldom co-localizes with collagenase-1 mRNA in migrating keratinocytes in wounds, suggesting that keratinocyte-derived collagenase-1 acts without impedance from TIMP-1 [13]. The presence of TIMP-1 beneath the epidermis may provide a mechanism to contain collagenolytic activity to the epidermal front. The failure of wounds to heal due to excess proteinases corresponds to the elevated MMPs levels in various forms of chronic ulcers. In many samples of pyogenic granuloma, pyoderma gangrenosum, decubitus and stasis ulcers, the levels of collagenase-1 mRNA are markedly higher and the transcripts are seen over much longer distances of the basal epidermis than the levels and pattern seen in normally healing wounds [13]. In addition, the levels of gelatinases, mostly gelatinase B released from neutrophils, in chronic wound fluids are elevated compared to the levels seen in fluid collected from normal wounds. Consequently, excess MMPs impair wound healing by indiscriminately degrading matrix, cytokines, and other components of the wound environment that are needed for repair. It has been shown that the stromelysins are produced by the same populations of basal keratinocytes in both normal and chronic wounds [14, 16]. TIMP-1 levels are markedly reduced in chronic ulcers [14], suggesting that a proteinase–antiproteinase imbalance contributes to excess damage and impaired healing in many lesions. Whereas overexpression of collagenase-1, coupled with decreased production of TIMP-1, may impair healing, insufficient proteinase activity may lead to the accumulation of wound-associated tissue and delays in wound closure [17].

MMP-13 additionally degrades type IV, IX, X, and XIV collagens, the large tenascin C isoform, fibronectin, laminin, aggrecan core protein, fibrillin-1, and serine proteinase inhibitors, being abundantly expressed in osteoarthritis, rheumatoid arthritis [2], chronic cutaneous ulcers, intestinal ulcerations [15], chronically inflamed periodontal tissue, atherosclerotic plaques, aortic aneurysms, breast carcinomas, squamous cell carcinoma of the head and neck and vulva, chondrosarcomas, and malignant melanomas [2].

Stromelysins are also involved in ECM breakdown and tissue remodelling. MMP-3 (Stromelysin 1) has an increased expression in the following pathological tissues: osteoarthritic cartilage, rheumatoid synovium (B-cells) and serum, herniated intervertebral disk, cholesteatoma epithelium, atheroma, aneurysmal abdominal aorta, proliferating basal keratinocytes, injured respiratory epithelial cells, gastrointestinal lesions (Crohn's disease, peptic ulcer, ulcerative colitis), colorectal cancer (stromal cells), head and neck carcinoma, basal cell carcinoma, bronchial and lung squamous carcinoma, and esophageal squamous carcinoma [2].

MMP-10 (Stromelysin 2) is involved in basal keratinocytes migration (in contact with dermal matrix), and in head and neck carcinoma [2, 13].

Although indirectly involved in ECM degradation, by degrading serine protease inhibitors, α1-proteinase inhibitor and α1-antitrypsin, MMP-11 is produced in breast cancer, uterus, placenta, and involuting mammary gland [2, 18].

Matrilysins (MMP-7 or matrilysin 1 and MMP-26 or matrilysin 2) are involved in degradation of fibronectin, laminin, nidogen, type IV collagen, proteoglycans, β4-integrin [2], being produced by gastrointestinal, prostatic, endometrial and breast carcinomas [19].

MMP-7 is involved in the degradation of ECM elastin, type IV collagen, fibronectin, vitronectin, aggrecan, proteoglycans, serum IGFBP-1, -2, -3, -4, -5, -6 and plasminogen, in the activation of lymphocytes and tumor cells ADAM28 and of Paneth cells intestinal α-defensin (cryptidin) into mature defensin [1, 19, 20].

Proteolytic modification of IGFBPs (IGF binding proteins), performed by MMP-7, MMP-3, MMP-9, and MMP-19 [21–24], increases the bioavaibility of IGFs, with mitogenic and antiapoptotic effects, in early carcinogenesis [22–26].

Secreted form ADAM28, a member of the disintegrin and metalloproteinase (ADAM) family, activated by MMP-7, digest IGFBP-3 in both free and complex forms with IGF-I or IGF-II [27]. MMP-7 is involved in ectodomain shedding of TNF-α precursor [28], Fas-ligand (Fas L) [29], HB-EGF (heparin-binding epidermal growth factor-like) precursor, E-cadherin, and β4-integrin [1].

Soluble TNF-α cleaved by the cell surface by MMP-7, with a 30-fold lower specificity constant relative to TNF-α converting enzyme (TACE) [28], increases apoptosis, by binding to the TNF-receptor 1.

As MMP-7 is expressed by tumor cells of epithelial and mesenchymal origin in esophagus, stomach [30], colorectum [31], liver [32], pancreas [33, 34], lung, skin, breast, endometrium, prostate, head and neck [35], it may be used as a biological marker of an aggressive

phenotype [32–34, 36] and as a target of therapeutic intervention. Furthermore, the immunohistochemical staining of MMP-7 is different in neoplastic glands compared to that of tumor cells of the invasive front, apically *vs.* basolaterally respectively, corresponding to its direct role in matrix degradation [1, 31]. It was also demonstrated that the functional polymorphism in MMP-7 promoter (-181A/G) is associated with the susceptibility to esophageal, gastric and lung carcinoma [37].

MMP-12 (macrophage metalloelastase) degrades elastin, type IV collagen, type I gelatine, fibronectin, laminin, vitronectin, proteoglycans, myelin basic protein, and α1-antitrypsin, being expressed by alveolar macrophages in pulmonary emphysema and by fibroblasts in intestinal and skin granulomatous diseases [15].

Gelatinases (MMP-2 or gelatinase-A and MMP-9 or gelatinase-B) degrade type IV, V, VII, X, XI, and XIV collagens, gelatine, elastin, proteoglycans core proteins, myelin basic protein, fibronectin, fibrillin-1, and precursors of TNF-α and IL-1β, being produced by several types of transformed cells [2].

Membrane-type MMPs (MT1-MMP or MMP-14, MT2-MMP or MMP-15, MT3-MMP or MMP-16, MT4-MMP or MMP-17, MT5-MMP or MMP-24, and MT6-MMP or MMP-25) are furin-activated. MT1-MMP activates proMMP-2 and cleaves type I, II, III collagen, gelatine, fibronectin, laminin-1, vitronectin, cartilage proteoglycans, and fibrillin-1 [2], being expressed by malignant epithelial cells. In glioblastomas, overexpression of MT1-MMP together with correlated activation ratio of pro-MMP-2 and tumor grades was observed [19]. These data suggest that MMP-2 and MT1-MMP contribute to the glioma cells invasion probably through degradation of brain proteoglycans and glial limitance external, consisting of type I, III, and IV collagens.

MT2-MMP activates proMMP-2 and proMMP-13, degrades laminin, fibronectin, and tenascin, being also correlated with pro-MMP-2 activation in gliomas [19]. MT3-MMP activates proMMP-2 and hydrolyzes gelatine, casein, type III collagen, and fibronectin [19].

MMPs in tumor invasion and metastasis

The importance of proteolytic enzymes in facilitating invasive tumor growth had been recognized some considerable time before these enzymes were isolated and characterized, with a hypothetical secretion by fibroblasts [38], and subsequently identified as hyaluronidases, serine proteinases, and matrix metalloproteinases.

According to the "three-step" hypothesis of tumor cell invasion, key events in the process of tumor invasion are tumor cell adhesion to ECM structures, ECM degradation by proteolysis, and then tumor cell migration into the degraded area. Acting by tissue breakdown and remodeling during tumor invasion, intravasation into circulation, extravasation, and migration to metastatic sites [39] and tumor angiogenesis [25], MMPs are overexpressed in a wide range of malignant tumors, with demonstrated correlation between their overexpression, tumor aggressiveness, stage, and prognosis [1].

The pattern of MMP expression is now known to be more complex than simple secretion by tumor cells and in many human malignancies it is characterized by the induction of metalloproteinase expression in "host" stromal cells [40], including the fibroblasts described in Gersch and Catchpole's original postulate. It is also clear that the MMP activity around a tumor is a feature of increased tissue remodeling as much as increased tissue degradation.

Initially, MMP-9 was considered a key MMP in the invasion and metastases, overexpressed by cancer cells and induced by several cytokines, growth factors, and oncogene products [41], its inhibition resulting in loss of metastatic phenotype. Subsequent studies demonstrated that the activation ratios of pro-MMP-2, not of pro-MMP-9, correlate with lymph node metastasis in breast, lung, thyroid, and digestive tract carcinomas [19]. Gelatinase A has been shown to play a key role in promoting invasiveness of both normal and neoplastic cells. BMs form physical barriers that separate and define epithelial compartments from the mesenchymal tissue compartments, routinely crossed by inflammatory cells in mediating immune surveillance, during glandular morphogenesis, angiogenesis, and tumor cell invasion. Furthermore, it was demonstrated that pro-MMP-2 overexpressed by stromal fibroblasts in cancer tissues or derived from serum is captured and activated on the cancer cells by MT1-MMP. Consequently, an overexpression of MT1-MMP was associated to that of MMP-2 in studied carcinomas [19]. In accordance with the biochemical data that TIMP-2 is required for the efficient activation of pro-MMP-2 by MT1-MMP, their co-localisation in epithelial carcinomatous and adjacent stromal cells was demonstrated by immunohistochemistry and by *in situ* zymography [19].

Although modifications or degradation of the ECM by tumor-derived proteases was originally thought to destroy physical barriers to cell migration, more recently profound effects on cell adhesion and migration were identified, related to gelatinase A activation on various tissue culture substrates including fibronection, gelatin, and vitronectin [42]. In contrast, inhibition of the endogenous gelatinase A with either neutralizing antibody or TIMP-2 results in enhanced attachment to these substrates when compared to untreated cells. Altering the production of TIMP-2 modulates not only proteolysis of the ECM, but also the adhesive and spreading properties of the cells and results in modified cell morphology.

Gelatinase A activity induces the migration of breast epithelial cells by cleaving and regulating the function of a specific ECM component, laminin-5 (Ln-5) [43]. BMs also contain collagen type IV, laminin-1 (Ln-1), and fibronectin (Fn) [44, 45]. Cells adhere or migrate on these ECM substrates using integrin receptors [46]. The effects of gelatinase A, gelatinase B, and plasmin on selected BM components show that the active form of gelatinase A cleaves the Ln-5 subunit at residue 587, exposing a putative cryptic pro-migratory site that triggers cell motility but not cell adhesion. Alternatively, this cleavage may mask a site that suppresses cell motility. The fact that the pro-motility cryptic site does

not support adhesion suggests that the proteolytic activity of gelatinase A may provide a signaling mechanism for cells to begin migration during mammary gland morphogenesis, as demonstrated by an enhanced migratory response to gelatinase A modification of Ln-5, in the neoplastic breast epithelial cell line, MCF-7 [47].

Cell adhesion and migration are markedly influenced by local levels of gelatinase A activity [42, 43], proteolytic degradation of the ECM being supplemented by stimulation of cell movement by proteolytic fragments generated by protease activity. As a result, gelatinase A activity creates a gradient of chemotactic stimuli that promotes and directs cell migration [42, 43].

Recent findings also suggest that excessive proteolysis may inhibit this process by impairing tumor cell adhesion or disrupting and degrading the cell-matrix interactions or matrix signals required for migration and invasion, demonstrating the existence of a certain critical range or a balance between gelatinase A and TIMP-2 expression in order for both tumor invasion and angiogenesis to occur [48].

Gelatinase A is not tumor cell specific, and is involved in ECM remodeling in a wide range of non-neoplastic processes, including embryonic development, trophoblast invasion, angiogenesis, T-cell transmigration, and wound healing [49-53].

Evidence for the enhanced expression of gelatinase A in human tumors comes from many experimental studies correlating enzyme expression and tumor grade, including carcinomas of the colon, pancreas [54], prostate, bladder, skin (squamous and basal cell carcinomas), breast, and ovary [1]. In contrast, benign proliferative disorders of these tissues usually show no activity of gelatinase A. By activating pro-MMP-2, MT5-MMP possesses a high expression in brain tumors [55].

Cellular localization in carcinomas indicates that gelatinase A mRNA appears to be localized to the stromal fibroblasts adjacent to the sites of tumor invasion. Exceptions to this observation include prostate cancer and carcinomas of the lung, where both mRNA and protein were detected mainly in the neoplastic epithelium, as well as carcinomas of the hypopharynx [54] and pancreas [56], in which gelatinase A mRNA was localized to both neoplastic cells and tumor stroma. For carcinomas of the colon, breast, and ovary, gelatinase A mRNA can only be detected in the connective tissue stroma. This stromal localization of the gelatinase A mRNA contrasts with the predominant localization of gelatinase A in the malignant epithelium that is seen with immunohistochemical techniques. Explanations for this discrepancy could be that the half-life of the gelatinase A mRNA, and/or the efficiency of translation, predominant synthesis in the tumor stroma, and recruitment by receptors on the surface of neoplastic epithelial cells.

Indeed, expression of both individual matrix metalloproteinases and their endogenous inhibitors (TIMPs) has been correlated with tumor progression and clinical outcome, with an increased level of TIMP-s in all cancer tissues [19]. Consequently, TIMP-1 was considered as a growth promoting factor and/or antiapoptotic factor to the cancer cells [57].

This is perhaps most clearly demonstrated in colorectal cancer where high expression of gelatinase B mRNA has been shown to be associated with early relapse and poor survival [58] and high levels of TIMP-1 correlate with lymph node and distant metastatic spread [59]. Other studies have reported an association between poor prognosis and high levels of expression of stromelysin-3 in breast cancer [60], activated gelatinase A in gastric cancer [61], TIMP-1 in small cell lung cancer [62], and TIMP-2 in bladder cancer [63].

An imbalance between MMPs (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-13) and TIMP-1 and TIMP-2, without correlations between the molar ratio and cancer progression or metastases is reported [19]. The high expression of both proteinase and inhibitor associated with poor prognosis probably reflects the need for some regulation of the increased metalloproteinase activity. The tumor must "remodel" the local tissue, by generation of a modified vasculature and the generation of supportive stromal tissues, the stroma acting as a "collaborator" with induction of proteinases and inhibitors by the adjacent tumor cells resulting in angiogenesis and invasive growth [19].

The growth of the MMP family has inevitably contributed to a more complex role in tumor growth and spread. The relative contribution of individual enzymes is important for the design of "selective" inhibitors. Gelatinase A, gelatinase B, stromelysin-3, matrilysin, MTI-MMP, collagenases [64], stromelysin-1 [65] seem to be associated most closely with the invasive phenotype. Analysis of the involvement of gelatinase B is complicated by the fact that in many tumors this enzyme is produced by inflammatory cells, as part of the "host" defense. However, the association of gelatinase B [58] and, in some cases, inflammatory infiltrate [66] with poor prognosis suggests a dual action.

In cancer, MMPs are believed to promote tumor progression by enhancing growth via angiogenesis, disrupting local tissue architecture to allow tumor growth, and breaking down BM barriers to allow metastatic spread. While some MMPs, such as matrilysin, collagenase-3, and gelatinase A, are expressed by tumors themselves, most other MMPs are predominantly produced by surrounding stromal host cells in response to cytokines released by the tumors, and promote tumor progression. For example, gelatinase A binds to endothelial cell, promoting tumor angiogenesis [67].

Application of hydroxamates, specific MMP zinc chelating agents, is effective in inhibiting growth of several primary tumors and metastases in animal models [68], and are currently undergoing clinical trials.

MMP inhibitors appear to be most effective in models using nude mice and least effective in inhibiting growth of tumors with tumor-associated macrophages. This may be due to unique properties of macrophage elastase (MME). Unlike other MMPs, MME is expressed exclusively in host macrophages. A study of MMP expression in human breast cancer demonstrated MME as the single MMP expressed in macrophages associated with tumors [69]. MME has recently been implicated as the enzyme responsible for maintenance of dormancy in lung metastases in the murine Lewis lung cell carcinoma model [70]. The removal of certain primary tumors,

in both experimental models and clinical practice, can be followed by rapid growth of dormant metastases. The experiments resulted in the isolation of an angiogenesis inhibitor termed angiostatin, a fragment of plasminogen containing kringle regions 1–4. Application of angiostatin prevents growth of lung metastases following resection, leading to the conclusion that primary tumors generate angiostatin which is lost upon resection resulting in tumor growth. Subsequently it was demonstrated that generation of angiostatin is not caused by the tumor cells but is associated with the presence of macrophages in the primary tumor. Furthermore, angiostatin activity is correlated with the presumably presence of MME [70]. MME generates angiostatin from plasminogen, resulting in inhibition of endothelial cell proliferation and "tube formation" [71]. Together these findings lead to the conclusion that MME-induced generation of angiostatin may be responsible for tumor growth inhibition. The local production of MME by macrophages in the lung, induced by soluble factors from the primary tumor (perhaps GMCSF) may be responsible for the effects on metastases growth [71]. These data suggest that MME prevents tumor metastases growth, while other MMPs promote tumor progression. MMPs spatially associated with the neovasculature, and capable of interacting with endothelial cells, may promote tumor angiogenesis. In contrast, macrophage elastase expressed by macrophages around the tumor comes in contact with and cleaves plasminogen and perhaps other molecules such as thrombospondin (Ts) or type XVIII collagen generating anti-angiogenic molecules; angiostatin, Ts fragments, and endostatin, respectively. Additionally, MME may have more potent proteolytic capacity and higher binding affinity for elastin and other matrix components than other MMPs, perhaps further enhancing this property. The free C terminal of MME may compete with "pro-angiogenic" MMPs for endothelial cell binding [72].

As the tumor progresses to lymphatic and visceral metastases, the local stromal environment may result in a quite different metalloproteinase profile and dependency [73].

The types of MMP expressed in tumor cells and the surrounding stromal cells depending on the types of tumor [48]. In breast cancer, MMP-3 is expressed in tumor stroma of invasive cancer in a widespread pattern along with MMP-1, MMP-2, MMP-11, and MT1-MMP [40]. In contrast, MMP-9, MMP-12, and MMP-13 are more focal: MMP-9 is expressed at endothelial cells, MMP-13 is associated with isolated tumor cells and MMP-12 with macrophage-like cells [40]. Head and neck carcinoma expresses both MMP-3 and MMP-10 along with MMP-1 and MMP-7. Esophageal squamous cell carcinomas express MMP-2 and MMP-3. Colon and gastric carcinomas express MMP-7 and stromal component of some colorectal carcinomas expresses MMP-3. Elevated MMP-3 transcripts were also detected in the foci of normal mucosa adjacent to bronchial lesions, inter-epithelial lesions, and squamous carcinomas of the lung [48]. In microinvasive and invasive bronchial lesions, the expression of MMP-3 is primarily in the stroma. Cutaneous basal cell carcinomas from patients with nevoid basal cell carcinoma syndrome, an autosomal dominant disorder, overexpress MMP-3 mRNA [48].

The conversion from a squamous cell carcinoma to an aggressive, metastatic spindle cell carcinoma, in mouse skin tumor progression model is associated with the expression of MMP-3 mRNA in the tumor cells as well as in adjacent normal stroma, whereas MMP-3 transcripts are found only in stroma in squamous cell carcinomas. Differences in expression of MMP-3 and MMP-10 in transformed rat embryo cell lines are noted, suggesting a correlation between invasive behavior of tumor cells and the expression of stromelysins [48].

Experiments on transgenic mice demonstrate that the expression of a single matrixin, MMP-3, is insufficient for the progression of mammary tumors to an invasive and metastatic phenotype, but the degradation of matrix would alter the basic process of cell proliferation and apoptosis [48].

Formation of the MT1-MMP-TIMP-2- progelatinase A trimeric complex [74] may lead to subsequent activetion of progelatinase A. The role of TIMP-2 in these interactions is a controversial matter, and while some investigators have shown that TIMP-2 inhibits the cell surface activation of progelatinase A, others suggest it may enhance activation through formation of progelatinase A-TIMP-2 complex, which facilitates binding of the progelatinase A to the cell surface. Some tumor cell lines have been shown to bind progelatinase A without activating the enzyme, suggesting that there is a receptor for progelatinase A that may be distinct from MT1-MMP, such as integrin avfi3 observed on the surface of cultured melanoma cells [67].

Employing sensitive and specific sandwich ELISAs, the levels of total TIMP-2 and gelatinase A-TIMP-2 complex in conditioned media samples from different tumor cell lines established from human melanoma, fibrosarcoma, breast carcinoma, lung carcinoma, and pancreatic carcinoma [48] showed that TIMP-2 may be present in significant excess over the levels of gelatinase A in the culture fluid of many human tumor cells. The results indicate that the ratio of secreted TIMP-2 to gelatinase A in culture varies greatly from one tumor cell type to another, and as such may have a variable influence on the invasive behavior of tumor cells. Model experiments results suggest that most of the progelatinase A produced by cells is immediately complexed with TIMP-2 and that the species that must be activated by the cell is the progelatinase A–TIMP-2 complex.

TIMP-2 selectively binds to progelatinase A and inhibits the protease activity of gelatinase A. Consequently, TIMP-2 can suppress invasion, metastasis, neovascularization, and growth of some rodent and human tumors. TIMP-2 expression is found to be lower in advanced gastric cancers, while gelatinase A expression is either increased or maintained. In addition, patients who died from their primary gastric tumor had higher a percentage of gelatinase A-positive cells and a lower percentage of TIMP-2 positive cells, compared to survivors. In urothelial cancer patients with muscular invasion or with lymph node metastasis, the mean

gelatinase A: TIMP-2 ratio in patients with recurrence is significantly higher than that in patients without recurrence ($p<0.05$) [75]. The disease-free survival of patients with high gelatinase A:TIMP-2 ratios is extremely poor compared with that of patients with lower ratios ($p<0.01$) [75]. The imbalance of the gelatinase A:TIMP-2 ratio examined in lymph-node-positive breast carcinomas as measured by RT-PCR [76] shows a lower ratio between gelatinase tumor/normal and TIMP-2 tumor/normal than in carcinomas without lymph-node involvement. In contrast, in patients with lymph-node metastasis a much higher ratio was observed, suggesting enhanced gelatinase A expression relative to TIMP-2. However, the magnitude of this ratio is not related to the frequency of positive lymph nodes, nor to relapse status at follow-up. These studies indicate that evaluation of gelatinase A: TIMP-2 mRNA balance and/or serum level may constitute an early prognostic indicator in various types of human cancer [76].

MMP-7 and MMP-3, by releasing soluble E-cadherin, a cell adhesion transmembrane protein, inhibit its function, in a paracrine way, acting as a promoter of the tumor cell migration and invasion [77].

MMP-7 transforms the receptor activator of nuclear factor-kappa B-ligand (RANKL) to a soluble form that promotes osteoclast activation in metastatic prostate cancer [77].

Several experiments using transgenic or knockout mice demonstrated the complex and somehow paradoxical effect of MMPs system related to tumor carcinogenesis. Skin carcinogenesis is stimulated by Haptoglobin-MMP-1, by hyperkeratosis and acanthosis and by the absence of MMP-8, by defective inflammatory response, and inhibited by the absence of MMP-9 (probably involved in prolonged contact dermatitis [25, 78, 79]. Mammary carcinogenesis is increased by MMP-3 (involved in epithelial cell apoptosis) and by MMP-14 (involved in mammary hyperplasia), and reduced by the lack of MMP-11 (involved in the mechanism of neointimal formation after vessel injury). Pancreatic carcinogenesis is inhibited by the absence of MMP-2, by a process of reduced angiogenesis, and by the lack of MMP-9 [25, 78, 79]. Tumor growth is reduced by the absence of MMP-2, MMP-7, MMP-11, and metastatic processs is reduced by the lack of MMP-9, and accelerated by the absence of MMP-11 [25, 78, 79].

Although acting through an uncertain mechanism, MT4-MMP is expressed in breast carcinoma and breast cancer cell lines [25].

MMPs roles in tumor invasion by interaction with non-ECM proteins

Although it was initially considered that degradation of ECM represents the main mechanism of MMPs involvement in tumor invasion, intravasation into circulation, extravasation, and migration to metastatic sites [20], there is growing evidence of their complex role in creating and maintaining a facilitating microenvironment for tumor growth at primary and metastatic sites [1].

Complex MMPs roles are performed by regulation of cytokines, growth factors, and cell adhesion molecules. MMP-3, MMP-7, MMP-9, and MMP-19 release IGF (insulin-like growth factor) stimulating tumor proliferation [1]. Surfaced-anchored MMP-9 activates TGF-β (transforming growth factor-β), facilitating tumor invasion and angiogenesis [80]. MMP-2, MMP-3, MMP-7, MMP-9, MMP-12, MMP-13, and MMP-20 release angiostatin, and MMP-3, MMP-7, MMP-9, and MMP-19 release VEGF (vascular endothelial growth factor), stimulating tumor angiogenesis [1]. Another process that can alter cell surface signalling by proteolytically cleavage of a transmembrane molecule in the juxtamembranar region of the extracellular domain is that of ectodomain shedding. This process provokes a cell–tissue interaction by subsequent release of a soluble ectodomain into the pericellular space. The mechanism is involved in tumor proliferation and angiogenesis, by HB-EGF shedding by MMP-3 and MMP-7, in tumor invasion, by E-cadherin shedding by MMP-3 and MMP-7, in tumor apoptosis by TNF-α (tumor necrosis factor-α) shedding by MMP-7 [1].

MMPs roles in angiogenesis

Angiogenesis is important for vascular remodelling and wound healing and its anomalies occur in numerous pathological conditions, such as rheumatoid arthritis, diabetic retinopathy, psoriasis, hemangiomas, and cancer [81]. Angiogenesis is based on migration of endothelial cells into surrounding connective tissues, MMPs possessing a complex role, degrading ECM, releasing ECM-sequestered proangiogenic substances, processing growth factors, integrins, and adhesion molecules [81], and creating a balance between proangiogenic and antiangiogenic effects.

Several pro-angiogenic factors, such as VEGF, basic fibroblast growth factor (bFGF) or transforming growth factor-β (TGF-β) are activated by MMPs, facilitating tumor angiogenesis [80, 82].

MMPs have been shown to have direct effects on endothelial cells, being necessary for cell migration and tube formation [81]. MT1-MMP is mainly involved in endothelial cell migration and invasion of fibrin barriers, due to its fibrinolytic activity [81]. MT1-MMP also processes the αv integrin into two disulfide-linked fragments that retain RGD-ligand binding and this processing enhances integrin signalling through focal adhesion kinase, contributing to enhanced adhesion and cell migration on vitronectin [81].

MMP-2 is responsible by the cleavage of type IV collagen, binding via its PEX domain to exposed cryptic $\alpha v\beta 3$ binding sites (by loss of binding to integrin $\alpha 1\beta 1$ integrin that promotes angiogenesis in tumor blood vessels. Conversely, soluble PEX domain inhibits MMP-2 binding to $\alpha v\beta 3$ and blocks angiogenesis [81]. The key role played by gelatinase A in angiogenesis was demonstrated by experiments using cultures of endothelial cells on matrigel, followed by an increase of the formation of tube networks by the addition of recombinant gelatinase A, and an inhibition both by neutralizing antibody and TIMP-2, suggesting that

network formation is limited by enzyme activity. However, excess enzyme activity is deleterious to this complex morphological change. These results suggest that while gelatinase A activity has an important role in angiogenesis, excessive levels of activity will inhibit the process. Furthermore, inhibitors such as TIMP-2 may both block or facilitate an angiogenic response depending on the level of protease expression [81].

MMP-7 enhances endothelial cell proliferation, up-regulates endothelial expression of MMP-1 and MMP-2, and induces angiogenesis *in vivo* [83].

Membrane vesicles containing MMP-2, MMP-9, and MT1-MMP are contained in endothelial cells, sometimes located near pseudopodia, and angiogenic stimulation of cells with bFGF and or VEGF results in shedding of vesicles. bFGF stimulation by MMP-9 enhances endothelial cell growth *in vitro* [84].

MMP-2, MMP-9, and MMP-7 are expressed in vascular endothelial cells of tumors [85]. Supplementary, MMP-7 stimulates vascular proliferation, as shown by several experiments [83]. Oppositely, matrilysin-specific antisense oligonucleotides inhibit tumoral angiogenesis.

MMPs cleave the ectodomain of VE-cadherin, disrupting cell-to-cell adhesion [81]. MMP-3, MMP-7, MMP-9, and MMP-19 cleave matrix-bound isoforms of VEGF, a potent mediator of angiogenesis, releasing it from the matrix as soluble fragments [86]. Soluble VEGF is less effective than matrix-bound VEGF in angiogenesis though they act through the same cell surface receptor (VEGFR2) [86].

Recent findings have opened new connections between MMPs and angiogenesis generated by the induction of MMP-9 in tumor macrophages and endothelial cells [48]. Contrary to these proangiogenic roles of MMPs, these enzymes also negatively regulate angiogenesis. MMP-2, MMP-7, MMP-9 [85], and MMP-12 [71] generate antiangiogenic polypeptides, by cleavage of plasminogen into angiostatin. Angiostatin inhibits endothelial cell proliferation and promotes endothelial apoptosis.

MMP-3, MMP-7, MMP-9, MMP-13, and MMP-20 generate endostatin (another endogenous inhibitor of angiogenesis), by cleavage of collagen type XVIII [87, 88]. Also, MMPs are able to cleave the precursor of endostatin. Moreover, MMP-7 generates by proteolysis on collagen XVIII, neostatin-7, the C-terminal 28-kDa endostatin-spanning proteolytic fragment [89]. MMP-9 may generate tumstatin, by proteolysis of type IV collagen, with suppression of angiogenesis and tumor growth [90].

MMPs roles in apoptosis

MMPs have the ability to act on substrates that influence apoptosis.

MMP-3 has pro-apoptotic activity on epithelial cells [25].

By shedding the ectodomain of proHB-EGF, mature HB-EGF is formed, promoting cellular proliferation by activating ErbB4 receptor and inhibiting apoptosis. MMP-7 sheds the ectodomain of membrane-bound mFasL from cell membranes to generate soluble FasL (sFasL) [29] that increases apoptosis through activation of Fas, excepting cancer cells that are refractory to this proapoptotic signal, because of proteic abnormalities in the signal transduction cascade [29]. Concomitantly FasL protects cancer cells from chemotherapeutic drug toxicity [91].

MMP-11 suppresses tumor cell apoptosis but, paradoxically, transgenic mice experiments demonstrated another, yet unknown mechanism of action to decrease the rate of metastasis [92].

MMPs roles in escaping mechanisms

MMPs have been associated with a variety of escaping mechanisms that cancer cells develop to avoid host immune response [93]. MMP-9 acts by the suppression of T-lymphocytes proliferation through the disruption of the IL-2Rα signaling [94]. MMP-11 decreases tumor cells sensitivity to natural killer (NK) cells by producing a bioactive fragment from α1-proteinase inhibitor [25].

MMPs roles in antitumor defensive mechanism

MMPs may be beneficial by stimulating protective immune responses.

MMP-8 deficient mutant mice experiments showed that prolonged accumulation of inflammatory cells results in chronic inflammation that facilitates genomic instability and promotion of tumor growth [93].

MMPs role in arthritis

The erosion of articular cartilage accompanies both rheumatoid arthritis and osteoarthritis. The cartilage matrix is composed of collagens (primarily type II collagen with minor collagen types IX and XI) and proteoglycans. Although a number of proteinases have been found in the arthritic joints, matrixins are considered to be key enzymes for the degradation of cartilage matrix [95].

MMP-3 (along with MMP-1 and TIMP-1) was immunolocalized in lining B cells of rheumatoid synovium and the elevated expression of their transcripts was identified by *in situ* hybridization. Both the concentration of MMP-3 in synovial fluid and serum levels of MMP-3 are significantly higher in osteoarthritic patients than in normal individuals [95], and they correlate with the erythrocyte sedimentation rate and C-reactive protein levels [95]. In osteoarthritis, the destructive enzymes largely originate from chondrocytes. The production of MMP-3 in osteoarthritic cartilage is elevated and the immunostaining level of MMP-3 correlates directly with the histological score. The mRNA level for MMP-3 is elevated in arthritic cartilage but not MMP-1 mRNA, suggesting MMP-3 involvement in arthritis [95].

MMP-3 cleaves the core protein of aggrecan and link protein, telopeptides of type II collagen, where intermolecular cross-linking occurs and type IX collagen *in vitro*, acting as a depolymerizer of cartilage collagens [95].

The major enzymes that cleave type II collagen in cartilage are thought to be collagenases (MMP-1, MMP-8, and MMP-13); however, MMP-3 and other MMPs cleave the core protein of aggrecan at the Asn 341–Phe 342 bond located in the region between the G1 and G2 globular domains. On the other hand, the treatment of cartilage explants with IL-1 generates fragments resulting from the cleavage of the Glu 373–Ala 374 bond, 32-amino-acid C-terminal to the major MMP cleavage site. Synovial fluids from patients with osteoarthritis and knee injury indicated that the cleavage of the Glu 373–Ala 374 bond increases [48].

The enzyme responsible for this cleavage, called aggrecanase, is yet to be identified, but inhibition of the Glu 373–Ala 374 bond cleavage by a peptidyl-hydroxamate MMP-inhibitor suggests that it may be a MMP type metalloproteinase. Recent studies indicated that fragments generated by the cleaveage of the Asn 34–Phe 342 bonds are present in synovial fluids in osteoarthritic patients [96]. The G1 fragments generated by MMPs and aggrecanase were immunolocalized in articular cartilage from patients with osteoarthritis and rheumatoid arthritis as well as in normal cartilage. These studies suggest that both MMPs and aggrecanase have the *in vivo* function in releasing aggrecan, under physiological and pathological conditions, possibly by two independent pathways [48].

MMPs in atherosclerosis

In atherosclerotic plaques, MMP-3 was detected in both smooth muscle cells and macrophages. The production of MMP-1, MMP-2, and MMP-9 [97] are also seen primarily in macrophages and smooth muscle cells in the lesion. They are thought to participate in weakening the connective tissue matrix in the intima, which leads to plaque rupture, acute thrombosis, and smooth muscle cell proliferation and migration. The polymorphism analysis of the MMP-3 gene identified a common variant in the promoter, the 6A6A genotype, significantly associated with greater progression to coronary atherosclerosis than other genotypes. It is postulated that reduced levels of MMP-3 expression in the 6A homozygotes contribute to a net increase in matrix deposition, leading to more rapid chronic growth of the atherosclerotic plaque [48].

MMP in pulmonary emphysema

Pulmonary emphysema is a major component of the morbidity and mortality of chronic obstructive pulmonary disease. Emphysema is defined as enlargement of peripheral air spaces of the lung including respiratory bronchioles, alveolar ducts, and alveoli, accompanied by destruction of the walls of these structures. Inherited deficiency of al-AT, the primary inhibitor of neutrophil elastase, predisposes individuals to early-onset emphysema, and intrapulmonary instillation of elastolytic enzymes in experimental animals causes emphysema. Together, these findings led to the elastase:antielastase hypothesis for the pathogenesis of emphysema, which has been the prevailing hypothesis for more than 30 years. However, macrophages, not neutrophils, are the most abundant defensive cell in the lung both under normal conditions and in the lungs of smokers [19].

The capacity of macrophages to degrade elastin and, hence, to contribute to emphysema, was controversial until the identification of elastolytic cysteine proteinases. Subsequently, elastolytic MMPs produced by alveolar macrophages were identified. MME (MMP-12), nearly undetectable in normal macrophages, is expressed in human alveolar macrophages of cigarette smokers. MME may be detected by immunohistochemistry and *in situ* hybridization in macrophages of patients with emphysema, but not in normal lung tissue. MME is required for both macrophage accumulation and emphysema resulting from chronic inhalation of cigarette smoke. Cigarette smoke induces constitutive macrophages to produce MME, which cleaves elastin-generating fragments chemotactic for monocytes. This positive feedback loop perpetuates macrophage accumulation and lung destruction [19].

MMPs in endometriosis

MMPs are also important in the etipathogeny of endometriosis, being involved in the ECM invasion of the endometriotic cells in the ectopic situs. Consequently, increased levels of MMP-1 [98], MMP-2 [99], MMP-7 [100], and MMP-9 [101] were detected in peritoneal fluid of patients with endometriosis. They are stimulated by TNF-α and IL-1. TNF-α seems to decrease the expression of TIMP *in vitro*, increasing the imbalance between MMP and TIMP. The low levels of TIMP-1 (MMP-1, MMP-3, MMP-9 or pro-MMP-9 regulator), by forming complexes, is characteristic for the peritoneal fluid of patients with endometriosis [102]. It was postulated that hemopexine domain expressed by the majority of MMP, excepting MMP-7, is recognized by autoantibodies T-like, in patients with endometriosis [103].

Similarly to cancers, in endometriosis MMP-activity is increased [104], associated with cathepsin D and plasminogen [105].

Intercellular adhesion anomalies, especially produced by β-catenin mutations, are involved both in female genital cancers [106] and in endometriosis (especially produced by E-cadherin mutations) [107–109]. In therapy, catenin alteration, cadherin signalization and selective MMP inhibitors may be useful in endometriosis management [110].

Synthetic MMPs inhibitors

Although MMP involvement in pathology is more than simple excessive matrix degradation, MMP inhibition may be of therapeutic benefit. The first disease target was rheumatoid arthritis but the range of potential applications has broadened to the treatment of cancer, now in the stage of clinical testing [73].

The first synthetic MMP inhibitor was developed in the early 1980s, as a pseudopeptide derivative, based on the structure of the collagen molecule at the site of initial cleavage by interstitial collagenase. Compounds designed from the Ile–Ala–Gly and Leu–Leu–Ala sequences on the right-hand side of the cleavage site have

emerged as the most promising drugs. The inhibitor binds reversibly at the active site of the matrix metalloproteinase in a stereospecific manner. The zinc-binding group, in this case, hydroxamic aid (–CONHOH), is then positioned to chelate the active site zinc ion. Modification of the stereochemistry of the molecule results in loss of inhibitory activity. Compounds, typified by the hydroxamate batimastat, showed broad specificity for members of the MMP family but displayed little detectable activity against other classes of metalloproteinase such as angiotensin-converting enzyme and enkephalinase [68]. Early studies with batimastat investigated the effect of the inhibitor on the growth of different types of tumors [111, 112]. Although fibrotic changes have been observed in some models, in others there are no histopathological clues as to why the rate of tumor growth has been reduced. Inhibition of angiogenesis has been suggested as a mode of action. The batimastat treated tumors are less frequent and smaller than the controls and are characterrized by an enlarged necrotic center. This increase in necrosis may be the result of antiangiogenic activity. Alternatively, it is possible that in certain tumors constriction of invasive growth by MMP inhibitors results in increased interstitial pressure. This in turn leads to compression of blood vessels in the center of the tumor causing ischemia and subsequent necrosis [113]. Recurrence of human breast carcinoma at the site of primary tumor resection could be significantly inhibited by batimastat, as could the number and size of lung metastases [114]. Batimastat has also been shown to inhibit the growth of secondary lesions, in the form of lung metastases and lymphatic metastases [115].

The next steps in the design of synthetic MMP inhibitors were made with two principal objectives: the development of compounds with improved oral bioavailability and the development of compounds with selective inhibitory activity against individual matrix metalloproteinases, assisted by X-ray crystallography data on the three-dimensional structure of the collagenase active site [116]. Marimastat was one of the first inhibitors with improved oral bioavailability, being different from its predecessor batimastat by the groups adjacent to the hydroxamate and at the P2'-position. In both positions a small substituent, hydroxyl and *t*-butyl respectively, replaces a larger cyclic group. The substitutions may reduce the compound's susceptibility to peptidases, improve absorption, or reduce first-pass metabolism [117].

More selective compounds have also been developed, showing a selective loss of activity against one or more of the enzymes. A series of compounds takes advantage of differences in the active site of gelatinase A and B that allow larger hydrophobic groups at the PI'-position. These compounds show greater than 1000-fold selectivity for gelatinases over interstitial collagenase but are also quite potent inhibitors of stromelysin-1, like CDP-845 (Celltech), a potent selective gelatinase inhibitor, Ro32-3555 (Roche), a hydroxamate-based inhibitor with relatively weak activity against gelatinase A and stromelysin-1, but good activity against interstitial collagenase, developed as a treatment for rheumatoid arthritis [118].

Hydroxamates, low-molecular-weight MMP inhibitors, have also been developed from natural products, such as BE16627B (Banyu) and matlystatin B (Sankyo) [119].

Inhibitors have also been obtained through chemical modification of the tetracycline family of molecules where it has been possible to separate the antibiotic and protease inhibitory activities [120]. These compounds can block human fibrosarcoma cell invasion through reconstituted BMs and inhibit lung colonization by mouse melanoma cells, leading to a focus on these compounds as antimetastatic agents.

However, a broader anticancer activity, by reducing the rate of collagen degradation and simultaneously stimulating collagen biosynthesis, lead to the concept of "encapsulation" of either secondary or primary lesions. It has been proposed that MMP inhibitors could inhibit tumor growth either by encouraging the development of fibrotic tissue around the tumor, thereby preventing invasive growth, or by inhibiting angiogenesis [73].

Synthetic MMP inhibitors have also been shown to reduce the growth rate of subcutaneously implanted tumors, such as hydroxamate inhibitors GI168 and GI173 (GlaxoWellcome) [121]. Histological analysis revealed no obvious effect on tumour angiogenesis although treatment was associated with increased tumor necrosis. Both inhibitors are generally broad-spectrum agents although GI168 is a relatively poor stromelysin-1 inhibitor and GI173 shows a preference for gelatinases.

Studies with BE16627B, a natural MMP inhibitor with a broad spectrum of activity, have shown that it can inhibit the subcutaneous growth of tumors composed of cells with detectable metalloproteinase activity *in vitro*.

The related batimastat compound, ilomastat (GM6001, Glycomed), inhibits angiogenesis in experimental models. In studies where antiangiogenic activity has been examined there have been no clear changes in blood vessel density [114, 121] but in these models the tumors that do develop may be resistant to the effects of metalloproteinase inhibitors.

However, although cancer models provide an indication of what might be expected in the clinic, they are applied early when the tumor diameter is still only a few millimeters and they lack one important element of human malignancy, namely, stromal tissue, which is known to be the source of most MMP activity. It is possible, therefore, that effects in the clinic may be more pronounced than the activity seen in animals.

The development of low-molecular-weight MMP inhibitors with good oral bioavailability represents an important milestone in chronic conditions requiring long-term treatments over months and years. Replacement of specific groups with the intention of shielding the peptide backbone and of increasing hydrogen bonding has led to the development of inhibitors with improved oral bioavailability, without loss of inhibitory potency.

Six MMP inhibitors are during clinical trials in patients as oral treatments: AG3340 (Agouron), CGS-27023A (Novartis), BAY-9566 (Bayer), and marimastat

(British Biotech) in cancer patients and D5410 (Chiroscience) and Ro32-3555 (Roche) in patients with rheumatoid arthritis [73, 117, 118].

A series of studies in patients with advanced malignancy examined the effect of different doses of marimastat on the serum cancer antigens CA125, CEA, PSA, and CA19-9, considered surrogate markers of disease progression, observing a dose related reduction of their serum concentration [73]. In advanced gastric cancer, treatment with marimastat may be associated with changes in the macroscopic and histological appearance of the tumors consistent with an increase in the quantity of fibrotic stromal tissue, similar to those seen in various cancer models, and alterations in tumor: stroma ratio [73]. Marimastat is also being studied in combination with carboplatin in patients with advanced ovarian cancer [73], preclinical studies suggesting increased therapeutic activity of the combination between MMP inhibitors and cytotoxic agents [122].

Matrixins are almost completely inhibited by chelating agents such as EDTA, EGTA, or 1,10-phenanthroline. About 50% inhibition of MMP-3 is observed with cysteine or dithiothreitol. Examples of synthetic MMP-3 inhibitors include phosphonamidate compounds, pseudopeptide-hydroxamate compounds [123], and non-peptide hydroxamate inhibitors [124].

Until 2006, at least 56 matrix metalloproteinase (MMP) inhibitors have been pursued as clinical candidates. The two primary indications that have been targeted are cancer (24 drugs) and anti-arthritis (27 drugs). Cardiovascular disease was listed as an indication for 10 drugs. Forty-six MMP inhibitors (MMPi) have been discontinued, seven remain in clinical development, and only one (PeriostatR for periodontal disease) has been approved [125]. To date, only three MMPi's have been assessed clinically for cardiovascular indications: doxycycline (MIDAS – Metalloproteinase Inhibition with submicrobial doses of Doxycycline to prevent Acute coronary Syndromes and doxycycline Hyclate-PeriostatR for acute coronary syndrome), batimastat (BRILLIANT–EU – Batimastat antiRestenosis trIaL utiLiz-Ing the BiodivYsio locAl drug delivery PC steNT or broad-spectrum inhibitor batimastat), eluting stents for restenosis, and PG-116800 (PREMIER – PREvention of MI Early Remodeling) for the prevention of post-ischemic left ventricular dilation. In all three studies, there was no significant difference between the drug and non-drug treated groups. Moreover, MMP inhibitors, which are potent zinc chelators such as batimastat and PG-116800, appear to induce the musculoskeletal syndrome, manifested as a tendonitis-like fibromylagia, the major side-effect limiting dose selection [125].

Marimastat was chosen as a reference agent because it is the only MMPi with sufficient clinical information available to illustrate the therapeutic index and to test the hypothesis that clinical dose selection may have been below the minimal effective dose. Marimastat works preclinically, improving median survival time and suppressing tumorigenesis in a variety of mouse cancer models [125].

The affinity of most MMPi's depends primarily upon two factors: a chelating moiety that interacts with the catalytic zinc ion, and hydrophobic extensions protruding from the catalytic site in the hydrophobic S1V-subsite, a deep pocket containing the catalytic zinc ion. The specificity of MMPs results from the shape of the hydrophobic S1'-pocket. The S2'-subsite is a shallow pocket open to solvent with minimal protein-inhibitor interactions. Drug design has focused on utilizing S2V-interactions to build scaffolds of MMPi's to enhance physicochemical and pharmacokinetic properties without affecting MMP binding affinity. The S3'-subsite is located on the periphery of the MMP active site, and drug design around this site typically produces little gain in inhibitor affinity. To date, MSS has appears to be intrinsic to MMP inhibitors which chelate zinc. Recently, micromolar inhibitors have been described which occupy the S1V pocket of MMP-13 that does not interact with catalytic zinc. Nanomolar pharmacophores have been described which occupy the S1V-pocket without interacting the catalytic zinc based on crystal structure analysis. In addition, one of the MMP-13 inhibitors has 50% oral bioavailability thus indicating that it may be suitable for *in vivo* use [125].

Continuous researches on the MMP/TIMP involvement in different diseases may develop new and revolutionary therapies.

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