Chapter 10
Matrix Metalloproteinases in Cancer Metastasis: An Unsolved Mystery

Shravanti Mukherjee, Argha Manna, Minakshi Mazumdar, and Tanya Das

Abstract Tumor progression is a complex, multistage process by which a normal cell undergoes genetic changes that result in phenotypic alterations and acquisition of the ability to spread and colonization to distant sites in the human body. Understanding the molecular mechanisms of metastasis is crucial for developing novel therapeutic strategies to combat metastatic cancers. Early studies established the importance of the extracellular matrix on tumor cell growth and differentiation. With time, the role of the extracellular matrix and matrix metalloproteinases (MMPs), a family of degradative enzymes, in the regulation of tumor invasion, metastasis, and angiogenesis was recognized. Initially, it was believed that the major role of MMPs in metastasis was to facilitate the breakdown of physical barriers to metastasis, thus promoting invasion and entry into and out of blood or lymphatic vessels (intravasation, extravasation). However, recent evidence suggests that MMPs may have a more complex and divergent role in metastasis as well as in cancer stem cell maintenance. In the present review, the role of MMPs and their functional contribution in metastasis have been revisited and discussed. Upcoming approaches target MMPs and their inhibitors, e.g., tissue inhibitors of metalloproteinases (TIMPs), genetically or pharmacologically, suggesting that MMPs are key regulators of growth of tumors, both at primary and metastatic sites. These evidences present MMPs as the important candidates in creating and maintaining an environment that supports the initiation and maintenance of growth of primary and metastatic tumors. Future endeavors to target matrix metalloproteinases would be important in the development of novel therapeutic strategies against metastatic cancers.

Keywords Cancer stem cell • Epithelial to mesenchymal transition • Invasion • Matrix metalloproteinases • Metastasis • Migration • Tissue inhibitors of metalloproteinases

S. Mukherjee • A. Manna • M. Mazumdar • T. Das (✉)
Division of Molecular Medicine, Bose Institute, P-1/12, CIT Scheme VII-M,
Kolkata 700054, West Bengal, India
e-mail: tanya@boseinst.ernet.in; akm@tezu.ernet.in

N.S. Dhalla and S. Chakraborti (eds.), *Role of Proteases in Cellular Dysfunction*,
Advances in Biochemistry in Health and Disease 8, DOI 10.1007/978-1-4614-9099-9_10,

1 Introduction

Cancer originating from mutations in genes that regulate essential pathways of cell function leading to uncontrolled outgrowth of tissue cells [1] seems to be one of the leading causes of disease and mortality worldwide [2]. The tumors are complex structures of malignant cancer cells embedded in vasculature and surrounded by a dynamic tumor stroma consisting of various nonmalignant cells, such as fibroblasts and myeloid cells. The milieu of the tumor microenvironment is akin to the inflammatory response in a healing wound, which promotes angiogenesis, turnover of the extracellular matrix (ECM), and tumor cell motility. Understanding the molecular mechanisms of this complex interplay between malignant cancer cells and the surrounding nonmalignant stroma represents one of the major challenges in cancer research. However, the past two decades of biomedical research have yielded an enormous amount of information on the molecular events that take place during carcinogenesis and the signaling pathways participating in cancer progression. Our laboratory has also marked a significant contribution to elucidate the key molecular machineries responsible for carcinogenesis along with the therapeutic approaches using dietary polyphenols [3–8].

Metastasis is a cascade of linked sequential steps involving multiple host-tumor interactions [9]. To successfully create a metastatic colony, a cell or group of tumor cells must be able to leave the primary tumor, invade the local host tissue, enter the circulation, arrest at the distant vascular bed, extravasate into the target organ interstitium and parenchyma, and proliferate as a secondary colony. During invasion, tumor cells disobey the social order of organ boundaries and cross into tissues where they do not belong. The mammalian organism is divided into a series of tissue compartments separated by the extracellular matrix unit consisting of the basement membrane and its underlying interstitial stroma [10]. The basal cells of the epithelium or organ parenchymal side of this unit are attached to the basement membrane. On the opposite side, the interstitial stroma contains stromal cells, fibroblasts, and myofibroblasts. During all types of benign tissue remodeling, proliferative disorders, and carcinoma in situ, the cell populations on either side of this connective tissue unit do not intermix. Only during the transition from in situ to invasive carcinoma do tumor cells penetrate the epithelial basement membrane and enter the underlying interstitial stroma to interact with the stromal cells. Thus, a definition of the behavior of the metastatic tumor cell is the tendency to cross tissue compartment boundaries and intermix with opposite cell types [11]. The continuous basement membrane is a dense meshwork of collagen, glycoproteins, and proteoglycans which normally does not contain any pores large enough for passive tumor cell transversal. Consequently, invasion of the basement membrane must be an active process. Once the tumor cells enter the stroma, they gain access to lymphatics and blood vessels for further dissemination.

Interactions of the tumor cell with the basement membrane can be separated into three steps: attachment, matrix dissolution and migration. The first step is binding of the tumor cell to the basement membrane surface-mediated cell-surface receptors

of the integrin [12] and non-integrin [13] variety. Matrix receptors recognize glycoproteins such as laminin, type IV collagen, and fibronectin in the basement membrane. Two to eight hours after attachment, a localized zone of lysis is produced in the basement membrane at the point of tumor cell contact. Tumor cells directly secrete degradative enzymes [14] or induce the host to elaborate proteinases to degrade the matrix and its component adhesion molecules. Matrix lysis takes place in a highly localized region close to the tumor cell surface [15], where the amount of active enzyme outbalances the natural proteinase inhibitors present in the serum, those in the matrix, or that secreted by normal cells in the vicinity. Locomotion is the third step of invasion which propels the tumor cell across the basement membrane and stroma through the zone of matrix proteolysis.

During metastatic dissemination, cancer cells activate a complex molecular machinery to migrate through the surrounding extracellular matrix (ECM) and intravasate into blood or lymphatic vessels [16]. To negotiate barriers to cell migration, cancer cells secrete their own proteolytic enzymes or induce their expression in other cells through the release of cytokines (e.g., endothelial cells, tumor-infiltrating fibroblasts or leukocytes) [17]. In particular, matrix metalloproteinases (MMPs) are considered key players in tumor progression because of their ability to remodel the ECM and cleave/activate membrane-bound and matrix molecules, and cytokines that stimulate cancer cell migration and proliferation [18].

Studies conducted over more than 40 years have revealed mounting evidence supporting that extracellular matrix remodeling proteinases, such as matrix metalloproteinases (MMPs), are the principal mediators of the alterations observed in the microenvironment during cancer progression. MMPs belong to a zinc-dependent family of endopeptidases implicated in a variety of physiological processes, including wound healing, uterine involution and organogenesis, as well as in pathological conditions, such as inflammatory, vascular and auto-immune disorders, and carcinogenesis [19]. MMPs have been considered as potential diagnostic and prognostic biomarkers in many types and stages of cancer [20]. The notion of MMPs as therapeutic targets of cancer was introduced 25 years ago because the metastatic potential of various cancers was correlated with the ability of cancer cells to degrade the basement membrane. Subsequently, a growing number of MMP inhibitors (MMPIs) have been developed and evaluated in several clinical trials. Recent report from our laboratory also suggested that downregulation of MMPs like MMP-2 and MMP-9 is prerequisite for the anti-migratory effect of the flavins in breast cancer cells [21].

2 Matrix Metalloproteinases: What Is So "Mysterious" About These Enzymes?

The matrix metalloproteinases (MMPs), also called matrixins, are a group of genetically distinct but structurally related calcium-dependent zinc-containing endopeptidases that are involved in the degradation and repair of major macromolecular components of extracellular matrix (ECM), connective tissue, and cell-surface-bound

molecules. They are naturally occurring proteolytic enzymes found in most mammals that are secreted especially by mesenchymal cells, macrophages, and polymorphonuclear leukocytes [22]. A large set of experimental data indicated that MMPs play essential roles in the processes of tissue remodeling and repair, morphogenesis, angiogenesis, embryonic development, apoptosis, ovulation, neural development, wound healing, chemotherapy-induced alimentary tract (AT) mucositis, cell adhesion and proliferation as well [23]. Moreover, these enzymes have frequently been detected in human tumor specimens and their production and/or misregulation has been associated with the tumor aggressiveness and poor prognosis [24, 25].

Under normal physiological conditions, the expression and activity of these enzymes are very low and strictly controlled by endogenous specific tissue inhibitors of metalloproteinases (TIMPs). Generally, there are a total of four TIMPs (TIMP-1, -2, -3 and -4) and these four protein inhibitors are able to control the proteolytic activity of all MMPs and mediate the stability of cells. That is, overexpression or high activation of MMPs has been causally linked with the pathological destruction of connective tissue and the ensuing pathological disorders characterized by the breakdown of ECM components or connective tissues [26]. These diseases include cancer, osteoarthritis (OA), rheumatoid arthritis (RA), angiogenesis, chronic periodontitis, pulmonary emphysema, skin ulceration, atherosclerosis, gingivitis, central nervous system disease, type I diabetes, myocarditis and dilated cardiomyopathy, coronary artery disease, multiple sclerosis (MS), congestive heart failure and cardiovascular disease [27]. On the basis of their primary roles in various oncologic events, the MMPs have been a highly active set of targets for the design of therapeutic agents to intervene the MMP-related pathological states, such as carcinogenesis and arthritis [28]. Here, we review the recent advances in our understanding of MMP-driven regulation of the tumor invasion.

2.1 Domain Structures of Matrix Metalloproteinases

Matrix metalloproteinases belong to a family of zinc- and calcium-dependent endopeptidases called metzincin. The endopeptidases belong to the wide metzincin group, in turn constitutes one of several metalloendopeptidase families; according to their structural characteristics, all the metzincins are mainly subdivided into astacins, ADAMs/adamalysins/reprolysins, serralysins, matrix metalloproteinases/matrixins, snapalysins, leishmanolysins, and pappalysins [29]. All the metzincins are mostly multidomain proteins with approximately 130–260-residue globular catalytic domains showing a common core architecture characterized by a long zinc-binding consensus motif, HEXXHXXGXX(H/D), and a methionine-containing Met-turn. Metzincins have been characterized to participate in unspecific protein degradation such as digestion of intake proteins and tissue development, maintenance, and remodeling, but they are also involved in highly specific cleavage events to activate or inactivate themselves or other (pro)enzymes and bioactive peptides [30]. Among these proteinases, the matrix-degrading metalloenzymes are the most common enzymes, mainly named

matrixins or matrix metalloproteinases (MMP). They form a multigenic family of proteolytic calcium-/zinc-dependent enzymes (expressed as 26 distinct proteins), functioning at neutral pH, secreted in their latent form (proenzymes or inactive zymogens or pro-MMPs), and requiring proteolytic activation [31].

2.2 Structural Classification of Matrix Metalloproteinases

MMPs are a family of zinc-dependent endopeptidases first described almost half a century ago [32]. They play a crucial role in various physiological processes including tissue remodeling and organ development [33], in the regulation of inflammatory processes [34], and in diseases such as cancer [35]. The general structural blueprint, shared by 23 MMPs, shows three domains that are common to almost all MMPs - the pro-peptide, the catalytic domain, and the hemopexin-like C-terminal domain that is linked to the catalytic domain via a flexible hinge region. MMPs are initially expressed in an inactive state due to the interaction of a cysteine residue of the pro-domain with the zinc ion of the catalytic site. Only after disruption of this interaction by a mechanism called cysteine switch, which is usually mediated by proteolytic removal of the pro-domain or chemical modification of the cysteine residue, does the enzyme become proteolytically active. The pro-domain contains a consensus sequence and requires proteolytic cleavage by convertases, which, depending on the sequences, occurs intracellularly by furin or extracellularly by other MMPs or serine proteinases such as plasmin [36].

Structurally related members of MMPs can be broadly classified into five subfamilies based on the variation in their primary structure and function, substrate specificity, as well as their cellular sources: collagenase group (MMP-1, -8, -13, -18), gelatinase group (MMP-2, -9), stromelysin group (MMP-3, -10, -11), membrane-type (MT)-MMP group (MMP-14, -15, -16, -17), and a nonclassified group (MMP-7, -12) [37] (Fig. 10.1). This superfamily shares a conserved structural topology comprising a catalytic domain containing three histidines that constitutes the zinc-binding site and a "methionine-turn" motif that lies beneath the active site zinc ion. The ion-binding motif reads HEBXHXHBGBXHZ, where histidine (H), glutamic acid (E), and glycine (G) are invariant, B is a bulky hydrophobic residue, X is a variable residue, and Z is a family-specific amino acid (serine in MMPs). All MMPs have an N-terminal hydrophobic signal sequence, i.e., predomain, which leads their secretion into the extracellular space after the synthesis in the endoplasmic reticulum. Predomain is followed by a 77–87 amino acid-long pro-domain that constitutes the N-terminus of the secreted enzyme and maintains it in its latent form until its removal or disruption. The prodomain keeps the enzyme inactive through a mechanism identified as "cysteine switch" where the unpaired cysteine in the highly conserved "Pro-Arg-Cys-Gly-X-Pro-Asp" sequence forms a bridge with the catalytic zinc, thereby preventing enzymatic activity. The enzyme acquires total proteolytic capacity when the prodomain becomes chemically removed by cleavage [38]. The active site is of great importance: it specifically binds to selective substrates by

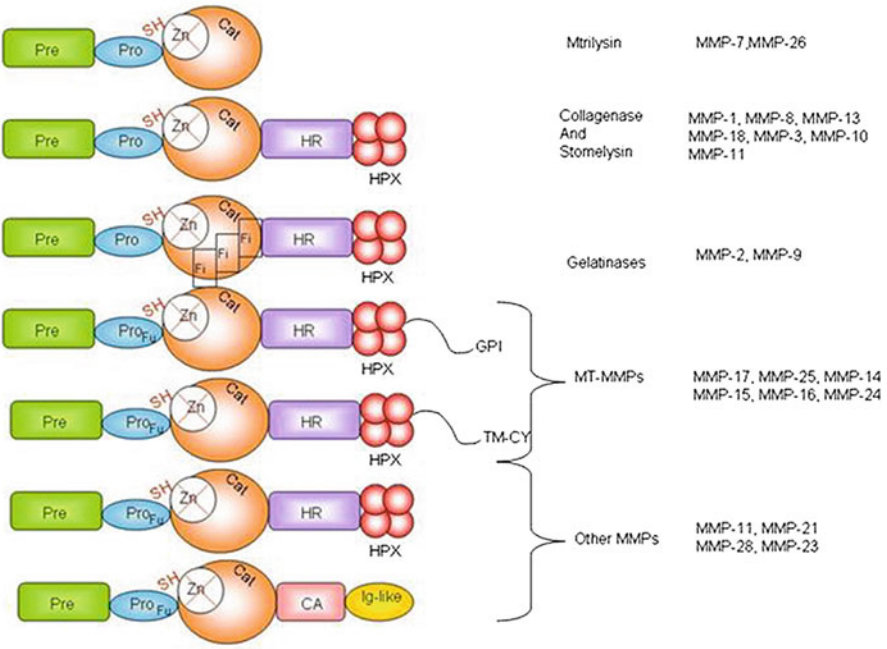

Fig. 10.1 Conserved domain structures of different groups of MMPs. *SH* zinc interacting thiol, *Fi* insert that resemble collagen-binding type II repeats of fibronectin, *Fu* furin cleavage site, *Zn* zinc, *HR* hinge region, *CA* cysteine array, *Cat* catalytic domain, *HPX* hemopexin domain, *GPI* glycosylphosphatidylinositol, *TM* single span transmembrane domain, *CY* short cytoplasmic domain, *MMP* matrix metalloproteinases, *MT-MMPs* membrane-type metalloproteinases, *Ig* immunoglobulin

means of its active site cleft, through specificity subsite pockets that bind amino acids adjacent to the scissile peptide bond, and through secondary substrate binding exosites located outside the active site [39]. These domains represent the minimal structure of MMPs found in MMP-7 (matrilysin) and MMP-26 (endometase/matrylisin-2) which lack any other domain. All the other MMPs have a hinge region varying in length and composition which also influences substrate specificity, and a four-blade structure representing the hemopexin/vitronectin-like domain [40, 41]. Two metalloproteinases, MMP-2 and MMP-9 (also named gelatinase A and B), are further characterized by the presence of three head-to-tail cysteine-rich repeats within the catalytic domain [42]. This structure resembles the collagen-binding type II repeats of fibronectin and is necessary for the binding and cleavaging activities of these MMPs. Not all MMPs are secreted enzymes; membrane-type (MT) MMPs have been identified to contain a single-pass transmembrane domain and a short cytoplasmic C-terminal tail or to be anchored to the cell membrane by a glycosylphosphatidylinositol anchor [43].

Most MMPs are secreted as latent precursors (zymogens) that are proteolytically activated in the extracellular space (Fig. 10.2), with the exception of MMP-11 and

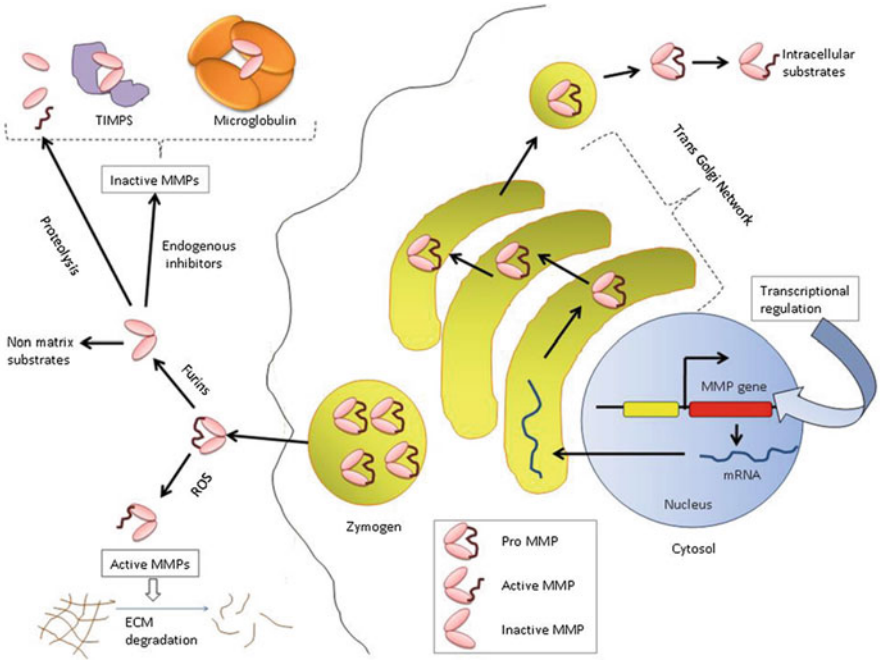

Fig. 10.2 General overview of activation and inactivation mechanism of MMPs within cytoplasm and extracellular space. *TIMP* tissue inhibitor of metalloproteinases, *ECM* extracellular matrix, *ROS* reactive oxygen species, *MMP* matrix metalloproteinases

MT1-MMP, which are activated prior to secretion by Golgi-associated, furin-like proteases. The activity of MMPs in extracellular space is specifically inhibited by tissue inhibitors of metalloproteinases (TIMPs), which bind to the highly conserved zinc-binding site of active MMPs at molar equivalence.

3 Regulation of Matrix Metalloproteinases Activity in Tumor Milieu: The Mysterious Interdependence

The complexity of the tumor microenvironment allows a variety of regulatory cascades to determine the functions of the diverse MMPs expressed. Proteolytic activity of MMPs can be regulated at different levels, i.e., gene expression, compartmentalization, conversion from zymogen to active enzyme, and, finally, the presence of specific inhibitors. While judging the patho-physiological relevance of increased expression of proteinases in tumor tissues, it is important to judge whether endogenous inhibitors or activating/converting enzymes are present in the microenviornment. A key step in regulating MMP activity is the conversion of the zymogen into an active proteolytic enzyme (Fig. 10.3). There are several proteinases that mediate

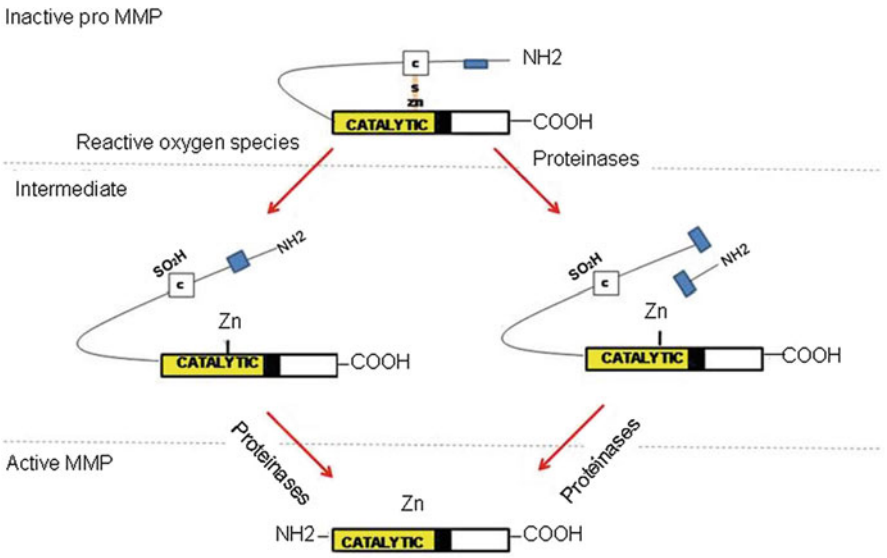

Fig. 10.3 Molecular mechanism of conversion of inactive pro-MMPs into active form of MMPs. *Zn* zinc, *MMP* matrix metalloproteinases, *S* sulfhydryl group

MMP activation, such as plasmin, furin, or active MMPs [44, 45]. The function of MMPs can also be influenced by reactive oxygen species (ROS). The inflammatory response at the tumor site creates large amounts of ROS that are produced by activated neutrophils and macrophages. These oxidants initially activate MMPs via oxidation of the pro-domain cysteine [46] but, eventually, in combination with the enzyme myeloperoxidase contributed by inflammatory cells, inactivate MMPs by modification of amino acids of the catalytic domain by hypochlorous acid [47].

The localization or compartmentalization of MMPs under physiological conditions often dictates their biological function. Several MMPs interact with surface receptors such as integrins or localize to specific areas of the ECM, which potentiates MMP activity by increasing their local concentration and also may interfere with accessibility to endogenous inhibitors [48]. The binding of MMP-2 to integrin αvβ3 via its hemopexin domain is crucial for mesenchymal cell invasive activity [49]. Likewise, high local concentrations of active MMP-14 on the cell membrane of metastatic cancer cells play important roles in cell migration [50]. However, there may also be additional mechanisms to concentrate extracellular proteinases in specific sites in the microenvironment.

Mechanical forces contribute to tumor progression [51], potentially by modulating proteolysis of ECM components. These forces may unwind the conformation of MMP substrate proteins, thus allowing recognition and cleavage by proteinases. For example, the ECM component fibronectin is unfolded by mechanical forces in the ECM of living cells and this unfolded fibronectin then acts as potent MMP substrates [52]. Tumor progression is frequently characterized by increased tissue stiffness, elevated interstitial fluid pressure, and altered blood flow conditions [53].

Thus, it is conceivable that similar mechanisms involving mechanical force are regulatory factors for MMP function in the tumor microenvironment.

The expression and activity of MMPs is regulated at the transcriptional level by cytokines and growth factors and after secretion by endogenous natural inhibitors. The tissue inhibitors of matrix metalloproteinases (TIMPs) provide a negative control of MMP-activity. Four various inhibitors of metalloproteinases characterized so far, are designated as TIMP-1, TIMP-2, TIMP-3, and TIMP-4. Among them, TIMP-1 and TIMP-2 have been characterized most extensively. The TIMPs inhibit active MMPs by forming 1:1 stoichiometric non-covalent complexes with the endopeptidase [54]. TIMP-1 and TIMP-2 are capable of inhibiting the activities of all known MMPs except MT-MMPs, and play a key role in MMP-driven different physiological processes. Moreover, TIMP-1 can also complex with the precursor of MMP-9 [55], whereas TIMP-2 and TIMP-4 can bind to the zymogen form of MMP-2, a 92-kDa type IV procollagenase [56]. TIMP-3 inhibits not only the activity of MMP-1, -2, -3, -9 and -13 [57], but also the activity of MT-MMPs as well as TNF-α converting enzyme. However, the role of TIMPs is not restricted to the inhibition of MMPs. They possess growth-promoting activities for various cell types as well as having antiangiogenic and proapoptotic properties [58].

4 Matrix Metalloproteinases in Cancer Progression: The Mystery Revisited

During development of carcinogenesis, tumor cells participate in several interactions with the tumor microenvironment involving extracellular matrix (ECM), growth factors and cytokines associated with ECM, as well as surrounding cells (endothelial cells, fibroblasts, macrophages, mast cells, neutrophils, pericytes and adipocytes) [59]. Four hallmarks of cancer that include migration, invasion, metastasis and angiogenesis are dependent on the surrounding microenvironment. Critical molecules in these processes are MMPs because they degrade various cell adhesion molecules, thereby modulating cell–cell and cell–ECM interactions.

The emerging view, reflected by several studies, reveals that the expression and role of MMPs and their natural inhibitors, i.e., tissue inhibitor of metalloproteinases (TIMP), is quite diverse during cancer development. The overexpression of MMPs in the tumor microenvironment depends not only on the cancer cells, but also on the neighboring stromal cells, which are induced by the cancer cells in a bidirectional paracrine manner. Cancer cells stimulate host cells (e.g., fibroblasts) and are themselves stimulated by host cell (e.g., neutrophil) to constitute an important source of MMPs through the secretion of interleukins and growth factors and direct signaling through extracellular MMP inducer [60, 61].

Recent studies show that members of the MMP family exert different roles at different stages during cancer progression (Fig. 10.4). In particular, they may promote or inhibit cancer development depending among other factors on the tumor stage, tumor site (primary, metastasis), enzyme localization (tumor cells, stroma), and substrate profile.

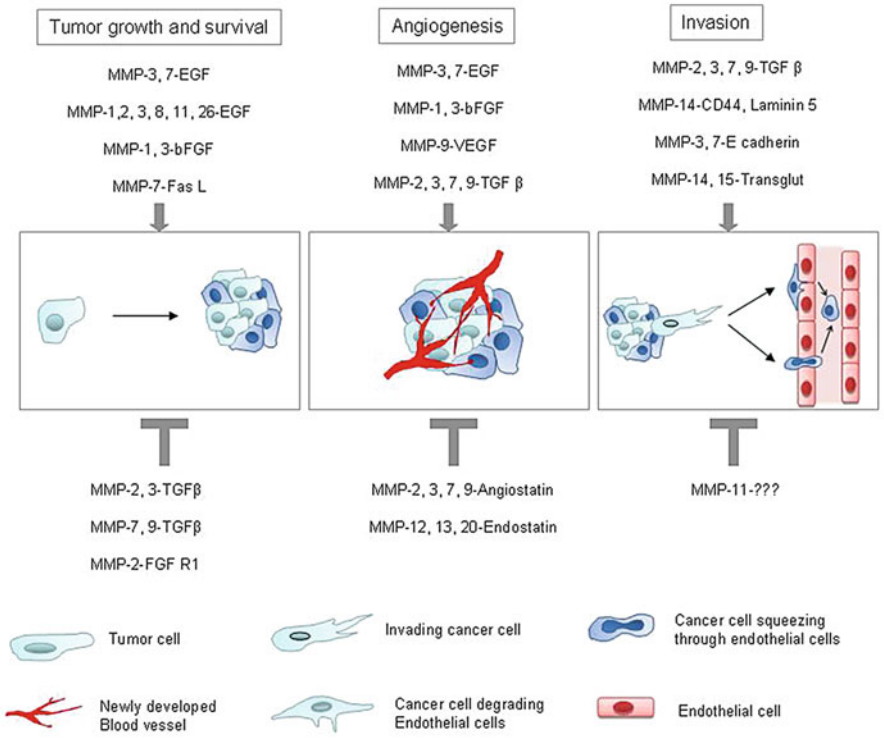

Fig. 10.4 Different stages of metastatic tumor progression (tumor growth and survival, angiogenesis, intravasation and extravasation) are positively and negatively regulated by different MMPs. *EGF* epidermal growth factor, *IGF* insulin-like growth factor, *TGF β* transforming growth factor-beta, *bFGF* basic fibroblast growth factor, *FGF-R1* fibroblast growth factor receptor 1, *MMP* matrix metalloproteinases, *VEGF* vascular endothelial growth factor, *FasL* Fas ligand, *Transglut* transglutaminase

5 Matrix Metalloproteinases in Tumor Metastasis: A Tale of the Mysterious Mediators

The extracellular matrix holds cells together and maintains the three-dimensional structure of the body. It also plays critical roles in cell growth, differentiation, survival and motility. For a tumor cell to metastasize from the primary tumor to other organs, the collagen-rich ECM and basement membrane that are the physical barriers for cell migration must be degraded. The key enzymes responsible for ECM breakdown are MMPs that actively fuel the progression of cancer from localized growth to the invasion of surrounding tissues and the development of distant organ metastasis. Also there is a cooperation between these two components, i.e., ECM and MMPs, enabling the tumor cell to reach its target organ and survive.

However, the classic view that these enzymes simply provide a mechanism for the breakdown of connective tissue barriers has been challenged.

5.1 Matrix Metalloproteinases in Extracellular Matrix Degradation and Distant Metastasis

Although all five major classes (serine, aspartic, cysteine, threonine, and metalloproteinases) are involved in metastasis, a great deal of emphasis has been placed on the type IV collagenases, MMP-2 and MMP-9 [62]. Type IV collagen is a major structural protein in the basement membrane and ECM. A number of studies have linked elevated MMP-2 and MMP-9 levels with an increased metastasis. The conclusions which can be drawn thus far are that the number and the relative levels of MMPs increase with tumor progression.

Several recent studies have been done to try and characterize the phenotypic and enzymatic profiles of more aggressive tumor cell lines. Selection of progressively more invasive human lung carcinoma cells from an established CU cell line revealed that the more invasive cells had a higher expression of MMP-9. These cells had a four- to sixfold increase in invasive activity over the parental and had an increased metastatic potential in vivo. MMP-9 has also been shown to be overexpressed in advanced stage melanoma cells and in breast cancer cell lines. Other tumor models involving MMP-9 in their invasive phenotype include human non-Hodgkin's lymphoma cells and human giant cell tumors [63]. MMP-2 has also been observed to be overexpressed in more aggressive tumor cells. The level of pro-MMP-2 vs. active MMP-2 also plays a role in determining invasive and metastatic capacity of pancreatic tumor cells towards regional lymph node [64–66]. Cell lines displaying an intermediate level of activation were the most invasive while those cells with a high level of activation were the least invasive. This is probably due to the balance required between MMPs and TIMP to create a controlled proteolytic system.

Although the major role of MMPs in metastasis has been inferred from the in vivo and in vitro data presented above to be breakdown of the ECM, recent studies have proposed additional roles for the MMP family. Most of the in vivo and in vitro assays designed to examine the role of MMPs on tumor invasion measure the end results, as in the number of micrometastases formed. The mechanism, however, remains unknown. Intravital video microscopy (IVVM) allows for the observation of the metastatic cascade by following the tumor cell through the microcirculation [67]. The results from these experiments suggest that the destruction of tumor cells in the circulation and during extravasation do not contribute as much as previously thought to the inefficiency of metastasis. Rather, the growth of the individual tumor cell once in the target organ appears to be the rate-limiting step. Tumor cells engineered to overexpress TIMP-1 were shown to extravasate at rates equal to wild-type cells but were unable to form proliferative colonies within the target organ [68]. Although these data suggest that MMPs may play a role in tumor cell growth, the

studies of MMP involvement in ECM degradation and basement membrane invasion still support the core role of MMPs in metastatic invasion.

5.2 Matrix Metalloproteinases in Epithelial to Mesenchymal Transition

Epithelial–mesenchymal transformation (EMT) is a critical step in malignant transformation of epithelial cells into carcinoma [69]. Loss of the homotypic cell–cell adhesion molecule E-cadherin and nuclear translocation of β-catenin are common features of EMT and are associated with the progression of most epithelial cancers [70, 71]. Several MMPs, including MMP-3, MMP-7, and MT1-MMP, cleave E-cadherin releasing a soluble 80 kDa peptide with motility stimulatory activity, which suggest that MMPs could actively contribute to EMT [72]. Interestingly MMPs like MMP-7 and MT1-MMP are also transcriptionally upregulated by β-catenin LEF/TCF complexes [73], suggesting the existence of an MMP-dependent positive feedback mechanism by which E-cadherin degradation by MMPs also results in an increase in MMP expression.

5.3 Matrix Metalloproteinases in Invadopodia Formation

Invadopodia (podosomes) are specialized cell-surface structures that have been identified on transformed malignant cells and are composed of a meshwork of actin ring, microfilaments and metalloproteinases, involved in degradation of underlying matrix. Invadopodia utilize proteases to degrade a variety of immobilized substrates including fibronectin, laminin, type I and IV collagens and other ECM components. Several integral membrane enzymes of different classes have been identified as important functional components of invadopodia [74]. These include the serine proteases, seprase (surface-expressed protease), and dipeptidyl peptidase IV, which must form oligomeric structures for expression of proteolytic activity and also MT-MMP (Fig. 10.5). Plasma membranes shed vesicles containing densely clustered MMP-9 and MMP-2, which might facilitate directional proteolysis of the ECM during cell migration and especially during cancer invasion [75].

5.4 Matrix Metalloproteinases in Cancer Dissemination

A positive correlation between tumor progression and the expression of multiple MMP family members (MMP-1, MMP-2, MMP-7, MMP-9, MMP-11, and MT1-MMP) in tumor tissues has been demonstrated in numerous human and animal studies [76, 77]. On the basis of numerous studies, it was proposed that pharmacologic targeting of MMP activity might provide a mechanism to prevent cancer

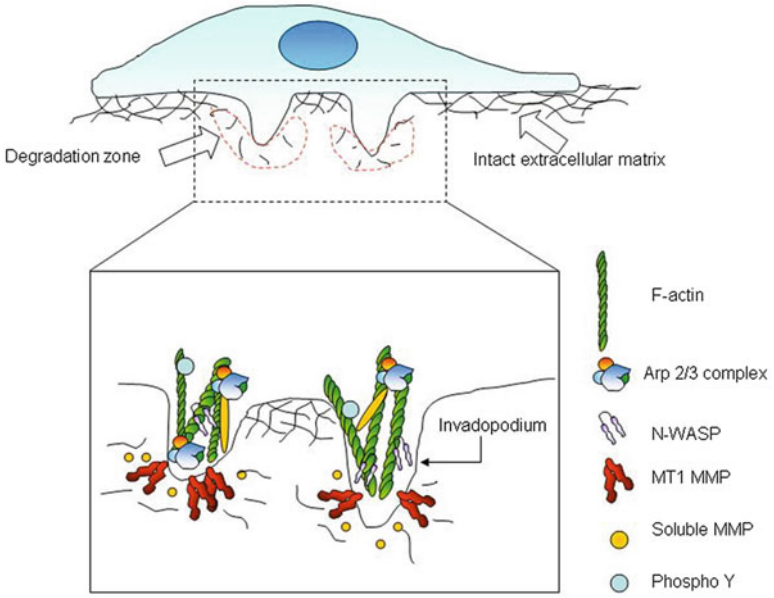

Fig. 10.5 Localization of membrane-bound MMP (MT1-MMP) at invasive bodies of tumor cell (invadopodium). *Arp* actin-related protein, *phospho* phospho tyrosine, *MT1-MMP* membrane-type 1 matrix metalloproteinases, *N-WASP* neuronal Wiskott–Aldrich syndrome protein, *F-actin* filamentous actin

dissemination [78]. Further support for the role of MMPs in cancer dissemination came from the demonstration that TIMPs can interfere with experimental metastasis [79]. However, the role of MMPs and TIMPs in cancer is far more complicated than suggested initially. For example, increased TIMP-1 levels in human cancer tissues have been associated with poor prognoses [80]. It is uncertain whether this reflects the growth-potentiating properties of TIMPs or some other undetermined property of TIMPs [81]. Other experimental studies demonstrated that MMPs act primarily to alter the extracellular environment to allow sustained cancer cell growth in an ectopic site, as opposed to having a specific role of allowing the cells to extravasate from the blood stream [82]. Furthermore, in some experimental tumor systems, increased MMP production did not correlate with increased metastasis [83]. One potential explanation of this finding is that excess proteolysis might degrade matrix signals and receptors, thereby disrupting cell–matrix interactions and inhibiting migration [84].

5.5 *Matrix Metalloproteinases Contribute to Intravasation and Extravasation*

Metastasis is the most devastating event associated with cancer because it heralds an irreversible stage of progression that responds poorly if at all to current therapeutic regimens. Cancer metastasis is a complex, multistage process, which includes cell

detachment from the primary tumor mass, migration through the ECM, degradation of the vascular endothelial basement membrane and penetration into the vascular lumen, survival within the circulation, proliferation on distal vascular endothelia, and finally penetration into a new host tissue microenvironment and establishment of a relationship with the local stroma that is conducive to new tumor colony outgrowth. Several if not all of these steps depend at least in part on MMP activity.

Detachment of cells from the primary tumor mass requires the downregulation of cell–cell adhesion mechanisms. Role of several MMPs in this aspect (especially E-cadherin downregulation), has been discussed before. Migration of tumor cells through the host tissue stroma requires partial degradation of the ECM and coordinated sequential attachment to and detachment from the ECM scaffold. Recent work using two-photon microscopy has provided spectacular real-time evidence that MMP proteolytic activity causes controlled degradation of collagen fibrils that are in contact with the invading tumor cell surface, leaving trail of released cell-surface molecules in the cell's wake [14]. Interestingly, inhibition of MMPs does not result in abrogation of tumor cell migration through the collagen gel but rather transforms the crawling movement associated with collagen fibril cleavage into amoeboid movement that leaves the collagen lattice intact. This model strongly supports the notion that the MMP activity relevant to ECM degradation is associated with the tumor cell surface.

As already discussed, cleavage of ECM components by MMPs generates proteolytic fragments that enhance tumor cell migration. Thus, cleavage of laminin-5 by MMP-2 and -14 results in laminin fragments that trigger migration signals in cells [85], and cleavage of collagen IV discloses cryptic sites that are recognized by integrins and contribute to migration stimuli [86]. MMPs also cleave adhesion receptors responsible for cell–matrix interaction, thereby presumably participating in the detachment of cells from the ECM. The cell-surface hyaluronan receptor and facultative proteoglycan CD44 is cleaved by MMP-14, and its cleavage promotes migration. Expression of CD44 containing a mutation of the proteolytic cleavage site abrogates cell migration on ECM [87].

Intravasation, the process whereby tumor cells penetrate the vascular endothelial wall, has been proposed to be a rate-limiting event in metastasis. Although it is likely that a variety of MMPs may be involved in the degradation of the vascular endothelial basement membrane, MMP-9 has thus far been shown to play a potentially leading role [88].

Survival in the face of the immune response is key for the ability of tumor cells with metastatic potential to establish new colonies. Among the wide range of mechanisms that have been proposed to explain tumor cell evasion of immune surveillance, several are MMP dependent. Tumor cells typically interact with neutrophils, macrophages, cytotoxic T cells (CTLs), and natural killer (NK) cells. T cell proliferation is controlled in large part by the engagement of the interleukin-2 receptor (IL-2R) by its natural ligand IL-2. MMPs, including MMP-9, have been shown to cleave the α-chain of IL-2R [89], resulting in the inhibition of T cell proliferation. MMP-9-mediated activation of latent TGF-β may also contribute to immune suppression, since TGF-β is a potent inhibitor of T cell function [90]. Recent evidence indicates

that MMP-9-mediated shedding of cell-surface ICAM-1 may block the ability of CTLs and NK cells to interact with target cells, thereby reducing the effectiveness of their cytotoxicity. Interestingly, an MMP-11 cleavage product of *α1-proteinase* inhibitor reduces the sensitivity of tumor cells to NK-mediated killing [91].

Recent evidence indicates that MMPs cleave a variety of chemokines in ways that can either enhance or block their function. SDF-1/CXCL12, which is inactivated by several MMPs [92], is a ligand for the CXC chemokine receptor 4 (CXCR4) on leucocytes and breast carcinoma cells. Inhibition of CXCR4 engagement by its ligand using monoclonal antibodies reduces metastasis from breast to lung and lymph nodes in vivo [93].

Extravasation was believed to be a key step in cancer metastasis. However, increasing evidence indicates that extravasation is not a rate-limiting step. This process does not appear to require the proteolytic action of MMPs but results from the mechanical disruption of blood vessels by locally growing tumor cells.

The final step in metastasis is the establishment of tumor colonies at sites distant to that of the origin, which relies on interactions between the tumor cells and host tissue stroma. Invading tumor cells may have their own repertoire of MMPs, but it is becoming increasingly clear that they direct, either by physical contact or in paracrine manner, MMP expression and secretion by stromal cells, including fibroblasts, endothelial cells, and leucocytes [94]. MMPs produced by the stroma augment the release of ECM sequestered growth factors, which may help enhance tumor survival, promote angiogenesis, and contribute to further tumor dissemination. A key question is whether reliance on MMP activity lasts throughout metastatic tumor growth or whether MMP-dependent events serve to initiate colony development, which may then proceed in the absence of further MMP-mediated proteolysis. This is an important consideration for therapeutic strategies targeted toward controlling MMP activity and one that remains to be adequately addressed.

5.6 Matrix Metalloproteinases Help Cancer Cells to Communicate with Distant Organ Cells to Form "Metastatic Niche"

Certain organs such as lung, liver, or bone are the preferential sites for the formation of metastases. MMPs and other proteinases are crucially involved in the formation of receptive environment at distant site, known as "metastatic niche". Soluble factors released from the primary tumor appear to trigger the formation of a metastatic niche that is induced initially by the expression of embryonic-type fibronectin, which is most likely produced by fibroblasts at these sites. This event takes place before disseminated tumor cells are detectable at these distant organs, hence, these authors name this process the formation of a "premetastatic niche" [95]. Increased fibronectin production at these sites allows for the infiltration of VEGFR1-positive, bone marrow-derived progenitor cells, which then establish a metastasis-supporting microenvironment. Interestingly, the production of MMPs, namely MMP-3 and -10,

is upregulated together with the angiogenic modulator angiopoietin-2 in premetastatic lung tissue even before myeloid cells are recruited to these sites [96]. These findings imply an important role of extracellular proteolysis in premetastatic niche generation.

6 Matrix Metalloproteinases and the Risk for Recurrence of Metastasis: The Mysterious Contributors

MMPs can be used as markers to predict tumor recurrence in several cancer types. High preoperative serum levels of MMP-2 or MMP-3 predicts recurrence in patients with advanced urothelial carcinoma [97]. Similarly in ovarian cancer, high expression levels of MMP-2 in tumor cells can predict tumor recurrence [98]. Kuniyasu et al. [99] found that a high ratio of gelatinase expression (MMP-2 or MMP-9) to E-cadherin expression in tumor cells can predict recurrence and death in pancreatic cancer. Similarly, expression of activated MMP-2 is related to regional lymph node and distal metastasis as well as to postresection recurrence of the same tumor [100]. The expression of certain MMPs in primary tumor can predict the risk of metastasis. Expression of MMP-1 is associated with lymph-vascular invasion and lymph node metastasis in stage IB cervical cancer [101] and peritoneal metastasis in gastric cancer [102]. Expression of MMP-2 in tumor cells can indicate increased risk of metastasis in uveal melanoma and in SCC of tongue [103]. Similarly, increased MMP-9 expression by tumor cells in colorectal cancer is associated with advanced Dukes stage and presence of distant metastases [104]. Interestingly, MMP-2 and MMP-9 expression levels are especially high in lung carcinomas and melanomas metastasizing to the spine, suggesting that they contribute to enhanced invasive properties of metastatic spinal tumors [105]. MMP determinations from patient serum have shown predictive value in estimation of metastasis risk. High serum levels of MMP-2 correlate with the presence of metastases in lung cancer or to disease progression in patients with prostate cancer [106], and a high serum MMP-9/E--cadherin ratio can predict metastasis of renal cell carcinoma [107].

7 Matrix Metalloproteinases in Cancer Stem Cell Maintenance: Escalating the Mystery

Matrix metalloproteinases (MMPs) stimulate tumor invasion and metastasis by degrading the extracellular matrix. Here we reveal an unexpected role for Mmp10 (stromelysin 2) in the maintenance and tumorigenicity of mouse lung cancer stem-like cells (CSC). MMP-10 is highly expressed in oncosphere cultures enriched in CSCs and RNAi-mediated knockdown of MMP-10 leads to a loss of stem cell marker gene expression and inhibition of oncosphere growth, clonal expansion, and transformed growth in vitro. Interestingly, clonal expansion of Mmp10-deficient

oncospheres can be restored by addition of exogenous MMP-10 protein to the culture medium, demonstrating a direct role for MMP-10 in the proliferation of these cells [108]. Oncospheres exhibit enhanced tumor-initiating and metastatic activity when injected orthotopically into syngeneic mice, whereas MMP-10 deficient cultures show a severe defect in tumor initiation. Conversely, oncospheres implanted into syngeneic non-transgenic or MMP-10/MMP-2 mice show no significant difference in tumor initiation, growth, or metastasis, demonstrating the importance of MMP-10 produced by cancer cells rather than the tumor microenvironment in lung tumor initiation and maintenance. Analysis of gene expression data from human cancers reveals a strong positive correlation between tumor MMP-10 expression and metastatic behavior in many human tumor types. Thus, MMP-10 is required for maintenance of a highly tumorigenic, cancer-initiating, metastatic stem-like cell population in lung cancer. Our data demonstrate for the first time that MMP-10 is a critical lung cancer stem cell gene product and novel therapeutic target for lung cancer stem cells.

8 Matrix Metalloproteinases as Targets for Anti-metastatic Therapy: Aiming Toward Unraveling the Mystery

The data from model systems, reviewed above, suggest that MMPs are involved in most phases of carcinogenesis from initiation to metastasis. Inhibition of these proteinases might thus lead both to prevention of cancer development and to inhibition of dissemination.

Two main types of MMP inhibitor exist: the TIMPs and low-molecular-weight synthetic inhibitors [109]. Because of their protein nature and multiplicity of actions, it is unlikely that TIMPs will be widely used as anticancer molecules. Because of this, most research in recent years has focused on the synthetic inhibitors. Many of these are peptides and are similar to the cleavage site in collagen [110]. Some of the zinc-binding groups that are currently being investigated in model systems include the hydroxamates, carboxylates, amino carboxylates, and sulfhydryls [111]. Some of these inhibitors (e.g., the hydroxamates) are presently undergoing clinical trials in patients with advanced cancers [112]. We are unaware of any studies so far in human breast cancer, however.

Although MMP inhibitors are currently being evaluated in patients with metastatic cancers, there are still many unanswered questions concerning the use of these compounds. Some of these are as follows. Is it better to use a broad-spectrum or specific matrix metalloproteinases inhibitor? In order to answer this question, it will be necessary to establish which are the MMPs whose involvement in the different phases of cancer progression is critical. If the action of MMP inhibitors is blocking of MMP activity only, these compounds may not induce the type of tumor shrinkage that is seen with the traditionally used cytotoxic agents. Conventional approaches that are used to assess tumor regression may thus not be possible. A novel approach taken to address this issue has been to monitor the rate of rise in levels of serum tumor markers. The use of these tests in phase 2 trials has shown a dose-dependent

decrease in rate of rise after treatment with the MMP inhibitor marimastat [108]. Furthermore, this decreased rate of marker rise appeared to correlate with extended patient survival.

It is reported that MMPs have functional overlap with other proteases, e.g., plasmin, and arrest of invasion will require inhibition of plasmin as well as of the MMPs [113] thereby making the targeting of matrix metalloproteinases in cancer therapy even more challenging.

9 Therapeutically Targeting EMT-Promoting Matrix Metalloproteinases: The Mystery Unfolded

An obvious point for intervention in MMP-induced or -mediated EMT is the catalytic inhibition of MMPs themselves. Unfortunately, clinical trials of first- and second-generation small-molecule MMP-inhibiting drugs in breast cancer and other cancers proved disappointing [114]. A phase III trial of the MMP inhibitor marimastat in patients with metastatic breast cancer found no therapeutic benefit [115], while phase II trials of marimastat and rebimastat in patients with early-stage breast cancer concluded that large adjuvant trials with these agents were not feasible due to musculoskeletal toxicity and failure to achieve therapeutic plasma levels [116]. Many of the problems with the MMP inhibitors tested to date appear to stem in large part from a lack of specificity; the drugs employed simply target too many enzymes. This is a critical problem, because some MMPs appear to protect against tumor progression at certain stages of breast cancer development, and inhibition of these MMPs at the wrong time can lead to increased tumor aggressiveness [117]. For example, high levels of MMP-8 have been shown to suppress breast cancer metastasis [118, 119] significantly. Ribozyme-mediated knockdown of MMP-8 in a nonmetastatic, high MMP-8 breast cancer cell line conferred metastatic competence [120]. Thus, pharmacological inhibition of MMP-8 along with invasion- and metastasis-promoting MMPs would be anticipated to reduce or limit the potential benefit of the therapy.

As another consequence of poor specificity, clinical trials of MMP inhibitors were plagued by the serious side effect of musculoskeletal syndrome (MSS). This dose-limiting toxicity frequently resulted in failure to achieve targeted plasma levels, and in patients withdrawing from treatment. The specific molecular target responsible for these side effects has not been conclusively identified. Remaining candidate mediators of MSS include MT1-MMP, metalloproteinases outside of the MMP and ADAM families [121], or nonprotease metalloproteins. To minimize off-target effects, well-tolerated MMP-directed therapeutics will need to achieve selectivity for the MMP family in preference to other metalloenzymes, as well as the ability to distinguish among MMPs. In the arena of more highly selective small-molecule MMP inhibitors, slow progress is being made. These synthetic compounds typically feature a zinc-chelating group such as hydroxamate derivatized with peptidic or nonpeptidic groups designed to mimic a peptide substrate; they target the MMP active site zinc and substrate binding site [122]. Structure-based design of selective

inhibitors has been hampered by the close structural homology of active sites and overlapping substrate specificities among the MMPs, and by the elastic and flexible nature of the MMP active site, which further complicates computational drug design even when high-resolution crystal structures are available [123, 124]. Current approaches to small-molecule MMP inhibitors include optimization of compounds based on an array of different zinc-binding groups to yield more selective inhibitors toward a variety of MMPs, as well as the development of non-zinc-binding inhibitors that selectively target unique aspects of the MMP-13 active site. A less conventional approach has pursued development of irreversible mechanism-based inhibitors, selective for gelatinases MMP-2 and MMP-9 that covalently modify the catalytic glutamate residue of the MMP active site. In yet another approach, several groups have attempted to exploit the selective substrate binding exosites present on MMP accessory domains to develop selective allosteric inhibitors of MMPs; while a promising concept, this approach has yet to yield highly potent and selective drug leads. Thus, the challenges are clear: while some MMPs facilitate breast cancer development and could potentially be targeted for therapeutic benefit, others are essential for basic physiological processes, interference with which can have serious negative consequences. We now need better understanding of which MMPs to target and when, as well as new generation technologies to target specific matrix metalloproteinases for regression of metastatic cancer.

10 Conclusions

MMPs are associated with multiple human cancers; hence they were early considered as drug targets to treat cancer. The first drug development programs based on the notion of blocking MMP-mediated angiogenesis and metastasis were started about 25 years ago and led to a number of small-molecule metalloproteinases inhibitor (MPI) drugs in phase III clinical trials. The effects of MPIs in these trials turned out to be disappointing as they failed to increase the survival rate of cancer patients. Possible reasons for the failure of MPIs have been extensively discussed previously. Indeed, the clinical studies were suboptimally designed with respect to the stage of cancer, so the question remains whether MPIs might have proven more effective when used in earlier stages of the disease.

Part of the rationale to use MPIs as anticancer drugs was to block interstitial migration of metastatic cancer cells. However, recent analyses have shown that cancer cells can switch to an amoeboid-like protease-independent migration mode by forming actin-rich protrusions and “squeezing” through the ECM. This would render MPIs impotent to inhibit the migratory behavior of metastatic tumor cells. Whether this alternative mode of migration is actually relevant for cancer cell migration, under in vivo conditions, in the presence of a naturally cross-linked collagen matrix, currently remains questionable.

The cytostatic potential attributed to MPIs is certainly in keeping with the numerous studies describing MMP-mediated regulation of cell growth signals, such as the

activation of TGF-β by MMP-2, -9 and -14, the proteolytic release of soluble EGFR ligands, or the degradation of E-cadherin by MMP-3 or -9. Moreover, MMPs interfere with apoptosis induction, especially after chemotherapy, by cleaving Fas ligand from the surface of cancer cells as shown for MMP-7. In the clinical trials, MPIs were administered to patients with advanced cancer, which was most likely too late to exert any beneficial effect on survival.

Interfering with the tumor vasculature is regarded as one of the most promising strategies to inhibit tumor growth and has motivated the development of drugs like Bevacizumab (Avastin, anti-VEGF monoclonal antibody), which has been FDA approved for the treatment of metastatic cancers in combination with chemotherapy. Many studies also support a dominant role of MMP-9 in the angiogenic switch by regulating the bioavailability of VEGF tumors, suggesting a beneficial effect of MPI on tumor angiogenesis. However, in other cancer models, MMP-9 generates ECM fragments like tumstatin, a potent suppressor of tumor vasculature formation, resulting in increased tumor growth in MMP-9-deficient mice. This illustrates that one MMP can have opposing effects in different tumor types and highlights that the use of MPIs has to be carefully considered and evaluated for each specific kind of cancer.

Certainly, the complexity of the mode of action of MMPs has expanded considerably from proteinases that simply degrade the ECM, to specific modulators of angiogenesis as well as fine-tuners of cell signaling pathways and the inflammatory response. One of the major, recent advances in MMP research is the discovery of specific regulatory effects of MMPs on the stromal cells in the tumor microenvironment. MMPs regulate the course of the inflammatory reaction in multiple ways and facilitate the recruitment of inflammatory cells by altering the function of chemokines and the bioavailability of important proinflammatory cytokines. Regarding the link between inflammation and cancer, the interference with MMP-mediated immunoregulatory functions could prove beneficial for cancer patients. For example, given that TNF-α contributes to progression of several sorts of cancer, inhibiting TNF-α activation using MPIs might dampen the inflammatory milieu at the tumor microenvironment.

Effects of MMPs on myeloid cells may well be implicated in the generation of the premetastatic niche. In fact, MMP-2, -3 and -9 have already been shown to contribute to the establishment of metastasis-prone sites at tumor-distant organs. These insights argue for the use of MPIs at early stages of malignant disease prior to the full initiation of tumor-associated inflammation and before the soil have been primed for metastasis in distant organs.

The tumor-suppressing function of these MMPs is probably another reason for the failure of broad-spectrum MPIs as anticancer drugs. The inflammation-suppressing function of MMPs accounts for increased incidence of cancer development in *MMP-8* knockout mice and for the link between MMP-8 loss-of-function mutations and melanoma in humans. Also, MMP-12 delivered by macrophages can suppress the growth of lung metastases, which appears to involve regulation of the tumor vasculature. Apart from that, some MMPs carry out biological functions other than proteolytic, mediated by specific binding to certain target molecules, for instance, via their hemopexin domain. Small-molecule MMP inhibitors as used in clinical trials are certainly ineffective to interfere with a nonproteolytic role of MMPs.

One of the major tasks for the future is the development of active site-directed inhibitors or antibodies that are specific for single MMPs and show little or no cross-reaction with other MMPs. Antibodies could also target functional noncatalytic domains of MMPs. Moreover, MMP activity can be exploited to activate cytotoxic agents such as anthrax toxin to target the tumor vasculature. New activity-based imaging probes specific for MMPs will facilitate monitoring the effect of MPIs on the function of MMPs in vivo. Imaging activity of specific MMPs in vivo will further advance our understanding of the time frame of MMP function during the progression of certain tumors. Like the development of tailor-made therapies and medications based on individual oncogenic pathway signatures in human cancers, expression patterns of MMPs in cancer patients could facilitate a fully rational decision about when and in what combination MPIs and anticancer drugs should be used in the future.

Acknowledgment Authors acknowledge the financial supports from CSIR and DST, Govt. of India.

References

1. Renee T (2005) Cancer Surpasses Heart Disease as Leading Cause of Death for All But the Very Elderly. J Natl Cancer Inst 97: 330-331
2. Joyce JA, Jeffrey W (2009) Pollard Microenvironmental regulation of metastasis. Nat Rev Cancer 9: 239-252
3. Saha B, Adhikary A, Ray P et al (2012) Restoration of tumor suppressor p53 by differentially regulating pro and anti p53 networks in HPV 18 infected cervical cancer cells. Oncogene 31: 178–186
4. Mazumdar M, Adhikary A, Chakraborty S et al (2013) Targeting RET to induce medullary thyroid cancer cell apoptosis in antagonistic interplay between PI3K/Akt and P38MAPK/caspase 8 pathways. Apoptosis 18: 587-604
5. Sen GS, Mohanty S, Bhattacharyya S et al (2011) Curcumin enhances the efficacy of chemotherapy by tailoring p65NFkB-p300 cross-talk in favor of p53-p300 in breast cancer. J Biol Chem 286: 42232–42247
6. Das T, Sa G, Saha B et al (2010) Multifocal signal modulation therapy of cancer: Ancient weapon, modern targets. Mol Cell Biochem 336: 85–95
7. Lahiry L, Saha B, Chakraborty J et al (2010) Theaflavin targets Fas/Caspase 8 and Akt/pBad pathways to induce apoptosis in p53 mutated human breast cancer cells. Carcinogenesis 31: 259-268
8. Lahiry L, Saha B, Chakraborty J et al (2008) Contribution of p53 mediated Bax transactivation in theaflavin induced mammary epithelial carcinoma cell apoptosis. Apoptosis 13: 771-781
9. Kinzler K W, Vogelstein B. The genetic basis of human cancer, 2nd edition, New York: McGraw-Hill, Medical Pub, 3-7, 2002
10. Katherine N, Weilbaecher T A, McCauley K. (2011) “Cancer to Bone” A fatal attraction. Nat Rev Cancer 11: 411-425
11. Frantz C, Stewart KM, Weaver VM (2010) The extracellular matrix at a glance. J Cell Sci 123: 4195-4200
12. Stetler-Stevenson WG, Aznavoorian S, Liotta LA (1993) Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Biol 9: 541-573
13. Wolf K, Wu YI, Liu Y et al (2007) Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol 9: 893-904

14. Kenneth A Iczkowski (2011) Cell adhesion molecule CD44: its functional roles in prostate cancer Am J Transl Res 3: 1-7
15. Rundhaug JE (2003) Matrix Metalloproteinases, Angiogenesis, and Cancer. Clin Cancer Res 9 (2): 551-554
16. Cavallo-Medved D, Rudy D, Blum G et al (2009) Live-cell imaging demonstrates extracellular matrix degradation in association with active cathepsin B in caveolae of endothelial cells during tube formation. Exp Cell Res 315: 1234-1246
17. Chua KN, Poon KL, Lim J et al (2011) Target cell movement in tumor and cardiovascular diseases based on the epithelial–mesenchymal transition concept. Adv Drug Deliv Rev 63: 558-567
18. Kimura YN, Watari K, Fotovati A, et al (2007) Inflammatory stimuli from macrophages and cancer cells synergistically promote tumor growth and angiogenesis. Cancer Sci 98: 2009-2018
19. Albert R. Davalos, Jean-Philippe Coppe, Judith Campisi (2010) Senescent cells as a source of inflammatory factors for tumor progression. Cancer Meta Rev 29: 273-283
20. Ram M, Sherer Y, Shoenfeld Y (2006) Matrix metalloproteinase-9 and autoimmune diseases. J Clin Immunol 26: 299-307
21. Adhikary A, Mohanty S, Lahiry L et al (2009) Theaflavins retards human breast cancer cell migration by inhibiting NFkB via p53-ROS crosstalk. FEBS L 584: 7-14
22. Sewon N, Jung Joon J, Jung M et al (2012) Body fluid MMP-2 as a putative biomarker in metastatic breast cancer. Onco Lett 3: 699-703
23. Opdenakker G, Philippe E. Van den Steen et al (2001) Gelatinase B functions as regulator and effector in leukocyte biology. J Leuko Biol 69: 851-859
24. Vu TH, Werb Z (2000) Matrix metalloproteinases: effectors of development and normal physiology. Genes & Dev 14: 2123-2133
25. Audic Y, Hartley RS (2004) Post-transcriptional regulation in cancer. Biol Cell 96: 479-498
26. Lochter A, Srebrow A, Sympson CJ et al (1997) Misregulation of Stromelysin-1 Expression in Mouse Mammary Tumor Cells Accompanies Acquisition of Stromelysin-1-dependent Invasive Properties. J Biol Chem 272: 5007-5015
27. Wysocki AB, Staiano-Coico L, Grinnell F (1993) Wound Fluid from Chronic Leg Ulcers Contains Elevated Levels of Metalloproteinases MMP-2 and MMP-9. J Invest Dermatol 101: 64-68
28. Malemud CJ (2006) Matrix metalloproteinases (MMPs) in health and disease: an overview. Front in Biosc 11: 1696-1701
29. Fingleton B (2007) Matrix Metalloproteinases as Valid Clinical Targets. Curr Pharmac Des 13: 333-346
30. Gomis-Rüth FX (2003) Structural aspects of the metzincin clan of metalloendopeptidases. Mol Biotech 24:157-202
31. Gomis-Rüth FX (2009) Catalytic Domain Architecture of Metzincin Metalloproteases. J Biol Chem 284: 15353-15357
32. Overall CM, López-Otín C (2002) Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat Rev Cancer 2: 657-672
33. Eisen A, Jeffrey J, Gross J (1968) Human skin collagenase. Isolation and mechanism of attack on the collagen molecule. Biochim Biophys Acta 151: 637–645
34. Fontana V, Coll TA, Sobarzo CM et al (2012) Matrix metalloproteinase expression and activity in trophoblast-decidual tissues at organogenesis in CF-1 mouse. J Mol Histol 43(5): 487-496
35. Parks WC, Wilson CL, López-Boado YS (2004) Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 4: 617-629
36. Fridman R (2006) Metalloproteinases and cancer. Cancer Metastasis Rev 25: 7-8
37. Van Wart H, Birkedal-Hansen H (1990) The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci USA 87: 5578–5582
38. Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2: 161-174

39. Pei D, Kang T, Qi H (2000) Cysteine array matrix metalloproteinase (CA-MMP)/MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation. J Biol Chem 275: 33988–33997
40. Morgunova E, Tuuttila A, Bergmann U et al (1999) Structure of Human Pro-Matrix Metalloproteinase-2: activation mechanism revealed. Sci 284: 1667-1670
41. Bode W (1995) A helping hand for collagenases: the haemopexin-like domain. Struct 3(6): 527–30
42. Kojima K, Ogawa H, Matsumoto I, Yoneda A (1998) Characterization of the ligand binding activities of vitronectin: interaction of vitronectin with lipids and identification of the binding domains for various ligands using recombinant domains. Biochem 37: 6351–6360
43. Massova I, Kotra LP, Fridman R, Mobashery S (1998) Matrix metalloproteinases: structures, evolution, and diversification. FASEB J 12: 1075–1095
44. Kojima S, Itoh Y, Matsumoto S (2000) Membrane-type 6 matrix metalloproteinase (MT6-MMP, MMP-25) is the second glycosyl-phosphatidyl inositol (GPI)-anchored MMP. FEBS Letter 480: 142-146
45. Monea S, Lehti K, Keski-Oja J, Mignatti P et al (2002) Plasmin activates pro-matrix metalloproteinase-2 with a membrane-type 1 matrix metalloproteinase-dependent mechanism. J Cell Physiol 192: 160-170
46. Park BC, Thapa D, Lee YS et al (2007)1-furan-2-yl-3-pyridin-2-yl-propenone inhibits the invasion and migration of HT1080 human fibrosarcoma cells through the inhibition of proMMP-2 activation and down regulation of MMP-9 and MT1-MMP. Eur J Pharmacol. 587: 193-197
47. Brooke C. Henderson, Suresh C. Tyagi (2006) Oxidative mechanism and homeostasis of proteinase/antiproteinase in congestive heart failure. J Mol and Cell Cardiol 41: 959-962
48. Fu X, Kassim SY, Parks WC (2003) Hypochlorous Acid Generated by Myeloperoxidase Modifies Adjacent Tryptophan and Glycine Residues in the Catalytic Domain of Matrix Metalloproteinase-7: An Oxidative: Mechanism for Restraining Proteolytic Activity during Inflammation. J Biol Chem 278: 28403-28409
49. Visse R, Nagase H (2003) Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases :Structure, Function, and Biochemistry. Circul Res 92: 827-839
50. Rupp PA, Visconti RP, Czirók A et al (2008) Matrix Metalloproteinase 2-Integrin αvβ3 Binding Is Required for Mesenchymal Cell Invasive Activity but Not Epithelial Locomotion: A Computational Time-Lapse Study. Mol Biol Cell 19: 5529–5540
51. Zarrabi K, Dufour A, Li J, Kuscu C et al (2011)Inhibition of matrix metalloproteinase 14 (MMP-14)-mediated cancer cell migration. J Biol Chem. 286: 33167-33177
52. Levental KR, Yu H, Kass L et al (2009) Matrix Crosslinking Forces Tumor Progression by Enhancing Integrin Signaling. Cell: 891–906
53. Smith ML, Gourdon D, Little WC, Kubow KE, Eguiluz RA et al (2007) Force-Induced Unfolding of Fibronectin in the Extracellular Matrix of Living Cells. PLoS Biol 5: e268
54. Wirtz D, Konstantopoulos K, Searson PC (2011) The physics of cancer: the role of physical interactions and mechanical forces in metastasis. Nat Rev Cancer 11: 512-522
55. Würtz SØ , Schrohl AS, Sørensen NM (2005) Tissue inhibitor of metalloproteinases-1 in breast cancer. Endocr Relat Cancer 12: 215-227
56. Ogata Y, Itoh Y, Nagase H (1995) Steps Involved in Activation of the Pro-matrix Metalloproteinase 9 (Progelatinase B)-Tissue Inhibitor of Metalloproteinases-1 Complex by 4-Aminophenylmercuric Acetate and Proteinases. J Biol Chem 270: 18506-18511
57. Seo DW, Li H, Guedez L et al (2003) TIMP-2 Mediated Inhibition of Angiogenesis: An MMP-Independent Mechanism. Cell 114: 171-180
58. Basu R, Fan D, Kandalam V et al (2012) Loss of Timp3 leads to abdominal aortic aneurysm formation in response to angiotensin II. J Biol Chem 287: 44083-44096
59. Tummalapalli CM, Heath BJ, Tyagi SC (2001) Tissue inhibitor of metalloproteinase-4 instigates apoptosis in transformed cardiac fibroblasts. J Cell Biochem 80: 512-521
60. DeBerardinis RJ. (2012) Good neighbours in the tumour stroma reduce oxidative stress. Nat Cell Biol 14: 235-236

61. Mishra P, Banerjee D, Baruch AB (2011) Chemokines at the crossroads of tumor-fibroblast interactions that promote malignancy. J Leukoc Biol 89: 31-39
62. Bekes EM, Schweighofer B, Kupriyanova TA (2011) Tumor-recruited neutrophils and neutrophil TIMP-free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. Am J Pathol 179: 1455-1470
63. Schmalfeldt B, Prechtel D, Härting K et al (2001) Increased Expression of Matrix Metalloproteinases (MMP)-2, MMP-9, and the Urokinase-Type Plasminogen Activator Is Associated with Progression from Benign to Advanced Ovarian Cancer. Clin Cancer Res 7: 2396-2404
64. Kuittinen O, Apaja-Sarkkinen M, Turpeenniemi-Hujanen T (2003) Gelatinases (MMP-2 and MMP-9), TIMP-1 expression and the extent of neovascularization in aggressive non-Hodgkin's lymphomas. Eur J Haematol 71: 91-109
65. Koshiba T, Hosotani R, Wada M (1998) Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. Cancer 82: 642-650
66. Choi JY, Jang YS, Min SY (2011) Overexpression of MMP-9 and HIF-1α in Breast Cancer Cells under Hypoxic Conditions. J Breast Cancer 14: 88–95
67. Kedrin D, Gligorijevic B, Wyckoff J et al (2008) Intravital imaging of metastatic behavior through a mammary imaging window. Nat Meth 5: 1019-1021
68. Koop S, Khokha R, Schmidt EE et al (1994) Overexpression of metalloproteinase inhibitor in B16F10 cells does not affect extravasation but reduces tumor growth. Cancer Res 54: 4791-4797
69. Royer C, Lu X (2011) Epithelial cell polarity: a major gatekeeper against cancer? Cell Death Differ 18:1470-1477
70. Vernon AE, LaBonne C et al (2004)Tumor Metastasis: A New Twist on Epithelial–Mesenchymal Transitions. Current Biol 14: 719-721
71. Brabletz T, Herrmann K, Jung A et al (2000)Expression of Nuclear β-Catenin and c-myc Is Correlated with Tumor Size but Not with Proliferative Activity of Colorectal Adenomas. Am J Pathol 156: 865–870
72. Lynch CC, Vargo-Gogola T, Matrisian LM et al (2010) Cleavage of E-Cadherin by Matrix Metalloproteinase-7 Promotes Cellular Proliferation in Nontransformed Cell Lines via Activation of RhoA. J of Oncol 2010: 530745
73. Liu P, Yang J, Pei J, Pei D, Wilson MJ (2010) Regulation of MT1-MMP activity by β-catenin in MDCK non-cancer and HT1080 cancer cells. J Cell Physiol 225: 810-821
74. Frittoli E, Palamidessi A, Disanza A (2012) Secretory and endo/exocytic trafficking in invadopodia formation: the MT1-MMP paradigm. Eur J Cell Biol 90(2-3): 108-114
75. Nascimento CF, Gama-De-Souza LN, Freitas VM (2010) Role of MMP9 on invadopodia formation in cells from adenoid cystic carcinoma. Study by laser scanning confocal microscopy. Microsc Res Tech 73: 99-108
76. Mizutani K, Kofuji K, Shirouzu K (2000) The significance of MMP-1 and MMP-2 in peritoneal disseminated metastasis of gastric cancer. Surg Today 30: 614-621
77. Yang EV, Sood AK, Chen M (2006) Norepinephrine Up-regulates the Expression of Vascular Endothelial Growth Factor, Matrix Metalloproteinase (MMP)-2, and MMP-9 in Nasopharyngeal Carcinoma Tumor Cells. Cancer Res 66: 10357-10364
78. Szabova L, Chrysovergis K, Yamada S (2008) MT1-MMP is required for efficient tumor dissemination in experimental metastatic disease. Oncogene 27: 3274-3281
79. Davies B, Miles DW, Happerfield LC (1993) Activity of type IV collagenases in benign and malignant breast disease. Br J Cancer 67: 1126-1131
80. DeClerck YA, Imren S (1994) Protease inhibitors: role and potential therapeutic use in human cancer. Eur J Cancer 30A: 2170-2180
81. Lu XQ, Levy M, Weinstein IB et al (1991) Immunological quantitation of levels of tissue inhibitor of metalloproteinase-1 in human colon cancer. Cancer Res 51: 6231-6235
82. L Guedez, W G Stetler-Stevenson, L Wolff et al (1998) In vitro suppression of programmed cell death of B cells by tissue inhibitor of metalloproteinases-1. J Clin Invest 102: 2002–2010

83. Zucker S, Lysik RM, Malik M et al (1992) Secretion of Gelatinases and Tissue Inhibitors of Metalloproteinases by Human Lung-Cancer Cell-Lines and Revertant Cell-Lines - Not an Invariant Correlation with Metastasis. Int J Cancer 52: 366-371
84. Parks WC (1999) Matrix metalloproteinases in repair. Wound Rep Regen 7: 423-432
85. Packard BZ, Artym VV, Komoriya A, Yamada KM (2009) Direct visualization of protease activity on cells migrating in three-dimensions. Matrix Biol 28: 3-10
86. Giannelli G, Pozzi A, Stetler-Stevenson WG et al (1999)Expression of matrix metalloprotease-2-cleaved laminin-5 in breast remodeling stimulated by sex steroids. Am J Pathol 154: 1193-1201
87. Xu J, Rodriguez D, Petitclerc E et al (2001) Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo. J Cell Biol 154: 1069-1080
88. Okamoto I, Tsuiki H, Kenyon CL et al (2002) Proteolytic Cleavage of the CD44 Adhesion Molecule in Multiple Human Tumors. Am J Pathol 160: 441-447
89. Jaime G. de la Garza-Salazar, Abelardo Meneses García, Claudia Arce-Salinas. Inflammatory Breast Cancer, 1st edition, Springer, 29-50, 2012
90. Paiva De CS, Yoon KC, Pangelinan BS (2009) Cleavage of functional IL-2 receptor alpha chain (CD25) from murine corneal and conjunctival epithelia by MMP-9. J Inflamm 6: 31-42
91. Becker C, Fantini MC, Neurath MF (2006) TGF-beta as a T cell regulator in colitis and colon cancer. Cytokine and growth factor reviews 17: 97-106
92. Kataoka H, Uchino H, Iwamura T (1999) Enhanced Tumor Growth and Invasiveness in Vivo by a Carboxyl-Terminal Fragment of α1-Proteinase Inhibitor Generated by Matrix Metalloproteinases. Am J Pathol 154: 457–468
93. Webster NL, Crowe SM (2006) Matrix metalloproteinases, their production by monocytes and macrophages and their potential role in HIV-related diseases. J Leukoc Biol 80: 1052-1066
94. Stephanie J. Mail G, Kurschat N, Drenckhan A (2012) Involvement of CXCR4 Chemokine Receptor in Metastastic HER2-Positive Esophageal Cancer. PLOS One 7: e47287
95. Davalos AR, Coppe JP, Campisi J et al (2010)Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metast Rev 29: 273-283
96. Pengfei L, Weaver MV, Werb Z (2012) The extracellular matrix: A dynamic niche in cancer progression. J Cell Biol 196: 395-406
97. Huang Y, Song N, Ding Y et al (2009) Pulmonary vascular destabilization in the premetastatic phase facilitates lung metastasis. Cancer Res 69: 7529-7537
98. Song N, Sung H, Choi JY et al (2012) Preoperative serum levels of matrix metalloproteinase-2 (MMP-2) and survival of breast cancer among Korean women. Cancer Epidemiol Biomarkers Prev 21(8):1371-1380
99. Demeter A, Sziller I, Csapo Z et al (2005) Molecular Prognostic Markers in Recurrent and in Non-recurrent Epithelial Ovarian Cancer. Antican Res 25: 2885-2890
100. Kuniyasu H, Lee ME, Douglas B et al (1999) Relative Expression of E-Cadherin and Type IV Collagenase Genes Predicts Disease Outcome in Patients with Resectable Pancreatic Carcinoma. Clin Cancer Res 5: 25-33
101. Yamamoto H, Itoh F, Shouhei I et al (2001) Expression of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Human Pancreatic Adenocarcinomas: Clinicopathologic and Prognostic Significance of Matrilysin Expression. J Clin Oncol 19: 1118-1127
102. Moser PL, Kieback DG, Hefler L et al (1999)Immunohistochemical detection of matrix metalloproteinases (MMP) 1 and 2 and tissue inhibitor of metalloproteinase 2 (TIMP 2) in stage IB cervical cancer. Anticancer Res.19: 4391–4393
103. Inoue T, Yashiro M, Nishimura S et al (1999) Matrix metalloproteinase-1 expression is a prognostic factor for patients with advanced gastric cancer. Int J Mol Med 4: 73-80
104. Yoshizaki T, Maruyama Y, Sato H et al (2001) Expression of tissue inhibitor of matrix metalloproteinase-2 correlates with activation of matrix metalloproteinase-2 and predicts poor prognosis in tongue squamous cell carcinoma. Int J Cancer 95: 44–50

105. Zeng ZS, Huang Y, Cohen AM et al (1996) Prediction of colorectal cancer relapse and survival via tissue RNA levels of matrix metalloproteinase-9. J Clin Oncol 14: 3133–3140
106. Gokaslan ZL, Chintala SK, York JE et al (1998) Expression and role of matrix metalloproteinases MMP-2 and MMP-9 in human spinal column tumours. Clin Exp Metastasis 16: 721-728
107. Gojhi K, Fujimoto N, Hara I (1998) Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. Int J Cancer 79: 96-101
108. Slaton JW, Inoue K, Perrotte P et al (2001) Expression levels of genes that regulate metastasis and angiogenesis correlate with advanced pathological stage of renal cell carcinoma. Am J Pathol 158: 735–743
109. Verline J, Regala RP, Tseng CI (2012)Matrix Metalloproteinase-10 Is Required for Lung Cancer Stem Cell Maintenance, Tumor Initiation and Metastatic Potential. PLoS one 7: e35040
110. Coussens LM, Fingleton B, Matrisian LM (2012) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Sci. 295: 2387–2392
111. Talbot DC, Brown P (1996) Experimental and clinical studies on the use of matrix metallo proteinases inhibitor for the treatment of cancer. Eur J Cancer 32A: 2528-2533
112. Nemunaitis J, Poole C, Primrose J et al (1998) Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: Selection of a biologically active and tolerable dose for longer-term studies. Clin Cancer Res 4: 1101-1109
113. Sparano JA, Bernardo P, Stephenson P et al (2004)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 E2 196. J Clin Oncol 22: 4683–4690
114. Miller KD, Saphner TJ, Waterhouse DM et al (2004)A randomized phase II feasibility trial of BMS-275291 in patients with early stage breast cancer. Clin Cancer Res 10: 1971–1975
115. Overall CM, Kleifeld O (2006) Tumour microenvironment—opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer 6: 227–239
116. Montel V, Kleeman J, Agarwal D et al (2004) Altered metastatic behavior of human breast cancer cells after experimental manipulation of matrix metalloproteinase 8 gene expression. Cancer Res 64: 1687–1694
117. Gutierrez-Fernandez A, Fueyo A, Folgueras AR et al (2008) Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. Cancer Res 68: 2755–2763
118. Sternlicht MD, Lochter A, Sympson CJ et al (1999) The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis Cell 98: 137–146
119. Moy FJ, Chanda PK, Chen J et al (2002) Impact of mobility on structure-based drug design for the MMPs. J Am Chem Soc 124: 12658–12659
120. Jacobsen JA, Major Jourden JL, Miller MT et al (2010) To bind zinc or not to bind zinc: an examination of innovative approaches to improved metalloproteinase inhibition. Biochim Biophys Acta 1803: 72–94
121. Ikejiri M, Bernardo MM, Meroueh SO et al (2005) Design, synthesis, and evaluation of a mechanism-based inhibitor for gelatinase A. J Org Chem 70: 5709–5712
122. Sela-Passwell N, Rosenblum G, Shoham T, et al (2009) Structural and functional bases for allosteric control of MMP activities: Can it pave the path for selective inhibition? Biochim Biophys Acta 1803: 29-38
123. Williamson RA, Hutton M, Vogt G et al (2001) 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: 32966–32970
124. Lee MH, Atkinson S, Rapti M et al (2009) 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: 114-122