ROLE OF MMPS IN METASTATIC DISSEMINATION: IMPLICATIONS FOR THERAPEUTIC ADVANCES

Diego Raffo[1], Osvaldo Pontiggia[1] and Marina Simian[1,2]

[1]Instituto de Oncología "Ángel H. Roffo". Avda San Martin 5481, Buenos Aires, Argentina.

[2]Corresponding author:

Marina Simian,

Instituto de Oncología "Ángel H. Roffo",

Avda. San Martin 5481,

Buenos Aires (C1417DTB), Argentina.

Email: marina.simian@galuzzi.com.

Tel: +54-114-504-7884

Fax: +54-114-580-2811

Abstract

Matrix metalloproteinases (MMPs) have been implicated in both normal and pathologic processes. In cancer in particular, in vitro and animal studies showed an involvement of MMPs in many stages of cancer progression. This led to the development of MMP inhibitors that in most cases failed in clinical trials. In this review we go over the role of MMPs in the different stages of cancer progression and try to understand why the early generation of MMP inhibitors failed. The analysis of the lessons from this first experience, plus the review of the current knowledge that shows that MMPs may be pro-or anti-tumorigenic may set the stage for a future success for this therapeutic strategy in cancer.

Keywords: cancer, clinical trials, matrix metalloproteinases, MMP inhibitors, therapy.

Introduction

Malignant tumors are characterized by the ability of cancer cells to invade other tissues and spread to distant organs, with fatal consequences in most cases. The metastatic cascade is defined as the series of steps that enable a tumor cell to form these secondary tumors. Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases that share a similar structure and which collectively have the capacity to degrade virtually every component of the extracellular matrix (ECM), have been shown to be key players in metastasis. As such the development of therapeutic agents that block MMP activity is today a major area of basic and clinical research. In this article we will review the current knowledge regarding the involvement of MMPs in the different stages of metastasis together with the current stage of anticancer trials using MMP inhibitors.

MMPs

Structure

MMPs are a family of zinc-dependent endopeptidases currently consisting of over 24 different members in humans [1]. MMPs are secreted as inactive zymogens [2] that have the ability to degrade most ECM components when activated. They are grouped according to their modular domain structure (Figure 1).

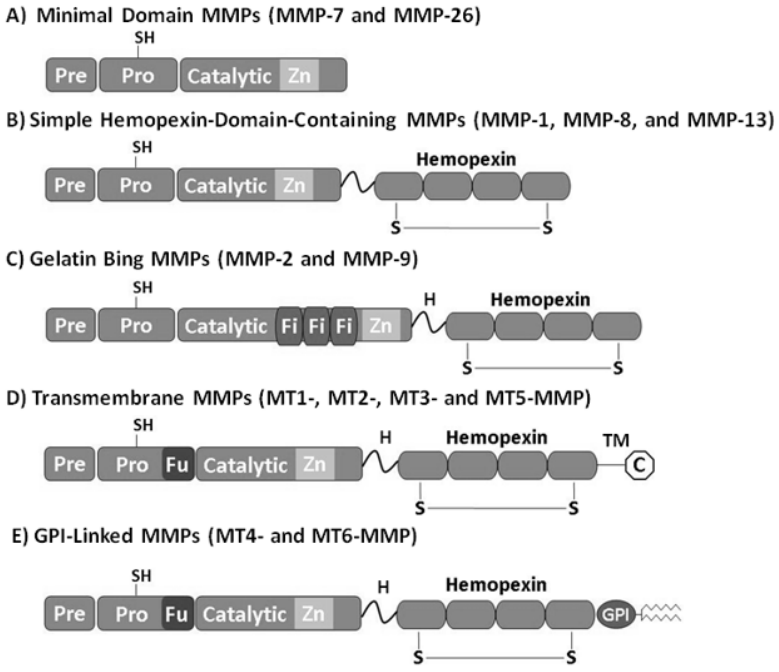

Figure 1: Structural domains of MMPs

Pre: signal sequence; Pro: propeptide with a free zinc-ligating thiol (SH) group; Fu: furin susceptible site; Zn: zinc-binding site; Fi: collagen-binding fibronectin type II inserts; H: hinge region; TM: transmembrane domain; C: cytoplasmic tail; GPI: glycophosphatidyl inositol-anchoring domain. The hemopexin/vitronectin-like domain contains four repeats with the first and last linked by a disulfide bond.

All MMPs have a pre-domain consisting of an N-terminal signal sequence that is removed after their synthesis has been directed to the endoplasmic reticulum. A propeptide pro-domain maintains enzyme latency until it is removed. A signal peptide directs their secretion from the cell, and a C-terminal hemopexin domain contributes to substrate

specificity and to interactions with endogenous inhibitors [3]. The catalytic domain contains a conserved zinc-binding site and dictates substrate specificity through its active site cleft, through specificity sub-site pockets that bind amino acid residues immediately adjacent to the scissile peptide bond, and through secondary substrate-binding exosites located outside the active site itself. The classical MMP structure is found in the subgroup of secreted proteases composed by the three collagenases (MMP-1, MMP-8, and MMP-13), the two stromelysins (MMP-3 and MMP-10), and four additional MMPs with distinctive structural characteristics (MMP-12, MMP-19, MMP-20, and MMP-27). The two matrilysins (MMP-7 and MMP-26) do not have the hemopexin domain [4] and the two gelatinases (MMP-2 and MMP-9) contain three fibronectin type II modules that confer a compact collagen binding domain [5]. There are six membrane-type (MT)-MMPs localized at the cell surface through a C-terminal transmembrane domain (MT1-, MT2-, MT3- and MT5-MMP) or by a glycosylphosphatidylinositol anchor (MT4- and MT6-MMP) [6]. The MT-MMPs also have an additional insertion of basic residues between the propeptide and the catalytic domain that is cleaved by furin-like serine proteases leading to the intracellular activation of the proenzymes [6].

Regulation of MMP activity

In normal physiological conditions MMPs are tightly regulated and expressed at very low levels. However, during tissue remodeling MMPs are rapidly transcribed, secreted (except in the case of MT-MMP) and activated. A classical example is mammary gland involution, where MMP activity plays a key role in the remodeling process that leads to the end of lactation [7-8]. The situation is different in epithelial cancers, given that MMP activity is highly upregulated. Tumor associated MMP expression is attributed not only to expression

by cancer cells themselves, but to the surrounding stromal, vascular and inflammatory cells. Understanding the stages of MMP regulation is thus critical to shed light on strategies that may contribute to their inhibition.

Transcription

Most MMPs respond to stimuli at the transcriptional level. A number of MMP promoters have been characterized, revealing a variety of cis-acting elements such as NFκB, AP-1, Sp1, Tcf/Lef-1, RARE and PEA3 [9]. The response at transcriptional level usually involves delayed kinetics over a time frame of several hours and requires ongoing translation. This suggests that they are components of genetic programmes such as the wound repair response, in which they are downstream targets of immediate-early response genes that are induced within minutes of cell stimulation and in the absence of new protein synthesis. Amongst these immediate-early genes are the Fos and Jun genes that comprise activator protein-1 (AP-1) [10]. Upstream of Fos and Jun are several cytokines and growth factors, including interleukins, interferons, EGF, KGF, NGF, basic FGF, VEGF, PDGF, TNF-α, TGF-β, and the extracellular matrix metalloproteinase inducer EMMPRIN [11]. Recently, Yan and Boyd [12] used the basic promoter conformation to assign MMPs to one of the following three groups: (1) those which contain TATA boxes at around−30 bp with AP- 1 sites around−70 bp (MMP -1, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12, MMP-13, MMP-19 and MMP-26); (2) those which contain a TATA box, but no promoter proximal AP-1 site (MMP-8, MMP-11 and MMP-21); and (3) those with no TATA box nor proximal AP-1 (MMP-2, MMP-14 and MMP-28).

Although AP-1 complexes play a critical role in the regulation of several MMP genes, other factors are also involved. For example for MMP-9, promoter sequence analysis showed a STAT3 binding site where a c-Jun/Fra-1 and Stat3 complex was identified on this region of the promoter [13]. In the MMP-1 promoter, the proximal AP-1 site has been reported to be within a composite element with at least a peroxisome proliferator-activated receptor gamma (PPARγ) consensus sequence [14-15]. MMP-11 appears to be unique amongst characterized MMP promoters in having a retinoic acid response element (DR1-RARE) in its proximal promoter. A β-catenin-regulated LEF/TCF recognition site are found upstream of the promoters of MMP-7 and MMP-14 [16-17]. A functional p53-binding site has been identified in the MMP-2 gene promoter [18], and wild-type p53 downregulates basal and inducible MMP-1 gene expression in human fibroblasts and osteogenic sarcoma cells, whereas some mutant forms do not [19]. The MMP-13 gene is regulated in part by a promoterproximal Runx2 (aka Cbfa1, AML3) site which directs its expression in osteoblasts and hypertrophic chondrocytes [20-21].

Levels of MMP expression are also regulated by genetic variations such as single nucleotide polymorphisms (SNPs). These differences in genotype may alter promoter function and consequently gene regulation. Examples of SNPs affecting MMP levels are a −1575 bp G to A polymorphism altering binding of oestrogen receptor [22] and a −1306 bp C to T polymorphism altering binding of Sp1 [23] in the MMP-2 promoter. An MMP-3 SNP is located 1171 bp upstream of the transcription start site and contains a run of five or six adenosines [24]. In this case, the 6A allele binds an 89-kDa nuclear factor more readily than the 5A allele and has 50% less transcriptional activity. Upstream of the MMP-9 transcription start site a SNP contains either a cytidine or thymidine [25]. Thus

transcriptional regulation of MMP levels depend on the combinatorial effect of genetic polymorphisms and the cross-talk of various signaling pathways.

Activation

ProMMPs are kept catalytically inactive by the interaction between the thiol of the conserved prodomain cysteine residue and the zinc ion of the catalytic site. The thiol-Zn2+ interaction must be disrupted for a proMMP to become catalytically active. This mechanism is referred to as the "cysteine switch". Latent MMPs can be activated by three mechanisms: 1) direct cleavage of the pro-domain by another proteinase; 2) reduction of the free thiol by oxidants or by nonphysiologic reagents such as alkylating agents, heavy metal ions, and disulfides [26]; and 3) allosteric perturbation of zymogen. Thiol reduction and allosteric controls would lead to inter or intramolecular autolytic cleavage of the prodomain. Proteolysis of the prodomain is considered to be the final step in MMP activation in vivo.

Direct proteolysis is considered to be the principal way that proMMPs are activated. About one-third of MMPs – including all membrane-bound MMPs – contain an RXKR or RRKR sequence between the pro and catalytic domains which serves as a target sequence for proprotein convertases or furins. Furin is a type 1 membrane subtilisin-like serine protease present in the trans- Golgi network [27]. MMPs with a furin cleavage site are processed intracellularly before secretion [28]. For the other MMPs, the mode of activation is more presumed than proved, and the in vivo mechanism for activation of most non-furin cleaved proMMPs is unknown.

Certain MMPs can cleave the prodomain of other MMP zymogens leading to activation in vitro. However, in vivo considerations should be complied for this to occur: 1) potentially activating MMPs should be co-expressed and co-localized with their potential substrate proMMPs; 2) if co-localized, the concentration of the activating MMP should be in high enough relative amounts to activate the zymogen, especially in a microenvironment where other substrates compete for the active MMP. The best described non-furin activation of an MMP is that of proMMP-2 by MMP-14; proMMP-2 requires cooperative action of MMP-14 and TIMP-2 (tissue inhibitor of metalloproteainse-2) for activation [29]. The right amount of TIMP-2 stimulates MMP-2 activation whereas an excess is inhibitory. TIMP-2 enables the formation of a ternary complex bringing MMP-2 and MMP-14 close enough for pro-MMP-2 activation.

Plasmin and other serine proteinases have been implicated as activators of MMPs. For example, plasminogen-null mice (plg−/−) present defects in wound healing after myocardial infarction, apparently due to diminished activation of proMMP-2 and proMMP-9 in the absence of plasmin [30]. However, other evidence from mice lacking various components for the plasmin activation cascade suggests that plasmin does not function in vivo as an MMP activator. For example, in aortic extracts and conditioned medium of tissues and fibroblast from u-pa−/−, t-pa−/−, plg−/− and pai-1−/− mice, the amount of active MMP-2 and MMP-9 do not differ among genotypes [31], MMP-dependent activity in wound healing is not affected by a lack of plasmin or the plasminogen-activating enzyme [32].

Oxidants can both activate and inactivate MMPs depending on their concentration. These are usually produced by leukocytes or other cells providing an additional mechanism of

control of proteolytic activity. In vitro, a number of proMMPs are activated by reactive oxygen species, which can modify the cysteine thiol group and activate proMMPs via autolytic cleavage. However, in vivo this mechanism remains to be clearly demonstrated [33].

Allosteric activation occurs when the zinc-thiol interaction is disrupted, allowing the zymogen to become active. This leads to transitional active state, however, in vivo, as mentioned above removal of the prodomain is considered as the final step of MMP activation [33].

Inhibition of MMP activity

TIMPs (tissue inhibitors of metalloproteinases) are endogenous MMP inhibitors consisting of 21–34 kDa proteins all possessing 12 conserved cysteine residues forming six disulfide bonds that fold the protein into two domains. In humans at least four members of this family have been identified: TIMPs 1, 2, 3 and 4 [34]. They inhibit MMP activity by forming non-covalent 1:1 stoechiometric complexes that are resistant to heat denaturation and proteolytic degradation [35]. Although TIMP-3 is found tightly bound to the matrix, the rest of the TIMPs are present in solution. TIMPs bind active MMPs, but also have the ability to attach to proMMPs, regulating their activation process. Individual TIMPs differ in their ability to inhibit various MMPs. TIMP-1 forms a preferential complex with proMMP-9 [36], TIMP-3 binds with both proMMP-2 and proMMP-9 [37]. TIMP-4 can also binds to the C-terminal domain of proMMP-2 [38]. TIMP-2 and TIMP-3, unlike TIMP-1, are also effective inhibitors of MT-MMPs. Paradoxically, TIMP-2 binds to proMMP-2 and facilitates enzyme activation as explained above [39-40].

TIMPs have been found to have multiple functions on cell behavior that are independent of their MMP inhibitory activity. For example, TIMP-1 and -2 have been identified as growth factors in a number of cells. TIMP-1 can bind to breast cancer cells through an 80-kDa transmembrane protein inducing a dose-dependent mitogenic effect. Overexpression of human TIMP-4 in rat vascular smooth muscle cells and in transformed, but not wild-type, cardiac fibroblasts on the other hand, induces apoptosis [41-42]. TIMP-1 and TIMP-2 seem to have, on the contrary, anti-apoptotic effects. In vivo experiments have shown contradictory results on the role of TIMPs in tumorigenesis. In certain tumor models gene delivery of TIMPS has led to stimulation of tumor growth. This was observed, for example, by systemic delivery of TIMP-4 that led to a significant stimulation of in vivo mammary tumorigenesis [43]. Moreover, two breast carcinoma cell lines overexpressing TIMP-1 show increased tumor growth [44]. In the clinic contradictory results have also been found where increased TIMP levels correlate with worse prognosis. This is the case, for example, in colorectal polyp cancer where increased levels of TIMP-1 and -2 are observed in carcinomatous epithelium [45], or in breast cancer where tissue and serum levels of TIMP-1 are associated with poor prognosis [46].Taken together, these observations suggest that TIMPs have a wide range of biological functions extending far beyond their ability to inhibit metalloproteinases.

The metastatic cascade

Most cancer deaths are due to metastasis, the spread of cancer to distant organs. The metastatic “cascade” is defined as the series of steps that a cancer cell must carry out to leave the primary tumor and establish at a new site where a new tumor will grow. These steps are: localized invasion, intravasation, transport through the circulation, arrest in

microvessicles in distant organs, extravasation, formation of micrometastasis and colonization which implies formation of the macrometastasis or distant tumor. Recent evidence suggests that the formation of a pre-metastatic niche at the target organ is necessary for tumor cells to establish at the new site. In the following section we will review the current knowledge related to the role MMPs play in each of the steps of the metastatic cascade (Figure 2).

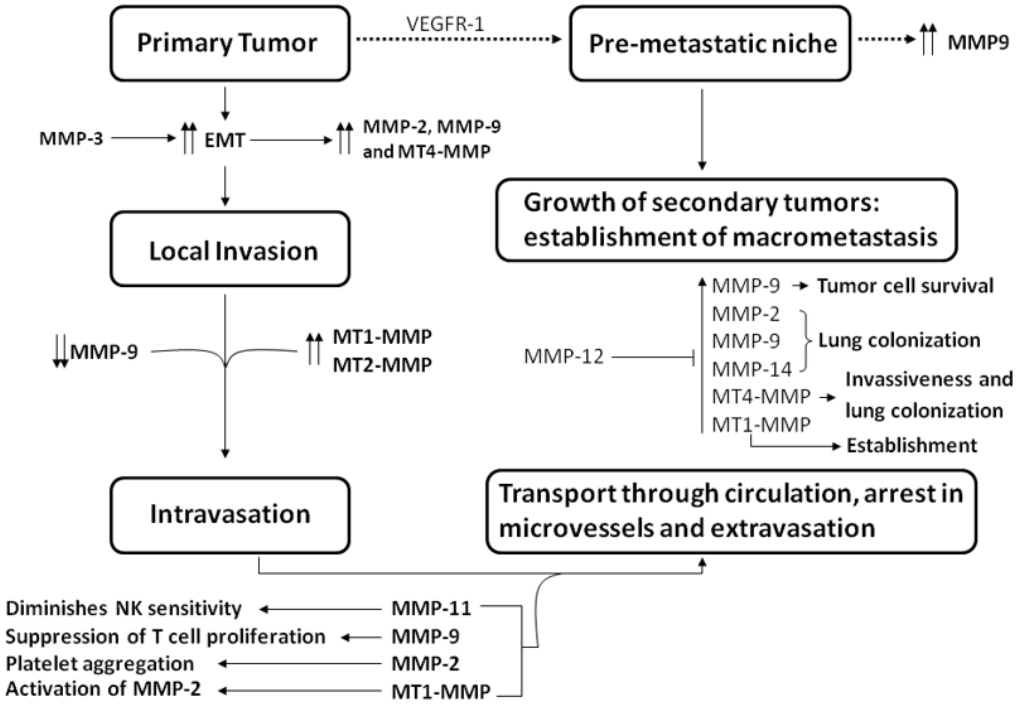

Figure 2: Involvement of MMPs in different stages of metastasis

MMPs are involved in the different steps of metastasis. Contribution of particular MMPs in the different steps of metastasis is summarized in the scheme, where MMPs that positively contribute to metastasis are next to upper-pointing arrows and those that have an inhibitory effect are next to arrows pointing downwards.

Formation of the pre-metastatic niche

Increasing evidence suggests that tumor cells require a permissive microenvironment to colonize a new organ and form a macrometastasis. This implies that the target organ must be prepared before the arrival of the circulating tumor cells. This hypothesis is based mostly on data collected from mouse models, and thus needs further clinical confirmation. Bone marrow hematopoietic cells have been shown to localize to metastatic sites before the arrival of the tumor cells [47]. These cells are characterized by the expression of the vascular endothelial growth factor receptor 1 and the fibronectin receptor α4β1. Sites of pre-metastatic niche formation are characterized by an increased expression of fibronectin [47]. The mobilization of the myeloid cells is thought to be the result of soluble factors secreted by the primary tumor and inflammatory chemokines [47]. At the pre-metastatic niche myeloid cells join forces with stromal cells and endothelial cells residing in the tissue parenchyma. Together they provide a platform of chemokines, growth factors, matrix degrading enzymes and adhesion molecules, thereby accelerating assembly of the metastatic lesion [48]. Matrix metalloproteinases seem to be increased in the pre-metastatic niche. So far MMP9 has been shown to be specifically induced in premetastatic lung endothelial cells and macrophages by distant primary tumors via VEGFR-1/Flt-1 tyrosine kinase (TK) and significantly promotes lung metastasis [49]. Using the B16/F10 tumor model other authors have shown that MMP3 and MMP10 regulate the premetastatic lung leading to increased permeability of pulmonary vasculatures promoting the extravasation of the circulating tumor cells [50].

Localized invasion

Most solid tumors originate in epithelial tissues, where sheets of cells are separated from the stroma by the basement membrane. Cell-cell interactions are strong amongst epithelial cells and are mediated by adhesion complexes mediated by molecules such as E-cadherin. During the transition from an in situ to an invasive lesion, epithelial tumor cells are released from their neighbors and the basement membrane is breached. This process is characterized by epithelial-mesenchymal transition (EMT), where epithelial cells loose the expression of E-cadherin, occludin, cytokeratins and catenins to gain mesenchymal markers such as N-cadherin, vimentin, tenascin C, laminin β1 or collagen VI α. This change in cellular phenotype leads to the acquisition of a spindle like cell shape and increased motility [51-52]. Upregulation in the expression of proteinases is also characteristic of EMT, enabling the degradation of the basement membrane and invasion. EMT is induced in epithelial cells by heterotypical signals released by the mesenchymal cells that constitute the stroma of the neoplastic tissues. The transforming growth factor beta (TGF-β) family of cytokines have been extensively implicated in the induction of EMT [53]. Several receptor tyrosine kinases are mutated and constitutively active in diverse cancer types and play a role in EMT [54]. Many EMT-inducing pathways are key players in stem cell self renewal and development [55-56] such as the transcription factors of the snail family and ZEB family (ZEB1, ZEB2), as well as TWIST1, TWIST2 and E12/E47 control EMT [57-61].

Different MMPs have been shown to be regulated by regulators of EMT. For example, MMP-2 and MMP-9 are regulated by SNAIL [62-63]. SLUG, a zinc-finger transcriptional factor that induces EMT through suppression of E-cadherin expression [64-65], regulates MT4-MMP expression, further supporting the correlation between EMT and MMPs [66].

Among the known MMPs, only MMP-3 has been shown to induce EMT through Rac1b activity and increased concentrations of reactive oxygen species [67]. In contrast to MMP-3, MT4-MMP was unable to induce EMT in cancer cells. This result suggests that MT4-MMP is located downstream of the EMT signal pathway and that it mediates cancer metastasis [66].

Intravasation

The process of how cells enter de blood and lymphatic vessels is called intravasation. Efficient intravasation of tumor cells is believed to occur through angiogenic blood vessels rather than through the pre-existing vasculature. There is evidence that tumor-induced angiogenic capillaries are dilated, have no blood flow and are not supported by continuous basement membrane and pericyte envelopment, all of which might assist tumor cell intravasation. A role for MMPs was first directly demonstrated by Ossowski and collaborators who designed the chick embryo/human cancer cell metastasis model [68] that combined with a PCR based method for detection of human cancer cells allowed them to develop the first semiquantitative assay for the analysis of human tumor cell intravasation in a xenogeneic host. They showed that cells producing high levels of uPAR/uPA that also produced MMP-9 were capable of efficient intravasation [69]. However, further experiments by other groups have shown that the relationship between MMPs and intravasation is not always straight forward. When specifically only tumor cell MMP-9 is downregulated with siRNA in the highly intravasative HT-1080 cell variant HThi/ diss, a two-to-three fold increase in levels of intravasation and metastasis was observed [70]. It is possible that an enhancer of intravasation is produced in the tumor tissue, which is susceptible to catalytic degradation or inactivation by MMP-9. As a result, down-regulation

of MMP-9 would up-regulate the levels of such an enhancer and increase the levels of intravasation [70]. MT1-MMP and MT2-MMP have also been implicated as mediators of intravasation. Using the CAM model together with MCF-7 and MDA-MB-231 cells the authors found that siRNA-specific silencing of MT1-MMP and MT2-MMP completely ablated the ability of Snail1 to drive cancer cell intravasation [71]. In patients few papers have addressed the relationship between intravasation and MMPs. For examples in colorectal cancer, no link was found between the levels of MMP-2 and MMP-9 in the primary tumor and intravasated cells [72].

Transport through the circulation, arrest in microvessicles and extravasation

Once cancer cells enter the lymphatic or blood circulation they encounter a new microenvironment. The blood is a very hostile environment for cancer cells, as they must survive numerous challenges such as destruction by mechanical stress, lack of attachment that may lead to anoikis, and surveillance by immune cells. There is controversy on how long tumor cells actually remain in the vasculature. Some studies suggest that, due to their large size, they are trapped in the first or second capillary they encounter [73]. Others however state that a high proportion of cells die as soon as they enter the vasculature [61]. A role for MMPs in the survival of cancer cells in circulation has been shown to cover multiple mechanisms: for example MMP-11 was shown to generate a bioactive fragment from α1-proteinase inhibitor which diminishes natural killer sensitivity, thereby enhancing tumor growth and invasiveness in vivo [74]. MMP-9 suppression of T cell proliferation through disruption of the signaling mediated by IL-2Rα leads to immunosuppression [75]. The interaction of tumor cells with platelets is also important for the survival of cells in the circulation. Formation of tumor cell aggregates facilitated by platelets protects also against

anoikis and sheer stress inside the vasculature. The ability of tumor cells to induce platelet aggregation correlates with the metastatic potential of tumor cells [76]. MMP-2 released from platelets and cancer cells is involved in platelet aggregation as shown for human fibrosarcoma HT-1080, lung carcinoma A549, breast adenocarcinoma MCF7 and colon adenocarcinoma Caco-2 cells [77-79]. Moreover, the aggregating effects of MMP-2 are dependent upon the activation of proMMP-2 to MMP-2 by MT1-MMP [77]. Platelets themselves are able to upregulate MMP production by cancer cells, thus promoting cancer invasion: intact platelets and their releasates up-regulate both the activity and expression of MMP-9 in various tumor cell lines leading to increased invasiveness of cancer cells [80]. Moreover, it has been shown that platelet-derived microvesicles are able to induce an up-regulation of MT1-MMP and MMP-9 in several lung carcinoma cell lines [81].

Tumor cells adhere to the vasculature of secondary organs and through the process of extravasation go through the blood vessel wall to colonize the new site. Intravascular proliferation of tumor cells frequently occurs before extravasation, creating a considerable tumor mass that can eventually obliterate the adjacent vessel wall by pushing aside the barrier composed of endothelial cells, pericytes and smooth muscle cells that previously separated the vessel lumen from the surrounding tissue [82]. Although one would imagine that MMPs would be essential players in the extravasation process given that tumor cells must breach the endothelial barrier, this issue is still poorly understood. Results implicating MMPs are few and contradictory. For example, the MMP inhibitor batimastat did not affect the extravasation of B16-F1 melanoma cells from the liver circulation in an early study [83]. However, in the experimental lung colonization model by i.v. injection of the same mouse B16-F10 melanoma cells, daily administration of MMI270 for 2 weeks significantly

decreased the number of colonies in the lung compared with the control without affecting the size of colony. Micrometastasis was monitored day 7 post-inoculation by measuring the melanin content in the lung as well as by microscopic examination of the lung tissue sections. Even only twice administrations of MMI270 on the first day after tumor injection significantly inhibited micrometastasis in the lung. In the spontaneous metastasis model using B16-BL6 melanoma cells, lung metastasis was not affected by a continuous administration of MMI270 using a mini osmotic-pump. On the contrary, when mice were subjected to popliteal lymphadenectomy on day 7 after the cell inoculation in the footpad subdermis, the continuous administration of MMI270 significantly suppressed the lung metastasis. These results suggest that the tumor cell extravasation in the target organ is the most critical step where MMPs can play their significant role in the experimental metastasis, and that the lymphatic metastasis process is less susceptible to MMI270 than the hematogenic metastasis process in the spontaneous metastasis model [84]. Moreover, transfection of the C127 breast cancer cell line by MMP-2, but not by MMP-1 or MMP-3, gave rise to an invasive and metastatic phenotype which depended not only on the catalytic properties of MMP-2 but also on properties associated with the MMP-2 non-catalytic C-terminal domain. Experiments with a synthetic gelatinase inhibitor revealed that a single dose could prevent the lungs of nude mice being colonized by the MMP-2 transfectants, and that the inhibitor had to be administered during or shortly after injection of the cells, indicating that an early event, such as the extravasation of the cells into the lung, was gelatinase-dependent in this system [85]. Indirect evidence from Timp-3-/- mice also support a role for MMPs in extravasation [86]. Multiple tissues of timp-3-/- mice were more susceptible to metastatic colonization. Overall, a 5–14- fold increase in liver and kidney colonization occurred by EL-4 lymphoma cells, and a twofold increase upon

targeting B16F10 melanoma cells to the bone or lung of timp-3-/- mice. Analysis of lungs showed that proliferation or angiogenesis were unaltered within the metastatic colonies. Lung-trap assays revealed that initial tumor cell trapping was similar in the lung vasculature of Timp-3-/- and wild-type mice. However, more tumor cells were found in timp-3 -/- lungs at 48 and 96 h after tumor cell injection indicating more efficient extravasation and initial proliferation. Activation of pro-MMP-2 was greater in timp-3-/- lungs at these time points, suggesting that Timp 3 regulates MMP-2 activation to limit tumor cell extravasation [86].

Growth of secondary tumors: establishment of macrometastasis

Clonal outgrowths are thought to give origin to secondary tumors [87]. This is a very inefficient step in metastasis, as cells may die or remain dormant for long periods of time. Cell cycle arrest and immune surveillance are two factors that have been implicated in tumor dormancy [88]. Moreover, the inability to induce an effective angiogenic response is also thought to affect tumor dormancy. However, when the microenvironmental inhibitory influences are removed, the cells rapidly regain growth capacity. MMPs have been implicated in several stages of colonization. For example, MMP-9 derived from bone marrow derived cells contributes to survival but not growth of tumor cells in the lung microenvironment, in a model system utilizing MMP-9 null mice and Lewis Lung Carcinoma [89]. However, in mice deficient in macrophage elastase (MMP-12) another group observed that significantly more gross Lewis lung carcinoma pulmonary metastases developed, than in their wild-type counterparts both in spontaneous and experimental metastasis models. The numbers of micrometastases between the two groups were equivalent; thus, it seems that MMP-12 affected lung tumor growth, and not metastasis

formation, per se. MMP-12 was solely macrophage derived being expressed by tumor-associated macrophages and not by tumor or stromal cells. The presence of MMP-12 was associated with decreased tumor-associated microvessel density in vivo and generated an angiostatic>angiogenic tumor microenvironment that retarded lung tumor growth independent of the production of angiostatin. These data define a role for MMP-12 in suppressing the growth of lung metastases [89]. In another recent study the authors found that lack of an anti-inflammatory protein in mice (Uteroglobin) led to increased lung colonization by B16-F10 melanoma cells and this was associated to an increase in MMP-2, MMP-9 and MMP-14 by the cancer cells [90]. The hypoxic microenvironment also regulates metastasis by modulating expression of MMPs. Hypoxia, or overexpression of hypoxia-inducible factor-1α (HIF-1α) induced MT4-MMP expression in human cancer cells. Activation of SLUG, a transcriptional factor regulating the EMT process of human cancers, by HIF-1α was critical for the induction of MT4-MMP under hypoxia. MT4-MMP promoted invasiveness and pulmonary colonization through modulation of the expression profile of MMPs and angiogenic factors. Finally, coexpression of HIF-1α and MT4-MMP in human head and neck cancer was predictive of a worse clinical outcome. These experiments were carried out using hypopharyngeal squamous cell carcinoma cell line FADU, tongue squamous cell carcinoma cell lines SAS and OECM-1, and the embryonic kidney 293T cell line [66]. Another recent and interesting study shows that while MT1-MMP is not required for the initial steps of melanoma cell homing to the lungs, it does plays a major role in the establishment of the metastasis [91].

Paradoxical effects of MMPs on tumor progression

Increased MMP expression either by tumor cells or the surrounding stroma has traditionally been associated to tumor progression. However, this relationship does not seem to be that straight forward as recent work has shown that in certain cases MMPs may provide a protective effect, or have dual roles depending on the tissue context. MMP-8 was the first MMP whose activity was associated to anti-tumor properties [92]. Studies in MMP-8 null mice showed that the absence of the protease increased the incidence of tumors on mice [92]. In this same line of evidence, MMP-3 null mice have increased sensitivity to chemically induced squamous cell carcinoma, which conferres an overall protective role for MMP-3 in skin carcinogenesis. Interestingly tumors from MMP-3 null mice were much more progressed by the end of the study [93]. Furthermore expression of MMP-3 in keratinocytes has been shown to enhance differentiation and prevent tumor establishment [94]. As mentioned above, MMP-12 which is mainly produced by macrophages has been linked to decreased tumor growth. Additionally studies have demonstrated that this MMP inhibits the growth of lung metastasis both in spontaneous and experimental metastasis models [95]. Another example is given by MMP-26 that is also produced by macrophages and polymorphonuclear leukocytes. High expression levels of MMP-26 correlate with a favorable clinical outcome in hormone-regulated tumors [96]. We have previously shown that regression of breast tumors in mice in response to endocrine therapy is accompanied by a dramatic increase in MMP-2, MMP-9 and MMP-3 activity together with a high degree of extracellular matrix remodeling [97]. In patients several studies have shown that increased MMP expression or activity is not always linked to enhanced malignancy. In patients with colorectal cancer, the MMP-1 gene promoter polymorphism associated with enhanced MMP-1 transcriptional activity correlated with a more favorable prognosis [98]. Clinical studies revealed that increased levels of MMP-9 were associated to favorable prognosis in

patients with node-negative breast cancer [99] and inversely correlates with liver metastasis in patients with colorectal cancer [100]. In another independent study low pre-operative serum levels of MMP-9 increased the risk of relapse 3.0 fold during the first 10 years of follow up in primary breast cancer [101]. MMP-11 and MMP-19 are examples of proteases with dual roles. MMP-11 for example, promotes primary breast tumor progression but inhibits the development of metastasis [102], whereas MMP-19 seems to be positively involved in the development of skin tumors but paradoxically acts as a negative regulator of the early steps of tumor invasion and angiogenesis [103].

MMPs as clinical targets

As reviewed above, our knowledge on the role of MMPs in cancer biology has increased exponentially in the last ten years. Originally, the aim of developing MMP inhibitors was based on the idea that they would inhibit extracellular matrix degradation and thus cancer invasion and metastasis. Today we know that MMPs are involved in many aspects of cell biology and that in some cases these effects are protective against cancer development. The first generation of MMP inhibitors as anti-cancer drugs were Batimastat (BB94) [104] and Ilomostat or Galardin (GM6001) [105] that had low water solubility and thus were not orally available. The second generation of MMP inhibitors such as Marimastat (BB-2516) [106] that were orally available failed in several phase III clinical trials and were commonly associated to musculoskeletal syndrome [107].

The development of the third generation of MMP inhibitors is founded on the current knowledge of the three-dimensional structure of the enzyme active site. These structure based inhibitors include Tanomastat (BAY 12-9566), Prinomastat (AG3340) and BMS-

275291[108], that although not as broad spectrum as the first generation of MMP inhibitors, still aim at inhibiting several enzymes simultaneously. Structure based inhibitors targeting a single MMP have been described for MMP-12 and MMP-13 [108-110]. Other compounds have been shown to have anti-MMP activity like statins [111], bisphosphonates [112] and chemically defined tetracyclines which do not possess antibiotic activities [113]. Current clinical trials where MMP inhibitors are being tested against different types of cancer have recently been summarized by Roy et al. [108]. However, in most cases positive results have not been obtained at the clinical level so far, although biomarkers sometimes suggest the opposite. One example of success is given by Neovastat (an inhibitor of MMP-2, and to a lesser degree of MMP-7, -9, and -13 [114]) significantly lengthened the median survival time in a phase II clinical trial of patients with refractory renal cell carcinoma [115]. Nevertheless, a phase III clinical trial of patients with non-small cell lung cancer has failed to show any positive effect for this new drug [116].

Concluding remarks and future directions

Great expectations were deposited on the inhibition of MMP activity as a possible road to the eradication of cancer. However, clinical trials have shown so far that this path was not as straight forward as initially assumed. The reasons for the current failure of MMP inhibition as a general treatment for cancer lie at various levels.

The first and probably the most conceptual of all was the belief that proteolytic activity only participated in cancer progression, and not in other aspects of cell biology that could restrain tumor growth. As mentioned above, MMPs have been shown to provide a protective effect against cancer in certain contexts and to even participate in tumor

remission [97]. Therefore the development of inhibitors that target only one MMP, and not a broad spectrum of proteases may allow us to distinguish between those MMPs that contribute to cancer progression from those that are beneficial to the host [107]. Second, administration of MMP inhibitors have shown to have adverse effects such as musculoskeletal syndrome that manifested as pain and immobility in the shoulder joints, arthralgias, contractures in the hands and an overall reduced quality of life for patients [117]. This led to a reduction in the administered doses of the drugs or withdrawal of the patients from the clinical trials [117]. Third, most pre-clinical studies were carried out in animals in the early fases of tumor development or metastatic disease, whereas patients recruited for clinical trials were mostly in the advanced metastatic stage. As reviewed above, MMPs are involved in most stages of tumor progression and there may be a chance that inhibition of proteolytic activity in early stages of tumor development may be a more effective clinical strategy.

The success of MMP inhibition as an effective therapy against cancer metastasis is still in its early stages. However, a serious consideration of the hallmarks that have led to the current failure in most clinical trials may set the basis for the development of a new rational which may determine what types of compounds need to be developed and how and when they should be administered. The MMP inhibitor experience clearly demonstrates that cancer biology is extremely complicated and that the translation from animal models to patients needs to be revisited if we aim at developing effective treatments.

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