Modifying the soil to affect the seed: role of stromal-derived matrix metalloproteinases in cancer progression

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Abstract In the 1980's, as the importance of matrix metalloproteinases (MMPs) in cancer progression was discovered, it was recognized that in most tumors these proteases were abundantly and sometimes exclusively expressed not by tumor cells, but by normal host-derived cells like fibroblasts, vascular endothelial cells, myofibroblasts, pericytes or inflammatory cells that contribute to the tumor microenvironment. Later experiments in mice deficient in specific MMPs revealed that host-derived MMPs play a critical role not only in tumor cell invasion, but also in carcinogenesis, angiogenesis, vasculogenesis and metastasis. Tumor cells secrete many factors, cytokines and chemokines that directly or indirectly increase the expression of these MMPs in the tumor microenvironment where they exert extracellular matrix (ECM) degrading and sheddase activities. The knowledge of the complex role that stromal-derived MMPs play in the interaction between tumor cells and stromal cells should allow us to consider specific windows in cancer treatment when MMP inhibition could have a valuable therapeutic effect.

Keywords MMP · Stroma · Microenvironment

1. Introduction

In the 1980's as new MMPs were identified, characterized and cloned and their expression examined in human cancer tissues, it rapidly became apparent that in many cases MMPs were more abundantly expressed by stromal cells than by tumor cells. These observations were in apparent contradiction with the three step theory of cancer invasion and accepted dogma at the time that MMPs were primarily made by cancer cells to degrade the surrounding (ECM) and basement membrane, in order to invade and metastasize [1]. The simplistic nature of this concept is now clear as it is demonstrated that although involved, MMPs are not required for tumor cells to invade tissue as they can invade and move through an intact ECM by adopting amoeba-like movements [2, 3]. Over the last several years we have also become increasingly aware of the oversimplified nature of a second concept in regard to the role of MMPs in biology. We have now fully realized that MMPs do much more than degrading ECM proteins as they can proteolytically process a large number of growth factors, growth factor receptors, cytokines, chemokines and precursor proteins of biologically active fragments [4, 5]. In this chapter, following a review of the literature on the expression of MMPs by stromal cells in cancer, preclinical evidence supporting a functional role of stromal-derived MMPs in cancer progression is reviewed, and the mechanisms of action by which stromal-derived MMPs affect cancer initiation and progression are discussed. We then address the mechanisms by which tumor cells specifically stimulate the expression of MMPs in the tumor stroma, and how on the basis of this knowledge we should consider testing the therapeutic efficacy of MMP inhibitors at specific phases of cancer progression and treatment. In this chapter we have included under the definition of stromal cells any non-malignant cell that contributes to the tumor microenvironment, including fibroblasts, myofibroblasts, pericytes and vascular smooth muscle cells, microvascular endothelial cells and inflammatory cells. Also included in the definition of stroma are ECM proteins which represent the non-cellular

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component of the tumor microenvironment and have a profound influence on tumor progression.

2. Stromal expression of MMPs in human cancer

2.1. MMPs are expressed by stromal cells in tumors

Among the first members of the MMP family to be cloned and characterized were the type IV collagenases (MMP-2 and MMP-9). The existence and production of these basement membrane-degrading MMPs by metastatic tumor cells was postulated in the early 1980's as being critical factors for tumor cell intravasation and extravasation [6]. This was consistent with the fact that these enzymes were isolated, purified and cloned from malignant cells and SV-40 transformed cells [7, 8]. As a more detailed examination of their expression in human tumor tissues became possible with the availability of nucleotide probes for *in situ* hybridization and antibodies for immunolocalization, it became evident that non-malignant cells were often the major source of production of these MMPs in human tumors [9–12]. Similar observations were then made for other MMPs as they were identified and cloned. For example, MMP-11 (stromelysin 3) was identified and cloned as a gene specifically expressed in stromal cells surrounding invasive breast carcinoma cells [13, 14]. Because these MMPs are secreted in soluble precursor forms that become activated in the extracellular milieu, their expression by stromal cells was not inconsistent with a postulated predominant role in the degradation of the ECM surrounding invasive tumor cells. However, when MT1-MMP (MMP-14), a membrane associated protease initially identified at the surface of invasive tumor cells [15], was found expressed at the surface of stromal cells in many cancers such as gastrointestinal carcinoma, hepatocellular carcinoma and colon and breast carcinomas, it raised the possibility that stromal cells may also use MMPs to contribute to tumor prognosis [16–19]. The contribution of stromal cells to the expression of MMPs in human tumors does not seem to follow any particular pattern and varies among the types and subtypes of tumors. For example, in colorectal carcinoma, MMP-7 is predominantly expressed by malignant epithelial cells whereas MMP-2, MMP-3, MMP-9 and MT1-MMP are predominantly although not exclusively expressed by stromal cells [10, 11, 20–23]. In human glioma and in neuroblastoma, MMP-2 is expressed by tumor cells and stromal cells whereas MMP-9 is prominent in the stroma [24, 25]. In breast cancer, MMP-3, MMP-11, MMP-12 and MMP-13 are expressed by stromal cells and MMP-2 by both tumor cells and stromal cells [26–28]. MT1-MMP is expressed at the surface of tumor cells, where it plays a critical role in invasion and MMP dependent motility [29] but also at the surface of stromal cells [18, 19].

2.2. Cellular origin of stromal expression of MMPs in human tumors

A large variety of host-derived cells express MMPs in human cancer. Among these are vascular endothelial cells which express MMP-2, MMP-9 and MT1-MMP [30, 31]. Some of these MMPs are transcriptionally upregulated under hypoxic conditions by transactivating factors like hypoxia inducible factor I α [32]. Pericytes that surround vascular endothelial cells also express MMPs, in particular MMP-9, and are a prominent source of this protease in breast cancer [33]. The expression of these MMPs by endothelial cells and pericytes contributes to angiogenesis (see Chapter 9). Fibroblasts and myofibroblasts are a major source of MMPs in many cancers, in particular cancers of the breast, where myofibroblasts adjacent to breast cancer cells express MT1-MMP and MMP-13 [18, 19, 34, 35]. The contribution of hematopoietic-derived inflammatory cells like monocytes, macrophages, mast cells and neutrophils to the expression of MMPs and in particular MMP-9 in the tumor microenvironment was recently emphasized. That the expression of this MMP by bone marrow-derived mast cells could play a role in tumor initiation was initially recognized in a mouse transgenic model of squamous cell carcinoma [36]. MMP-9 is expressed by CD68 positive macrophages along the invasive margin of metastatic colorectal cancer cells [37, 38] and in melanoma [39]. In our laboratory, we recently demonstrated that in human neuroblastoma tumors, MMP-9 is predominantly expressed by CD45 positive bone marrow-derived cells and that its expression correlates with an absence of Schwannian stroma, a histological feature of unfavorable outcome [40].

3. What evidence do we have that stromal-derived MMPs play a contributory role in cancer progression and initiation?

3.1. Stromal-derived MMPs contribute to cancer progression

Convincing evidence that stromal-derived MMPs actively contribute to tumor progression comes from preclinical studies in mouse models in which stromal-derived MMPs are either overexpressed or suppressed. For example, evidence that increased expression of stromal-derived MMP-9 can affect tumor progression is illustrated in experiments performed in integrin alpha-1 deficient mice. These mice overexpress MMP-9 and have high plasma levels of this enzyme. As a result they also have high plasma levels of the angiogenesis inhibitor angiostatin that is generated from the cleavage of plasminogen by MMP-9 [41]. Increased MMP-9 expression in this model has a paradoxical effect on

tumor growth as these mice exhibit a reduction of growth of the primary tumor as a consequence of the inhibitory effect of angiostatin on angiogenesis, but an increase in the number of lung metastatic nodules that, however are of smaller size and less vascularized [42, 43]. These data illustrate the complex role of stromal-derived MMPs in cancer progression that includes a suppressive effect on angiogenesis and a promoting effect on metastasis. The contribution of stromal-derived MMPs to tumor angiogenesis and metastasis is also demonstrated in transgenic MMP knock-out mice models. In MMP-2 and MMP-9 null mice, injection of B16 melanoma cells into the tail vein results in a decrease in the number of metastatic nodules compared to mice in which the host has normal levels of these MMPs [44, 45]. In our laboratory, we showed that implantation of human neuroblastoma cells in the adrenal gland of immunodeficient mice lacking MMP-9 results in smaller tumors that have a defect in angiogenesis and vascular structure characterized by smaller and pericyte-deficient vessels [46]. That stromal and not tumor-derived MMP-9 is responsible for this effect was then demonstrated by transplanting MMP-9 null mice with bone marrow-derived cells obtained from wild type mice. In these transplanted mice, re-expression of MMP-9 by bone marrow-derived inflammatory cells restored the original vascular phenotype of the tumor and the recruitment of pericytes along endothelial cells [40]. Implantation of human ovarian cancer cells in the peritoneal cavity of immunodeficient MMP-9 null mice results in a lower tumor incidence and a decreased tumor growth when compared with MMP-9 expressing mice [47].

3.2. Stromal-derived MMPs contribute to tumor initiation

Experiments in MMP-deficient mice have pointed to a contributory role of MMPs not only in metastasis and angiogenesis, but also in carcinogenesis. Absence of MMP-2 or MMP-9 expression in knockout mice retards the formation of pancreatic islet tumors and skin tumors in transgenic models of pancreatic carcinoma and squamous cell carcinoma [36, 48]. In MMP-11 null mice, exposure to the carcinogen DMBA results in the formation of less malignant tumors suggesting that a lack of MMP expression in host tissue may impair malignant transformation. In this study, convincing evidence that lack of expression of the enzyme in stromal cells and not in transformed cells is responsible for this effect is elegantly provided by the demonstration that mixing MCF-7 breast cancer cells with MMP-11 deficient embryonic fibroblasts decreases the ability of MCF-7 cells to develop mammary tumors upon injection into the mammary fat pad [49].
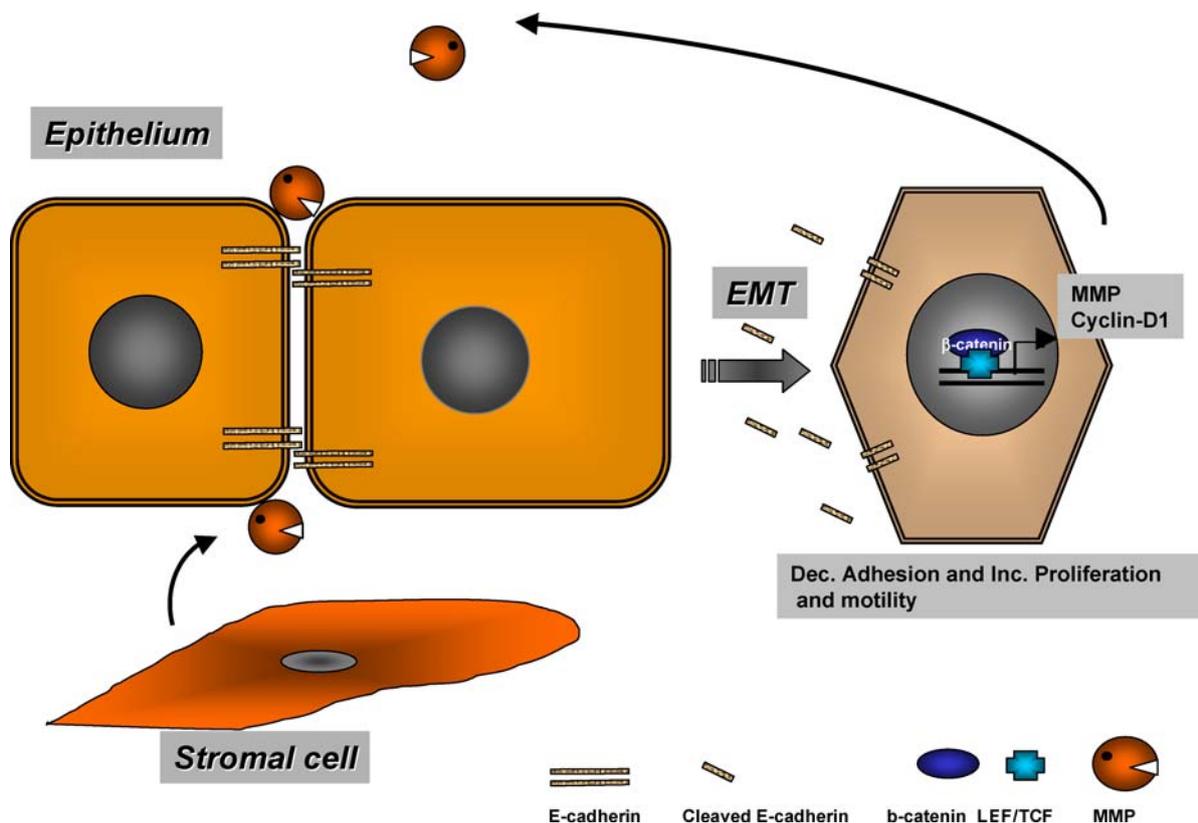
4. Mechanisms of action of stromal-derived MMPs in cancer progression and initiation

4.1. MMPs contribute to epithelial-mesenchymal transformation (EMT)

Epithelial-mesenchymal transformation is a critical step in malignant transformation of epithelial cells into carcinoma [50]. Loss of the homotypic cell-cell adhesion molecule E-cadherin is a common feature of EMT and is associated with the progression of most epithelial cancers [51]. Loss of E-cadherin expression at the cell surface results in the translocation of β-catenin from the cell membrane to the cytosol and the nucleus, where through the formation of β-catenin LEF/TCF complexes, β-catenin activates the expression of tumor promoter genes like c-Myc and cyclin D1 [52, 53]. The observation that several MMPs, including MMP-3, MMP-7 and MT1-MMP cleave E-cadherin releasing a soluble 80 kDa peptide with motility stimulatory activity suggests that MMPs could actively contribute to EMT [54, 55]. Interestingly, several MMPs like MMP-7 and MT1-MMP are also transcriptionally upregulated by β-catenin LEF/TCF complexes [56, 57], 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 (Fig. 1). Whether stromal-derived MMPs could cleave E-cadherin expressed by epithelial cells and promote EMT has not been demonstrated so far, however the contribution of stromal cells to epithelial carcinogenesis is well demonstrated [58]. It is therefore conceivable that over-expression of stromal-derived MMPs by carcinogens like phorbol esters known to upregulate several MMPs [59] could induce epithelial transformation through this mechanism.

4.2. Stromal-derived MMPs contribute to angiogenesis and vasculogenesis

It is now well documented that stromal-derived MMPs contribute to the formation of the tumor vasculature. One mechanism of action is through the solubilization of angiogenic factors, in particular Vascular Endothelial Growth Factor (VEGF). For example, MMP-9 cleaves isoforms of VEGF (165 and 121) that have a heparin-binding domain. The elimination of this domain by MMP cleavage decreases the binding of VEGF to proteoglycans present in the ECM surrounding the tumor and increases the bioavailability of VEGF. A similar mechanism of solubilization is also responsible for osteoclast recruitment [60]. Solubilization of VEGF is a necessary step to initiate the angiogenic switch needed for the formation of pancreatic and skin tumors in transgenic mice overexpressing the large T-Ag in a tissue specific manner [48, 61]. Expression of MMP-9 by the stroma

Fig. 1 MMPs contribute to epithelial-mesenchymal transformation. Several MMPs can cleave E-cadherin, releasing a soluble 80 kDa fragment with motility activity. Cleavage of E-cadherin promotes the translocation of β-catenin to the nucleus where by forming complexes with LEF/TCF it increases the transcriptional activation of genes like cyclin D1 and also MMP, further promoting EMT. Whether stromal derived MMP can initiate such processes has, however, not been demonstrated so far

may therefore be a critical regulatory step controlling tumor dormancy. Stromal-derived MMPs also contribute to angiogenesis in established tumors. The expression of MMPs in endothelial cells is enhanced by angiogenic factors and MMPs are critical for endothelial cell migration and tube formation *in vitro* [62, 63]. Mice deficient in a specific MMP like MMP-2 or MMP-9 develop smaller tumor that grow at a slower rate, exhibit increased apoptosis and decreased mean vascular density and vessel size. The mechanism is complex and involves impaired endothelial cell invasion and motility and inhibition of pericyte recruitment by endothelial cells [46, 47, 64, 65]. MMPs also contribute to tumor vasculogenesis (Fig. 2). In contrast to angiogenesis, which consists of the invasion of a tumor by adjacent mature endothelial cells, vasculogenesis involves the recruitment by the tumor of endothelial precursor cells (EPC) that are released from the bone marrow niche into the blood circulation and become part of the tumor vasculature [66]. Circulating EPCs are recruited to the primary tumor site by chemokines like stromal-derived factor-1 (SDF-1/CXCL12) or monocyte chemotactic protein-1 (MCP-1/CCL2) that are secreted by tumor cells [67]. A small percentage of these cells (2–5%) then contribute to the tumor vasculature by integrating into

vessels. SDF-1 induces MMP-9 expression [68]. Interestingly, there is recent evidence suggesting that MMP-9 plays a critical role in the mobilization of hematopoietic precursors cells and EPCs from the bone marrow niche. MMP-9 expression is upregulated by SDF-1 and causes shedding of membrane associated mKitL in its soluble form sKitL. This enhances the recruitment of hematopoietic precursor cells and EPCs [69]. Consistent with this concept, a lack of MMP-9 expression by bone marrow-derived cells is associated with decreased mobilization of hematopoietic stem cells into the peripheral blood and decreased colonization of CD45 positive bone marrow-derived cells in xenotransplanted tumors [40, 69]. Recent evidence suggests that these bone marrow-derived hematopoietic precursor cells can also home in tumor-specific pre-metastatic sites forming cellular clusters that precede the arrival of malignant cells [70].

4.3. Stromal-derived MMPs contribute to metastasis

It has been generally assumed that tumor cell-derived MMPs were important to allow tumor cells to invade the ECM, penetrate the basement membrane and metastasize. This concept was supported by experimental evidence demonstrating

Fig. 2 Stromal derived MMPs contribute to angiogenesis and vasculogenesis. MMP-9 produced by CD45 positive bone marrow cells promotes the mobilization of hematopoietic stem cells from the bone marrow to the peripheral blood by solubilizing the mKit-L. Among these cells are EPCs which contribute to vasculogenesis. In the tumor, MMP-9 increases the solubilization of growth factors like VEGF platelet-derived growth factor (PDGF) and Heparin bound epithelial growth factor (HB-EGF), increasing the tumor vasculature and its normalization by promoting pericyte recruitment along the endothelium
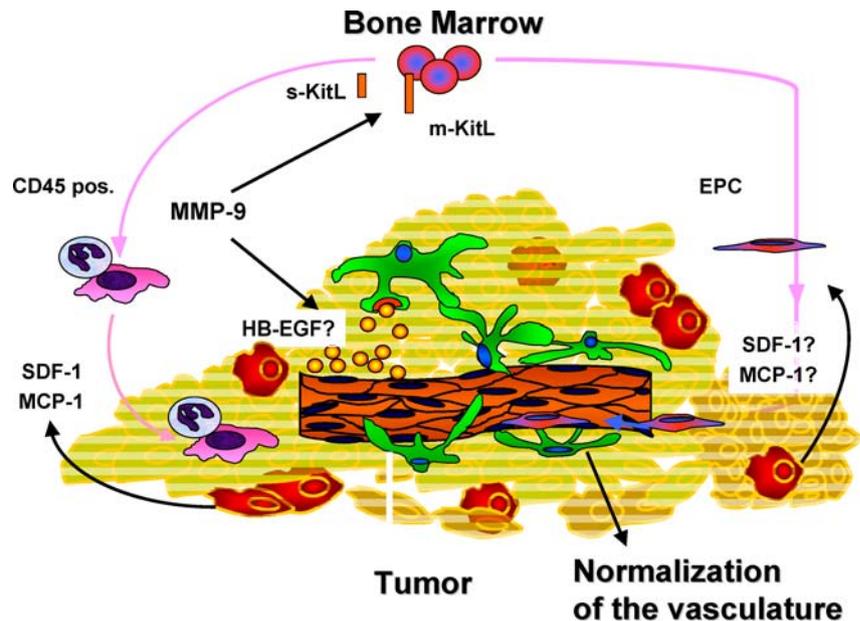
that upregulation of MMP expression in tumor cells promotes their metastatic potential, and that downregulation of MMPs or upregulation of MMP inhibitors in tumor cells inhibits metastatic potential. However the experiments in transgenic mice described above clearly indicate that stromal-derived MMPs also play a role in tumor metastasis [44]. A decrease in the number of metastatic nodules in MMP-9 deficient mice but an increase in MMP-7 deficient mice was reported recently when these mice were injected intravenously with Lewis lung carcinoma cells [71]. Using an orthotopic metastatic xenotransplanted model of neuroblastoma developed in our laboratory, we observed a decrease in spontaneous lung metastasis but no effect on liver or bone metastasis in MMP-9 deficient mice [46]. The mechanism by which stromal-derived MMPs contribute to tumor metastasis is unclear, however MMP-9 seems to play a critical role in the early survival and establishment of tumors in the lung rather than on subsequent growth [71]. Alternatively, considering the recent evidence that hematopoietic stem cells can colonize tissue before the establishment of metastasis [70], it is conceivable that MMPs like MMP-9 may play a role by promoting the recruitment of these cells to future sites of metastasis, thus "priming" the soil to accept the seed.

5. How do tumor cells increase the expression of MMPs by stromal cells?

5.1. EMMPRIN, AN MMP specific inducing protein

In the early 1980's, Biswas et al. postulated that tumor cells expressed a factor that specifically induced the production of collagenase in stromal fibroblasts [72]. In 1989, her laboratory isolated a collagenase stimulatory factor from human lung carcinoma cells [73]. This factor was subsequently cloned and characterized and was renamed extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147 or basigin. It is a member of the immunoglobulin superfamily of proteins that is present on the surface of several tumor cells [74, 75]. It is a potent stimulator of the expression of MMPs located adjacent to tumor cells [76, 77]. In tumors, EMMPRIN stimulates the expression of MMPs in stromal fibroblasts and endothelial cells as well as in tumor cells themselves by a mechanism involving close interactions between EMMPRIN molecules on apposing cells or on neighboring cells after shedding of EMMPRIN [74, 75]. MMPs can solubilize cell-associated EMMPRIN, enhancing its biological activity by eliminating the need for cell-cell contact for its effect [78]. EMMPRIN stimulates the expression of MMP-1, MMP-2 and MMP-3 in endothelial cells [79, 80] and induces the expression of these MMPs and MT1-MMP in fibroblasts [81]. In co-cultures of tumor cells and fibroblasts, overexpression of EMMPRIN in tumor cells induces the expression of MMPs and VEGF in fibroblasts [80]. In human breast cancer, positive EMMPRIN staining significantly correlates with other histopathological risk factors of poor prognosis and is associated with a decrease in tumor-specific survival [82]. EMMPRIN is also expressed on approximately 90% of micrometastatic cells in bone marrow samples of patients with breast cancer [83]. The mechanisms by which EMMPRIN stimulates MMP expression have not been completely elucidated. However recent data show that upon homophilic interaction, EMMPRIN upregulates MMPs in a genistein-sensitive manner, suggesting that its signaling mechanism depends on tyrosine kinase activity. Analysis of tyrosine phosphorylation-dependent MAP

kinases ERK 1/2, SAPK/JNK, and p38 in stromal fibroblasts cultured in the presence of EMMPRIN-expressing tumor cells reveals that the activity of p38 but not that of the other 2 kinases becomes elevated in response to EMMPRIN [84]. Induction of MMP-2 expression by soluble EMMPRIN produced by breast cancer cells involves activation of phospholipase A(2) and 5-lipooxygenase, linking their production to prostaglandins and leukotriene production [85].

5.2. Stimulation of MMP expression by tumor-derived cytokines

Several soluble growth factors and cytokines produced by tumor cells are potent inducers of the expression of MMPs by stromal cells. Among those is VEGF, which induces the expression of several MMPs in endothelial cells. Interleukin-6 (Il-6) and its soluble agonist receptor (sIL-6R) stimulate the expression of MMP-1 and MMP-2 in bone marrow mesenchymal cells and this mechanism is involved in promoting myeloma progression [86]. MMP-9 expression in fibroblasts is also induced by tumor cell-derived TNF-alpha and TGF-beta and this stimulatory effect is dependent on Smad-, Ras-, and PI3-kinase-signaling. It is also modulated by Hepatocyte growth factor (HGF)- and EGF-mediated signaling [87]. In the bone, SDF-1 increases MMP-9 expression and MMP-9-mediated dependent transcollagen migration in osteoclast precursor cells [87–89]. Although primarily expressed by stromal bone marrow cells, SDF-1 is expressed by some tumors like prostate cancer cells and therefore could play a role in prostate cancer bone metastasis by stimulating MMP-9 expression in osteoclasts [90]. Chemokines like MCP-1/CCL2, MIP-1 alpha/CCL3, and RANTES/CCL5 stimulate the release of monocyte-derived MMP-9 in a TNF-alpha dependent manner [88].

5.3. Mechanisms of recruitment of inflammatory cells

Tumor cells recruit MMP expressing stromal cells and in particular CD45 positive inflammatory cells within the tumor tissue. The contribution of inflammatory cells to cancer progression has been increasingly recognized and many of these cells like CD45 positive neutrophils and macrophages do express MMPs including MMP-2 and MMP-9. Among the factors that promote the recruitment of these cells to the tumor microenvironment is Colony Stimulating Factor-1 (CSF-1), a major regulator of the mononuclear phagocyte lineage. CSF-1 is expressed in more than 70% of human breast cancers and its expression is correlated with poor prognosis. Studies of CSF-1 null mutant mice demonstrate that CSF-1 plays an important role in normal mammary ductal development as well as in mammary tumor progression to metastasis [91]. CSF-1 may promote metastatic potential by regulating the infiltration and function of tumor-associated macrophages at

the tumor site, where these cells contribute to tumor progression by being an abundant source of stromal-derived MMPs [92]. Chemokines like SDF-1/CXCL12 and MCP-1/CCL2 not only can stimulate the release of MMPs but also attract CD45 positive bone marrow-derived inflammatory cells. The recent indication that MMP-9 is also critical for the successful recruitment of inflammatory cells into the tumor suggests that stromal-derived MMP-9 has a dual-site of action, in the bone marrow where it promotes the mobilization of bone marrow cells and at the tumor site where it promotes angiogenesis.

6. Conclusion

6.1. MMP inhibitors have failed in clinical trials

In the 1990's, on the basis of promising *in vitro* and *in vivo* preclinical data supporting a positive contributory role of MMPs in cancer progression, several synthetic inhibitors of MMPs developed by the pharmaceutical industry went rapidly into clinical trials. In most trials these inhibitors were tested in patients with end stage disease, in the absence of any tool to measure the biological activity of these inhibitors in patients and without a complete knowledge of the complex mechanism of action of MMPs in cancer (for review, see [93]). For example, that MMPs could inhibit rather than stimulate angiogenesis through the generation of angiostatin was not fully appreciated at the time clinical trials with MMP inhibitors were initiated. The chronic administration of several of these inhibitors was also associated with a musculoskeletal syndrome characterized by muscle and bone pain and the formation of connective tissue nodules that necessitated the removal of the drug [81]. As a result, MMP inhibitors are currently not considered as viable anticancer agents and all clinical trials on their use have been terminated.

6.2. Are there still windows of opportunity to target stromal-derived MMPs in cancer therapy?

Clinical trials with MMP inhibitors were done with the assumption that MMPs were mainly produced by tumor cells and/or endothelial cells contributing to invasion, metastasis and angiogenesis. We now realize that many other cells are a source of these MMPs and in particular that MMPs like MMP-9 may be critically involved in controlling the recruitment of bone marrow-derived inflammatory cells and EPCs. There are also recent suggestions that the recruitment of these cells is enhanced post chemotherapy [94]. Inhibition of MMPs post intensive chemotherapy may have a valuable antitumor effect by preventing or inhibiting the recruitment of inflammatory cells and EPCs by the primary tumor without having the side effects associated with long term MMP

inhibition. With a better knowledge of the biological function of MMPs in cancer and the contribution of stromal cells to their expression, it is now possible to return to preclinical models that more closely mimic specific phases of cancer treatment in patients in order to test such a hypothesis.

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