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The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia

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Contents

Abstract

In the past decades, a lot of effort has been put in identifying the role of matrix metalloproteinases (MMPs) in cancer. The main role of MMPs in angiogenesis, tumor growth and metastasis is degradation of extracellular matrix (ECM) and release and/or activation of growth factors through their degradative activity. The degradative activity finally results in cancer progression. MMP-inhibitors (MMPIs) have already been designed and tested, based on the degradative role of MMPs in cancer progression. First clinical trials with MMPIs have been performed

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with disappointing results, showing that in order to use MMP-inhibition the mechanisms underlying MMP-expression in cancer have to be further elucidated. This paper reviews the mechanisms of MMPs on molecular and cellular level and discusses the role for MMPs and MMP-inhibition in cancer with special focus on acute leukemia.

Keywords: Matrix metalloproteinase; Angiogenesis; Acute myeloid leukemia; Acute lymphoblastic leukemia

1. Introduction

Angiogenesis is a process that requires proteolysis of the extracellular matrix (ECM), migration as well as proliferation of endothelial cells, and synthesis of new matrix components [1]. Angiogenesis plays a role in non-pathological conditions, like the reproductive cycle in women, wound healing as well as placental development. In these conditions, angiogenesis is strictly regulated [2]. Dysregulation of angiogenesis may lead to pathological conditions like: rheumatoid arthritis, diabetic retinopathy and psoriasis [3,4]. Moreover, angiogenesis is essential for cancer progression. It has been demonstrated that tumors cannot grow beyond several mm^3 without new vessel formation [5].

Because of their capacity to degrade the extracellular matrix, resulting in, e.g. migration of endothelial cells, matrix metalloproteinases (MMPs) (especially MMP-2 and MMP-9) are known to play a role in angiogenesis, tumor growth and metastasis [6–10]. Recently, MMPs are being used as target molecules in anti-angiogenesis therapies, to increase the overall survival in several solid tumors.

In patients with acute leukemia increased angiogenesis was found, which disappeared if complete hematological remission was achieved [11–14]. The increased vessel density found in AML bone marrow correlated with vascular endothelial growth factor (VEGF)-expression [15]. Therefore, a role for other angiogenic factors, like matrix metalloproteinases is hypothesized.

In this review, we will focus on the mechanisms of MMPs on the molecular and cellular level. The role of MMPs in cancer progression will be discussed with a focus on their degradative capacity and the effect on angiogenesis, metastasis and tumor growth. Also an overview of current literature on MMPs in acute leukemia and the possible roles of MMP-expression in acute leukemia will be given. Finally, currently used drugs for MMP-inhibition (MMPI) and a possible role for interfering with MMP-activity in acute leukemia will be discussed.

2. Matrix metalloproteinases

Matrix metalloproteinases are a family of endopeptidases excreted by a number of cell types, capable of cleaving several macromolecules of the extracellular matrix. MMPs expose a great diversity in their domain structures and substrate specificities, while they still have shared features. These common features are: (1) the protein is able to hydrolyze a protein or proteoglycan component of the extracellular matrix; (2) the protein contains calcium and zinc ions necessary for catalytic activity; (3) the protein is excreted as a latent activatable protein (in a soluble or membrane bound form); and (4) the protein can be inhibited by its natural inhibitor, tissue inhibitor of matrix metalloproteinases (TIMPs) [16]. Over 60 MMPs have been sequenced to date, of which at least 17 are human MMPs [17].

2.1. MMP classification

Based on their structure and substrate specificity, the human MMPs are roughly divided into five groups: collagenases, gelatinases, stromelysins, membrane-type (MT) metalloproteinases and the others [7,18,19].

The main substrates for the collagenases are collagens types I, II and III; gelatinases mainly hydrolyze components of the basal lamina, gelatin and collagen type IV; and stromelysins mainly hydrolyze elastin and collagens types IV, V, XI and X. The membrane-type MMPs are not grouped for their substrate specificity, they are grouped because they are all membrane bound. All remaining MMPs (“others”) are MMPs that have been found, but are not fully characterized yet [8,18–20].

2.2. Structure of matrix metalloproteinases

The primary structures of MMPs contain three common structurally well-preserved domain motifs: (1) a catalytic domain; (2) an amino-terminal domain; and (3) a carboxy-terminal domain.

The catalytic domain of matrix metalloproteinases is responsible for substrate hydrolysis [21–23]. The structure of the catalytic domains of MMPs is quite similar with subtle structural differences among the five substrate groups. Together with the differences in domain structure, these subtle structural differences in the catalytic domains are thought to control the characteristic specificity for substrates of MMPs [23,24].

The amino-terminal domain, also called the propeptide domain, is about 80 amino acids large and responsible for the latency of the enzyme. The propeptide domain contains a unique PRCG(V/N)PD sequence [16,23]. The cysteine residue in this sequence interacts with the catalytic zinc atom in the active site, prohibiting activity of the MMPs. To activate the MMPs, the cysteine–zinc interaction has to be disrupted by a water–zinc interaction (called the cysteine

switch) [16,25]. In vitro activation of MMPs can be achieved by several methods. Two commonly used methods are organomercurial treatment with 4-aminophenyl mercuric acetate (APMA) or proteolysis. Treatment with APMA results in cysteine-modification, a permanent disruption of the cysteine–zinc interaction. Whereas proteolysis results in removal of the propeptide domain, that contains the cysteine residue [16,23,25].

Finally, the C-terminal domain or hemopexin domain of metalloproteinases has a four-bladed propeller structure, with a disc like structure in the middle containing a calcium ion [22]. The function of the hemopexin domain is not fully understood but it is thought to contribute to substrate specificity [26]. Other functions for the hemopexin domain are also known. In membrane-type metalloproteinases, the hemopexin domain contains a transmembrane domain for anchoring the protein in the membrane, whereas the hemopexin domain in MMP-2 also has a function in the activation of the enzyme [22,27].

2.3. Regulation of matrix metalloproteinases

2.3.1. Transcriptional regulation of MMPs

MMPs are highly regulated proteins and this regulation includes at least three different levels: transcriptional regulation, activation of latent MMPs and inhibition of MMPs [7,28]. The first level of regulation is transcriptional regulation. The basal gene-expression level and mRNA stability can be rapidly changed when remodeling of the extracellular matrix is required. This change can be achieved by change of extracellular matrix components or by growth factors or cytokines [7,19,23,28,29]. Not all MMPs contain well-defined transcriptional elements, one of these MMPs is MMP-2. Formerly, it was thought that MMP-2 could only be post-transcriptionally regulated, nowadays a number of well-known transcriptional elements have been identified in the MMP-2 promoter [29–33].

2.3.2. Activation of MMPs

The second essential form of regulation of MMPs is activation of latent MMPs. All MMPs are secreted as inactive zymogens and are activated by cleavage of the N-terminal pro-domain [7]. Most MMPs are activated by tissue or plasma serine proteases, like plasmin. The propeptide sequence is cleaved at basic amino acid motifs and subsequently autocatalytical activation of the MMPs is induced. Exceptions for this activation cascades are the membrane-type MMPs (MT-MMPs) and proMMP-2. MT-MMPs are activated intracellular before transport to the cell surface, resulting in an active membrane protein. ProMMP-2 however lacks a basic amino acid motif and can therefore not be activated by serine proteases [7,16,18,23,25].

Three mechanisms for proMMP-2 activation have been described at this moment; autocatalytical activation, TIMP-2 dependent activation and cell-surface associated urokinase-type plasminogen activator (uPA)/plasmin system dependent activation [34–40]. The mechanisms differ substantially, but have a characteristic phenomenon. That is, all three mechanisms of activation involve processing of proMMP-2 to an intermediate form and the intermediate form is then further processed to the active MMP-2.

However, mechanisms by which proMMP-2 can be activated are still under investigation, resulting in the elucidation of other mechanisms for proMMP-2 activation. Examples hereof are proMMP-2 activation by thrombin in a MT1-MMP dependent manner [41] and activation of proMMP-2 by MT2-MMP in a TIMP-2 independent manner [42]. The complexity of the different activation mechanisms indicates however, that there is a complicated network regulating extracellular matrix degradation. Another process that makes regulation even more complicated is inhibition of MMP-activity.

2.3.3. Inhibition of MMPs

A third form of regulation of MMPs described so far occurs via natural endogenous inhibitors of MMPs. Several natural inhibitors of MMPs exist, but the most common inhibitors are TIMPs and α_2-macroglobulin (α_2M) [8,28].

TIMPs are a group of endogenous proteins capable of inhibiting MMPs. Four TIMPs (TIMP-1–4) have been identified so far [7,8,17,23,28,43,44]. In general, binding of TIMPs to the active site of the MMPs results in an efficient reversible inhibition of enzymatic activity, but TIMPs do differ in their ability to inhibit the various MMPs. Also the tissue distribution of TIMPs is very diverse [23,28,45]. Together this might result in specific inhibition patterns of MMPs. TIMPs also have other activities next to influencing angiogenesis and tumor growth through MMP-inhibition. Examples hereof are reduction of cell growth and induction of apoptosis [23,46,47].

Another inhibitor of MMPs is α_2-macroglobulin. α_2M is a non-specific protease inhibitor, that is commonly expressed in the human plasma and serum. α_2M is a homotetramer, built up of two pairs of non-covalently associated dimers. Each subunit contains a “bait domain” with cleavage sites for almost all known endopeptidases and a receptor-binding domain. When endoproteinases cleave the four “bait domains” α_2M undergoes a structural change. This structural change results in the irreversible entrapment of one or two molecules of the endoproteinase. At the same time, the receptor-binding domains are exposed on the surface and the total complex undergoes rapid endocytosis by binding to the low-density lipoprotein receptor-related protein. It is known that this protease inhibitor is capable of capturing inactive MMPs [48].

A third known natural inhibitor of MMPs is endostatin. Endostatin is a cleavage product of type XVIII collagen. Under physiological conditions, it is known to circulate at low levels, whereas levels are increased during cancer progression. The reason for this rise in endostatin levels is not clear yet, but increased MMP-activity might play a role [49–56]. Endostatin was described to be able to inhibit proMMP-2

activation and inhibit MMP-2 activity in vitro. Endostatin is able to form a complex with MMP-2, but the precise mechanism of inhibition is not known [57,58]. Endostatin however does in this way provide a negative feedback loop of MMP-activity, because it is generated by MMPs and it inhibits MMPs. Not all regulatory mechanisms of MMPs are known yet, but in the next years, the complexity of MMP-regulation will be further elucidated.

3. The extracellular matrix

The extracellular matrix is a network of molecules supporting and dividing tissues, that is also able to influence and interact with cells in the environment. It consists of structural proteins (e.g. collagen and elastin), specialized proteins (e.g. fibronectin and laminin) and proteoglycans and polysaccharides (e.g. perlecan) [59,60].

The precise composition of the ECM is related to the characteristics of the specific tissues, so that each tissue seems to have slightly different types of ECM. For example, the ECM of bone marrow is mainly made up of collagens (types I, II, IV–VI), fibronectin, vitronectin, laminin, thrombospondin and glycosaminoglycans/proteoglycans [61].

Another form of specialized ECM is the basement membrane (BM). The BM is a thin flexible mat that separates epithelial cells from the underlying stroma. In this way, it provides a first barrier against cell invasion and therefore the BM is implicated in angiogenesis, tumor growth and metastasis. The basement membrane primarily consists of collagen type IV, fibronectin, vitronectin, laminin and proteoglycans, and its composition is the same throughout the body [8,60].

The differences and/or similarities in ECM compositions not only have an impact on the different matrix metalloproteinases needed, but can also influence cell behavior through cell–matrix interactions. In the next paragraph, we will briefly discuss cell–matrix interactions that are known to play a role in tumor growth and angiogenesis, with a focus on $\alpha_v\beta_3$-integrin.

3.1. Cell–matrix interaction

ECM proteins can interact directly with cells through receptor-mediated signaling with cytokine receptors, growth factor receptors and other cell-adhesion receptors [61]. All of these receptors are important for regulating biological functions like proliferation, apoptosis and migration. Besides these direct interactions, indirect interactions can occur through changing of growth factor availability and co-localization of growth factors and cells [62–65]. In this paragraph, we focus on cell-adhesion receptor-mediated signaling in relation to MMPs. A large group of cell-adhesion receptors capable of signaling via receptor-mediated signaling are the integrins.

Integrins are a family of heterodimeric cell-surface receptor proteins, consisting of α and β subunits, that can activate a variety of protein tyrosine kinases like focal adhesion kinase (FAK) and Src-family kinases. Integrins are involved in a direct interaction of cells with matrix and each combination of αβ-subunit has its own binding specificity. Next to regulation of cell migration by providing cell–matrix interactions integrins are also thought to regulate degradation of ECM [63,66].

An integrin receptor strongly involved in angiogenesis and tumor growth is $\alpha_v\beta_3$-integrin [67–69]. This integrin can bind vitronectin and fibronectin and various other proteins with an arginine-glycine-aspartic acid (RGD) (Arg-Gly-Asp) sequence, like fibrinogen, von Willebrand factor and denatured collagen [62,66]. Different reports have correlated expression of integrins with more invasive phenotypes of tumor cells and activation of MT1-MMP and MMP-2 [67,69–71]. An explanation for the activation of MMP-2 is its capacity to bind $\alpha_v\beta_3$-integrin through its fibronectin like repeats. This binding of MMP-2 to $\alpha_v\beta_3$-integrin results primarily in localization of MMP-2 at the membrane. But it is also suggested that this binding enhances the expression of MMP-2 due to activation of the integrin [72,73]. It is known that integrin receptors are expressed on activated endothelial cells [66,74–76], but it is not known whether leukemic cells can express these integrins. In cell migration and cell–matrix interactions, $\alpha_v\beta_3$-integrin is localized to focal adhesion sites and promotes cell spreading and migration on various RGD-motif containing substrates [62,65,77].

4. The role of MMPs in cancer

First evidence for the role of MMPs in cancer came from knock-out mice. Knock-out mice for MMP-2 show reduced tumor angiogenesis resulting in reduced tumor growth [78–80]. In several tumors, MMPs have been linked to tumor growth, metastasis and clinical outcome [7,10,18–20,23,81–83]. It has also been shown that proMMP-2 and proMMP-9 expression is increased in malignant cancers compared to benign cancers [84–86].

Moreover, MMP-2 has been shown to be a prognostic factor in different malignancies such as ovarian cancer, gastric carcinoma and stage I non-small cell lung carcinoma [87–89]. In the next paragraphs, we will highlight the role of MMPs in cancer progression.

4.1. The role of MMPs in cancer progression

In the process of cancer, a number of cell-types are known to express MMPs. Besides tumor cells, stromal, endothelial and inflammatory cells are also capable of expressing MMPs.

The role of MMPs in the process of cancer can be mainly attributed to their degradative capacity. This results in numerous products that are capable of affecting the process of cancer. To illustrate the involvement of MMPs in the process

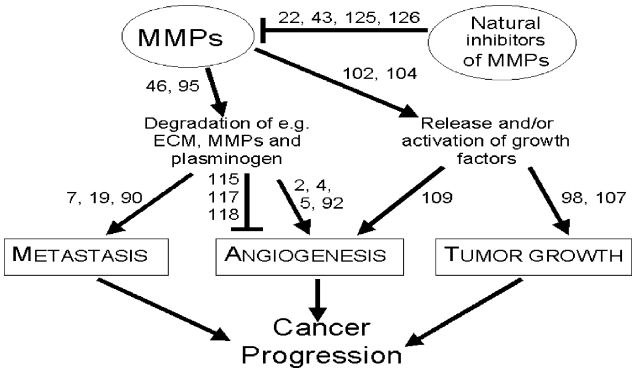

Fig. 1. A schematic overview of cancer progression by MMPs. Some key references are given (⊣: negative regulation; →: positive regulation).

of cancer, the process can be roughly divided into three main processes: (1) tumor angiogenesis; (2) tumor growth; and (3) metastasis, albeit that these processes are intermingled with each other. MMPs have been identified to play a role in all of these processes. In Fig. 1, we have schematically depicted the role of MMPs in the process of cancer. With help of this figure, we will discuss the link of MMPs with tumor angiogenesis, tumor growth and metastasis in the next paragraphs.

4.2. The role of MMPs in tumor angiogenesis and tumor growth

An important part in the process of cancer is tumor angiogenesis. It is known that angiogenesis is a prerequisite for tumor growth, since tumors cannot grow beyond several mm^3 when new vessel formation is blocked [5].

Angiogenesis is a process in which new vessels are formed from existing vessels. One of the beginning steps of tumor angiogenesis is degradation of ECM, performed by the MMPs. This enables endothelial cells to migrate followed by proliferation, finally resulting in new vessel formation [66,78,90–92]. Through the years, great progress has been made in elucidating the factors involved in angiogenesis. To date many angiogenic and anti-angiogenic factors have been described. Several of these factors are primarily involved in migration, proliferation and/or survival of endothelial and tumor cells [3,4,93,94].

Matrix metalloproteinases, especially MMP-2 and -9 are known to be involved in tumor angiogenesis mainly through their degradative capacity [2,92,95]. Degradation of ECM can result in tumor cell as well as endothelial cell migration due to loss of cell–matrix contacts and cell–cell contacts. For example, degradation of laminin-5 (Ln-5) by MMP-2 results in the exposure of a Ln-5 cryptic site that enhances endothelial cell migration [2,92,96,97]. Besides degradation of ECM, MMPs are also capable of releasing growth factors from ECM and/or inactive complexes, cleaving growth factor receptors and activating growth factors excreted as pre-pro-enzymes, like transforming growth factor (TGF)-α, TGF-β, macrophage-colony stimulating factor (M-CSF), insulin like growth factor (IGF) and fibroblast growth factor receptor (FGFR)-1 [4,98,98–109]. As pointed out, above MMPs can directly or indirectly induce tumor angiogenesis through degradation of ECM and release or activation of growth factors, like TNF-α and TGF-β. Moreover, the release and/or activation of growth factors, such as IGF and TGF-α, might also lead to tumor growth independent of angiogenesis [110–114].

However, MMP-activity may not always lead to a positive effect on tumor angiogenesis. Numerous ECM-fragments (e.g. tumstatin and fragments of collagen type IV chains), fragments of plasminogen (e.g. angiostatin) or fragments of MMPs (e.g. PEX) are associated with inhibition of tumor angiogenesis [115–124]. Some mechanisms of action of tumstatin and endostatin have recently been elucidated. It was found that tumstatin mainly inhibits tumor angiogenesis via inhibition of endothelial cell proliferation and promotion of endothelial cell apoptosis, whereas endostatin inhibits endothelial cell migration [125]. The mechanism of action of PEX has not been elucidated yet, but it is known that PEX inhibits angiogenesis both in vitro and in vivo [123,124].

Other well-known inhibitors of tumor angiogenesis are the inhibitors of MMPs like TIMPs and α_2-macroglobulin (see Section 2.3.3). TIMPs can interfere with tumor angiogenesis in different ways. Firstly, the TIMPs can inhibit tumor angiogenesis by inhibiting the degradation of ECM by MMPs [43]. Secondly, the TIMPs, especially TIMP-2, can also interfere with tumor angiogenesis through their role in activating MMPs (mainly MMP-2), as described in chapter 2 [22,126]. Moreover, TIMPs are known to have several effects on tumor cell proliferation and apoptosis, independent of their capacity to inhibit MMP-activity [43,44].

Next to MMP-expressing tumor cells, stromal, endothelial and inflammatory cells are also associated with tumor angiogenesis. Inflammatory cells expresses a number of angiogenic factors, like metalloproteinases, cytokines and growth factors. For instance, mast cells are able to release stored and newly synthesized inflammatory mediators, including MMPs, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor. However, not only mast cells are capable of excreting MMPs and other pro-angiogenic factors, also tumor associated macrophages (TAMs), neutrophils and activated T lymphocytes can contribute to angiogenesis by releasing MMPs and angiogenic factors [127–130]. In a number of cancers, inflammatory cell infiltration correlated with the degree of tumor angiogenesis [131–134]. The role of inflammatory cell derived MMPs is largely the same compared to tumor cell derived MMPs. They mainly affect tumor angiogenesis through their degradative capacity. The relationship between inflammatory cells and the process of cancer has been excellently reviewed by Coussens and co-workers [128,129].

Summarized, in tumor angiogenesis, the main role for MMPs is degradation of ECM and release and/or activation of pro-angiogenic growth factors, resulting in enhanced

new vessel formation locally. Almost simultaneously negative regulators of angiogenesis, including MMP-inhibiting fragments are formed through ECM degradation, to control the local process of new vessel formation. Systemically, MMPs can play a role as negative regulator of angiogenesis by releasing fragments that function as negative regulators of angiogenesis, like angiostatin and endostatin. These fragments are known to circulate systemically and can be up-regulated in the process of cancer.

Numerous studies have shown that in vitro inhibition of MMPs does not have cytotoxic effects on tumor cells. However, using the same inhibitor in in vivo studies with tumors implanted in mouse-models decreases tumor growth [9,17,135–142]. It is not ruled out that the in vivo results might be obtained by negatively affecting tumor angiogenesis, resulting in decreased tumor growth.

Moreover, the MMP-dependent release of growth factors from ECM and/or inactive complexes and activation of pre-pro-growth factors resulting in tumor growth might also be blocked by MMP-inhibitors in vivo. The role of MMPs in activating pre-pro-growth factors is under discussion. More scientific evidence implicates the adamalysin family of metalloproteases (ADAMs, e.g. tumor necrosis factor-α converting enzyme (TACE)) in the activation of pre-pro-growth factors. ADAMs are known to be very efficient in activating pre-pro-growth factors [143–145]. The confusion between MMPs and ADAMs could arise since early studies for the role of MMPs in substrate cleavage were largely conducted with TIMPs and it is known now that TIMPs are also capable of inhibiting ADAMs [146,147]. Furthermore, MMPs have been implicated in protecting tumor cells from immune responses and apoptosis via influencing death receptor/death ligand pathways, resulting in tumor cell survival and tumor growth. For instance, MMP-7 is capable of cleaving Fas ligand (FasL), resulting in soluble FasL (sFasL). This sFasL can then bind to Fas receptor on tumor cells and the complex is rapidly internalized, resulting in down regulation of Fas-receptor [148].

It is clear that MMPs play an important role in regulating tumor angiogenesis and thereby tumor growth, mostly through their degradative activity of ECM and/or activation of pre-pro-growth factors. Besides the role of MMP in tumor growth via tumor angiogenesis, MMPs have a role apart from angiogenesis in tumor growth.

4.3. The role of MMPs in metastasis

Next to their role in angiogenesis and tumor growth MMPs are also known to play a role in the process of metastasis. Metastasis can be roughly subdivided into several phenomena: (1) dissociation of metastatic tumor cells from the bulk tumor; (2) migration to blood and/or lymph vessels; (3) penetration of vascular lumen; (4) escape from immune cells; (5) adhesion to vascular endothelium at distant sites; (6) extravasation from vessels into new host tissue; and (7) tumor cell survival and growth in the new host environment. So far no single gene has been implicated as a metastasis specific gene, but it is known that the metastatic process depends on tumor angiogenesis [7,95,149–151]. The main role of MMPs in the process of metastasis again is their degradative capacity, which enables tumor cells to migrate and colonize host tissues.

MMP-2 and -9 are the most studied MMPs in the processes of cancer, including the process of metastasis. However, research to identify the role of other MMPs, like MT1-MMP, in cancer and metastasis is increasing [152,153]. A number of studies have linked elevated MMP-2 and -9 expression to increased metastasis and tumor stage, e.g. malignant versus benign breast tumors and advanced ovarian tumors versus benign [84–86]. In several invasive cell lines, MMP-9 expression was increased compared to non-invasive counterparts [154,155]. Also evidence for a role of MMP-2 and -9 in the process of metastasis has come from experiments with MMP-overexpression, recombinant TIMPs or synthetic MMP-inhibitors [156–160]. For instance, MMP-2 or MMP-9 overexpression increased the incidence of metastasized diseases in immunocomprized mice [161–163].

Most of the performed research presented above has been done with endpoint studies, in which tumors were implanted in mice and the number of metastases were determined. These studies clarify that MMPs are involved in metastases, but in which steps of the process of metastasis do they play a role? Other techniques besides endpoint-studies in metastases research implicated that MMPs might be involved throughout the whole process of metastasis [157,164–170].

Although the precise role of MMPs in the process of metastasis remains to be elucidated, it appears to be depending on the degradative capacity of MMPs, as with tumor angiogenesis and tumor growth. In conclusion, the role of MMPs in cancer progression is degradation of ECM and releasing and/or activating growth factors. The resulting products of MMP-degradation are capable of affecting cancer progression.

5. The role of MMPs in acute leukemia

A possible role for MMPs in acute leukemia has been hypothesized, because of the role of MMP-2 and -9 in tumor angiogenesis. In acute leukemia, increased vessel density is found at diagnosis, disappearing when complete remission is achieved [12,15,171–173]. In this paragraph, an overview of current literature on MMP-2 and -9 expression in acute leukemia will be given.

5.1. Acute lymphoblastic leukemia (ALL)

MMP-2 expression of lymphoblastic cell lines correlated with the ability to invade matrigel in vitro and with the capacity to invade and metastasize in a SCID mouse model [174]. Also, MMP-9 expression in lymphoblastic cell lines was

found to be important for invasion and metastasis [175]. It was also shown that Burkitt's lymphoblastic cells implanted in SCID mice could invade the central nervous system if the implants contained MMP-9 of mice origin [176].

So far, only Kuittinen et al. [177] have studied the role of MMP-2 and -9 expression in the clinical course of ALL. A remarkable difference could be found in expression of MMP-2 and -9 between pediatric and adult ALL; in adult ALL 65% of the cases showed positive staining in blast cells for MMP-2 and 25% for MMP-9. In contrast, in the pediatric ALL cases, only 12.7% of the cases showed a positive reaction for either of the antibodies.

A correlation could be found between MMP-2 expression and an extramedullary disease pattern (spleen, liver, nodal or bone lesions and/or testicular, central nervous system or lung involvement) in adult ALL. Moreover, a trend towards worse survival could be observed in cases with MMP-9 positive blasts. In pediatric ALL, there seemed to be no correlation between MMP-expression and survival [177].

In conclusion, the studies performed so far have focussed on the role of MMP-2 and -9 in the invasive behavior of ALL cells and were mostly performed in vitro and in mouse models. As in solid tumors, one could hypothesize not only a role of MMPs in invasive behavior and metastases, but also in tumor growth. However, none of the studies so far have investigated this possible role of MMPs. Further studies of the biological roles of MMPs in ALL are still warranted.

5.2. Acute myeloid leukemia (AML)

In the past decade, a number of articles have investigated MMP-2 and -9 expression of myeloid cells. Variable expression levels of MMP-2 and -9 could be detected in conditioned media of myeloid cell lines [177–183]. The capability of penetrating matrigel in an invasion assay by KG-1 could be partially blocked by TIMP-2 and an MMP-2 antibody, but not by an MMP-9 antibody, indicating that in vitro invasion largely depends on MMP-2 activity [181].

In isolated myeloid blasts of AML patients at diagnosis variable levels of MMP-2 and-9 expression could be detected ex vivo [15,178,180,184]. A significantly lower expression level of MMP-9 in AML-patients compared to normal controls was found when the expression of MMP-9 in bone marrow plasma was measured. At complete remission, MMP-9 levels had the same range as in normal controls, with a drop in MMP-9 levels in case of relapse [185]. Upon these results, it was suggested that MMP-9 measured in bone marrow plasma might be a surrogate marker of leukemic status in AML-patients.

MMP-9 expression might be related to the normal expression in mature monocytic and granulocytic cells. Moreover, normal $CD34^+$ bone marrow mononuclear cells are capable of expressing MMP-9, with an adequate stimulus [61,179,186–188]. Some articles have suggested that MMP-9 expression might be involved in physiological processes, e.g. the differentiation process in hematopoiesis [189,190]. However, the differences in MMP-9 expression between AML and normal bone marrow cannot be explained by the maturation stage of the leukemic cells in the bone marrow. In plasma of more mature AML cells (FAB classification M4/M5), MMP-9 levels are still far below the levels found in normal bone marrow cells. Moreover, other studies showed MMP-9 expression in AML cells as well as in normal bone marrow cells. An explanation might be that most MMP-9 secretion originates from other non-leukemic bone marrow cells, like stromal cells, endothelial cells and fibroblasts. Low MMP-9 levels at diagnosis of AML suggest that the lack/decrease of MMP-9 can eventually play a role in cancer/leukemic progression.

In normal bone marrow mononuclear cells, no MMP-2 expression could be detected [15,61,178,180,181,184]. Primary AML blasts are able to invade Matrigel dependent on their MMP-2 expression [181]. MMP-2 expression might also play a role in the increased vessel density found in bone marrow of AML-patients at diagnosis by facilitating in vitro endothelial cell migration [15]. These data indicate that MMP-2 might be involved in leukemic progression.

So far two studies have been published that studied the role of MMP-2 or -9 expression in clinical course of AML. One study showed that patients with lower MMP-9 levels tended to have longer survival times [185]. A second study surprisingly showed that 82% of the MMP-2 positive patients survived for over 3 years, whereas all MMP-2 negative patients relapsed within 13.5 months of diagnosis in a Kaplan-Meier analysis [180]. Compared to solid tumors this finding is surprising, as increased MMP-expression in solid tumors is often related with a more biological aggressive and invasive phenotype (see Section 4). However, in studies performed so far mostly MMP-protein and mRNA levels have been measured in stead of active MMP-2. This might explain some of the discrepancy between MMP-2 protein expression and good prognosis in AML.

In conclusion, most of the studies performed so far have focussed on the role of MMPs in the invasive behavior of AML-cells. The role of MMP-expression in increased angiogenesis and leukemic cell growth have not been extensively investigated, warranting further studies of the role of MMPs in tumor angiogenesis and tumor growth in AML.

6. MMP inhibition in cancer

6.1. Current used interventions in MMP-inhibition

To inhibit the growth and metastasis of tumors a lot of effort is put in development of angiogenesis inhibitors. As described in chapter 4, one way of inhibiting angiogenesis is MMP-inhibition. Therefore, endogenous inhibitors of MMPs, like TIMPs and α_2-macroglobulin might be interesting candidates for development of angiogensis inhibitors. TIMPs however are not yet applicable because of their short half-life [7]. In the future, TIMPs might be used in, for

instance, site directed gene therapy, resulting in TIMP delivery and activity at the desired place [136]. Recently, a lot of effort has been put in designing synthetic MMP-inhibitors as an alternative approach for inhibition of angiogenesis.

The engineered MMP-inhibitors can be roughly divided into four pharmacological groups: (1) collagen peptidomimetics; (2) non-peptidomimetics; (3) tetracyclinederivatives; and (4) bisphosphonates [9,18,135,136].

Collagen peptidomimetic MMP inhibitors are protein derivatives that have been synthesized so that the structures mimic the structure of the MMP-binding site and cleavage site on the different collagens. Two well-known inhibitors of this group are Batimastat and Marimastat. Batimastat was the first inhibitor used in clinical trials for cancer patients [6,9,17,18,135,136,191].

Non-peptidomimetic MMP inhibitors do not contain a peptide backbone in contrast to the peptidomimetics. They are synthesized based on the crystal structure of the active site of MMPs overcoming the problem of a-specificity of the peptidomimetics. Two of these compounds AG3340 (prinomastat) [139] and BAY 12-9566 [192] have been synthesized to specifically inhibit MMP-2. Pre-clinical studies with BAY 12-9566 seemed very promising. In vivo, the administration of BAY 12-9566 reduced angiogenesis and inhibited the formation of lung metastases in a mouse model [17].

The tetracycline derivatives inhibit MMPs in a bifunctional way; not only the activity is inhibited, but also the production of MMPs. An example of a tetracycline derivative capable of inhibiting MMPs is Col-3 (or CMT-3 or metastat). In various cancer cell lines, it was shown to be capable of inhibiting the expression of MMP-2 and -9. Col-3 could also prevent proliferation, invasion, tumor growth and metastasis in a metastatic prostate cancer model [9,136,193].

Bisphosphonates are medications that have been developed for use in disturbed Ca^{2+}-homeostasis. The mechanism of action of the bisphosphonates is not known yet, but they have various inhibiting effects on MMPs, including inhibition of MMP-activity. One of the most used and well-known bisphosphonates is clodronate. Clodronate is a bisphosphonate that has the capability to inhibit the expression of MT1-MMP at both mRNA and protein levels in fibrosarcoma cells [136].

However, most of the currently used MMP inhibitors are not very selective. Therefore, inhibitors are developed that are selective for specific MMPs based on the structure of the catalytic site and by means of random peptide libraries. In this way, a number of new inhibitors have been developed that are selective for MMP-2 and -9, like the cyclic peptide (CTTHWGFTLC) [136,160]. The cyclic peptide (CTTHWGFTLC) was able to effectively inhibit MMP-2 and -9 activity, and was specific for MMP-2 and -9. In vitro, the peptide inhibits cell migration, tumor neoplasia and tumor growth in a gelatinase dependent manner [160]. In vivo, studies in mouse models have shown that the peptide possesses an anti-tumor activity and that it can inhibit invasive growth. Advantages of these cyclic peptides are that they are very stable due to their cyclic form and that, in the mouse models, there were no cytotoxic side effects [194].

6.2. Clinical trials with MMP-inhibitors

Several MMP inhibitors have been tested in phase I/II trials. Trials with batimastat were stopped after development of marimastat, an oral MMPI variant of batimastat. But data from early clinical trials of MMPIs like marimastat are difficult to interpret. This has several reasons: (1) often trials have been reported in abstract form only [9,191]; (2) a number of trials were conducted not as classical clinical trials but as combination phase I/II studies; (3) clinical activity has been reported primarily in terms of change in the rate of rise of tumor markers pre- and post-treatment; (4) mechanisms underlying toxicity of the MMPIs and measures to prevent or minimize the toxicity are often poorly understood [191]. In the meantime, phase III studies have been started for several MMPIs.

In 1999, two phase III clinical trial with BAY 12-9566 in cancer were stopped [6,195]. The patients receiving BAY 12-9566 had a poorer survival than the patients in the control group [6,8,196]. Also a phase III trial with prinomastat was stopped in 2000 due to the lack of effectiveness in patients with a late stage cancer. Several clinical trials with prinomastat in earlier stages of cancer are still continued (see Auguron Pfizer Press release website: http://www.agouron.com/Pages/press_releases). Phase III studies with marimastat and other MMPIs are also ongoing [9].

There could be several reasons for failure of the phase III clinical trials. (1) Both BAY 12-9566 and prinomastat were designed to inhibit MMP-2 and MMP-9. It could be that the efficiency of inhibiting MMP-2 and -9 is not enough to inhibit the process of cancer. Many other MMPs and enzymes might take over part of the activities of MMP-2 and -9. (2) Inhibition of the MMPs also inhibits processing of anti-angiogenic factors like endostatin and angiostatin, resulting in a decrease of these factors. (3) The stopped trials were all designed for late stage tumors, whereas the pre-clinical data showed that the best effects were reached in early stage [6,8]. (4) The trials were not optimally designed for MMPIs. Pre-clinical studies have shown little cytotoxic effects. Also the classical endpoints of phases I and II studies have not been reached in the MMPI studies. Dose-limiting toxicity does not occur, resulting in an incapability of defining one single phase II dose. And also the classical phase II study endpoint, tumor regression, will probably not be reached with MMPIs. It is important to design clinical trials optimal for MMPIs, because the potential anti-tumor effect of MMPIs might be underestimated.

7. Conclusions/future perspectives

Matrix metalloproteinases are a family of endopeptidases excreted by a number of cell types. In spite of the great

diversity in domain structures, MMPs have characteristic features. They are able to hydrolyze a protein or proteoglycan component of the extracellular matrix, contain calcium and zinc ions necessary for catalytic activity, are excreted as a latent activatable protein (in a soluble or membrane bound form) and they can be inhibited by their natural inhibitors tissue inhibitor of matrix metalloproteinases [16].

In cancer matrix, metalloproteinases are known for their degradative activity. MMPs are capable of cleaving several (angiogenic) factors like TGF-β, FGF and FGFR-1, next to degradation of ECM [98,109,197]. The degradative activity of MMPs can result in cancer progression by affecting tumor angiogenesis, tumor growth and/or metastasis. Therefore, high MMP-expression is often related to poor clinical outcome [7,10,18–20,23,81–83].

The precise role of MMP-expression in acute leukemia is still not clear. So far, it is known that leukemic cells are capable of expressing MMP-2 and/or -9. Many studies show a reflection of MMP-2 and/or -9 with invasive behavior in ALL and AML cell lines [15,174–180,184,187]. No MMP-2 expression can be detected in normal bone marrow mononuclear cells. However, MMP-9 expression in normal bone marrow is higher compared to MMP-9 expression in acute leukemia.

The role of MMP-2 and -9 in acute leukemia is most likely dependent on its degradative capacity. It is known already that MMP-2 can affect tumor angiogenesis in acute leukemia by facilitating endothelial cell migration in vitro. In addition, MMP-2 in acute leukemia might also be involved in leukemic cell growth through changing cellular signaling and functions by the release of several growth factors, such as VEGF, resulting in enhanced leukemic cell survival. Finally, MMP-2 might affect the process of metastasis as in vitro MMP-2 dependent Matrigel invasion has been found. However, the precise role for MMP-2 in clinical outcome remains to be elucidated. Taken together these data indicate that there might be a role for MMP-2 in acute leukemia. However, additional functional analysis of MMP-activity and RNAi model systems will help to clarify the role of MMPs in acute leukemia.

Clinical trials with MMP-inhibitors have been started in solid tumors. However, the results were not always positive. There are several reasons for failure of the trials. Dose-limiting toxicity does not occur and the classical endpoint of phase II trials has to be redefined.

In acute leukemia, MMP-inhibitors combined with other treatment strategies can be hypothesized. However, in order to use MMP-inhibition approaches it is important to identify the processes and mechanisms underlying MMP-expression and function in cancer, e.g. acute leukemia.

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Biography

Eveline de Bont received her degree in Medicine in 1989 at the University of Amsterdam, The Netherlands. Until 1998, she was trained in pediatrics and pediatric oncology at the University Hospital of Groningen, The Netherlands. In 1994, she received the Young Investigator Award of the European Society of Pediatric Infectious Diseases for her research concerning diagnostic parameters in neonatal sepsis. In 1997, she worked for a year at the Department for Cancer Biology, Harvard School of Public Health, Boston, USA under supervision of Dr. L.H. Glimcher. She obtained her Ph.D. degree in Medicine in 1999 at the University of Groningen, The Netherlands. Currently, she is working as an academic staff member of the division of Pediatric Oncology and Hematology, Beatrix Children's Hospital, Groningen, The Netherlands.