MMPs in invasion and metastasis, and their use as targets for anti-cancer therapy

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Metastasis is the most frequent cause of death in cancer patients and is therefore important to target therapeutically. To prevent metastasis it is useful to target invasion. It has been suggested that inhibition of MMPs (matrix metalloproteases) is an effective way to target invasion, since MMPs have many functions in invasion. However, so far MMPIs have failed in clinical trials. The main reason for this failure is unspecific inhibition of MMPs. To be able to create more specific MMP inhibitors, MMP biology in cell migration and invasion should be investigated *in vivo*.

Preface

The spread of cancer cells from a primary tumor followed by formation of secondary tumors is termed metastasis, and is in cancer patients the most frequent cause of death. Prevention of metastasis is therefore of high importance. In this review I will discuss MMPs (matrix metalloproteases) as a target for anti-metastasis therapy. I will do that by first explaining the process of metastasis and invasion. Then I will explain the role of MMPs in invasion. Next, I will discuss MMP inhibitors and why they have failed so far. At last, I will give some future directions for the MMP field; what we should keep in mind when designing new MMP inhibitors, and in what direction research should go.

Introduction to metastasis

The metastatic process is thought to be a multi step process that involves 1. Cell invasion towards the blood vessel 2. Intravasation into the blood vessel or lymphatic system 3. Survival in the bloodstream 4. Arrest in a new organ 5. Extravasation from the blood vessel 6. Growth at a secondary site (Ian C. MacDonald, 2002).

To prevent the formation of secondary tumors (metastasis), inhibition of invasion or chemotaxis is thought to be a useful strategy. Cancer cell invasion refers to the process of cell migration through the surrounding tissue and chemotaxis refers to cell recruitment towards a specific place (fig 1). It is suggested that inhibition of invasion or chemotaxis might be a good way to prevent metastasis because many of the proteins involved in invasion and chemotaxis are upregulated in cancer. Furthermore, inhibition of several of these processes have shown promising results in animal models (Ali and Lazennec, 2007; Sahai, 2005). For example, overexpression of LIM-kinase, which suppresses invasion and motility, decreases metastasis of breast cancer cells (Wang et al., 2006). Moreover,

knockdown of the chemokine receptor CXCR4 (involved in invasion and chemotaxis) decreases breast cancer metastasis in mice (Smith et al., 2004). Thus, inhibition of invasion or chemotaxis is a promising strategy to prevent metastasis.

Invasion and chemotaxis

Inhibition of metastasis can be accomplished by inhibiting invasion or chemotaxis. To prevent cancer cells from invading the surrounding tissue, it is important to understand how exactly the invasive and chemotactic processes work, which is explained below.

As said, cancer cell invasion is the first step of metastasis, and it refers to cell migration through the extracellular matrix (fig 1). Before a cancer cell can become invasive, it needs to detach from its neighboring cells. This process of detachment is termed **EMT** (Epithelial to Mesenchymal Transition), whereby the cell undergoes a transition from an epithelial differentiation to a more mesenchymal differentiation. EMT is marked by the loss of polarity and an increase in cell motility. From an apical/basal polarization, the cell is altered to an elongated morphology, losing cell-cell contacts. Furthermore, molecular markers such as E-cadherin and cytokeratins are downregulated, and markers such as N-cadherin, vimentin and MMPs are upregulated.

Following EMT, a cell can start invading the tissue towards a blood vessel. On its journey towards the vessel, the cell encounters matrix with changing densities. It is thought that these densities determine the mode of cell migration.
Within the tumor, the matrix is not very dense, consisting mainly of collagen and laminin. The cell is easily able to migrate through this matrix, and is thought to use the matrix fibers as a “highway” along which it can migrate towards a blood vessel (fig 1)(Condeelis and Segall, 2003; Wang et al., 2002). It does so, most likely, via **amoeboid-like migration**. Several tumor cell lines have been shown to be able to migrate via an amoeboid movement *in vitro* on loose matrix, including A375m2 melanoma cells, MTLn3E highly metastatic breast cancer cell, walker carcinoma cells and MDA-MB-435 cells (H. U. Keller, 1996; Kitzing et al., 2007; Sahai and Marshall, 2003; Wyckoff et al., 2006). Amoeboid movement is characterized by a round morphology, bleb like membrane protrusions and is protease independent. It is not only observed in cancer cell migration, but during development as well (Blaser et al., 2006). Cell blebs are formed upon rupture of F-actin, or upon dissociation of the PM from the cortex. Due to hydrostatic pressure the bleb expands and the actin cortex is reformed in the bleb. The advantage of this way of migration is that a cell is able to change direction quickly because it is not polarized. Furthermore, amoeboid migration is fast, because the cell hardly adheres to its surroundings. (Charras and Paluch, 2008; Fackler and Grosse, 2008).
When a cancer cell encounters a blood vessel, the matrix density changes to more dense matrix (also called the basement membrane) consisting of a tightly packed network of collagen IV, laminins, nidogen/entactin and perlecan (Kalluri, 2003). The cell cannot migrate through this matrix, unless it degrades the matrix. Matrix degradation is done by mesenchymal cells (fig 1). Interestingly, it is suggested that when a cell encounters a dense matrix, a cell changes its mode of migration from amoeboid towards **mesenchymal migration** (Friedl and Wolf, 2003). Mesenchymal migration is characterized by polarized cell movement and by matrix degradation. Degradation of the tight and dense matrix is essential to create a path for the cell to move through. The matrix is degraded by MMPs, and is thought to occur by specialized cell structures called invadopodia. Invadopodia are actin rich protrusions, and have been observed in cancer cells *in vitro*. Once the path is created, the cell can migrate. Migration requires, besides invadopods, other protrusions as well, namely

lammellipods and filopods (for a review about protrusions see (Chhabra and Higgs, 2007; Stylli et al., 2008)). Lamellipods and filopods are suggested to be required for sensing of the environment and binding to the underlying matrix. Lamellipods and filopods are located at the leading edge of a cell, and they bind to the underlying matrix via focal adhesions and integrins (fig 1). This binding is required for mesenchymal cell migration, since it allows actomyosin contraction at the rear of the cell to move the cell body forward. After the cell body has moved, the trailing edge of the cell detaches from the underlying matrix by uncoupling its focal adhesions. (Friedl and Wolf, 2003; Lauffenburger and Horwitz, 1996; Ridley et al., 2003; Vicente-Manzanares et al., 2005). This mesenchymal migration allows a cell to migrate through the BM and intravasate into a blood vessel.

During the invasive process, a cell needs to know where to go; it requires direction (for example towards a blood vessel). A cell receives this direction via chemotaxis (fig 1). Chemotaxis refers to the process of cell recruitment towards a specific place, which is mediated by chemoattractants such as chemokines or growth factors. The cell senses the chemoattractant via membrane receptors (i.e. GPCRs, RTKs) on the cell surface. This results in activation of motility pathways that allows a cell to migrate. In tumors, chemoattractants are derived from the tumor microenvironment; secreted by fibroblasts and leukocytes that are present. For example, tumor associated fibroblasts deduced from breast cancer tumors have been found to secrete the chemokine SDF-1 (stromal derived factor-1, also known as CXCL12) to promote tumor migration (Chen et al., 2003; Muller et al., 2001; Orimo et al., 2005). SDF-1 binds to CXCR4 chemokine receptors on the membrane of breast cancer cells, upon which the actin cytoskeleton is remodeled and protrusions are formed (Holland et al., 2006). Moreover, tumor associated macrophages secrete EGF, which is part of a CSF-1/EGF paracrine loop between mammary carcinoma cells and macrophages, allowing them to migrate together (Wang et al., 2005; Wyckoff et al., 2004; Yamaguchi et al., 2006).

In conclusion, the current *in vivo* invasion model is as follows; cells detach from the tumor via EMT. They then migrate quickly along ECM fibers towards a blood vessel via amoeboid migration. When the cells arrive at a vessel, they switch to a mesenchymal phenotype, which enables them to proteolyse the matrix and migrate into the blood vessel (intravasation). Cells are directed towards the blood vessel via chemotaxis (fig 1).

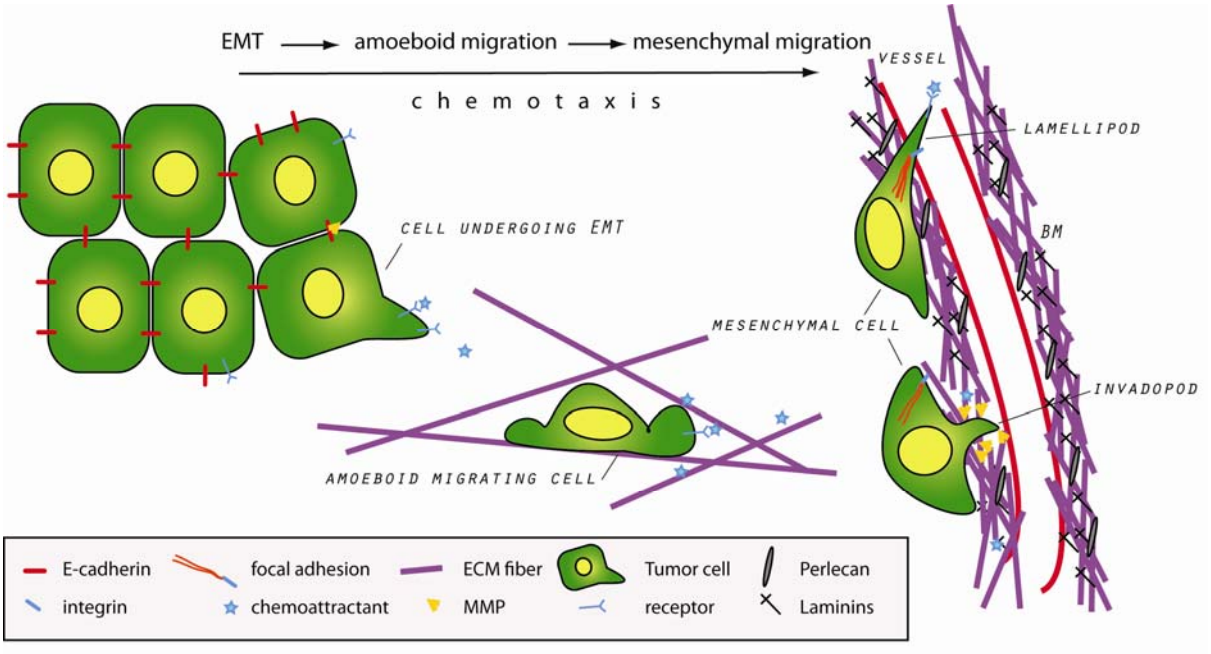

Figure 1 Invasion and chemotaxis **Tumor cells adhere to one another via E-cadherin. During invasion, a tumor cell undergoes EMT and loses E-cadherin cell-cell adhesions and starts polarizing. Once the tumor cell is detached from the tumor it can invade the tissue towards the blood vessel (probably) via amoeboid movement, making use of an ECM fiber to migrate along quickly. Once the cell encounters the blood vessel it needs to invade the dense matrix (BM) that surrounds the vessel. It degrades the BM as a mesenchymal cell. The mesenchymal cell makes a protrusion (most likely an invadopod) that uses MMPs to cleave the matrix. Once the BM is degraded, the mesenchymal cell enters the vessel, which is called intravasation. The cell adheres to the matrix via focal adhesions and migrates through the degraded BM into the vessel. Conclusion, invasion consists of EMT, amoeboid migration and mesenchymal migration. During this invasive process, the cell is attracted towards the vessel via chemotactic molecules like growth factors and chemokines that are present in the matrix.**

MMPs in chemotaxis and invasion

Invasion, as explained above, involves cell migration along matrix fibers, but also degradation of matrix fibers to create a path to be able to move through the matrix. Degradation of the matrix and matrix remodeling occurs by matrix metalloproteases (MMPs). MMPs have, besides their role in invasion, a role in all the steps of metastasis and tumor formation, and are therefore thought to be a good target to tackle metastasis. Furthermore, MMPs are upregulated in numerous cancers (Egeblad and Werb, 2002), and increased MMP expression is generally indicative of a poor prognosis due to increased metastasis and tumor aggressiveness (Orlichenko and Radisky, 2008). This relationship between tumor progression (increased growth, invasion and metastasis) and MMP overexpression is confirmed by several MMP overexpressing mice. The opposite (decreased growth, invasion and metastasis) was observed in MMP knock-out mice (Egeblad and Werb, 2002; Konstantinopoulos et al., 2008). This corroborates MMPs as a target for cancer therapeutics. Thus, can we target MMPs in invasion, migration and chemotaxis, to prevent the formation of metastasis?

We first need to know how MMPs induce the invasive process explained above. Thus, what exactly are the functions of MMPs in invasion and chemotaxis? MMPs are a family of at least 25 endopeptidases, whose function is to proteolytically cleave extracellular substrates. They have an important role in tissue repair, angiogenesis and organogenesis. Most MMPs are soluble and are secreted extracellular. However, 6 MMPs are membrane tethered and are expressed as cell surface enzymes. All MMPs contain a pre-, pro-, and a catalytic domain that contains a conserved zinc-binding region. The pre domain is removed short after synthesis. The pro domain keeps the enzyme

latent until it gets removed by other already activated MMPs or by serine proteinases. The catalytic domain exerts the proteolytic activity. MMPs can be expressed and secreted by tumor cells and by most of the cells present in the microenvironment of the tumor, such as fibroblasts, macrophages and other leukocytes. Tumor cells can recruit these secreted MMPs from other cells, and subsequently use them for their own proteolysis. MMPs are important for degradation and processing of numerous (mainly extracellular) substrates, including ECM proteins, growth factors, cell-cell adhesion molecules and MMPs themselves (Konstantinopoulos et al., 2008; Sternlicht and Werb, 2001). The exact role of MMPs in invasion and migration is now further discussed (see also figure 2).

Matrix remodeling

- The most well known function of matrix metalloproteases is matrix remodeling. Matrix remodeling increases invasion by **removing a physical matrix barrier**. The matrix consists of many different proteins, each being a substrate for specific MMPs for a precise list of ECM substrates of MMPs see (Egeblad and Werb, 2002; Sternlicht and Werb, 2001)). Hence, depending on the matrix composition that a cell encounters, different subsets of MMPs are required by the cell to effectively degrade the matrix.
- MMP matrix remodeling not only results in the removal of a physical barrier, it also results in the induction of chemotaxis via generation of **biologically active ECM fragments** and the release of bound signaling molecules. The ECM harbors cryptic fragments and neo-epitopes that can be released by MMP activity. Laminin-5 (ln-5) and Laminin-10 are two of those ECM components that contain cryptic fragments. Laminin-5, a component of the basement membrane, is a well studied example of an ECM component that contains several cryptic fragments that can induce migration (Pirilä et al., 2003). MMP14 and MMP2 release the laminin-5 γ2 chain domain III, which can activate the EGF receptor and induce migration in vitro (Schenk et al., 2003). The cryptic fragments are observed *in vivo* in the involuting mammary gland, and their presence is reduced in MMP-14 null mouse, suggesting that MMP-14 is important for the release of the laminin-5 γ2 fragment *in vivo* (Koshikawa et al., 2003). Laminin-10, a component of the basal membrane surrounding prostate cancers, contains a cryptic fragment that is released by MMP-14 and that can induce prostate cancer cell migration in vitro (Bair et al., 2005).
- In addition to the cryptic information, the ECM also functions as a depot for latent **growth factors** (like TGF-β) that can be released and activated by proteases and induce chemotaxis. TGF-β (transforming growth factor) is an example of such a latent growth factor that is maintained in a latent complex and which is bound to the ECM. MMP-1, -2, -3, -9, -13 and -14 can each activate the latent TGF-β, which is important for (among others) maintaining matrix integrity and stability (Annes et al., 2003). Activation of TGF-β by MMPs can result in tumor invasion, but also growth inhibition, depending on the tissue context and the protease. For example, MMP-9 activated TGF-β is associated with increased tumor invasion and angiogenesis (Yu and Stamenkovic, 2000; Yu and Stamenkovic, 2004). This is in contrast with MMP-14 activated TGF-β, which results in growth inhibition (Mu et al., 2002).

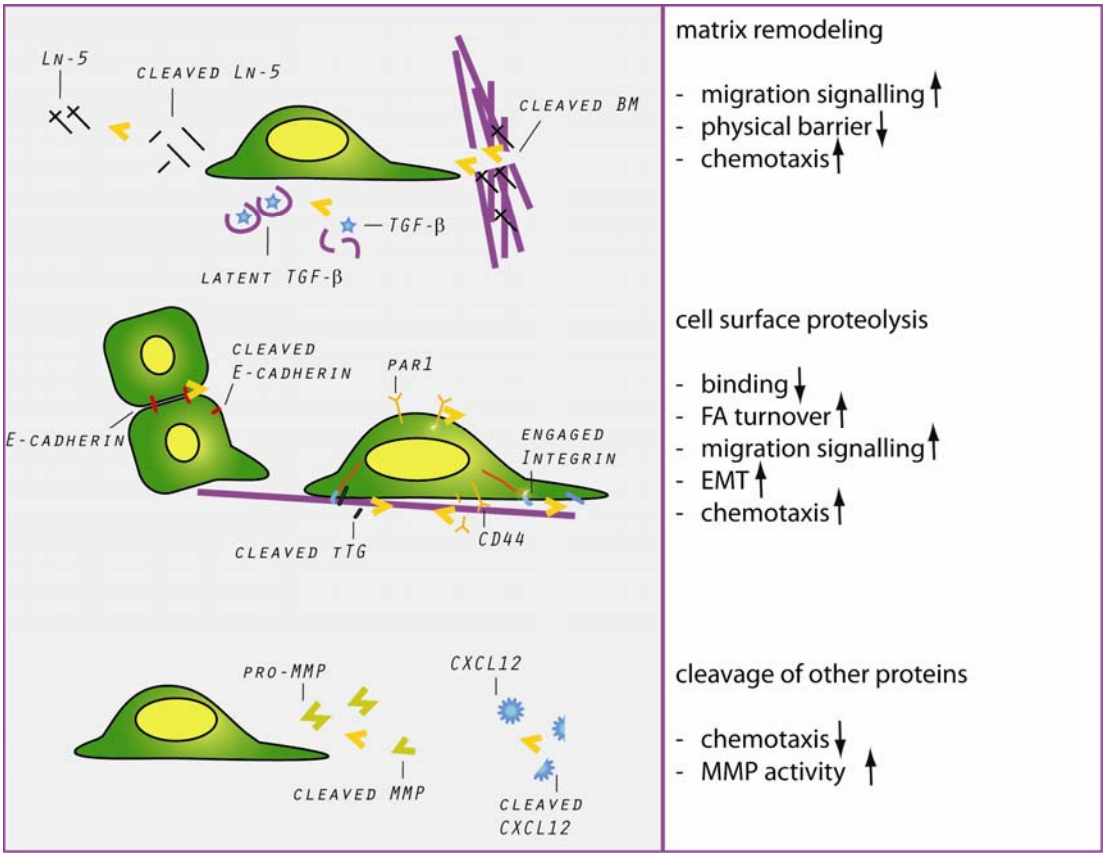

Figure 2 MMP functions in cancer cell migration **MMPs remodel the matrix, cleave cell surface proteins and degrade or process other proteins. This results in aberrant cell migration signaling, chemotaxis, binding to the ECM, FA turnover, EMT and changed MMP activity, which leads to increased cell invasion and metastasis.**

Cell surface protein degradation

A less well known but still important function of MMPs is degradation of cell surface proteins. Those cell surface proteins can be part of cell-matrix interactions, cell-cell interactions, or can be receptors involved in chemotaxis.

- Disruption of **cell-matrix interactions** by MMPs results in changes of cell migration because the cell is less attached to the matrix and therefore more motile. Disruption of cell-matrix interactions can also lead signaling inducing migration. MMP-14 (MT1-MMP), for example, processes integrin precursor $\alpha v\beta 3$ into a mature and more functionally active integrin (Elena I. Deryugina, 2000). This leads to increased adhesion and migration of MCF7 breast cancer cells onto fitronectin in vitro, most likely due to increased focal adhesion kinase (FAK) signaling (Deryugina et al., 2002). Moreover, engaged integrins can upregulate MMP expression (Ivaska and Heino, 2000), suggesting that there might be a positive feedback loop influencing migration and invasion. Besides the fact that integrins signal to increase migration and invasion, they can also bind to MMPs. The binding of MMPs by integrins is critical for invasion in vitro. Namely, Brooks and colleges showed that integrin $\alpha v\beta 3$ can bind and activate MMP-2, resulting in enhanced matrix degradation in vitro (Brooks et al., 1996). Since cells expressing integrin $\alpha v\beta 3$ are invasive cancer cells, it is tempting to speculate that he binding and activation of MMP-2 by the integrin $\alpha v\beta 3$ induces invasion via enhanced matrix degradation.

Besides integrins, there are other cell-matrix proteins that are affected by MMP processing leading to aberrant cell migration and invasion. An example is the integrin co-recptor tissue transgulaminase (tTG). tTG can be degraded by MMP14. This degradation leads to a block of cell adhesion and migration on fibronectin, but to stimulation of adhesion and migration on collagen I (Belkin et al., 2001). Moreover, cleavage of the hyaluronan matrix adhesion receptor CD44 by MMP14 is also required for cell migration, since kajita *et al* showed that mutation of the MMP14 cleavage site on CD44 prevents cell migration (Kajita et al., 2001). Finally, processing of β-dystroglycan by MMP2 and MMP9 which is associated with tumor aggressiveness and invasion (Jing et al., 2004; Shang et al., 2008), is another example of MMP processing at the cell-matrix interface that result in aberrant cell migration or invasion.

- When **cell-cell interactions** are cleaved by MMPs this can lead to aberrant cell invasion due to induction of EMT. The detachment of cancer cell via EMT can be induced via several mechanisms, including MMP proteolysis. Several MMPs have been shown to induce EMT in different tumor cell types. For example, MMP-2 and MMP-14 activity is essential to induce EMT in endocardial muscle cells. Expression of MMP-3 or MMP-9 in SCp2 mouse mammary epithelial cells and MMP-28 expression in A549 lung adenocarcinamo cells have also been shown to lead to EMT in those cells (ANDRÉ LOCHTER, 1998; Illman et al., 2006; Orlichenko and Radisky, 2008). MMP induced EMT occurs via different signaling pathways. Namely, MMP-3 induces EMT in SCp2 cells via enhanced expression of a Rac1b splice variant and increased ROS formation (Radisky et al., 2005). In contrast, MMP-28 has been shown to induce EMT via (most likely MMP-9 and MMP-14 induced) TGF-β activation (Illman et al., 2006). Since different tumor types have a different genetic program, it is not surprising that tumors have specificity for an EMT inducing MMP; for example, MMP-2 does not induce EMT in SCP2 cells, whereas MMP-3 and MMP-9 do induce EMT in those cells (Orlichenko and Radisky, 2008).

  Cleavage of cell-cell adhesion molecule E-cadherin can induce EMT as mentioned above, but the released extra cellular fragment of E-cadherin can also promote cell invasion independent of EMT. In the laboratories of Noe and McGuire it has been shown that E-cadherin can be cleaved by MMP3 (in vitro) and MMP7 (in vivo). The released E-cadherin fragment promotes cell invasion in *in vitro* assays (McGuire et al., 2003; Noe et al., 2001).
- An example of **cell surface receptor** cleavage that changes cell invasion involves a paracrine loop between fibroblasts and cancer cells. Hereby, the cancer cells secrete the chemokine Cyr61/CCN1 (Pendurthi et al., 2000) which induces MMP-1 production and secretion by the fibroblast. MMP-1 can then cleave a receptor at the cancer cell surface, PAR1. Cleavage of this receptor by MMP-1 appears to be an essential component for invasion and migration (Boire et al., 2005).

Cleavage of other proteins

The last group of proteins that lead to aberrant cell invasion when processed by MMPs are proteins that are present in the environment of the cancer cell.

- An example is **pro-MMPs**; MMP-7 (matrilysin) can cleave proMMP-2 and proMMP-9 in an immortalized ovarian cancer cell line. Increased proMMP-2 activation correlated with increased invasion, suggesting that in these cell lines invasion is induced in part by MMP-7 induced activation of proMMP-2 (Feng-qiang Wang, 2005).

- Another example is the **chemokine** CXCL12 (SDF-1), which can be cleaved by MMP-1, -3, -9, -13 and -14. Cleavage of CXCL12 leads to inactivation of this chemokine, thereby disabling the activation of CXCR4. As mentioned, CXCR4 activation induces invasion and metastasis in breast cancer cells, and blocking CXCR4 with antibodies reduces invasion in those cells. Therefore, invasion might be inhibited by MMP cleavage of CXCL12 (McQuibban et al., 2001; Muller et al., 2001).

From the above, we can conclude that MMPs are involved in promotion of metastasis through induction of invasion and chemotaxis via different processes including matrix remodeling, cell surface protein processing and cleavage of other proteins. Taken together, it is clear that MMPs fulfill an important function in chemotaxis and invasion. Hence, by inhibiting MMPs we would expect to prevent both chemotaxis and invasion, and thereby blocking metastasis. Based on this, we expect that inhibition of MMPs will be a promising anti cancer therapy.

MMPs as drug targets for cancer therapy

Because this relationship between tumor invasion and increased expression of MMPs was clear early on, farmaceutical companies started the development of MMP inhibitors (MMPIs) more than 25 years ago as a promising anti-cancer drug. Developed MMPIs showed MMP inhibition in experimental settings (in vitro and in vivo models), which validated MMPs as anti-cancer targets (Murphy and Willenbrock, 1995). Since then, many inhibitors have been tested in clinical trials, but unexpectedly, most fase III clinical trials failed to show any improvement in tumor regression or metastasis (vihinen et al., 2005). Moreover, there were reports showing MMPI treated patients were doing worse compared to the placebo treated patient group. This is probably due to inhibition of anti-tumorigenic effects (protective effects) some MMPs have now been associated with (Konstantinopoulos et al., 2008). But why did the MMPI fase III clinical trials fail? Several reasons have been proposed for this failure. Firstly, most clinical trials involved patients with an advanced cancer stage (malignant metastasis were already established). However, animal models have shown that MMPIs predominantly prevent metastasis in early stage disease (when no- or micro metastasis has occurred) (cousins 2002). Secondly, several MMPs (MMP-3, -8, -9, -12 and MMP-14) have been shown to exert anti-tumorigenic effects (protective effects) like inhibition of tumor growth, inhibition of metastasis, inhibition of angiogenesis and induction of the innate immunity against cancer (Konstantinopoulos et al., 2008). The MMPIs that were used in clinical trials were not specific but broadspectrum MMP inhibitors. The broadspectrum inhibitors were also targeting the tumor protective effects some MMPs have, thereby worsening the results of the trials. Thirdly, the unspecificity of the broadspectrum MMPIs also caused severe side effects in patients with high MMPI doses, which stopped the trials. However, the best beneficial results were obtained in those patients with a high MMPI doses. This suggests that MMPIs have a small therapeutic window. Therefore, patients with lower MMPI doses and no side effects might have had inadequate MMP inhibition, explaining the “no effect” outcome of many trials (king et al., 2003; Konstantinopoulos et al., 2008). At last, MMPIs have been shown to induce a change in the type of cell migration; from mesenchymal migration towards amoeboid migration. Amoeboid migration is MMP independent, and this change in migration type might have provided an escape mechanism for cells to metastasize without MMPs.

General future directions in MMP studies

Despite the fact that the initial failure of fase III clinical trials has cast a dark shadow over the MMP field, there is still a future for targeting MMPs as anti cancer therapy, since most of the above explained reasons for failure can be overcome. Moreover, there are a few examples of MMPIs that did have beneficial effects. To effectively use MMPs as a target to inhibit invasion, we need to keep the following in mind;

1. Animal models have shown that MMPIs predominantly work by prevention of metastasis in early stage animals. Therefore, we should set up clinical trials for patients with an early stage tumor (patients where no metastasis has been found yet) to prevent this tumor from metastasizing, and not try to regress an already highly metastasized tumor.

2. If tumor cells are indeed able to escape MMPIs by switching from mesenchymal migration to amoeboid like migration, we will have to explore how cells are able to switch between the two different types of migration. Furthermore, we need to investigate if this switching is a general mechanism that might be used by all tumor cell types.

3. Realizing that MMPs have both tumorigenic and non-tumorigenic functions, we will need selective inhibition of MMPs. Furthermore, it is clear that MMPs have context specific functions and specific tumors upregulate specific MMPs during specific stages. This indicates that MMPs should be context specifically targeted. An advantage of selective MMP inhibitors is that not only tumor cell migration will be better targeted; it might also reduce the side effects seen with the broad spectrum MMPIs.

In conclusion; MMPIs should be used as a cytostatic agent, not as a cytotoxic agent. Furthermore, MMP inhibitors should be more selective and additional research should be done to understand escape mechanisms.

Future research directions

In order to target MMPs more specific and thereby improve therapeutic efficiency, we need to understand the biology of MMPs. This means; which signaling pathways can induce MMP production and what set of MMPs is expressed by a specific tumor at a specific time, what are the substrates of those MMPs and how does this induce invasion. As shown above, a lot of research has already been done about MMPs and their function in invasion, and this knowledge should be used to specify MMP targets. However, much of this research has been done in vitro, with cell lines that are in culture for a while. The in vitro assays do not recapitulate the *in vivo* setting, because it lacks the microenvironment. And as shown above, it is the microenvironment that is so important in MMP biology. Therefore, it is important to verify MMP as targets in *in vivo* setting, using genetical models like double and triple knock-outs or in vivo imaging techniques like MRI and intravital imaging. Intravital imaging visualizes life tumor cell migration at a single cell resolution in mice (Condeelis and Segall, 2003), and can be used to monitor the requirements for specific MMPs and how these MMPs affect cell migration *in vivo*. Furthermore, intravital imaging will prove a good tool to better understand how cells can escape and metastasize despite MMPI treatment, because this is still a debate in the field. Several studies have now shown that cells are able to adapt a more rounded amoeboid like morphology upon MMPI treatment, and that these cells are still able to invade (Fackler and Grosse, 2008; Wolf et al., 2003). Since cancer cells have been shown to invade via

amoeboid movement, it is plausible that they switch back from mesenchymal migration to amoeboid migration upon treatment with MMPIs. However, most of the MMPI studies have been done in 3D matrices, which do not recapitulate the tumor microenvironment and which are much looser compared to BM that needs to be invaded to intravasate a blood vessel. Whether or not a mesenchymal to amoeboid switch takes place *in vivo* remains unknown. A study examining 4 different cell types showed that some were able to adapt a rounded morphology, whereas other cell types could not. This suggests that whether or not cells are able to switch between migration strategies depends on the cell type (Sahai and Marshall, 2003). If this is true, MMPIs will be more effective in tumor types that are not able to switch. Furthermore, it also remains elusive whether or not an amoeboid cell is able to cross the BM and intravasate without MMPs. *In vivo* imaging of the mesenchymal to amoeboid switch will provide better detail on whether or not this is a true escape mechanism that cells use upon MMPI treatment.

As for the future directions in MMPI studies, we can conclude that we need more specific targeting of MMPs and a different setup of the clinical trials is required. To accomplish this, knowledge of MMP biology obtained by in vitro assays should be used to determine specific targets, and this should be verified using in vivo models and imaging. Furthermore, the mesenchymal to amoeboid switch as a possible escape mechanism upon MMPI treatment should be investigated *in vivo*.

Conclusion

Metastasis is one of the most frequent causes of death in cancer patients, and prevention of metastasis is therefore high priority. To prevent metastasis we can inhibit invasion. This can most likely be accomplished by targeting MMPs, because MMPs have many functions in invasion, ranging from matrix degradation, to disruption of cell-cell contacts and generation of biologically active ECM fragments or chemoattractants. Furthermore, overexpression of MMPs strongly correlates with increased invasion. However, so far MMP inhibitors have failed in clinical trials. The reasons for this failure are a wrong set up of the trials, not enough specificity of the MMPIs and possible protease independent escape mechanisms of the cell. Thus, the future of MMPIs lies in a better understanding the biology of MMPs, predominantly *in vivo* model systems.

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