Tumor Metastasis: Matrix Metalloproteinase-9 (MMP-9)

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1. Introduction

Metastasis invariably indicates a poor prognosis even when the control of primary cancers is effectively achieved by surgery, chemotherapy or radiation therapy. Once a tumor mass is found in a distant tissue from the primary site, it often signals involvement of multiple organs (frequently involving bones and/or nerve plexus). The unresectable nature of the disseminated tumors and the levels of pain caused by the bone and nerve involvement shift the treatment plan from curative to palliative with an aggressive pain management plan.

Due to the biological and technical difficulties such as multi-step nature of metastasis, complex cross communication between metastatic and host stromal cells, and difficulties in studying metastatic steps using in vivo model systems, molecular mechanisms underlying each metastatic step are still poorly understood. Matrix metalloproteinase-9 (MMP-9), an enzyme synthesized and secreted by both metastatic and host cells, is one of the classic metastasis-promoting genes implicated with metastasis of many types of human cancers (Coussens & Werb, 1996; Deryugina & Quigley, 2006; Stamenkovic, 2000). MMP-9 has been initially suggested to contribute to tumor metastasis by cleaving various extracellular matrix molecules, which allows metastatic cells to be more motile and invasive (Chang & Werb, 2001; Egeblad & Werb, 2002). This review will discuss each step of metastasis and multiple roles of MMP-9 to hope to re-evaluate it as a promising target for the treatment and prevention of metastasis.

2. Metastatic Cascade

Tumor metastasis is a complex, multi-step process, which includes escaping from the primary tumor, invading surrounding extracellular matrix, entering the vascular system, traveling toward secondary sites, extravasating and forming secondary metastatic nodules (Figure 1) (Hanahan & Weinberg, 2000; Pantel & Brakenhoff, 2004; Steeg, 2006). Since this multiple cascade functions as physiologic and biological barriers to clear the tumor cells, the majority of tumor cells fail to survive and establish metastatic cascade, which termed metastatic inefficiency (Kim et al., 2004; Wong et al., 2001). Thus, it is tempting to
suggest that unlike the primary tumor cells, metastatic cells acquire additional genetic aberrations, which provide capacities to overcome multiple barriers and to complete all the metastatic steps (Hynes, 2003).

**Figure 1. The tumor metastasis cascade.** Highly motile metastatic cells escape from a primary tumor mass and invade toward adjacent lymphatic or blood vessels. Once intravasated inside blood vessel, metastatic cells disseminate into their preferred secondary organs including liver, lung and bone. Initial attachment and survival of metastatic cells in the capillary bed of secondary organs are followed by extravasation into connective tissues and formation of secondary metastatic nodules.
1) Invasion

The first characteristic of metastatic cells to initiate metastatic process is a capacity to invade and migrate. Invasive cells are characterized by a fine coordination of biological processes including altered adherence to the primary tumor cells, enhanced motility toward adjacent blood vessels and increased proteolytic degradation of extracellular components (Cavallaro & Christofori, 2001; Gupta et al., 2005; Pantel & Brakenhoff, 2004). A number of adherent molecules have been identified as modulators of metastatic invasion and migration. CD44 is a metastatic cell receptor binding to an ECM component, hyaluronan (Yu & Stamenkovic, 2000; Yu, Toole & Stamenkovic, 1997). Integrins family members bind to various proteins in ECM (Guo & Giancotti, 2004; Hynes, 2002; Wang et al., 2004). In addition to ECM-binding molecules, cadherin mediates cell-cell interaction. Decrease in tumor cell-tumor cell interaction by switching cadherin expression from E- to N-cadherin allows tumor cells to invade toward adjacent blood vessels (Cavallaro, 2004; Cavallaro & Christofori, 2004). Adherent molecules not only provide mechanical means for cellular invasion and motility, but also generate intracellular signal transduction, which modulates cellular polarity and movement. These include focal adhesion kinase, Rac, Rho and GTPase CDC42 (Cavallaro, 2004; Cavallaro & Christofori, 2001; Cavallaro & Christofori, 2004; Guo & Giancotti, 2004; Hynes, 2002; McLean et al., 2005).

2) Intravasation, Dissemination and Arrest

Once metastatic cells reach the blood or lymphatic vessels, cells enter into the vessels (intravasation) and travel throughout the circulation (dissemination), and arrest at the preferred distant organs such as liver, lung, bone and lymph nodes (Chambers, Groom & MacDonald, 2002). Survival of tumor cells circulating in the bloodstream is quite inefficient step due to a number of factors including mechanical forces from blood flow and size restriction of the capillary, lack of dependence on anchorage and host immune systems. Survived metastatic cells then, arrest and form a strong attachment to vascular endothelial cells in various organs (Al-Mehdi et al., 2000; Kim et al., 2004). Pulmonary metastasis is most common in various types of human cancers (Wong et al., 2002; Wong &
Bone metastasis is frequently derived from prostate and breast cancers (Kang et al., 2005; Minn et al., 2005a; Mundy, 2002). Liver is a major target for colon cancer metastasis (Steeg, 2006). Molecular mechanisms underlying organ preference for each type of cancers have not been clearly understood, yet recent studies suggest that an organ specific microenvironment characterized by distinct chemokines, surface adherent molecules and histological structure of capillary beds is one of the key determinants for the metastatic organ preference (Gupta & Massague, 2006; Kang & Massague, 2004; Minn et al., 2005b; Mueller & Fusenig, 2004). Stable and proper interaction with microenvironment provides secure adherence to endothelial cells through the organ-specific surface molecules, which confers essential survival and proliferative signals to metastatic cells.

3) Extravasation and Metastatic Colonization

Molecular mechanism underpinning extravasation is poorly understood. Quite a lot of disseminated cells (over 80% or I.V. injected cells) are successfully observed at their preferred secondary organs and escape from the bloodstream (extravasation) to form a microscopic metastatic nodule (Chambers et al., 2002; Steeg, 2006). It appears that extravasation through the blood vessel barrier is a critical step for final colonization in the secondary organs, but in certain experimental systems, arrested metastatic tumor cells in the pulmonary vasculature actively interact with pulmonary endothelial cells and grow within the blood vessels (Al-Mehdi et al., 2000; Wong et al., 2002). No viable extravasated cell was detected in a real-time intravital video microscopic assay. In contrast to earlier steps of metastasis, colonization is very inefficient process (metastatic inefficiency). Less than 0.1% of systemically injected metastatic tumor cells form micro-metastases; even much fewer numbers of macro-metastatic nodules are successfully developed. Multiple factors including organ-specific microenvironment, angiogenesis and host immune response have been suggested as determinant for metastatic colonization (Chambers et al., 2002; Gupta & Massague, 2006; Gupta et al., 2005; Hanahan & Weinberg, 2000; Joyce, 2005; Mueller & Fusenig, 2004; Steeg, 2006). Yet, cellular and molecular mechanisms underlying this final step of metastasis cascade are mainly unknown.
3. MMP-9 and Tumor Metastasis

MMP-9 is one of the two gelatinases (MMP-2 and MMP-9) that is produced and secreted by various types of malignant cells, host stromal cells and inflammatory cells. As illustrated in Figure 2, MMP-9 is a multi-domain enzyme including signal peptide domain, pro-domain, catalytic domain and hemopexin domain. MMP-9 is secreted and maintained as an inactive, latent form by a pro-domain. Proteolytic removal of the pro-domain activates enzymatic activity of MMP-9. Several enzymes including MMP-2 and mast cell chymase have been shown to cleave the pro-domain (Tchougounova et al., 2005; Winum et al., 2006). Catalytic domain has an active site for enzymatic activity that is composed of 3 histidines. Catalytic domain also contains a gelatin-binding region, which provides specific affinity for gelatin. In addition to gelatin, MMP-9 has various substrates including collagens, elastin, galectin3, entactin and ICAM-1 (Ram, Sherer & Shoenfeld, 2006).
Along with other MMPs, overexpression of MMP-9 particularly correlates with various cancer patients with poor prognosis and survival (Coussens & Werb, 1996; Deryugina & Quigley, 2006; Kupferman et al., 2000; Stamenkovic, 2000). MMP-9 has been associated with malignant progression, influencing both the early stages of conversion of carcinoma in situ to invasive disease as well as the latter steps of metastatic cascade. In addition, MMP-9 appears to contribute to proliferation and growth of primary tumors in prostate carcinoma, lymphoma, neuroblastoma and glioblastoma (Chang & Werb, 2001). Metastatic potential evaluated by pulmonary macro-metastatic colonization after intravenous injection correlated with MMP-9 expression in various types of malignant cells. Enforced expression of MMP-9 in fibrosarcoma cells or melanoma cells...
resulted in markedly enhanced metastasis in the experimental metastasis assay (Bernhard et al., 1995; Kupferman et al., 2000). Consistently, many studies demonstrated that inhibition of MMP-9 expression significantly reduced metastatic potential in various experimental systems including pulmonary metastasis of prostate cancers, fibrosarcomas and melanomas (Hua & Muschel, 1996; Sehgal et al., 1998).

Proteolytic removal or modification of extracellular matrix by MMP-9 and other MMPs appears to be essentially involved in the malignant transition from primary tumor into invasive metastatic tumor. It is not known, however, whether MMP-9 is involved in decrease in affinity between tumor cells, which allows them to escape from their primary tumor mass. Entrance of metastatic cells into the circulation of the chicken embryo correlated with MMP-9 expression suggesting that MMP-9 may contribute to intravasation of metastatic cells (Deryugina et al., 2005). Despite the consistent experimental data showing that MMP-9 is implicated with tumor progression and metastasis, how MMP-9 contributes to each step of metastatic progression at the molecular level remains to be determined.

Intriguingly, recent studies using transgenic mice, in which host MMP-9 expression is conditionally nullified or specifically induced in particular tissues such as hematopoietic lineage, provided compelling evidence that MMP-9 derived from host bone marrow stromal cells and infiltrating inflammatory cells affects tumor progression and metastasis (Coussens et al., 2000; Coussens & Werb, 2002; Kupferman et al., 2000; Lynch & Matrisian, 2002; Mueller & Fuseniq, 2004). These studies suggest that metastatic cell – host cell communication as well as tumor microenvironment are essentially involved in the metastatic cascade, which can be further explored for the new metastasis therapeutic targets (Joyce, 2005; Lubbe et al., 2006; Lynch & Matrisian, 2002; Overall & Kleifeld, 2006).

4. Clinical Relevance of MMP-9

Supporting evidence on the role of MMPs in tumor progression became available when endogenous tissue inhibitor overexpression reduced metastasis in an experimental system (Koop et al., 1994). In addition, small molecules that bind to the catalytic domain
of MMPs were found to reduce metastasis, further supporting MMPs as good cancer therapeutic targets (Whittaker et al., 1999).

Several candidate drugs that can inhibit MMPs were developed. In case of MMP-9, a broad spectrum hydromxamate inhibitor called barimastat was tested on a pancreatic cancer model as a prototypical antineoplastic agent. Clinical trials involving MMPs inhibitors have been largely disappointing with some major discrepancies between the human and animal data. There was some critical side effects identified during the phase I (safety as a function of dose escalation) trial not seen in the animal studies, which included hematologic, gastric, and musculoskeletal side effects (Molina et al., 2005; Whittaker et al., 1999). Musculoskeletal pain and inflammation, in particular, became apparent when patients were subjected to MMP inhibitors for a prolonged period of time. This problem was circumvented with a development of an MMP inhibitor (tanomastat) that can selectively attack MMP-2 and MMP-9 (Whittaker et al., 1999).

The efficacy of the MMP inhibitors was tested during phase II (small scale proof of principle study) and phase III (large scale study) of the clinical trials carried out by several groups. The result was disappointing at best with no or marginal improvement of survival (marimastat and prinomastat respectively). In case of tanomastat, the patients treated with the drug showed poorer survival than those who received placebo, further dampening the ominous prospect of using MMP inhibitor as a cancer treatment (Coussens, Fingleton & Matrisian, 2002).

However disappointing the result from the initial clinical trials was, it is imperative to carefully reevaluate the design of these studies in order to better understand why such treatments were or seemed to be so ineffective. Two important aspects of the clinical and pre-clinical studies worth mentioning are the endpoint discrepancy and point of intervention initiation. In pre-clinical studies, the end-point was defined as size and/or number of tumors. Such a measurement is often difficult, if not impossible, in clinical trials involving human subjects. In order to circumvent this problem, either reduction of serum markers or survival was used as the endpoint. The majority of the patients enrolled in all three phases of the clinical trials suffered from advanced stage cancer. In pre-clinical studies involving tumor bearing mice, MMP inhibitor treatment of the animals during early- or mid-stage of the disease provided measurable benefit, while failing to provide a
similar level of benefit when the drugs were administered to the animals in advanced stage of the disease (Bergers et al., 1999).

Taken together, these two confounding factors might have hampered our way of properly designing the study and analyzing the result to understand better the usefulness of MMP inhibitors in treating cancer.

5. Concluding Remarks

MMP-9 has been known for many years to augment metastasis, yet the molecular mechanisms underlying the effects of MMP-9 on each step of metastatic cascade still remain to be determined. Recent advances in imaging technologies including molecular fluorescence and intravital video microscopy (Al-Mehdi et al., 2000; Hoffman, 2005; Im et al., 2004; MacDonald, Groom & Chambers, 2002; Wong et al., 2002) will allow us to better delineate the roles of MMP-9 on each metastatic step. Despite the disappointing outcomes from the clinical trials of MMP inhibitors (Coussens et al., 2002; Drummond et al., 1999), better pinpointing the course of metastasis in which MMP-9 acts most critically will direct us to develop more promising metastasis prevention and therapy.

6. References


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