Matrix metalloproteinases in the process of invasion and metastasis of breast cancer

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INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases that are engaged, aside from various physiological processes such as embryogenesis, reproduction, uterine involution, angiogenesis, in pathological conditions, such as tumor invasion, by mediating degradation of basement membrane and remodeling of extracellular matrix. As invasiveness and metastasis are important biological characteristics of malignant tumors, these processes are extensively investigated and it has been shown that increased expression and activity of MMPs are among the earliest and most sustained events in tumor progression.

It has been established that the first stage of metastatic cascade involves the localized invasion of the host tissue. In order for this to happen, tumor cells must separate from each other, overcoming the usual restrictions imposed by homotypic cell adhesion and cell-cell contact inhibition. Normal cells are connected with each other and the surrounding stroma thus maintaining the architectural structure of epithelial tissues. Whereas the extracellular domain of E-cadherin mediates calcium-dependent homotypic cell-cell adhesion, down-regulation of cytokeratins, and their association with catenins. The loss of adhesive E-cadherin molecules and the E-cadherin–catenin complexes leads, morphologically, into impaired structure of the cytoskeleton and cytoskeletal domains by stromelysin-1 (MMP-13), a member of MMP-family that has been closely linked with tumor progression (4).

Constitutive expression of active stromelysin in mammary epithelial cells results in cleavage of E-cadherin and progressive phenotypic changes in vitro, including loss of catenins from cell-cell contacts, down-regulation of cytokeratins, and up-regulation of vimentin and MMP-9. These changes result in a stable epithelial to mesenchymal transition of cellular phenotype. In vivo stromelysin expression promotes mammary carcinogenesis that includes certain genomic changes (5). Thus, a decrease in cell-cell adhesion is associated with malignant conversion. Forced expression of E-cadherin in tumor cell lines results in reversion from an invasive to a benign tumor cell phenotype (1,2).

Therefore, in many carcinomas the loss of E-cadherin expression correlates with an invasive and undifferentiated phenotype. Furthermore, the normal function of E-cadherins depends on their association with catenins. The loss of adhesive E-cadherin molecules and the E-cadherin–catenin complexes leads, morphologically, into impaired structure of the cytoskeleton and allows ameboid movement and motility, but also deregulates the cell cycle and leads to cell proliferation. The observed decrease in adhesive force facilitates dispersion of carcinoma cells from the primary tumor mass. In addition to promoting passive dissemination of carcinoma cells, loss of E-cadherin function can also promote cell invasiveness (3).

Aside from these, there is yet another mechanism to alter E-cadherin function, its proteolytic modification. E-cadherin function can be disrupted by degradation of E-cadherin’s extracellular domains by stromelysin-1 (MMP-13), a member of MMP-family that has been closely linked with tumor progression (4).

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For most carcinomas, progression toward malignancy is accompanied by loss of epithelial differentiation and a shift towards a mesenchymal phenotype. This process, referred to as epithelial-to-mesenchymal transition (EMT), exacerbates motility and invasiveness of...
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many cell types and it is considered as a crucial event in late stage tumorigenesis and a prerequisite for tumor infiltration and metastasis (6). EMT provides mechanisms for epithelial cells to overcome the physical constraints imposed on them by intercellular junctions and adopt a motile phenotype. The transition from a well-differentiated epithelial phenotype to an invasive mesenchymal phenotype may involve diverse molecular mechanisms that may independently enhance motility and invasiveness. EMT is characterized by proteolytic loss of E-cadherin or Ecadherin/ N-cadherin “switching” and by increasing motility plays a crucial role during invasion and metastasis of carcinomas (3). Mesenchymal cells in the absence of tight junctions, lack an apical-basolateral polarity. Instead, these cells possess an elongated morphology with front-back asymmetry that facilitates motility and locomotion. Filopodial extensions at the leading edge of the mesenchymal cells are enriched with integrin family receptors that interact with the ECM and also contain MMPs that digest basement membranes and promote invasion. Transforming growth factor (TGF)-beta plays a pivotal role in inducing EMT of various epithelial cell types. Proteins upregulated during EMT, including Src kinase, integrin-linked kinase, integrin h-5, and MMP-11, MMP-12, and MMP-14, induce cytoskeletal remodeling and promote cell motility (7). The plasticity of malignant carcinoma cells due to diverse molecular mechanisms that contribute to EMT, also allows incomplete EMT, reversion to an epithelial phenotype and collective migration. As these mechanisms can manifest in a series of independent and reversible steps, EMT represents just one mechanism in the global metastatic carcinoma development process (8,9). Loss of p53 allows an upregulation of MMP-2 and downregulation of E-cadherin modulation of Slug and regulation of cancer cell invasion and metastasis (10,11).

The additional loss of heterotypic adhesion of endothelial cells, maintained by β1 and β2 integrins (e.g. VLA-1), as receptors for different components of ECM, BM, a highly specialized layer of the extracellular matrix composed of dense, interwoven fibrils of collagen type IV and glycoproteins, such as laminin and heparan sulfate proteoglycan, as well as similar components in the extracellular matrix (ECM), also allow detachment and invasion. A fundamental difference of neoplastic cells is the loss of anchorage requirement for cell survival and growth. The anchorage-independent growth of tumor cells may result from an uncoupling of cell survival signals transduced from the ECM by attachment, coupled with activation of cell-cycle progression is associated with neoplastic transformation (12).

TWO PROTEOLYTIC SYSTEMS

In the process of degradation of surrounding stroma tumor cells use two proteolytic systems. It has been proposed that a cascade of degradative enzymes clears a path at the advancing edges of invasive tumors, through which the tumor cells move. The inactive, pro-enzyme forms of the degradative enzymes (zymogens) are locally secreted, and become active by the action of their regulatory enzymes which are produced either by the tumor cells themselves or the surrounding stromal cells (fibroblasts, mast cells). Suchzymogens include pro-cathepsins, plasminogens and procollagenases, heparanases and matrix metalloproteinases (MMPs). Of these the best characterized in the process of invasion are MMPs and plasminogen activation system (13,14).

Plasminogen activation is an extracellular proteolytic system, which has been well characterized at the molecular level. Plasminogen activator molecules convert the inactive plasminogen into a trypsin-like serine protease plasmin. Plasmin has a very broad substrate specificity and can degrade matrix proteins and can convert other zymogens (i.e. MMPs) to their active forms by proteolysis. Two plasminogen activators have been defined, urokinase plasminogen activator (uPA) and tissue-type PA (tPA) (15). It has been shown that uPA is essential in cell migration and invasion. Therefore, manipulation with the levels of uPA activity can modulate tumor invasiveness and metastasis. Plasminogen activation by uPA occurs at the cell surface where the inactive proenzyme of uPA is bound by its cellular receptor uPAR. Pro-uPA is activated by an unknown mechanism but remains bound to its receptor (uPAR) where it is capable of activating plasminogen. uPA is often secreted, aside from tumor cells, by stromal fibroblasts. In this way, highly focalized centers exist where controlled areas of proteolysis can occur at the leading edge of the invading cell. The inhibition of receptor bound uPA by plasminogen inhibitors, PAI-1 and PAI-2, regulates the invasive process and influences the “proteolytic activity map”. The potential importance of u-PAR for the development of minimal residual disease in solid cancer and its biological relevance for tumor cell dormancy has been described due to its influence on the angiogenic switch (16).

The uPA-plasmin system has a role in the control of gelatinase (MMP-2, MMP-9) activity. MMP-2 and MMP-9 are secreted in the form of inactivezymogens that are activated extracellularly, a fundamental process for the control of their activity. Both gelatinases are associated with the cell surface, binding of uPA and plasminogen to the cell surface results in gelatinase activation without the action of other metallo- or acid-proteinases and the inhibition of uPA or plasminogen binding to the cell surface blocks gelatinase activation. Plasmin in soluble phase degrades both gelatinases and gelatinase activation and degradation occur in a dose- and time-dependent manner in the presence of physiological plasminogen and uPA concentrations.

MATRIX METALLOPROTEINASES

In tumor progression increased expression and activity of matrix metalloproteinases (MMPs) plays a role in angiogenesis, invasion and metastasis. They are a family of zinc metalloproteinases, secreted as latent proenzymes, which are activated by proteolytic cleavage and are inhibited by the tissue inhibitors of metalloproteinases (TIMPs) (17). There are 23 structurally related known members, which differ according to their individual substrate specificities, including stromelysin 1 and 2, gelatinase A and B, matrilysin, neutrophil collagenase and interstitial collagenase. Gene encoding stromelysin 3 has been identified and is specifically overexpressed in breast carcinomas. The gene was expressed from stromal cells in all of the invasive breast carcinomas tested and only in the direct vicinity of the neoplastic cells of the tumor. The transformed cells secrete a factor that induces expression of stromelysin 3 from the stromal fibroblasts and allows progression of the expanding tumor cell mass through the ECM into surrounding tissue (18).

MMPs have been subdivided into four subclasses based on their substrate specificity and cell localization:

1) Collagenases ( MMP-1 and MMP-8) degrade fibrilar collagen
2) Gelatinases (MMP-2 and MMP-9) have a preference for collagen type IV or denatured collagen, i.e. gelatin
3) Stromelysins (MMP-3, MMP-7, MMP-10) degrade a wide variety of ECM substrates such as proteoglycans, laminin and fibronectin
4) Collagenase-3 (MMP-13) has the widest spectrum of substrates
5) Membrane-type MMPs (MT-1 to MT-6-MMP) integral plasma membrane proteins capable of activating MMPs (20).

MMPs are secreted as soluble inactive enzymes which are activated by intrinsic Zn²⁺ ions, located in the center of the molecule and by extrinsic Ca²⁺ (19). Enzyme activity of MMPs is tightly regulated on several levels and dysregulation of MMPs activity can lead to uncontrolled degradation of ECM, a process important in tumor metastasis. The most commonly associated MMPs with the processes of metastasis are MMP-2 (gelatinase A) and MMP-9 (gelatinase B), due to their ability to degrade collagen type IV, a major component of vascular basement membrane (13).

Also, MMPs, specifically MMP-2 and MMP-9, are required for the switch to the angiogenic phenotype during tumor progression. MMP-2, unlike MMP-9, is expressed in early human breast carcinomas. Increased MMP expression required for the angiogenic switch and may predict when a non-angiogenic, microscopic, dormant tumor becomes angiogenic and invasive (21,22).

TIMPs are tight binding inhibitors of the active forms of MMPs. There are four of homologous proteins, referred to as the tissue inhibitors of MMPs (TIMP-1 to TIMP-4) (13). In addition to inhibiting matrix metalloproteinase (MMPs) activity, TIMP-2 has been shown to...
to suppress mitogenesis of both host (endothelial, fibroblast) and tumor cells via receptor tyrosine kinase (RTK) inactivation. Potent anti-tumor activity of TIMP-2 is composed of three separate activities. These are direct inhibition of MMP activity, MMP-independent antiangiogenic effects and direct down-regulation of tumor cell EGFR signaling (23).

Membrane-type matrix metalloproteinases (MT-MMP) constitute a subfamily of six distinct membrane-associated MMPs. Although the contribution of MT1-MMP during different steps of cancer progression has been well documented, the significance of other MT-MMPs is rather unknown. MT4-MMP overproduction accelerates in vivo tumor growth, induces enlargement of blood vessels, and is associated with increased lung metastases. MT4-MMP is identified as a new putative target to design anticancer strategies (24).

Activation of proMMP-2 by MT1-MMP is considered a critical event in cancer cell invasion. In the activation step, TIMP-2 bound to MT1-MMP on the cell surface acts as a receptor for proMMP-2. Subsequently, adjacent TIMP-2-free MT1-MMP activates the proMMP-2 in the ternary complex. Tumor size is associated with loss of both MT1-MMP and TIMP-4 for proMMP-2. Subsequently, adjacent TIMP-2-free MT1-MMP activates the proMMP-2 in the ternary complex. Tumor size is associated with loss of both MT1-MMP and TIMP-4 expression suggesting that the two markers could be specific for early stage ductal breast carcinomas (25).

Physiological activation of MMPs almost certainly involves cleavage by plasmin, itself activated at the cell surface by uPA. Therefore, the invading cell, which expresses uPAR, can activate an array of proteases at close proximity to a restricted region of the cell membrane, which is actively moving through the extracellular matrix. Degradation of the ECM is clearly a very tightly regulated process, which depends upon the relative concentrations of secreted proenzymes, their activators and inhibitors. Tumor cells acquire the ability to express matrix and invasion-promoting genes and if the ratio of invasion-promoting lytic enzymes/activators exceeds that of invasion-suppressing inhibitors, at the advancing edge of the tumor, invasion will proceed. It follows that analogues of the inhibitors of lytic enzymes known to be secreted by tumor cells may be useful in the treatment of tumors by inhibiting the invasion process.

It has recently been shown that overexpression of extracellular matrix metalloproteinase inducer (EMMPRIN or CD147), a member of the immunoglobulin family and a glycoprotein enriched on the surface of tumor cells, promotes invasion, metastasis, and growth and survival of malignant cells and confers resistance to some chemotherapeutic drugs (26).

Tumor-stroma interaction via EMMPRIN regulates, at least in part, the expression pattern of MMPs. MMP activity in tumor local environment results in proteolytic cleavage of membrane-associated EMMPRIN, releasing soluble EMMPRIN. Soluble EMMPRIN in turn acts in a paracrine fashion on stroma cells that are both adjacent and distant to tumor sites to further stimulate the production of MMPs (27).

**IMMUNOMODULATION OF MATRIX METALLOPROTEINASE EXPRESSION**

Activation of peripheral blood lymphocytes (PBL) to a locomotive state in order to invade target tissue requires production of proteases regulated by cytokines. Matrix metalloproteinases, aside from being involved in the process of angiogenesis and invasion, participate in transmigration of lymphocytes to the tumor site. MMP-2 and MMP-9 expression is modulated in a cell-type and stimulus-specific manner by a variety of immunological agents such as TGF-β, IFN-γ, IL-10, TNF-α. Effects of IL-12 such as up-regulation of VCAM-1 on the endothelial surface, recruits leukocytes to the tumor site, while inhibition of angiogenesis depends on the release of TNF-α and IFN-γ (28). Experimental models showed that systemic administration of IL-12 reduces MMP-9 expression in tumor tissue and that MMP inhibitors increase the therapeutic efficacy of IL-12 (29). On the other side, in healthy volunteers, IL-18 mediates MMP-9 production in PBL and induces VCAM-1, so MMP-9 production may constitute an additional mechanism that contributes to increased metastasis due to IL-18. Cytokines (IL-12, IL-18, IL-12 and IL-18 combination), by inducing production of MMP-2 and MMP-9, might contribute to PBL migratory ability and support their localization within metastasis. Th1 cytokines have the most pronounced effect on MMPs production in patients in clinical stage II, which suggests better immunomodulating effect of cytokine immunotherapy in improving cell mediated antitumor cytotoxicity effect in early stages of breast cancer.

ECM degradation during tissue remodeling requires a proteinase cascade that involves plasminogen activators and several MMPs (14, 30, and 31). This proteinase cascade can be modulated by specific interactions of both serine proteinases and MMPs with the cell surface. The comprehension of the molecular mechanisms that mediate gelatinase–cell surface interactions can provide useful tools for the development of novel strategies aimed at inhibiting type IV collagenase activity and controlling a variety of tissue remodeling processes, including tumor invasion and angiogenesis (13).

**METASTATIC CASCADE**

Although metastasis is the major cause of death in cancer patients, it is well established, that metastasis formation is an inefficient process. Studies have demonstrated that late events in metastasis formation are largely responsible for the inefficiency of this process. The number of circulating tumor cells and tumor emboli correlate with the size and age of the primary tumor. However, the number of circulating tumor cells does not correlate with the clinical outcome of metastases. The inefficiency of tumor cells in completing the metastatic cascade is the result of the fact that successful formation of metastatic foci consists of several highly complex and interdependent steps. Each step is rate limiting in that failure to complete any of these events completely disrupts metastasis formation.

The steps involved in metastasis formation are thought to be similar in all tumors and are characterized as follows:

1. **Tumorigenesis.** After the initial neoplastic transformation, the tumor cells undergo progressive proliferation that is accompanied by further genetic changes and development of a heterogeneous tumor cell population with varying degrees of metastatic potential. The initial growth of the primary tumor is supported by the surrounding tissue microenvironment, which eventually becomes rate limiting for further growth.

2. **Angiogenic switch.** As the tumor grows and central tumor cells become hypoxic, the tumor initiates recruitment of its own blood supply by a process called the “angiogenic switch” which involves the secretion of various angiogenic factors and the removal or suppression of angiogenesis inhibitors.

3. **Clonal dominance of invasive phenotype.** Continued genetic alterations in the tumor cell population lead to selection of tumor cell clones with distinct growth advantage and acquisition of an invasive phenotype. Invasive tumor cells downregulate cell-cell adhesion, alter their attachment to the ECM by changing E-cadherin, integrin expression profiles, and by proteolytically altering the matrix accomplish stromal invasion. Tumor cells lose “contact inhibition” that prevents normal cells from continuing to divide when they contact their nearest neighbors.

4. **Survival of tumor cells and tumor cell emboli in the circulation in vascular or lymphatic compartments, despite a variety of hemodynamic and immunologic challenges.**

5. **Tumor cell arrest in distant organs or lymph nodes.** This occurs by size trapping on the inflow side of the microcirculation, or by adherence of tumor cells through specific interactions.
with capillary or lymphatic endothelial cells, or by binding to exposed basement membrane. 6. Extravasation and growth at the secondary site. Arrested tumor cells proliferate in response to paracrine growth factors or become dormant. The poor growth of tumor cells after extravasation from the circulation is a major factor contributing to the inefficiency of the metastatic process. 7. Angiogenesis in metastatic foci. Finally, continued growth of the metastatic foci is also dependent on angiogenesis. The development of this neovascular network at the metastatic site enhances the metastatic potential of these cells just as it does for the primary tumor. 8. Evasion of immune response. Tumor cell evasion of immune response in metastatic foci includes antigenic modulation and immunosuppression and prevents their eradication. Therefore, detachment of single cells or clumps of cells from the tumor mass may be directly related to a decreased level of cell adhesiveness in tumor populations, regulated by a variety of cell surface molecules such as the cadherins and integrins.

Circulating tumor cells arrest at distant sites and by a repeated process of invasion may colonize a secondary site for growth. In some instances, this organ preference of metastasis can be explained simply in terms of the anatomical relationship of the organ with the site of primary tumor growth (Ewing theory of metastasis). Some cancers express organ preference that is not associated with a non-specific entrapment process, but rather with specific determinants, which actively promote the growth of the metastatic cells, thereby providing favorable soil (Padgett’s theory of metastasis). Fully metastatic behavior is attributable to the expression of only one, or a few, metastasis promoting genes (e.g. CD44) or to the loss of an equally small number of metastasis-suppressing genes (e.g. nm23).

After arriving in a secondary site metastatic cells begin proliferating, undergo apoptosis or remain as solitary dormant cells. The process of metastasis, although dangerous, is extremely inefficient with the majority of the cells undergoing apoptosis and thus becoming clinically irrelevant. The cells that begin proliferating and dormant cells are responsible for cancer recurrence (21). Tumor dormancy, a complex and still poorly understood phenomenon observed both in experimental models and in patients, has been associated with insufficient angiogenic capacity.

A defined event, termed “angiogenic switch” characterized by an imbalance between pro- and anti-angiogenic factors, by the influence of two proteolytic systems, uPAR and MMPs, often marks interruption of the dormant state, thus triggering invasive tumor growth (22).

CLINICAL CORRELATION AND PROGNOSIS

There is an urgent need for more efficient prognostic markers that can accurately and reliably identify breast carcinomas with potential to cause recurrence and/or death (25). There are data that show that markers for survival prognosis could be found among the proteins that participate or regulate the processes associated with cancer progression. Possible candidates include MMPs and their tissue inhibitors, TIMPs. Due to their specificity for a substrate, i.e. collagen IV, particularly abundant in basal membrane, MMP-2 and MMP-9 are the most involved in tumor initiation, growth and metastasis, especially in breast cancer (32). Recently obtained data show increases of the activity of MMP-2 and MMP-9 in tumor tissue of breast cancer patients and their correlation with TNM clinical stage of the disease (17,18), and significantly higher activity levels in tumor tissue than in surrounding tissue (33-35). The analysis of the activity of MMP-2 and MMP-9 in the serum of breast cancer patients shows significant clinical stage-dependent increase (36), with high level of MMP-9 sera activity being associated with a worse overall survival rate. Novel data of MMP-9/TIMP-1 complex activity in clinical stage IV of the disease implicates that MMP-9 overproduction is not balanced by equal amounts of inhibitors. The association of serum MMP-2 and MMP-9 activity and clinical stage suggests the usefulness of these parameters as markers, both, in the follow up and prognosis of breast cancer patients (36). Active estrogen receptors have resulted in tumor progression by stimulating cell growth and invasiveness via acceleration of the expression of MMPs, including MMP-9. In addition, a tendency of increased MMP-9 expression is found in ER receptor positive patients (37). As MMP-9 activation rate increases when lymphovascular permeation occurs, this suggests a possible role of MMP-9 in subendothelial basement membrane degradation and intravasation of cancer cells into vessels (38).

A aberrant expression of transcription factor activator protein (AP)-2 and HER-2 oncogene are related to disease progression and increased invasive capacity in breast cancer (39,40), which may be due, in part, to stromal MMP expression and activation caused by HER-2 in the early phase of breast cancer (41,42). The reported association of the increase in serum MMP-2 and MMP-9 activity and prognostic and therapeutic parameters suggests a role of MMP-2 and MMP-9 in prognostic stratification of breast cancer patients. This may be helpful in avoiding overtreatment with chemotherapy in the group of lymph node-negative patients.

CONCLUSION

Breast cancer may now be subclassified into luminal, basal, and HER2 subtypes with distinct differences in prognosis and response to therapy. Breast tumors that exhibit a basal phenotype are believed to be similar to the earliest mammary “progenitor cells” that are associated with a poor prognosis and are resistant to standard chemotherapy, supporting the hypothesis that therapeutic failure results from inefficient targeting of cancer stem cells (43). A recent publication examining the “wound response” signature, which includes genes involved in matrix remodeling and angiogenesis, found an association of this signature with basal-like subtype, suggesting other potential avenues of targeting. Angiogenesis is a process required not only for embryonic development but is encountered in wound healing and in pathological situations, such as tumor growth (44). Molecular programs reveal links between wound healing and cancer progression in a variety of common epithelial tumors. Many of these normally reparative processes may be constitutively active in the tumor milieu and critical for tumor engraftment, local invasion, and metastasis to distant organs (45,46).

It has been shown that neutralization of VEGF, as well as inhibition of matrix metalloproteases (MMPs), using the broad-spectrum MMP inhibitors, both, strongly reduced the tumor-induced tube formation. It is suggested that MMPs, in particular MT-MMPs, play a pivotal role in the formation of capillary-like tubular structures in a collagen-containing fibrin matrix in vitro and, thus, may be involved in angiogenesis in vivo.

Based on the data showing participation of MMPs in breast cancer invasion and metastasis, the new concept of “stromal-directed therapy” has been introduced with MMPs as targets of MMP inhibitors. The results obtained so far suggest that interrupting “tumor-stroma cell communication” by targeting MMPs may provide an alternative therapeutic approach for the treatment of invasive breast cancer. However, considering that MMPs, aside from their up-regulation in pathological processes, have important physiological roles in tissue homeostasis, as well as in cell-mediated antitumor immune response, it follows that further refining the stromal targets, more specific MMP inhibitors, and preferable testing in the bone metastasis setting, represent viable approaches to breast cancer therapy with inhibitors of MMP.

REFERENCES


