# Recent progress in the molecular mechanism underlying breast cancer invasion and metastasis

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ABSTRACT Invasion and metastasis is the most life-threat event in patients with cancer. The metastatic process involves a complex cascade of event. Many molecules such as cell adhesion molecules, proteinases and motility factors play important role in this process. In order to find new and effective treatments, it is essential to understand the mechanisms underlying cancer invasion and metastasis. This article reviews the recent insights into the roles of those molecules in breast cancer invasion and metastasis and the clinical implication of the findings.

**Key Words** breast neoplasms; invasion; metastasis

Breast cancer is the most common malignant tumor in women. The major threat to patients with breast cancer is tumor invasion and metastasis. In fact, metastasis is the primary cause of death in human breast cancer. The metastatic process involves a complex cascade of event including loss of cell-cell adhesion, attachment to basement membrane, degradation of extracellular matrix (ECM) and cell migration. An understanding of the molecular mechanisms involved in those steps of the metastatic cascade will be very important in designing new therapeutic approaches. This article discusses recent progress in the molecular mechanism underlying breast cancer invasion and metastasis and the clinical implication of the findings.

## Cell Adhesion Molecules (CAMs)

CAMs play an important role in cell-cell interactions, which regulate the development and maintenance of multicellular organisms. As an initial step in metastasis, tumor cell should detach from the primary site. This is thought to be due to changes in the cell-cell adhesive properties. Hence, CAMs are supposed to play a critical part in cancer invasion. There are four main groups of CAMs. These include the integrin family, the immunoglobulin superfamily, selectins and the cadherins, which are major cell-cell adhesion molecules.

## Cadherins

Cadherins are $Ca_2$+-dependant adhesion molecules that mediate homophilic cell-cell adhesion. The cadherin gene family encompasses E-cadherin, P-cadherin, and N-cadherin. A number of studies have found a close correlation between the expression of cadherins and the tumor characteristics in breast cancer.

E-cadherin exerts a potent invasion-suppressing role in tumor cell lines and in vivo tumor systems [1]. In breast cancer, generally speaking, partial or total loss of

E-cadherin expression correlates with loss of differentiation characteristics, acquisition of invasiveness, increased tumor grade, metastatic behavior and poor prognoses [2]. And taking into account the two major histological subtypes of breast cancer, however, different modes of E-cadherin expression modulation have been found. While infiltrating ductal breast cancers mostly show no or only heterogeneously reduced E-cadherin expression, associated with epigenetic transcriptional downregulation; infiltrating lobular breast cancers, which are typically completely E-cadherin-negative, often show inactivating mutations in combination with loss of heterozygosity of the wild-type E-cadherin gene (CDH1) allele [3]. Interestingly, inflammatory breast cancer (IBC), which is the most aggressive form of breast cancer, often shows strong expression of E-cadherin. It is challenging our current understanding of metastatic procession. Maybe both the intense angiogenesis and the strong E-cadherin expression

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may contribute to the highly metastatic phenotype of IBC[4].

Although reduced levels of E−cadherin are often associated with poorly differentiated breast cancers, recent studies show that expression of other cadherins such as N−cadherin and P−cadherin are actually elevated in invasive breast cancers and cell lines. The expression of P−cadherin show a strong inverse correlation with estrogen receptor (ER) expression in both types of breast cancer (in situ and invasive), and P−cadherin−positive and ER−negative tumors were related to a higher histologic grade, a high proliferation rate and expression of c−erbB−2. Maybe an estrogen −independent pathway regulates P−cadherin expression[5]. Rachel B et al. transfected a weakly metastatic and E −cadherin −expression breast cancer cell line, MCF−7, with N−cadherin and found N−cadherin promotes motility, invasion and metastasis even in the presence of the normally suppressive E−cadherin. Two properties maybe responsible for metastasis of N −cadherin −expressing cells: one is the increase in MMP−9 production by N−cadherin−expressing cells in response to a growth factor which may endow them with a great ability to penetrate matrix protein barriers, the other is the increase in their adherence to endothelium which may improve their ability to enter and exit the vasculature[6].

Catenins are a family of proteins including the α−(102kDa), β−(88kDa), γ−(82kDa) catenins. The cytoplasmic domain of E−cadherin binds directly to either β− or γ−catenin, whereas α−catenin links the E−cadherin−(β, γ)−catenin complex to the actin cytoskeleton. The integrity of the adhesion function of E−cadherin also depends on an intact catenin system. If one of these four proteins is downregulated in breast cancer, the function of the others in suppression metastasis is altered.[7] It became clear during the past decade that β−catenin is also a downstream signaling molecule in the Wnt signaling pathway, and it plays essential roles in development and tumorigenesis in a number of tumors, including breast cancer[8].

General speaking, The E−cadherin/catenin complex plays a critical role in establishing and maintaining the polarity and histological structure of cells. Dysfunction of the E−cadherin−mediated cell adhesion system plays an important role in tumor progression of the relatively benign tumor to invasive, metastatic carcinoma. Restoring this system may enable suppression of the metastatic spread of cancer. PP2, a specific inhibitor of Src family kinases, can activate the functioning of the E−cadherin−mediated cell adhesion system, which is associated with the suppression of metastasis in cancer cells. Thus, selective inhibition of Src activation may be potentially useful in the prevention of cancer metastasis[9].

**Integrins**

Integrins were originally characterized as a family of cell surface receptors that mediate the attachment of cell to cell and cell to ECM. In addition to their adhesive functions, recent findings implicate integrins also involve the regulation of tumor cell growth and survival, migration and invasion, angiogenesis, and extravasation.

Integrins are heterodimeric, which consist of two transmembrane glycoprotein subunits (α and β) that are noncovalently bound. Thus far the integrin family is composed of 18 α and 8 β subunits that form heterodimers to produce some 25 different αβ cell surface receptors. Each of these integrin heterodimers supports interaction with a unique set of extracellular matrix proteins and sometimes soluble ligand proteins in cell type specific manner.

The contributions of integrin receptors to breast cancer progression have been investigated in numerous immunohistochemical studies. In the normal breast, intense staining for many of the integrin subunits is seen concentrated at the basement membrane in the myoepithelial layer. However, in invasive carcinomas this cell layer is most often absent and the expression of integrin subunits on the carcinoma cells is diffuse. Although the expression of some integrin subunits is decreased, it is clear from many in vitro and in vivo studies that integrin receptors are expressed in breast cancer and that they contribute significantly to pathobiology of breast cancer [10]. In the following content we will talk about integrin α6β4 and αvβ3 as examples to explain the role of integrins in breast caner invasion and metastasis.

Expression of the α6β4 integrin, which is a receptor for laminin family of basement component, persists in breast cancer. And α6β4 expression has been correlated with poor prognosis for breast cancer patients[11]. Some studies provided evidence that α6β4 integrin promotes breast cancer invasion by stimulating lamellae formation and the chemotactic migration of breast cancer cells[12]. The formation of new blood vessels is essential for tumor growth and

also for the development of metastasis. Some integrins especially αvβ3 also play a critical role during tumor angiogenesis. The mechanism by which αvβ3 supports angiogenesis relies on binding of the activated form of metalloproteinase MMP-2 to endothelial αvβ3.Thereby αvβ3 recruits active MMP-2 to the invasive edge of the sprouting blood vessel cells [13]. And activated αvβ3 also cooperates with MMP-9 in breast cancer cell migration. These molecules cooperate to enhance breast cancer cell migration toward specific matrix proteins, and this may contribute to the strongly enhanced metastatic capacity of breast cancer cells that often express activated αvβ3.[14]

**Selectins**

Selectins are leukocyte adhesion molecules, which mediate leukocyte infiltration into the inflammatory foci. The selectin family is composed of three molecules named P- selectin, E- selectin and L-selectin. E-selectin is a cell surface adhesion molecule that is specifically expressed by activated endothelial cells. Recent studies have suggested that E-selectin participates in tumor progression and metastasis. E-selectin may enhance tumor angiogenesis and the adhesion of tumor cells to endothelial cells at distant sites. In breast cancer, circulating soluble E-selectin was significantly higher in patients with liver metastases than in patients without liver metastases[15]. Circulating sE-selectin concentration may be an important prognostic factor for overall and disease-free survivals in patients with node-negative breast cancer, and this prognostic value was higher than that of traditional parameters [16].

**IgSF**

The immunoglobulin superfamily (IgSF) of molecules contains a highly diverse group of proteins whose functions include intercellular adhesion and/or binding interactions that trigger cellular activation and differentiation. The intercellular adhesion molecule-1 (ICAM-1) and the vascular adhesion molecule-1(VCAM-1) are two important molecules of IgSF in tumor progression and metastasis. The findings of Regidor and his colleagues suggest that malignant breast cancer cells could induce neovascularisation with subsequent high expressions of ICAM-1 and VCAM-1. These upregulations of adhesion molecules might contribute to changes in invasive phenotypes by promoting endothelial cell adhesion and angiogenesis, as well as being responsible for the recognition of tumor cells by the human immune system[17]. Soluble ICAM-1 and VCAM-1 are elevated in patients with advanced disease and especially elevation in VCAM-1 has prognostic significance in patients with breast carcinoma[18].

**Proteinases**

Among the process of tumor invasion and metastasis, the degradation of the host tissue by proteolytic enzymes is absolutely necessary. At least four classes of proteinases exist and they are most frequently classified according to their catalytic types. These are the matrix metalloproteinases (MMPs or matrixins), and the serine, aspartic, and cysteine proteinases. The coordinated and synergistic action of these four classes of proteinases can completely degrade all ECM components. Of these four classes, the matrix metalloproteinases and the serine proteinase, urokinase-type plasminogen activator (uPA), are most extensively linked to cancer invasion and metastasis.

**MMPs**

MMPs are a family of zinc-dependent endopeptidases. Their primary function is degradation of proteins in the extracellular matrix. Currently, at least 20 members of this family are known to exist. Based on substrate specificity and domain organization, the MMPs can be loosely divided into four main groups: the interstitial collagenases, gelatinases, stromelysins and membrane-type MMPs. Many data from model systems suggest that MMPs are involved in breast cancer initiation, invasion and metastasis.

MMPs facilitate tumor cell invasion and metastasis by at least three distinct mechanisms. First, proteinase action removes physical barriers to invasion through degradation of ECM macromolecules such as collagens, laminins, and proteoglycans. Second, MMPs have the ability to modulate cell adhesion. For cells to move through the ECM, they must be able to form new cell-matrix and cell-cell attachments and break existing ones. Finally, MMPs may act on ECM components or other proteins to uncover hidden biologic activities. For example, the angiogenesis inhibitor angiostatin may be produced from plasminogen by MMPs action and laminin-5 is specifically degraded by MMP-2 to produce a soluble chemotactic fragment[19].

Recent data from model systems suggest that

MMPs are involved in not only breast cancer invasion and metastasis, but also tumour initiation and angiogenesis. MMPs may promote angiogenesis by at least two different mechanisms: by degrading barriers and thereby allowing endothelial cell invasion; and by liberating factors that promote or maintain the angiogenic phenotype. An example of the latter is the degradation of the ECM protein laminin-5 by MMP-2, which results in enhanced mammary epithelail cell growth. Similarly, both MMP-1 and MMP-3 have been shown to breakdown endothelial-derived perlecan, releasing basic FGF, a potent endothelial mitogen[20]. At the same time MMPs may also play a role in breast cancer initiation and growth. Evidence show that overexpression of stromelysin-1 in transgenic mice gave rise to preneoplastic and malignant mammary gland tumours[21].

Exceptionally MMP-3, one of the stromelysins, isn't behaving like the other members of MMPs. MMP-3 can inhibit breast tumour cell invasion in vitro by a mechanism involving plasminogen degradation to fragments that limit plasminogen activation and the degradation of laminin. So it would explain why MMP-3 expression, associated with benign and early stage breast tumors, is frequently lost in advanced stage, aggressive, breast disease[22].

MMPs are secreted from cells in a latent form and require activation extracellularly for proteolytic activity. The second level of control is the presence of specific proteinase inhibitors- TIMPs. Four endogenous specific inhibitors of MMPs have been described: TIMP-1, 2, 3 and 4. The TIMPs inhibit protease activity by forming high-affinity 1:1 stoichiometric, noncovalent complexes with the active MMPs. For proteolysis to occur, active proteinase concentrations must exceed those of their inhibitors. Therefore, it is probably the overall balance between the concentrations of each form of MMPs and TIMPs that will determine whether matrix degradation occurs at each stage of tumour invasion and metastasis [23]. And finally it will be associated with patients' overal survival[24].

**uPA**

The PA system consists of the urokinase-type and tissue-type PAs (uPA and tPA, respectively), a receptor for uPA, which focuses proteolysis, and the plasminogen activator inhibitors PAI-1 and PAI-2. Urokinase-type plasminogen activator (uPA) is a highly specific serine protease that is secreted as an enzymatically-inactive, single-chain proenzyme. Secreted uPA at the cell surface where it is more readily converted into an active two-chain form (tcuPA) by plaxmin, kallikrein, cathepsin B, and nerve growth factor-γ.

In breast cancer invasion and metastasis, uPA、PAI-1 and PAI-2 play a important role. Several studies assessing both mRNA and protein levels have found that increases in uPA, uPAR, and PAI-1 are associated with either aggressive tumor characteristics or a poor prognosis[25,26]. Interestingly, the increases of these components' levels in the stroma rather than in the tumor cells were found more relevant to patient's outcome[27]. These reflect the importance and hinting at the collaboration of the stroma and its components in the invasive process. And the study of Saluda-Gorgul and colleagues demonstrated the PAI-2 antigen levels growth was observed in histologically defined primary breast cancer forms, so he thought PAI-2 might be the most reliable marker for the identification of primary breast cancer[28].

What deserves to be mentioned is that PAI-1 is a multifaceted proteolytic factor. It not only functions as an inhibitor of the protease uPA, but also plays an important role in signal transduction, cell adherence, and cell migration. Thus--an apparent paradox considering its name--although it inhibits uPA during blood coagulation, it actually promotes invasion and metastasis. And recently novel therapeutic approaches targeting the PAI-1/uPA interaction are already in pre-clinical testing.

**Motility factor**

For invasion, a subpopulation of tumor cells must recognize the extracellular matrix barrier, modify the barrier, migrate through the barrier, and then proliferate in the adjacent but ectopic locale. Motility factor also plays a critical role in tumor invasion and metastasis. In many invasive tumors, upregulation of active motility is stimulated by growth factor receptor signaling, the EGF receptor being the most frequently implicated.

Hepatocyte growth factor (HGF) is a cytokine that is released after injury. It is a paracrine factor that is produced by mesenchymal cells; epithelial and endothelial cells respond to HGF through its receptor, the c-met protein. Hepatocyte growth factor induces cell growth and cell movement and is also highly angiogenic. Evidence from breast cancer patients suggests that HGF is a negative prognostic

indicator for breast cancer and is associated with invasive disease. The patients with high HGF levels exhibited a significantly shorter survival rate than those with low HGF levels. Circulating HGF levels may be a useful indicator for the progression of metastatic lesions and the prognosis of patients with metastatic breast cancer[29].

Besides HGF, keratinocyte growth factor (KGF) was also an active factor from mouse fibroblasts responsible for most of the motility response in breast cancer cells. KGF from stromal tissue surrounding a primary tumor mass can enhance tumor cell motility and may be an early signal in the progression of breast cancer cells to a more motile and metastatic phenotype[30].

Take together, tumor invasion and metastasis is a complex process. Many molecules, including CAMs、proteinases、cytokines and growth factors etc are involved in this process. Different molecules are expressed in different cascades. And some molecules interact with others, such as integrin and MMPs. Recent advances in cellular and molecular biology and oncology have rapidly imposed our understanding of molecular and cellular mechanisms underlying breast cancer invasion and metastasis. With further understanding of the mechanisms, we think it could yield potential insights for diagnostic and therapeutic applications.

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