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# Breast cancer brain metastases

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**Abstract** Breast cancer is the most common malignancy in woman in the USA. Metastasis is a major cause of morbidity and mortality in breast cancer patients. Total incidence of brain metastases of breast cancer is about 30%. Because of the improvements in control of systemic disease, for example the successful use of Trastuzumab, and the consequent prolonged life span, the incidence of brain metastases is increasing in breast cancer patients. The progressive neurological disabilities not only impair the quality of life, but also decrease the survival in patients. However, current treatments are of limited effectiveness. This is partially caused by the unique structure of the blood brain barrier. So far very little is known about the mechanisms how breast cancer metastizes to the brain. Some studies showed that ErbB2 overexpression is associated with the brain metastatic phenotype. Other molecules, like vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs) and chemokine receptor CXCR4 are also involved in the metastasis of breast cancer cell to the brain. The current review will briefly overview the clinical features of brain metastasis of breast cancer and discusses the relationship of blood brain barrier and ErbB2 signal pathway to brain metastasis in breast cancer.

**Keywords** Breast cancer · Brain metastasis · ZrbB2

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## 1 Introduction

Breast cancer is the most common malignancy in woman and the second major cause of death (after lung cancer) in the USA. It is estimated that nearly 178,480 women are going to be diagnosed for invasive breast cancer in 2007, which accounts for 12.67% of women population and 26% of all estimated new cancer cases in women in the USA. Approximately 40,460 breast cancer deaths are going to occur, which account for 15% of all estimated deaths from cancer in women. Metastasis is a major cause of morbidity and mortality in breast cancer patients. Five-year relative survival in local invasive breast cancer patients is 98.1%, while it is only 26% in patients with distant metastases (http://www.cancer.org). Because of the progressive neurological disability of brain metastases and the lack of effective treatment as seen in visceral or bone metastasis, brain metastasis of breast cancer is becoming an important problem, although brain metastases are less common in breast cancer patients than bone or visceral metastases.

## 2 Clinical features

### 2.1 Incidence

The incidence of clinically evident brain metastases among women with metastatic breast cancer is estimated to be 10 to 16% [1–4]. The median latency between the initial diagnosis of primary breast cancer and the diagnosis of symptomatic brain metastases is about 2 to 3 years. In most cases, brain metastases represent a late relapse in breast cancer patients who already have lung, liver or bone involvement [5]. In addition, the incidence of asymptomatic brain metastases is about 15%, showed by a screening in

155 metastatic breast cancer women without symptomatic brain metastases [6]. Therefore, the total incidence of brain metastases of breast cancer, including symptomatic and asymptomatic, is about 30%. This is also supported by the autopsies in which brain metastases were found in about 30% of breast cancer patients [2, 4, 7, 8].

The metastasis of breast cancer to the brain appears either within the brain parenchyma or along the leptomeninges. Majority of the brain metastases of breast cancer occur in the parenchyma, which typically follows a vascular distribution, indicating that parenchymal metastases are mainly though hematogenous spread [4]. In contrast, leptomeningeal metastases are less common, which arise via multiple pathways including hematogenous spread, direct extension, infiltration from vertebral metastases via venous plexus, and extension along nerves or perineural lymphatics [9].

### 2.2 Risk factors

Several risk factors of brain metastases have been reported. Young age appears to be an independent risk factor. The median age of patients with brain metastases is about 5 years younger than that without brain metastases. Other features such as nodal status (≥4 positive nodes), tumor grade (tumor grade 3), pathologic tumor size (>2 cm) are considered risk factors of brain metastases as well [10–13]. Hormone receptor status, especially estrogen receptor (ER) negative, is also associated with the incidence of brain metastases. Several retrospective studies showed at least twice of the incidence of brain metastases in ER-negative breast cancer patients compared to ER-positive patients [10–12, 14, 15]. In addition, epidermal growth factor receptor ErbB2 (also known as Her2/neu) is considered another independent risk factor for brain metastases [10, 11, 13, 15, 16]. Some other factors, like p53-positivity, lower bcl-2 expression, high EGFR expression and low CK5/6/19 expression, although less widely studied, were also reported to associate with brain metastases of breast cancer [12, 13, 15, 16].

### 2.3 Diagnosis

The most common clinical symptom of parenchymal brain metastases is headache, mental status changes and cognitive disturbances. Other manifestations that could reflect the location of metastatic lesion and the subsequent cerebral edema may also occur, including motor deficits, seizures, ataxia, and nausea or vomiting [17, 18]. Leptomeningeal metastases, on the other hand, are typically present with nonlocalizing symptoms, such as pain or headache and cranial neuropathies [19]. In many cases, neurologic examination will elicit deficits of which the patient is unaware [6]. Neuroimaging, i.e. gadolinium-enhanced magnetic resonance imaging (MRI) or contrast-enhanced computed tomography (CT), is more sensitive and informative that it can also detect clinically asymptomatic occult brain metastases and differentiate between solitary and multiple lesions [20]. Comparatively, MRI is more preferred than contrast-enhanced CT, because it is more sensitive for identifying both parenchymal and leptomeningeal disease [2, 21–24]. CSF cytology can be employed for the detection of leptomeningeal metastases. Studies showed that the sensitivity of CSF cytology is comparative to that of MRI (even higher with serial examinations) [25]. The advantage of CSF cytology is that it has higher specificity compared with MRI [9]. A brain biopsy is indicated in patients with an atypical clinical presentation, when the diagnosis is in question, and the results would influence management decisions [2, 26].

### 2.4 Prognosis

In most cases, central nervous system (CNS) involvement occurs in the late stage of metastatic breast cancer. Usually, patients are already found to have lungs, liver, or bone involvement by the time CNS metastasis is diagnosed. Mean survival from diagnosis of a brain metastasis varies from 2 to 16 months. The mean 1-year survival is estimated only about 20% [27, 28]. A prognostic index for brain metastases was formulated by the Radiation Therapy Oncology Group (RTOG), based on the study of 1,200 patients with a variety of solid tumors. Patients with age less than 65-years-old who have controlled primary tumor without extracranial metastases and a better general performance status (Karnofsky performance score greater than 70) usually have better outcomes, with a median survival of 7.1 months. Whereas, patients with an older age and their Karnofsky performance score less than 70 have much more poorer outcomes, with a median survival of only 2.3 months [29]. Other favorable prognostic factors include the presence of a solitary brain metastasis, and a longer disease-free interval [29, 30]. However, as systemic therapies improve, control of extra-cranial disease may no longer be an important predictive factor for breast cancer patients with CNS metastases. With the control of systemic disease, the incidence of CNS involvement increases, and more of these breast cancer patients are died because of the progressive CNS disease [31].

### 2.5 Treatments

Treatments for brain metastases of breast cancer include corticosteroids, whole brain radiation therapy, surgical resection, stereotactic radiosurgery and chemotherapy. Corticosteroids are used to relief symptoms by decreasing cerebral edema surrounding brain metastases [11].

Whole brain radiation therapy (WBRT) is considered the most common choice of treatment for patients who present with multiple brain metastasis. In addition, patients with solitary brain metastasis that are not qualified for either surgical resection or stereotactic radiosurgery are often treated with WBRT. WBRT is able to control neurologic symptoms and therefore improve the quality of life in approximately 75 to 85% of patients. In addition, WBRT is able to prolong the mean survival, compared with corticosteroids alone [32].

Surgical resection of brain metastases allows for pathologic diagnosis of the intracranial disease. Besides, it may improve neurologic symptoms and increase quality of life by immediate decompression of tumor mass effect. Surgical resection also improves the overall median survival compared to the supportive care alone. In patients with a single surgically accessible metastasis, good performance status, and stable or absent extracranial disease, there is a survival advantage for surgery, especially with the combination approach of surgery and WBRT, over WBRT alone [32]. However, in patients with multiple brain metastases, the role of surgical management is currently considered limited, unless there is an obvious symptomatic lesion [2, 33, 34].

Stereotactic radiosurgery (SRS) uses either a linear accelerator or multiple cobalt-60 sources to deliver focal radiation to areas smaller than 3.5 cm, which minimizes radiation exposure to the normal surrounding tissues. Because SRS is less invasive than surgical resection, it is given to patients who cannot tolerate surgery or have surgically inaccessible lesions. SRS involvement increases the overall median survival in breast cancer patients with brain metastases. Study showed that the combination approach of SRS and WBRT significantly improved the overall survival of patients with single brain metastasis. However, in spite of the fact that such combination approach could improve the overall performance, it has no survival advantage for patients with multiple brain metastases [32].

Generally, chemotherapy has not yet been considered a useful strategy in the management of brain metastases because the tight junction of blood brain barrier precludes the entry of most chemotherapeutic agents into the CNS. However, some drugs did show the promise in combination with radiation therapy [32]. For example, Efaproxiral, which can increase tumor oxidation and therefore increase the radiation sensitivity, demonstrated an improvement of median survival in breast cancer patients with brain metastases when used in combination with WBRT compared to those using WBRT alone [32, 35]. Several new techniques to deliver chemotherapeutic agents are now being studied. For example, an attempt to place BCNU (carmustine) in the resection cavity at the time of surgery is under investigation. BCNU is an impregnated polymer wafer that can protect hydroxylasing and allow slow releasing of chemotherapeutic agents. This technique has been shown successfully in brain primary tumors and is now being studied for metastasis cancers [32, 36]. Another newly developed technique is direct intracerebral microinfusion (convection-enhanced delivery). This approach has been tested in the animal study and showed the effectiveness [37]. However, human trials have not yet been conducted.

## 3 Blood brain barrier

Blood brain barrier (BBB) consists of astrocytes, pericytes, capillary endothelial cells and basement membrane. Astrocytes that form the blood brain barrier have tight junctions between each other, which can enclose the capillaries on all sides. Distinctive from endothelial cells of other organs, endothelial cells within the blood brain barrier is nearly leak-proof. They join together by connective elements or continuous tight junctions and are equipped with a selective substance permeability which allows only particles with a diameter of less than 20 nm to cross over (Fig. 1). The structure of blood brain barrier is so constructed in order to build an effective shield against higher molecular substan-

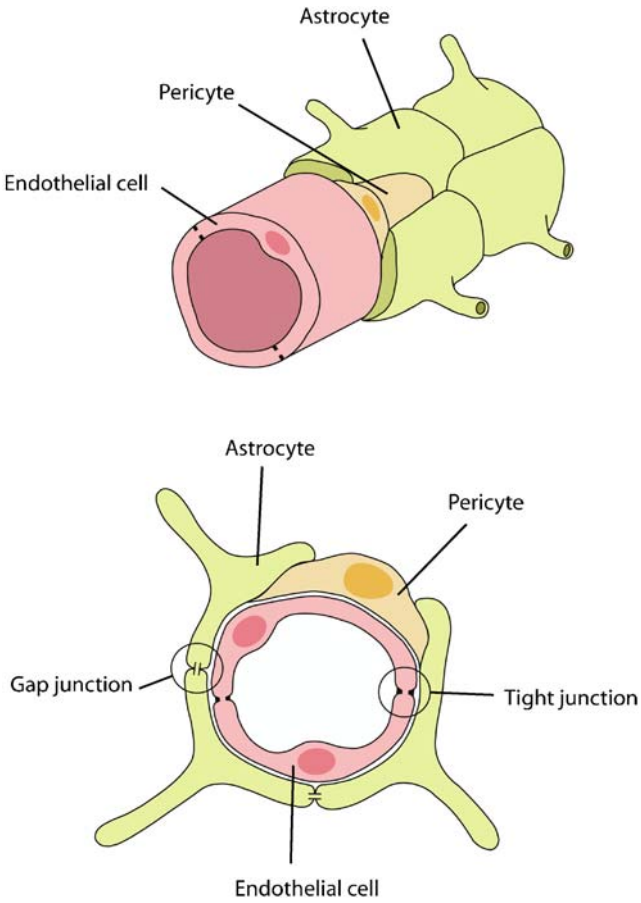


**Fig. 1** The structure of blood brain barrier (BBB)

ces and organisms which may be harmful to brain function and let only necessary small substances present in the blood to immediately pass through [38]. In cases of tumors burden, the tight junctions between the endothelial cells become stretched out and result in the increased vascular permeability, which allows circulating tumor cells to move out of the vessel and go into the brain. Studies showed that breast cancer cells that express high level of chemokine receptor CXCR4 can increase the permeability of brain endothelial cell and facilitate the invasion of these breast cancer cells into the brain [39]. However, even with the increased permeability of brain endothelial cells in the present of tumor burden, blood brain barrier is still a formidable diffusion barrier. As a result, most systemic chemotherapeutic agents are too large to cross the blood brain barrier and result in a poor drug delivery. Therefore, new therapeutic approaches for metastatic brain tumors could be either to increase the permeability of blood brain barrier or to develop small molecular weight drugs. Also, an improved understanding of the interactions between tumor and epithelial cells could assist in the development of prevention strategies which aims to block the invasion of tumor cells into the brain.

Human brain microvascular endothelial cell (HBMEC) is now widely used *in vitro* as a model system to mimic the *in vivo* human blood brain barrier. There are several characteristics of HBMEC indicating that it does maintain the signature properties of human brain endothelial cell that forms the blood brain barrier, including the formation of tubular-like networks on matrigel, the ability to uptake acetylated low-density lipoprotein (AcLDL) and to produce von Willebrand factor and γ-glutamyl transpeptidase endothelial-specific markers [39, 40]. In about 4 to 5 days after plating, HBMEC can form the tight junction between each other, which can be detected by increased measurements of trans-endothelial electrical resistance (TEER) [41]. HBMEC can be used in the transendothelial migration assay to study the invasion ability of tumor cells. In this assay, HBMEC is cultured in fibronectin-coated Boyden chambers for 5 days allowing the formation of tight junctions before the migration measurements. HBMEC can also be used to measure the adhesion ability of tumor cells [39]. Although lacking the contribution of astrocyte in this system, it is so far one of the best methods to mimic blood brain barrier *in vitro*.

## 4 ErbB2 signaling

The *ErbB2* gene (also known as *Her2* or *neu*) is one of the members in the epidermal growth factor receptor (EGFR) family, which encodes a 185-kDa transmembrane receptor tyrosine kinase [42, 43]. The importance of ErbB2 in human primary breast cancer is addressed by the fact that 20–30% of human breast cancers express elevated levels of ErbB2 due to the genomic amplification of *ErbB2* proto-oncogene and transcriptional upregulation of ErbB2 promoter [44–46]. Furthermore, its overexpression strongly correlates with a negative clinical prognosis in both lymph node positive and negative breast cancer patients: patients with breast cancer that overexpresses ErbB2 have a worse overall survival [43, 47, 48]. ErbB2 overexpression may be useful not only as a prognostic marker but as a predictive marker as well, as ErbB2 overexpression predicts tamoxifen resistance of the primary tumor [49].

It is known that upon activation, ErbB2 phosphorylates many downstream molecules that in turn activate a variety of signaling cascades, including the phosphatidylinositol-3-OH kinase (PI-3K)/Akt pathway, the Ras/MEK/MAPK (mitogen-activated protein kinase) pathway and Ras/MEK/Erk (extracellular signal-regulated kinase) pathway. By activating different pathways, ErbB2 causes alterations in a variety of gene expressions at different levels, including transcription, translation and protein stability. These alterations have been implicated in a variety of cellular processes, including cell growth, survival and metastases [50, 51]. For example, ErbB2 promotes cell growth by inducing cytoplasmic localization of $p21^{Cip1/WAF1}$ through activation of PI-3K/Akt pathway, and the combination of ErbB2 and $p21^{Cip1/WAF1}$ provides a better stratification of patients' survival than any single clinical pathological or biological marker [52, 53]. The activation of PI-3K/Akt by ErbB2 can mediate resistance to DNA-damaging agents through enhancing MDM2-mediated ubiquitination and degradation of p53 [54]. ErbB2 can also promote cell growth by stabilizing cyclin-dependent kinase inhibitor p27 through activating MAPK pathway [55], and by increasing β-catenin through activating MEK/Erk pathway [56]. In addition to cell growth and survival, ErbB2 signal is also involved in metastasis. For example, ErbB2 overexpression not only activates Src kinase activity, but also increases its translation and stability, which plays critical roles in ErbB2 mediated breast cancer metastases [57]. Besides, ErbB2 overexpression was found to increase membrane degradation and invasiveness of breast cancer cells through activating transcription and enhancing secreting of matrix metalloproteinases MMP9 [50]. ErbB2 can also increase invasiveness and targeted lung metastases of breast cancer though upregulation of chemokine receptor CXCR4 expression [58]. In addition, ErbB2 overexpression in breast cancers increases vascular endothelial growth factor (VEGF) production by activating p70S6K, which enhances angiogenesis and metastases [57]. Therefore, ErbB2 activates a variety of metastases related downstream signals, which contributes to the metastases potential of ErbB2 overexpressing breast cancer cells.

## 5 ErbB2 overexpression in breast cancer brain metastases

Interestingly, ErbB2 overexpressing breast cancers have been shown to spread to visceral organs, such as lung, liver and brain [59]. More and more evidence showed that *ErbB2* amplification is an important risk factor for brain metastasis [10, 11, 13, 15, 16]. Recently a retrospective analysis was performed in a cohort of 9,524 women with early stage breast cancer (42% node-negative) who were randomized in International Breast Cancer Study Group clinical trials between 1978 and 1999, and without taxane or trastuzumab treatment. Within 3871 cases whose ErbB2 status were available, the 10-year cumulative incidence of CNS disease as site of first relapse was 2.7% in patients with ErbB2 positive primary tumors, and 1.0% in patients with ErbB2 negative tumors ($P<0.01$). The 10-year cumulative incidence of CNS metastasis at anytime was 6.8% in patients with ErbB2 positive primary tumors, and 3.5% in patients with ErbB2 negative tumors ($P<0.01$). This result supports an inherent increased likelihood of CNS metastasis independent of taxane or trastuzuma therapy [11].

Trastuzumab (Herceptin™), a humanized monoclonal antibody against ErbB2 has been used as an anti-cancer treatment approved by FDA since 1998, and has shown an improved disease-free and overall survival when delivered together with cytotoxic chemotherapy to patients with ErbB2 overexpression matastatic breast cancer [60]. However, patients receiving Trastuzumab as first-line therapy for metastastic disease frequently developed brain metastases while responding to or stable on Trastuzumab at other disease sites. A series of retrospective reviews showed the brain metastases incidence of 25–50% among breast cancer patients treated with Trastuzumab. The median time from the administration of Trastuzumab to the development of brain metastases varied from 4 to 24 months. Furthermore, ErbB2 overexpression is considered a predictive factor for CNS relapse in breast cancer patients treated with Trastuzumab [14, 61–64].

The increased incidence of brain metastases in breast cancer patients treated with Trastuzumab dose not seem to result from a loss of ErbB2 overexpression in the brain metastases, although loss or gain of ErbB2 overexpression during tumor progression was demonstrated in some malignancies, including Trastuzumab-refractory breast cancer. The correlation between ErbB2 overexpression of primary breast cancers and subsequent brain metastases is 97% in a retrospective study in which all 13 patients with ErbB2 positive brain metastases had ErbB2 positive primary tumors, whereas 15 of 16 patients with ErbB2 negative brain metastases had ErbB2 negative primary tumors [65].

One explanation for the progression of brain metastases during Trastuzumab treatment is that improvements in systemic control and overall survival associated with Trastuzumab based therapy have led to an unmasking of brain metastases that would otherwise have remained clinically silent prior to a patient's death. This explanation is supported by the study in which 155 women with metastatic breast cancer but no symptomatic brain metastases was screened for the occult brain metastases before treatment, nearly 15% of the woman screened had occult brain metastases. ErbB2 overexpression in the primary tumor was showed as a predictive factor. And survival among patients with occult brain metastases was shorter than that of patients without brain disease but was similar to that of patients with symptomatic brain metastases [6].

More likely, the explanation for the progression of brain metastases during Trastuzumab treatment is the poor penetration of Trastuzumab into the brain through blood brain barrier. Trastuzumab is a relatively large protein with a molecular weight of 148-KDa. In a case report of a breast cancer patient with leptomeningeal involvement, the level of Trastuzumab in cerebrospinal fluid was about 300-fold lower than in the concomitant serum [66]. In another study in which six brain metastatic breast cancer patients were recruited, the ratio of median Trastuzumab level in the serum to that in the cerebrospinal fluid was 420:1. However, the ratio decreased to 76:1 after completion of radiotherapy in these patients [67]. These results suggested that blood brain barrier prevents the penetration of Trastuzumab and make the brain as a sanctuary site, increasing the permeability of blood brain barrier can increase the reach of Trastuzumab to the brain. This concept is further supported by the studies in the rat model. The study by Ira Berman et al. showed the inhibition of growth of cancer cells in the athymic rats implanted with human breast cancer cell lines after continuous intraventricular administration of 4D5, a monoclonal antibody that recognizes the extracellular domain of ErbB2 receptor, into cerebrospinal fluid [68]. In a more recent study by Peter M. Grossi et al., same kind of ErbB2 overexpressing human breast cancer cell line intracerebral implantation athymic rat model was employed. Trastuzuma or a control antibody was administered regionally directly into the tumor for 7 days. Animals treated with intracerebral administration of Trastuzumab had significantly improved survival compared to controls. However, systemic administration of Trastuzumab failed to deliver the drug to the brain and did not significantly affect survival [37]. These results also suggested that ErbB2 overexpressing breast cancer, which is growing in the brain, can be targeted with ErbB2 directed therapy if the drug can penetrate the blood brain barrier.

Therefore, ErbB2 inhibitors that are administrated systemically but are small enough to cross the blood brain

barrier are the new approach to treat brain metastases in patients. One example of an irreversible inhibitor of ErbB2 tyrosine kinase, which may cross the blood brain barrier, is Lapatinib. Lapatinib is a dual inhibitor of epidermal growth factor receptor and ErbB2. Phase I clinical trials have already finished and the results showed that the side effects of Lapatinib are favorable. Phase II studies showed that Lapatinib has promising clinical benefits in the setting of ErbB2 positive advanced breast cancer patients [69]. Although the result in patients with progressive, ErbB2 positive brain metastases was not as ideal as predicted, Lapatinib did show the responses in 2 of 39 patients [2]. Another example is CI-1033, which has already been tested in a phase I clinical trial and demonstrated acceptable side effects with doses that modulated target tyrosine kinase activity [70].

## 6 Microenvironment in breast cancer brain metastases

There is a growing body of evidence that the numerous interactions between the cancer cells and the host microenvironment play important roles in the progression and metastasis of cancers. The interactions of cancer cells with the primary microenvironment, including disruption of basement membrane and extracellular matrix, facilitate the metastatic cancer cells to escape from the primary tumor. While the interactions of cancer cells with a tissue microenvironment that is distant from the primary organ, for example the formation of new vascular networks and evasion of the host immune system, enable the colonizing in the distant site [71].

Studies have shown that angiogenesis is involved in the breast cancer metastasis to the brain. Xenograft murine models which directly injected human breast cancer cell into either the mammary fat pad or intracranial window showed that the brain metastatic tumor exhibited more angiogenesis but a lower vascular permeability compared to the primary breast cancer, suggesting that cranial environment is leakage resistant but proangiogenic [72]. Vascular endothelial growth factor (VEGF), a heparin-binding glycoprotein which is considered to be the most selective mitogen for endothelial cells and also a vascular permeability factor, is expressed about four-fold higher in the primary breast cancer patients with brain metastasis compared to those without brain metastasis [73]. *In vitro* study indicated that VEGF might contribute to breast cancer brain metastasis by enhancing the transendothelial migration of tumor cells through the down-regulation of endothelial integrity and increasing the adhesion of tumor cells onto the human brain microvascular endothelial cell (HBMEC) monolayer [74]. Brain metastasis variant breast cancer cells (MDA-231BR-1, 2, 3), which were selected after three cycles of injection into the internal carotid artery of nude mice and harvest of brain metastases, showed an increased potential for experimental brain metastasis and mice injected with these cells had significantly shorter mean survival than mice injected with the original cell line. Brain metastatic lesions of the selected variants showed a higher vascular density and released significantly more VEGF and IL-8 compared to the original cell line. Targeting endothelial cells with a VEGF receptor specific tyrosine kinase inhibitor reduced angiogenesis and restricted the growth of the brain metastases [75]. These studies indicate that angiogenesis, especially the function of VEGF, is involved in promotion of breast cancer brain metastasis.

Matrix metalloproteinases (MMPs) are a broad family of zinc-dependent proteinases that play a key role in extracellular matrix (ECM) degradation. Studies showed that MMPs might be involved in the metastases of breast cancer to the brain. A breast cancer brain metastases rat model was derived from injection of a carcinogen-induced mammary adenocarcinoma cell line in to left ventricle of rat. The micro-metastasis in the brain showed a significantly higher expression of MMP-2, -3 and -9 and an increasing in MMP-2 and MMP-3 activity compared to the normal brain tissue. Furthermore, the development of brain metastasis was significantly decreased by treatment with a selective synthetic MMP inhibitor [76]. This phenomenon was confirmed by another study in which human breast cancer cells overexpressed with MMP2 were inoculated into the left ventricle, a higher incidence of metastasis to brain was observed [77]. An *in vitro* study also showed that brain-seeking breast cancer cells have a higher total and active amount MMP-1 and MMP-9 with the higher migration and invasion capacity, which could be decreased by the application of MMP-1 and/or MMP-9 inhibitor [78].

## 7 Targeted metastasis of breast cancer to brain

Metastasis is a complex pathophysiological process that is highly organ selective. Chemokines and their receptors regulate leukocyte migration to inflammation sites and play an important role in the regulation of hematopoiesis, homing of hematopoietic stem cell in bone marrow and T and B lymphocytes in lymphoid tissue, and in the trafficking of dendritic cells. Recently, it was suggested that chemokines and their respective receptors are involved in the development of targeted metastases of primary tumors. In particular, chemokine stromal cell derived factor-1$\alpha$ (SDF-1$\alpha$, also known as CXC chemokine ligand 12, a kind of $\alpha$-chemokines), and its specific receptor CXCR4 (a G protein-coupled seven-transmembrane receptor). Chemokines such as SDF-1$\alpha$ are released in high amounts by certain organs, such as lung, bone, and liver. Malignant breast cancer cells, which express the chemokine

receptor CXCR4, invade the extracellular matrix and circulate in the blood and lymphatic vessels. The attraction between SDF-1α and CXCR4 causes breast cancer cells to leave the circulation and migrate into organs with large amounts of chemokines, where cancer cells proliferate, induce angiogenesis, and form metastatic tumors [79]. In the brain, SDF-1α is selectively expressed both in the developing and mature CNS. In addition, the expression of CXCR4 is consistently higher in primary breast tumor cells than in normal breast epithelial cells. *In vitro* study showed that SDF-1α could induce blood vessel instability, through an increased vascular permeability, resulted in the penetration of breast tumor cells through the human brain microvascular endothelial cells. Blockade of the CXCR4/SDF-1α pathway with anti-CXCR4 antibody decreased transendothelial breast cancer migration as well as vascular permeability [39]. Furthermore, it was shown that ErbB2 could induce CXCR4 expression. The ErbB2-induced CXCR4 expression is required for ErbB2-mediated *in vitro* invasion and *in vivo* lung metastasis, and the blockage of Akt activity inhibits metastatic potentials [58]. In the light of above information, it might be possible that ErbB2 may interact with CXCR4 and contributions to the brain metastasis.

In addition, a recent study suggests that chemokine receptor CX3CR1 with its ligand-- fraktalkine, which was originally found to mediate the chemo-attraction of macrophages and natural killer cells, are significantly associated with brain metastases in a set of 142 auxiliary node positive breast cancer patients [80].

Other chemokine and its receptor that might be involved in the breast cancer brain metastases are Slit and Robo. The Slit family of secreted proteins (Slit1, 2 and 3) and their corresponding receptors Robo (Robo1, 2, 3, and 4) play important roles in neuronal development. The slit proteins guide the directional migration of neurons in the brain and the olfactory system. Slit1 is predominantly expressed in the nervous system, while Slit2 and Slit3 are also expressed by other cells and tissues. Recently it was suggested that Slit/Robo1 signaling is also involved in the metastasis of breast cancer to the brain. *In vitro* study showed that Slit2/Robo1 signaling is capable of inducing directed migration and Slit2 acts as a potent attractor for breast cancer cells expressing Robo. Attracted by Slit, the circulating Robo expressing tumor cells will attach to vascular endothelial cells in the brain, where increased activities of MMP9 and VEGF facilitate penetration of the blood brain barrier [81].

## 8 Conclusion

The incidence of brain metastases of breast cancer is increasing as the improvement of systemic disease is achieved. Because of the progressive neurological disability of brain metastases and the lack of effective treatment due to the unique structure of blood brain barrier, brain metastases of breast cancer is becoming more important and urgent as it affects both the survival and quality of life of the patients. However, so far little is known about the mechanisms of breast cancer metastasis to the brain, as well as the interaction of the metastatic cancer cells with the surrounding microenvironment. An improved understanding of these mechanisms will help us to prevent the metastases of cancer cells to the brain, or to develop better therapeutic strategies for the brain metastases, therefore improve the survival and quality of life of breast cancer patients.

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