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# Pathogenesis of Breast Cancer Metastasis to Brain: a Comprehensive Approach to the Signaling Network

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**Abstract** There is a general consensus that breast cancer is a rising trend disease in the world. It is one of the most common cancer types and is the leading cause of death among women's cancers. There are several reasons for this high rate of mortality including metastasis which is responsible for about 90 % of cancer-related mortality. Therefore, recognition and understanding of metastatic process is important, and by considering the key role of pathophysiological route in metastasis as a multistep cascade of "invasion–metastasis," it might modify and improve our insight toward this complex phenomenon. Moreover, it can provide novel approaches for designing advanced targeted therapies. The present work aimed to review the published papers regarding molecular basis of metastatic process of breast cancer to brain metastasis, especially related genes and signaling network. Furthermore, the use of molecular aspects of metastatic breast cancer to brain was discussed in horizon of future treatment of breast cancer.

**Keywords** Breast cancer · Brain metastasis · Gene · Signaling pathways

## Introduction

Metastasis is responsible for more than 90 % of cancer-related mortality [1, 2]. The process of tumor metastasis is highly selective and consists of a series of sequential, interrelated steps [3]. During this process, cancer cells disseminate from the primary tumor to distant organs, or to other regions of the same organ to form secondary metastatic tumor(s), and establish long distant or local metastases, respectively [4]. Breast cancer (BC) is the most common malignancy and one of the major causes of cancer-related mortality among women [5]. The majority of deaths from breast cancer are due to *metastasis* [6]. Breast cancer has great economic and psychological burdens upon healthcare systems of patients, especially when metastasized to the *brain* [7]. Brain is the *fourth most common site* of breast cancer distant metastases, after the bone, lung, and liver [8]. Approximately 10–15 % of patients with breast cancer develop brain metastasis (BM), even though autopsy studies have shown much higher incidence [9]. There are many reports indicating the *increasing rate* of BM within recent years [7].

Recent increase in frequency and incidence of brain metastasis may be due to several factors, including increased aging population [10, 11] and increased awareness of the warning signs and risk factors [11]; however, improvement in treatment and advances in diagnostic methods are raising the chance of early detection [12]. Prognostic factors of breast cancer brain metastasis (BCBM) in the domain of central nervous system, focus on different insights including identification of high-risk individuals, and evaluation of therapeutic-programming and outcomes [13–16]; higher tumor size and grade [8, 9]; luminal B, triple-negative [17] and HER2-positive tumors [8, 9, 14, 15, 17]; overexpression of p53, p63, and Ki67 [8, 18]; multiple distant metastases [9, 17]; presence of lung metastases [14, 15, 18, 19]; lymph node involvement [8, 14]; and Karnofsky performance status (KPS) [16] (Fig. 1). Identification of risk factors and prognostic markers would provide specific surveillance, management of patients at risk [20], and designing therapeutic modalities to reduce the chance of metastasis [21].

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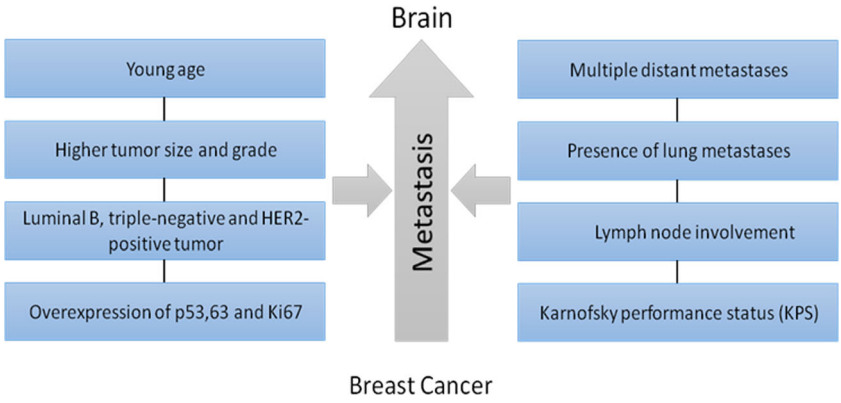


**Fig. 1** Predisposing factors in the occurrence of BCBM. Identification of risk factors would provide accurate diagnosis and better management of patients. [KPS: patient's ability to perform everyday tasks on a scale of 0 (dead) to 100, no symptom of disease]

## Cancer Stem Cells and Concept of Metastasis Cascade

Most tumors are heterogeneous and appeared to harbor a slight population of self-renewing and expanding stem-like cells, known as *cancer stem cells* (CSCs) [22–24]. CSCs share self-renewal, differentiation, organogenesis, and features of normal stem cells [24, 25]. Recent studies have shown that differentiated cancer cells can be *induced into* a CSC-like state through a biologic multistep process entitled *epithelial–mesenchymal transition* (EMT) [26]. In addition to tumor progression and metastasis, EMT is a main and essential part of embryogenesis and tissue regeneration [27]. EMT consists of a series of radical changes in cell phenotype, during which epithelial cells lose their cell–cell adhesion structures and therefore their polarity and rearrange their cytoskeleton. EMT is a critical pathway in the mesenchymal movement of single migratory cells, and therefore, cells that undergo EMT acquire mesenchymal phenotype and become isolated, motile, and resistant to apoptosis [26].

The EMT programs and early stages of cancer are regulated by several key signaling pathways and transcription factors, that lead to deregulation of the expression of epithelial markers (e.g., E-cadherin), and enhancement in the expression of mesenchymal markers (e.g., vimentin) [28]. SNAIL, TWIST, and ZEB families of transcription factors are master regulators of EMT [29]. On the other hand, TGF-β, Wnt, and Notch signaling pathways are among the most critical regulators of this process. Indeed, interactions between key transcription factors and signaling pathways form an autoregulatory network are believed to regulate different steps of metastasis [30]. The CSCs are capable to continue the metastatic dissemination process, known as metastatic cascade (3).

The metastatic cascade is a complex network of biological events [31, 32]. The most accepted model for metastasis, "seed and soil" theory, indicates that disseminated cancer cells (the seed) can thrive only in permissive tissues (the soil) [33, 34]. Looking at the pathophysiological process of metastasis as a series of distinguished steps, called "invasion–metastasis cascade," has increased our understanding of this complex phenomenon [35]. On the formation of metastases, tumor cell growth and secretion of angiogenic factors lead to an extensive vascularization within primary tumors [34]. Thereupon, cells locally invade through the surrounding extracellular matrix (ECM) and stromal cell layers, via induction of EMT, enabling them to leave the primary site [36]. Locally invasive tumor cells *intravasate* and enter into the lumina of blood vessels or lymphatic system to be disseminated [37]. Tumor cells must evade the host's immune system and apoptotic signals at the same time to survive. Henceforth, tumor cells must reach and attach to the vasculature of the brain, *extravasate* into the parenchyma, and pass through the blood–brain barrier (BBB) [38]. Interestingly, only a few tumor cells have a chance to survive [39] and reinitiate their proliferative program in foreign microenvironments and therefore form micrometastases. Next, tumor cells may further proliferate at metastatic site(s) and form secondary tumors. The latter step, called "metastatic colonization," is the most rate-limiting step of metastasis [40]. In almost all steps, dynamic interactions of tumor cells with their specialized niche have a profound effect and govern metastasis [30].

In stem cell biology, the tumor microenvironment, or niche, is a specialized network [41] that supports stem cell induction and maintenance and actively controls cell function and proliferation. The niche consists of various elements such as nutrients, soluble factors, vascular networks, stromal cells, and ECM architecture [42]. The sophisticated patterns of interactions between different cell populations determine tumor behavior and subsequently the outcome of the disease [43]. A favorable microenvironment known as "pre-metastatic niche" (PMN) is required to evolve in order to support the tumor cells for development of macrometastasis from micrometastasis [42]. Understanding the molecular aspects of the "pre-metastasis" niche generation and its role in supporting the organ-specific metastasis may

open new avenues toward achieving novel prognostic and therapeutic approaches in breast cancer management [44].

## Metastasis Organotropism of Breast Cancer

Studies have approved that distribution of metastases is a disproportional highly *selective* process, in which each primary tumor metastasize to a number of distinct organs [45]. This sophisticated phenomenon in metastasis, called *organotropism* (organ-specific metastasis) [46], has been shown in breast tumors with remarkable trend in metastasis to the bone, lung, liver, and brain [40]. Indeed, human breast tumors are heterogeneous and are classified according to the diverse gene-expression patterns. These molecular subtypes include luminal A and luminal B as the estrogen-receptor- positive (ER+) tumors, and types of estrogen-receptor-negative (ER−) including basal-like, human epidermal growth factor receptor 2 (HER2+/ER− or Erbb2) [47–51]. Sometimes, normal breast-like [52, 53] and luminal C [52] groups have been described as other molecular subtypes. This classification has a considerable clinical value, since some of the molecular subtypes show aggressiveness and poor prognosis such as HER2 and basal-like [49–52]. These subtypes are characterized with differential statue and overlapped gene expression that might determine the preferential site of relapse. Bone metastases which are the most common type of metastasis of breast cancer is more frequently originated from the luminal subtypes and are found less frequently in the basal subtype. Lung metastases are found less frequent in the luminal A subtype [48] but are common in basal-like and Erbb2 tumors [54]. The highest number of liver metastases was observed in the Erbb2 group and has been found with less frequency in luminal B subtype [48]. Erbb2, basal-like subtype or triple-negative breast cancer (TNBC) have higher risk of developing brain metastases among patients affected with breast cancer [55, 56]. The architecture of the vascular and/or lymphatic system also has key role in the dissemination pattern of circulating tumor cells (CTCs) and intricate tumor–stroma interactions at the target organ. Therefore, both the intrinsic features of cancer cells and the distant organ microenvironment play critical roles in determining the efficiency of organ-specific metastasis [45].

## Molecular Portraits of Breast Cancer Metastasis to Brain

Breast cancer brain metastasis is influenced by several genes and signaling pathways. Genes that mediate brain metastases may be excellent markers to predict the site of recurrence and afford targeted treatment for an individual patient [57].

*Chemokine signaling* plays important roles in cancer metastasis [58, 59] and seems to be a worthy biological support for the seed and soil theory [60]. *Migration*, which is one of the most pivotal involved mechanisms in metastasis, is controlled by chemokines [61–63]. The chemokines that are expressed at specific organs determine the metastatic tropism by promoting tumor cell adhesion to microvessels and facilitating angiogenesis, extravasation, tumor proliferation, survival, and subsequently metastatic colonization, through key signaling pathways such as PI3K-Akt [64]. Chemokines such as stromal-derived factor-1 (SDF-1α, also called CXCL12) and C-C motif chemokine ligand 21 (CCL21) and their corresponding receptors CXCR4 and CCR7 play pivotal roles in homing, motility, and proliferation of tumor cells at distinct sites of metastasis [65]. It may be possible to predict the site of metastasis by evaluating the expression pattern of chemokine receptors in primary breast cancers [60]. It was reported that CXCR4 had significantly higher expression in primary breast cancer compared to normal breast tissues. CXCR4 is the outmost chemokine receptor expressed in most cancers, while SDF-1α has revealed to be highly expressed in common metastatic sites of breast cancer [66]. There is compelling evidence that CXCR4 may be one of the critical mediators of metastatic breast cancer [65, 67]. Besides, by binding to CXCR4, SDF-1α could activate multiple signaling pathways, including phosphatidylinositol-3 kinase (PI-3K/AKT), mitogen-activated protein kinase (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), as well as Crk [66, 68]. It has been demonstrated that PI-3K/AKT signaling pathway activation via CXCR4/SDF-1α is indispensable for breast cancer cell migration through the BBB barrier [68]. AKT, which is a downstream target of PI-3K, plays a critical role in promoting tumor cell survival by inactivating the apoptotic machinery [66] and chemotherapy resistance [69].

Direct contact between astrocytes, protective cells of BBB, and tumor cells induces calcium sequestration [69] and subsequently activate the AKT/MAPK signaling pathways [70]. These pathways stimulates upregulation of interleukin six (IL-6), IL-8 [69], BCL2L1, TWIST1, and GSTA5. In fact, these anti-apoptotic genes are responsible for breast cancer metastases to the brain and chemotherapy resistance in tumor cells [70, 69]. In addition to calcium, phospholipid-binding proteins such as annexin A1 (ANXA1 or lipocortin) ignite CXCR4-mediated migration of breast cancer cells in response to SDF-1α [71]. Experimental studies have shown that cooperation of SDF-1α with CXCR4 leads to penetration of breast cancer cells into human brain microvascular endothelial cells (HBMEC). Suppressing the CXCR4/ SDF-1α-mediated signaling pathway can be considered as a

therapeutic approach for inhibition of breast cancer invasion and vascular permeability [66, 68]. The Cxcr4 and Cxcl12 signaling axis can be blocked by Slit family of secreted proteins (Slit1, 2, and 3) and their corresponding receptors (Robo1, 2, 3, and 4) [72]. Slits and Robos have critical roles in neuronal development and migration [73] and are candidate as tumor suppressor genes that are silenced in approximately 50 % of human breast tumors [72].

NF-κB regulates the motility of breast cancer cells through direct upregulation of CXCR4 expression. This complex, upregulates the expression of several prometastatic and proangiogenic genes including IL-6, IL-8, vascular endothelial growth factor (VEGF), and urokinase-type plasminogen activator (uPA) [65]. uPA convert plasminogen into plasmin which in turn inhibits the L1 cell adhesion molecule (L1CAM). L1CAM is an essential molecule for infiltration of metastatic breast cancer cells into brain capillaries and has a pivotal role in metastatic outgrowth of cancer cells. Furthermore, overexpression of anti-PA serpins (including neuroserpin and serpin B2), a family of protease-enzyme inhibitors, as brain metastatic cells originated from breast cancer tissues, can suppress plasmin and as a result provoke the metastatic process [39]. The uPA also degrades matrix components and activates matrix metalloproteinases (MMPs) through NF-κB activation [6, 74]. As one of the important biological markers in breast cancer [75], MMPs, which belong to a zinc-dependent endopeptidase family, are involved in different steps of tumor progression and facilitate cancer cell invasion and metastasis. These proteins act as enzymes that degrade structural components of the extracellular matrix. They are divided into two major types, soluble and membrane-MMP types. Based on substrate specificities and structural similarities, 28 human MMPs have been identified and categorized so far [76]. MMPs have also been broadly studied in context of breast cancer prognosis [77]. The mean messenger RNA expression of MMP-2, MMP-7, MMP-9, MMP14 genes besides tissue inhibitors of metalloproteinase-1 (TIMP-1) and TIMP-2 have shown to be *significantly higher* in breast cancer compared to normal tissue. This expression profile would be important in predicting the aggressive behavior of breast cancer cells [75].

There are many researches that highlighted the upregulation of MMPs in normal breast epithelium which was associated with invasive tumor formation through increase in genomic instability and EMT. Moreover, MMPs have critical roles in creating the pre-metastatic niche [74], and they induce growth factor signaling as well as TGF-β, FGF-2, and VEGF-A through enhancing their availability to corresponding receptors. It results in tumor evolution through stimulation of tumor fibroblasts and angiogenesis [74]. Among MMPs, MMP-2 and MMP-9 are known as type IV collagenases, or as an alternative gelatinase A and B, respectively [78]. Upon their function, MMP2/9 degrade type IV collagen, which is believed to be involved as a main component of the vascular basement membrane structure [75, 79]. Besides, MMP-2 is capable to hydrolyze other constituents of connective tissues such as elastin, laminin, fibronectin, proteoglycans, and fibrillin [79]. MMP-7 is upstream of MMP-2 and MMP-9 and turns them on to be critically involved in the degradation of the ECM components including type IV collagen [75]. Animal model studies have demonstrated that the MMP-2, MMP-3, and MMP-9 proteins expression is meaningfully higher in neoplastic compared to normal brain tissue. It has been proposed that MMP-2 [77, 80], MMP-3, and MMP-9 [77] might be active in the process of metastasis of breast cancer to the brain. It was confirmed that there is an association between MAPK pathway elements such as extracellular-signal-regulated kinase1/2 (ERK1/2), MMP expression, and/or astrocyte activity. It is assumed that astrocyte factors and the ERK1/2 signaling pathway may be associated with the development of BCBM. Animal studies have shown that ERK1/2 modulate the MMP2 to be modified by astrocyte factors [80].

Joyce and his colleagues confirmed the role of cathepsin S (CTSS) which encodes a lysosomal cysteine protease playing crucial role in metastatic seeding and outgrowth. They also demonstrated that CTSS modulates the organotropism and regulates BCBM through facilitating the transmigration of CSCs into BBB [81]. It was described that *cyclooxygenase-2* (*COX-2*, also known as *PTGS2*) alters membrane arachidonic acid into prostaglandins and is able to upregulate the MT1-MMP, which itself activates MMP-2 that may provoke angiogenesis. *COX-2* is well known to be involved in precursor lesions of various solid tumors and contributes to tumorigenesis by hindering the signaling pathways of the pro- and anti-apoptotic proteins [76]. *COX2* accompanies the epidermal growth factor receptor (EGFR) and the *alpha-2,6-sialyltransferase* (ST6GALNAC5), which are highly distinguished genes among involved genes in breast cancer brain metastasis [82]. These genes are active when the tumor cells enter into the brain through the BBB (extravasation). The MMP1 and angiopoietin-like four (ANGPTL4) [82] are other involved proteins in this way which play pivotal role in driving the TGF-β and Notch signaling [32] and they thus mediate *intravasation* and *extravasation* processes [40]. The latent TGF-β-binding protein (LTBP1) as a major modulator of TGF-β activation, fascin-1 or FSCN1, and retinoic acid receptor responder protein3 (RARRES3) are other involved proteins [82]. Except *ST6GALNAC5*, they are linked to breast cancer infiltration of the lungs, suggesting that they have these mediators in common with cerebral and pulmonary metastases [82]. Moreover, eukaryotic translation initiation factor two (EIF2S3), FABP7, NMDA receptor regulated 1(NARG1), zinc finger proteins [involved in transcription and translation], aldehyde dehydrogenase 1 family, member A1 (ALDH1A1)

[involved in metabolism], EGFR [involved in signal transduction], integrin alpha-6 (ITGA6), integrin, and laminin [involved in adhesion], ERGIC [involved in transportation] make a long list of proteins which have been shown to be expressed in breast cancer brain metastases [57].

Angiotensin II upregulates MMP2/MMP9 through which the sequential steps of cancer metastasis would be motivated. This progressive/ programming cascade includes promoting cancer cell adhesion to endothelial cells, transendothelial migration, and subsequently tumor cell migration across ECM, and subsequently facilitating the formation of metastatic foci at secondary sites [83]. MMP-9 is known as a major modulator of *HER2/neu* expression in human mammary epithelial cells. HER2/neu proto-oncogene or *erbB-2* belongs to the ErbB protein family and is a cell surface receptor tyrosine kinase (RTK) that is principally contributed in cell growth and differentiation [83, 84]. ErbB-2 has been shown to be upregulated in 20–30 % of human breast cancers [85], while 34 % of HER2-positive breast tumors have led to brain metastases. HER2 pathway starts to relay the signals of several signaling proteins and pathways including PI3K/Akt, when it is activated [86]. Overexpression of *HER2* upregulates the expression of MMP-9 and MMP-2 proteases [87], transmembrane proteins as well as plexin-B1 [86], and cluster of differentiation CD151 [88]. Accordingly, it was demonstrated that the CD151 and plexin-B1 play major roles in motility, invasion, and metastasis of cancer cells [88]. Of note, estrogen receptor beta (eRβ) and pea3 are among other proteins whose expression has been increased in response to *HER2* overexpression. Importantly, they lead to IL-8 upregulation, which belongs to the superfamily of CXC chemokines and becomes overexpressed when its promoter is bound with the latter proteins [89]. IL-8 is a major mediator of angiogenesis and is capable to induce this process through stimulating the proliferation and sprouting of endothelial cells [40, 90]. In addition, the potential effects of TGF-β on HER2 signaling have been demonstrated. It was shown that IL-6, TGF-β, and IGF receptors are actively involved in the progression of breast cancer cells to the brain [91]. Therefore, by blocking the TGF-β, HER2 crosstalk may restrain breast cancer cells from progression and metastasis [92].

ErbB2 overexpression can also be associated with over expression of VEGF in breast cancer cells [85, 87]. It is also known as a vascular permeability factor (VPF) and major regulator of new blood vessel formation (angiogenesis) during tumor development [93]. It was described that it stimulates the proliferation and transendothelial migration of tumor cells, which is a key event in cancer metastasis inducing the expression of metalloproteinases and plasminogen proteins [38]. Potential of breast cancer cells to form brain metastases is a main consequence of the latter mentioned inductions [90]. VEGF also promotes the growth of BM induced by breast cancer in nude mice, and targeting endothelial cells with a VEGF receptor-specific tyrosine kinase inhibitor can reduce angiogenesis and restrict the growth of brain metastases [90] (Fig. 2).

## Therapeutic Approach

BM represents a significant healthcare concern and has a drastic deleterious impact on patient mortality [94]. Of note, not all the brain lesions are considered as primary tumor and

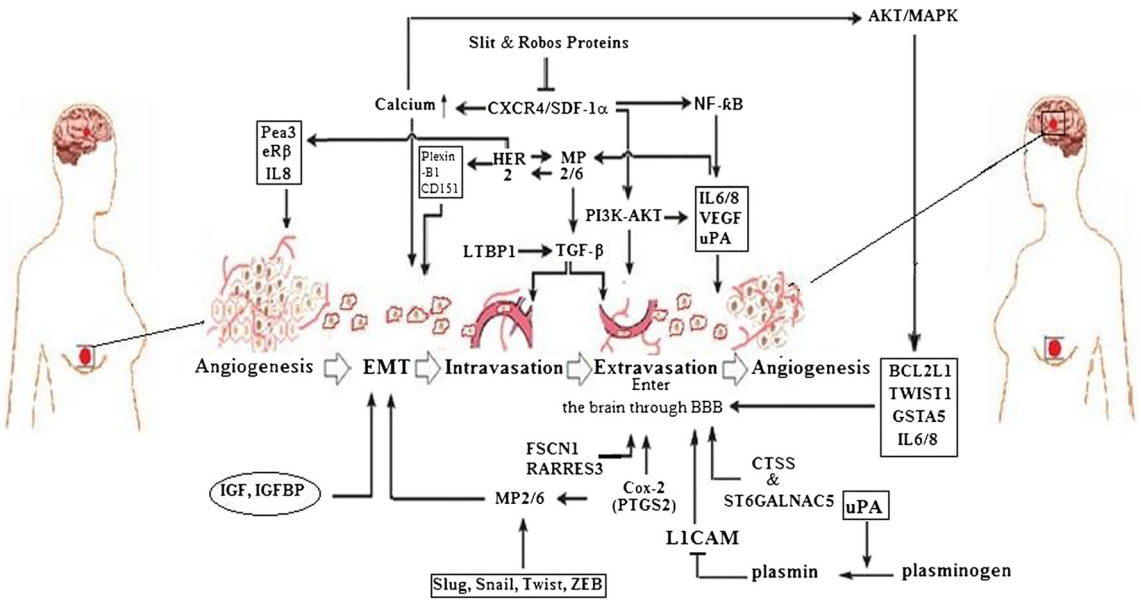


**Fig. 2** Multistep process and signaling network of BCBM. From the figure, we can see that BCBM is influenced by several genes and signaling pathways. Potentially, inhibition of these pathways can be a valuable therapeutic approach, especially early signaling pathways, because of this approach leads to inhibit more of accessory signaling pathways involved in progression of metastasis

the possibility of metastasis from other organs especially breast should always be noted [95]. The biology of the primary tumor, the number and location of metastatic lesions, and the phase of systemic disease are important considerations for the treatment of brain metastasis [34].

Appropriate diagnostic approaches are presently available such as computed tomography (CT) and contrast-enhanced magnetic resonance imaging (MRI). When it comes to the diagnosis of brain metastases, MRI is the ideal test [95]. Molecular diagnosis may also be useful, for example, miR-205 that is negatively regulated by *HER2/neu* overexpression and miR-342 that is involved in the breast tumor's invasive behavior, these may be used as potential biomarkers for diagnosis of triple-negative breast cancer [96]. Treatment options of brain metastasis for diagnosed patients include surgery, whole brain radiotherapy (WBRT), stereotactic radiosurgery (SRS) [97], and chemotherapy that among them WBRT is the most common selected choice [12, 98]. In patients with several brain metastases, studies have identified that the use of surgical resection simultaneously with radiotherapy is highly preferred to using of radiotherapy solely. Additionally, patients who take systemic chemotherapy after brain radiotherapy show significant higher curative outcome [99]. The other commonly used method, chemotherapy, has played trivial roles in the treatment of BM; however, the intact BBB blocks the passage of many chemotherapeutic drugs into the brain [16].

Targeted therapies are the *most attractive* molecular therapeutic approaches that have shown to be promising. In these methods, certain proteins and signal transduction pathways that are involved in BCBM are *specifically* targeted, for instance targeting the angiogenesis by anti-VEGF agent [100]. Targeting poly-adenosine diphosphate ribose polymerase (PARP), which plays an important role in DNA damage repair, and its corresponding pathway using specific inhibitors such as iniparib, olaparib, and veliparib may have a critical role in increasing the responses of tumor cells to chemotherapy and radiotherapy [94]. In addition, targeting HER2 tyrosine kinase by their specific inhibitors such as gefitinib, erlotinib, lapatinib, and trastuzumab constitute one of the effective strategies of targeted therapeutic approaches [12]. Notably, trastuzumab is a humanized monoclonal antibody that binds to a specific epitope of the HER-2/neu (c-erbB-2) protein. This interaction suppresses signal transduction pathways that regulate cell growth, survival, migration, differentiation, and angiogenesis, thereby decreases malignancy [16, 101] and also increases the sensitivity of tumor cells to both endocrine therapy and certain chemotherapeutic agents [16]. Lapatinib inhibits the dual epidermal growth factor receptor and HER2 tyrosine kinase [95] and corresponding downstream signaling proteins and therefore cell proliferation and migration [102]. In targeted therapy of metastasis, inhibition of early signaling pathways is important, because this approach would be associated with inhibition of more accessory signaling pathways involved in progression of metastasis. Certainly, inhibition of EMT leads to the prevention of early steps of metastasis. Recent studies on stem cell showed that gene expression profiling of cancer stem cells is similar to gene expression profiling of induced pluripotent stem cells. These cells can be changed into mesenchymal-like phenotype by enhanced gene expression including insulin-like growth factor (IGF) or its binding protein as transferrin (IGFBP) [103]. It is possible to hypothesize that EMT and generation of mesenchymal-like cells are inhibited by expression inhibition of *IGF* and *IGFBP* genes, and potentially, these genes can be a *more practical target therapy*. However, many experimental researches are warranted to confirm this hypothesis.

BM as one of the metastatic organotropism of breast cancer has a great unpleasant effect upon patients and their families. Hence, a more comprehensive understanding of molecular aspects of metastatic cascade is essential to achieve an appropriate strategy in accurate diagnosis and novel methods of therapeutics. Using this viewpoint, many results have been reported in studies of involved signaling pathways in BCBM, ranging from CXCR4/ SDF-1α, PI-3K/AKT, MAPK, NF-κb. Comparison between pathways involved in BCBM with other pathways leading to metastasis of breast cancer cells to other organs as well as lung can shed further light on a new set of genes that play critical role in BCBM, as well. These findings could be important because these may lead to provide a new target-based therapy. Beyond question, further research is required to explore the unknown aspects of signaling network in BCBM. As a final word, the main concern is whether the results achieved in vitro and/or in vivo are translatable in human.

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**Conflict of Interest** Both authors have no conflicts of interests.

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