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ABSTRACT

Breast cancer (BC) is one of the most frequently diagnosed cancers in women worldwide. It has surpassed lung cancer as the leading cause of cancer-related death. Breast cancer brain metastasis (BCBM) is becoming a major clinical concern that is commonly associated with ER-ve and HER2+ve subtypes of BC patients. Metastatic lesions in the brain originate when the cancer cells detach from a primary breast tumor and establish metastatic lesions and infiltrate near and distant organs via systemic blood circulation by traversing the BBB. The colonization of BC cells in the brain involves a complex interplay in the tumor microenvironment (TME), metastatic cells, and brain cells like endothelial cells, microglia, and astrocytes. BCBM is a significant cause of morbidity and mortality and presents a challenge to developing successful cancer therapy. In this review, we discuss the molecular mechanism of BCBM and novel therapeutic strategies for patients with brain metastatic BC.

1. Introduction

With 2.3 million newly diagnosed cases, breast cancer (BC) has now eclipsed lung cancer to become the most frequently diagnosed cancer type [1]. About 20–30% of women with early-stage BC are likely to develop distant metastases to other organs primarily the brain, liver, and lung [2]. Approximately 90% of BC patients die due to metastatic disease complications. Metastatic organotropism (metastases to specific organs) is the non-random spread of malignant cells to distant organs influenced by various factors, including cancer subtypes at the molecular level, the host immunological milieu, the anatomical location of the organs, the organ-specific niche of the metastatic sites, and its interaction with the tumor cells [3,4]. BC preferably metastasizes to bones and the brain, which are regulated by the host immune barriers such as specialized activities of the organ and constraints on how cancer cells break the barrier to extravasate to unique distant organs (Fig. 1) [5].

In 1889, Stephen Paget postulated the classical 'seed and soil' hypothesis to describe site-specific metastasis, according to which cancer cells infused with metastatic ingredients are referred to as "seeds" and the tissue microenvironment (TME) of any organ is the proverbial "soil" that provides a fertile ground for the growth of seeds [6]. Metastasis is a multistep signaling cascade where tumor cells detach from the primary tumor intravasate and survive into the systemic circulation, resist immune attacks, attach to capillaries, and extravasate to distant organs before colonization [7]. The colonization of the brain involves a complex interplay between the TME, metastatic cells, and brain cells like endothelial cells and astrocytes [8]. Moreover, BC sets the course for malignant progression via tumor heterogeneity and cell plasticity [9]. Interestingly, the TME plays an instrumental role in steering the cancer cells towards heterogeneity and metastasis via reprogramming of their transcriptome [10].

Recent advances in molecular technologies for the early detection of BC, as well as targeted chemotherapeutic agents, have increased survival rates and improved the life quality of BC patients [11–14]. Current gold standards for treating brain metastasis include whole-brain radiation therapy and stereotactic surgery [15]. A better understanding of the

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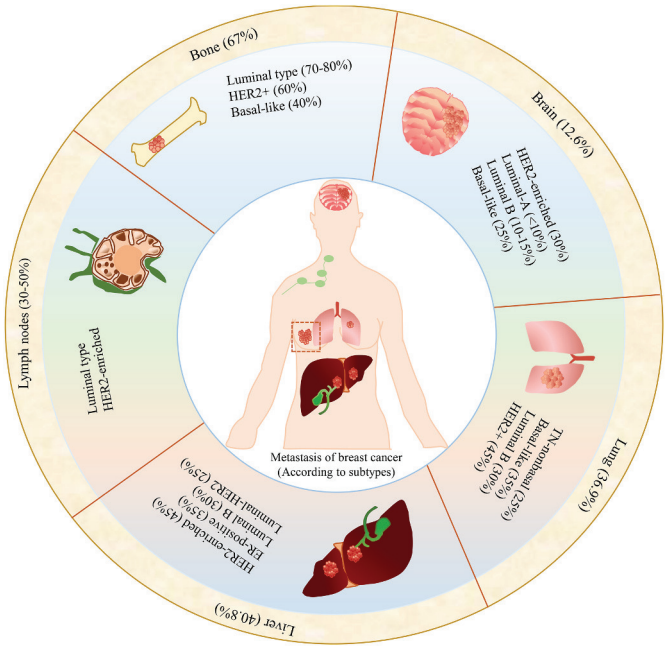

Fig. 1. Metastasis of breast cancer: Breast cancer can metastasize to different organ sites, including the bone, lung, liver, brain, and lymph nodes, depending on the molecular subtypes. Among these, bone is the first preferential site of metastasis, followed by the brain, liver, lung, and lymph nodes.

molecular mechanisms involved in brain metastases is critical for developing novel and effective treatments before making field clinical decisions for patients. This review describes a comprehensive overview of the existing knowledge base of molecular mechanisms and cell signaling pathways. Chemotherapeutic agents for treating BC patients with brain metastases will also be reviewed here.

2. Clinically relevant molecular subtypes

Brain and central nervous system (CNS) metastases account for approximately 30% of metastatic BC cases [16,17]. According to the expression of receptors on the cell surface, clinically relevant molecular subtypes of BC include estrogen receptor-positive (ER+) or progesterone receptor-positive (PR+) BC, human epidermal growth factor receptor-2-positive (HER2+) BC, and triple-negative BC (TNBC) [18, 19]. On the basis of gene expression, BC can be further subdivided into luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like subtypes [20,21]. The primary molecular subtypes significantly influence the risk for brain metastasis at diagnosis, which are commonly determined by the differences in the characteristics of breast carcinoma cells at various phases of the development of brain metastasis. Radiomic signals can predict BCBM receptor status noninvasively because receptor switching in BCBM is prevalent [22]. TNBC cells are much more capable of adhering to brain endothelial cells than luminal BC cells [23]. For instance, HER2+ and TNBC have a much higher risk of metastasizing to the brain than ER + or PR + BC [24]. Metastasis to the lung as the first metastatic site was found to be a risk factor for brain metastasis in a retrospective investigation of HER2+ve individuals [25]. The HER2+ve subtype shows a higher rate of overall survival and a lower rate of CNS metastasis, while the TNBC subtype is more prone to metastasis and portends a poor prognosis [26,27]. Also, the median time between diagnosis of primary BC and CNS metastases is the shortest in the case of TNBC [28]. On the other hand, luminal BC shows a relatively better patient prognosis but a higher risk of metastatic relapse as compared to other subtypes [29]. Multivariate logistic regression analysis recently implicated that there is an increased risk of BC metastasis to the brain in HER2-low BC patients [30]. The incidence of brain metastasis at the time of diagnosis and survival outcomes of metastatic BC in 2248 patients were estimated using the Surveillance, Epidemiology, and End Results (SEER) population database. HER2-ve molecular subtype (38.1%) showed the highest incidence rate of brain metastatic BC, followed by TNBC (17.5%), while patients with invasive lobular carcinoma (ILC), a histological subtype, are at a lower risk of developing brain metastasis at diagnosis (4.76%) [31]. Patients with invasive lobular BC who have a longer PFS are more likely to develop brain metastasis [32]. Brain metastases are associated with younger age, HER2-enriched, luminal-HER2, poorly differentiated tumors, basal-like, and TNBC subtypes, as well as four or more metastatic lymph nodes (Fig. 1) [33–35].

Aside from the expression of hormone receptors like HER2, ER, and PR as predominant prognostic indicators, gene panels using the power of next-generation sequencing are reinforcing decision-making in the clinical management of BC brain metastasis. Metastatic tumors in the brain possess different mutational and transcriptional signatures than their primary counterparts, as indicated by several studies [36]. Recent studies mapped subtype-specific transcriptional programs in brain metastatic tissue [37]. Several metastasis-associated genes in brain metastasized BC have been identified with the application of high throughput transcriptomics [38]. The expression of RAD51, TPR, and HDGF was also detected as a predictor of early brain metastasis [39].

Gene expression profiling of BC metastasis patients revealed the expression of different genes, including FGF1, GRIN1, NRCAM, CRYAB, SOX2, SOX10, CH13L1, ZIC2, GDF15, VEGFA, LEFTY2, RASGRF1, NRXN1, TTYH1, and SHC4 are upregulated in BCBM FFPE tissues. The CRYAB gene was also significantly associated with poor overall survival in patients with brain metastasis [40]. Microarray data from GEO datasets of primary vs. brain metastatic tissues, detected COL1A1, COL3A1, and periostin (POSTN) genes involved in a protein-protein interaction (PPI) network. These proteins were also significantly associated with the overall survival of BCBM patients. POSTN is an extracellular matrix protein that binds to integrins and regulates the migration of endothelial cells. POSTN has been linked to BC progression and metastasis [41]. Whether these genes can act as biomarkers in BCBM, remains to be elucidated in functional validation models [42]. RNA and DNA sequencing of BC subtype-specific brain metastases characterized clinically relevant mutations in the DNA repair pathway. Moreover, p53, NOTCH, and AKT pathways are enriched in luminal subtype-originated brain metastasis. In HER2+ve BCBM, focal adhesion processes are diminished and hypoxia, cell cycle pathway, and WNT signaling are enhanced [43].

3. Molecular signaling network of BC brain metastasis

During cancer progression, brain metastasis often manifests after systemic metastases have been formed in organs like the lungs, liver, and/or bone [15]. Owing to the peculiar lattice of blood vessel formation, parenchymal type of brain metastases take the lion's share (about 80%) as compared to leptomeningeal metastases [44,45]. Blood-brain-barrier (BBB) is a specialized structure that contains endothelial cells (ECs), pericytes, and astrocytic end feet, which govern CNS homeostasis [46]. It represents selective heterogeneous permeability at the level of the cerebral microvascular endothelium in the brain. Extravasation over the BBB is one of the most significant barriers to cancer cells colonizing the brain and one of the rate-limiting elements in clinically successful therapy [47]. The BBB is disrupted, which ultimately leads to the genesis of the blood–tumor barrier, which is more permeable than the previously existing BBB [48,49]. Circulating tumor cells (CTCs) must first breach the BBB to colonize the brain tissue. Cytokine and chemokine signaling, immune suppression, altered metabolomic signatures, and communication between brain resident cells and invading cancer cells are all molecular instruments associated with brain metastasis and are discussed in detail here [50,51].

3.1. Chemokines/cytokines in TME affect the metastasis of BC to the brain

Chemokines are chemoattractants and low molecular weight proteins with molecular weights ranging from 8 to 10kDa that mediate cellular processes culminating in organ-specific metastasis (organotropism). Chemokines and cytokines shape the tumor microenvironment, aiding cancer metastasis in solid cancers [52]. In TME, chemokines and cytokines are involved in tumor cell extravasation and disruption of the BTB (blood-tumor-barrier) [53]. Breast carcinomas have elevated levels of the proinflammatory C-X-C motif chemokine ligand 1 (CXCL1), which mediates the polarization of macrophages towards the M2 phenotype [54]. Metastatic cells differ in their secretome depending upon their destination sites [55]. Treating normal brain endothelial cells with brain-seeking MDA-MB-231-BR cells results in higher CXCL1 and lower thioredoxin-interacting protein (TXNIP) expression. CXCL1 and TXNIP are shown to promote angiogenesis and metastasis [23]. Antibody against CXCL1 diminishes the metastatic potential of a leukemia cell line [54].

TGF-β is a multi-pronged cytokine that facilitates cell apoptosis and growth arrest. TGF-β has tumor suppressor properties but drives metastasis by indirectly activating the AKT signaling pathway [56]. A research study showed that TGF-β signaling and the anti-tumor activity of T cells become flawed when $CD4^+$ and $CD8^+$ ROR1-CAR T-cells from healthy donors are co-cultured with MDA-MB-231 in a 3D tumor model [57]. It is well known that TGF-β is a negative regulator of CXCL1, and its expression is inversely related to CXCL1 [54].

Further, CXCL12 is a ligand for CXCR4, located on the surface of breast tumor cells that plays a critical role in tumor cell migration to the brain tissue. CXCL12 expression was significantly higher in cancer-associated fibroblasts from brain metastatic tissue than the fibroblasts derived from primary BC and normal breast tissues [58]. Increased levels of CXCR4 have already been linked with increased metastasis, resistance to therapy, and worse prognosis [52,59–61]. CXCR4-CXCL12 signaling activates PI3K/AKT, p38, and MAPK pathways, catalyzing cancer cell transmigration through the BBB and, ultimately, cancer cell survival [62,63]. Scala et al. were the first to report therapeutic targeting of CXCR4 with antagonist peptides to improve the survival of BC patients with brain metastases [64]. Thymoquinone, a bioactive compound from N. Sativa, abolishes the CXCR4-CXCL12 signaling axis and prevents bone and brain metastases in a mouse model of BC [65]. Thymoquinone also lowers the expression of NF-kB-regulated CXCR4 in brain metastatic tissue. NF-κB is a transcription factor responsible for the up-regulation of the cytokine CXCR4 and matrix metalloproteinases in BC cell lines. NF-κB binds directly to the promoter region of CXCR4. Further, studies showed that when the inhibitor of NF-κB is overexpressed in vitro, the expression of CXCR4 diminishes [66].

A recent study evaluated the prognostic value of a group of differentially expressed genes using functional enrichment analyses, which revealed CXCL8 as a prognostic biomarker significantly associated with brain metastases and infiltration of macrophages and neutrophils in TNBC [67]. Pro-metastatic cytokines like TNFα and IL-1β are highly expressed in TNBC cell lines. TNBC cells, co-cultured with mesenchymal and stromal cells, stimulate the release of CCL8/IL-8 and CCL5 [68]. It is important to note that the release of CXCL8 is primarily driven by the NF-κB/Notch1 pathway. Several cytokines, including oncostatin M (OSM), IL-6, and IL-1β control the extravasation of metastatic cells through the BBB during BCBM. Implicated in glioblastoma, OSM belongs to the IL-6 family of cytokines and is associated with Jak-STAT and NF-κB signaling pathways [69]. STAT3 inhibitors successfully subdue the OSM-mediated Jak-STAT pathway [70]. Cytokines and chemokines form a multibranched, involuted network of paracrine and autocrine cellular pathways within the TME and promote metastasis to the brain [71,72]. A deep understanding of these molecular pathways and downstream effector molecules is of the essence for developing novel chemotherapeutic agents against brain metastasis.

3.2. The two-faced immune system in metastasis

By using anti-tumor immunity mechanisms, the immune system can either eliminate metastasizing cancer cells or favors tumor progression through inflammation and immune suppression [73–75]. For example, tumor cells bearing cancer antigens bound to MHC class I are eliminated by cytotoxic T lymphocytes, whereas inflammation encourages tumor formation and metastasis by suppressing anti-tumor immunity and producing large quantities of pro-inflammatory cytokines [76]. Primed B cells and Th2 cells are actively involved in tumorigenesis by secreting growth-promoting cytokines like TGF-β, IL-6, and IL-10 in the tumor microenvironment, thus enhancing cell proliferation and survival [77,78]. Genes conferring immunity are differentially expressed among different metastatic sites in BC. The decrease in mRNA expression levels of immunity genes like CXCL5, CCL9, CXCR6, IDO1, CD8A, STAT1, and CD274 is seen in BC metastasizing to the brain, while CRYAB and NRCAM are overexpressed [40]. Quantifying gene expression of brain metastasis-specific genes might help choose a better chemotherapeutic agent to treat patients. More functional studies are required to understand if these genes can be used as biomarkers.

Astrocytes, the regulators of blood-brain permeability, have a pro-metastatic role as they secrete C–C motif chemokine ligand 2 (CCL2), which docks into the CCR2 receptor and promotes the migration of cancer cells in the mouse brain. CCL2 also binds to CCR4 receptors on cytotoxic T lymphocytes and Treg cells and blocking CCR4 or CCR2 results in decreased extravasation of tumor cells into the brain parenchyma [79]. A novel lncRNA has been shown to promote the expression of CCL2, which in turn drives the recruitment of macrophages in the brain tissue that increases oncostatin M and IL-6, activates the JAK/-STAT3 pathway, and leads to BC brain metastasis [80]. Interestingly, the loss of Tap73 is responsible for hyperactivating the NF-κB pathway, increasing inflammation, secreting CCL2, and regulating the recruitment of macrophages and monocytes into the brain via activation of the NF-κB pathway [81]. The Warburg effect, a hallmark of cancer, produces lactate in the cancer cell environment, which in turn increases inflammation and metastasis. It has been observed that when the lactate metabolic pathway was attenuated, metastasis to the brain was obstructed in mice. In brain metastatic cells isolated from mice, NK (natural killer) cells reduced significantly in number, size, and cytotoxicity upon lactate exposure [82]. Lactate also renders the TME immune-suppressed by educating M2 macrophages, eventually turning them into IL-6 and TGF-β secreting TAMs. These tumor-associated macrophages (TAMs) are known inducers of inflammation and angiogenesis. TME also confers metastasis by creating an immune-suppressive environment for tumor cells to escape immune surveillance [75]. Zinc finger E-box binding homeobox 1 (Zeb1) is a transcription factor aberrantly expressed in several cancers. Hypoxia induces the Zeb1 gene, which is a key inducer of resident macrophage polarization into M2-bearing TAMs in the TME by upregulating glycolysis and the PKA cell signaling pathway. Also, glycolysis, HIF1-α, and PI3K activity were reduced in mice lacking the Zeb1 locus [83]. TME-associated molecular pathways are angiogenesis, autophagy, hypoxia, and DNA repair, which are often upregulated in BC brain metastatic tissues. Thus, the Zeb1-linked glycolysis pathway can be therapeutically targeted in patients with invasive BC. A comparison of CCL19, CCL21, $CD4^+$ T cell, $CD8^+$ T cell, and M1 macrophage populations in primary breast tumors and matched brain metastasized tumor pairs revealed that CCL19, CCL21, $CD4^+$ T cell, $CD8^+$ T cell, and M1 macrophage populations decreased in brain metastatic tissue while CD163+, CD206+, and the M2 macrophage population increased [84].

A high number of M2 macrophages is associated with immune suppressive TME, and poor prognosis/overall survival in patients with brain metastasis, especially in the HER2+ve subtype. RNA-sequencing showed that the M2 macrophage population is increased and the total immune subset is decreased in matched brain-metastatic pairs of primary breast tumors [85]. TME must be targeted therapeutically since it

interacts with tumor cells at the local level.

3.3. Crosstalk between metastatic cells and the brain microenvironment

Astrocytic glial cells are abundant among microglial cells and oligodendrocytes in the brain tissue microenvironment. These astrocytes are engaged in cross-talk with the infiltrating BC cells, taking assistance from the gap junctions [86]. Astrocytes communicate with the brain microenvironment via the gap junction proteins known as connexin 43 [87]. They are non-proliferative in the normal physiological state; however, they can be activated during brain damage or invasion by metastatic cancer cells. Invading BC stem cells release IL-1β, activating the astrocytes that enhance cancer cell proliferation via the JAG1-Notch signaling axis [88]. By increasing matrix metalloproteinase expression and inducing neighboring cells to release VEGF, IL-6, IL-8, TNF-α, and TGF-β, IL-1β has been shown to increase cancer cell invading potential in an autocrine manner [88]. For example, reactive astrocytes secrete oncogenic signals such as IL-6, IL-10, and TGF-β, which promote cell migration and invasion [89]. Previously, it has been shown that the expression of ANGPTL4 is also spiked by TGF-β1 in BC cells metastasizing to the lung [90]. Another study revealed that connexin 43 also favors local inflammation and inhibiting connexin 43 may be a potential therapy for diseases occurring in the CNS [87]. CCL7, SCF, MMP2, MMP9, and TNF-α are also released by astrocytes, which are pro-angiogenic in nature and promote tumorigenesis [86].

Interestingly, brain infiltration of BC stem cells is facilitated by paracrine signaling by the inhibitor of differentiation 3 (ID3) to nuclear respiratory factor 1 (NRF1) [91]. The brain is the site of high oxidative phosphorylation (OXPHOS) activity, and its metabolic derivates serve as chemoattractants both in vitro and in vivo. Metastatic progenitor cells specifically migrate towards astrocytes when Cox7b is overexpressed, increasing OXPHOS activity in the brain [92]. ANX1A (Annexin-A1), the downstream activator of STAT3, is released by the 4T1 mammary cells, which activate microglial cell migration to the brain via paracrine signaling [93]. Microglial cells mount an anti-cancerous response, but also, upon migration to the TME, they release STAT3 to evade immune responses and favor metastasis to the brain. STAT3 leads to the transcription of pro-inflammatory cytokine, IL-6, and anti-inflammatory cytokine, IL-10. IL-6 has also been identified as the primary inducer of microglial cells promoting colonization into brain tissue by single-cell RNA sequencing in non-small cell lung cancer (NSCLC) [94]. Crosstalk between microglia and metastatic cells is important for colonizing primary BC in the brain. Microglial cells are also reported to promote brain metastasis via the STAT3 pathway in lung carcinoma [95]. Therefore, activated microglia can serve as an effective therapeutic target to treat brain metastatic cancer.

A recent study demonstrated that astrocytes activate PPAR-y signaling by furnishing polyunsaturated fatty acids to metastatic BC cells because astrocytes are the main wellspring of fatty acid synthesis in the brain tissue [96]. However, there is much information about molecular pathways regulating dormancy in brain metastasis. Intravital imaging of dormant and metastatic triple-negative BC cell lines shows that dormant disseminated tumor cells rest on astrocyte end feet, and their quiescence is driven by secretion of laminin-211 [97].

One study characterized the involvement of various brain-resident cells in mouse models. Microglia, like astrocytes, normally stay dormant, but once vitalized, they boost the invasion and colonization rates of metastatic BC cells by triggering the Wnt signaling pathway [98,99]. Reactive microglia bear major histocompatibility complex classes I and II and exhibit phagocytic activity. Finally, microglia are brain-resident macrophages with dual functions that kill cancer cells and promote tumor growth [100]. Single-cell RNA sequencing unveiled discrete gene signatures in brain-resident microglial cells in a lung cancer brain metastasis model [100]. In BCBM patients, microglial cells accumulate around the invading cancer cells, generating local inflammation and leading to cortical derangement.

Neurotransmitters, namely GABA transporters and GABA receptors, were found to be highly expressed in brain metastatic BC cells, resulting in increased NADPH production and increased tumor proliferation. By triggering the AKT/PI3K/mTOR, MAPK, and NF-κβ signaling pathways, tumor cells can modify the brain microenvironment to a great extent [101]. Gamma-aminobutyric acid receptor subunit alpha-3, or GABRA3, is an inhibitory neurotransmitter that binds to the GABA receptor and activates the AKT/PI3K/mTOR pathway. GABRA3 is not expressed in normal breast epithelial cells and is found to be overexpressed in BC cell lines. As a result of RNA editing, GABRA3 censors the tumor-promoting function of wild-type GABRA3 by arresting AKT/PI3K/mTOR signaling [102].

Pericytes are present in the CNS, lying along the walls of blood capillaries, and are vital for the maintenance of the blood-brain barrier. Pericytes provide the architectural framework for the blood capillaries and frequently play a role in brain metastasis. A reduced number of pericytes indicates a poor prognosis in patients with invasive ductal carcinoma and increased metastasis [103]. Pericytes secrete IGFR2, and ECM (extracellular matrix) proteins, affecting the adhesion and migration of TNBC cells [104,105]. Pericytes also communicate with the immune cells with the help of their cell adhesion receptors like VCAM and I-CAM [106]. Little is known about the molecular mechanisms used by the pericytes in establishing a pre-metastatic niche in brain tissue. A study reported that different populations of pericytes exist in the brain parenchymal tissue and deposit large amounts of collagen in the brain tissue [107]. Using immunofluorescence, the pericyte subpopulations were assessed, and increased permeability of the blood-tumor barrier was linked to the high expression of the desmin + subpopulation of pericytes [108]. Desmin and pericytes are also found near newly formed blood vessels, thereby promoting angiogenesis, and may serve as novel chemotherapeutic targets [109].

3.4. PI3K/AKT/mTOR signaling pathway

BCBM involves aberrant expression of genes and signaling pathways, which could serve as potential markers for predicting recurrence and providing therapy targets (Fig. 2). The mechanistic/mammalian target of the rapamycin (mTOR) signaling pathway is instrumental for tumorigenesis, increased cell division, and tumor cell metabolism. The PI3K/AKT/mTOR signaling pathway is the chief regulator in cancer, and several mutations aberrantly activate the PI3K/Akt signaling pathway in brain metastasis [110]. Genes of fatty acid biosynthesis, transport and uptake are upregulated in the case of BC. The SREBP pathway is central to lipid biogenesis and boosts tumor cell metabolism. PI3K/AKT/mTOR activates this pathway and dampens the SREBP pathway, which inhibits the synthesis of fatty acids and curbs tumor growth [110]. Some activating mutations result in abnormal amplification of mTOR signaling, which promotes tumorigenesis and sets the stage for mTOR inhibitors to be used as chemotherapy agents. Contributing PI3K and/or KRAS mutations resulting in erroneous activation of the mTOR pathway have also been reported in other cancers and are associated with brain metastasis in particular. Activating mutations or duplications in one of the PI3K genes, PI3KCA, have been sighted in 30% of the metastatic BC tissues [111]. PI3KCA encodes for p110, the catalytic subunit of the PI3K protein. The association between PI3KCA mutations and drug resistance, as well as decreased overall survival in patients with metastatic BC, lends credence to the findings [112]. A substantial number of patients bear concomitant PI3K and MAP3K1 mutations. MAP3K1 is a driver gene in the early stages of BC and sets the ERK/MEK pathway in motion [113]. The MEK inhibitor selumetinib has already been shown to curtail the population of CD44-positive cells and their mammosphere-forming efficiency in TNBC cell lines. Furthermore, treating the TNBC xenograft mouse model with a MEK inhibitor resulted in fewer cases of lung metastasis [114]. Dual targeting of BC using PI3K and MEK inhibitors might act as a novel treatment strategy in PI3K-mutated metastatic cancers. This appears to be consistent with the fact that the PI3K

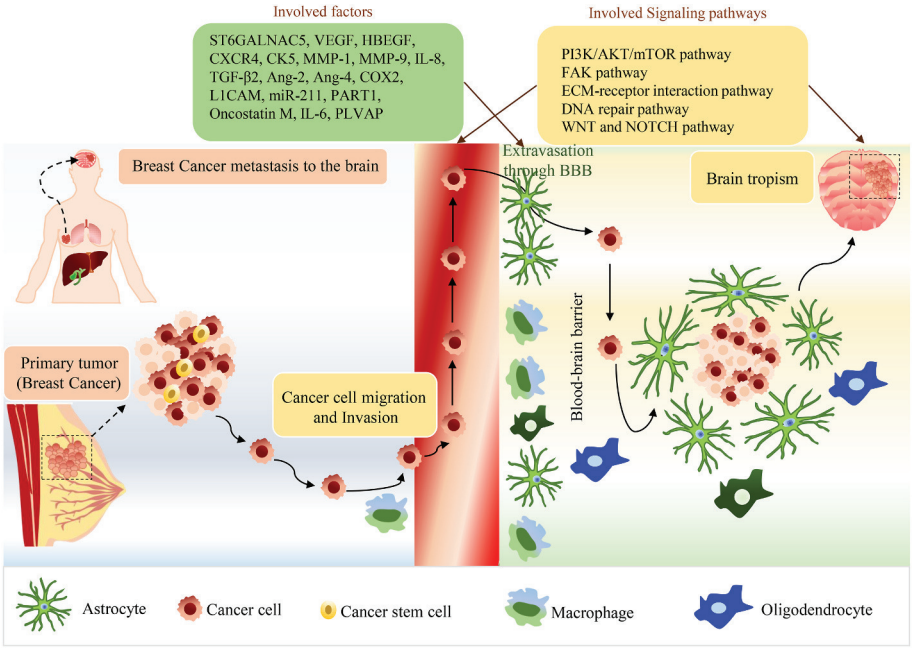

Fig. 2. Involvement of various factors and signaling pathways in the metastasis of breast cancer to the brain: During metastasis the primary tumor (breast cancer) detaches from the original site and enters blood circulation through migration and invasion process, then extravasate through BBB and establishes tumors in the brain (brain tropism). Various factors and signaling pathways are involved in this process as indicated.

signalling pathway is deregulated in cases of BCBM [115].

3.5. Focal adhesion kinase (FAK) pathway

FAK is a non-receptor tyrosine kinase that participates in cell signaling by a wide range of extracellular stimuli, including growth factors, G-protein coupled receptor agonists, cytokines, and other inflammatory mediators, in addition to its role as a primary mediator of integrin signaling. Accumulating evidence revealed that the FAK gene is amplified and overexpressed in a large fraction of BC specimens, indicating the association between FAK and breast malignancies. FAK promotes tumor development and metastasis through its actions on cancer cells and stromal cells in the tumor microenvironment. Interestingly, FAK also promotes angiogenesis, cell migration, and invasion in the BBB upon interacting with PI3K by forming the FAK-β4 integrin complex, leading to an increase in the number of FAK and β4 integrin-positive cells. In BCBM, differential gene expression analysis revealed genes enriched in PI3K/AKT and the focal adhesion pathway [115]. It was recently discovered that the FAK–NF–κβ pathway is activated by connexin 31, which serves to strengthen metastatic cancer cells' interaction with the astrocytes and increases tumor cell survival in the brain tissue. The FAK pathway also involves connexin 43 and LAMA4 in BCBM. On the contrary, inhibiting the FAK pathway resulted in decreased brain metastasis in vivo [116]. A FAK–NF–κβ signaling pathway is an attractive chemotherapeutic target in BCBM. To unravel novel potential molecular targets in metastatic cancer, researchers recently explored the chronological events in mouse models of BC with brain metastasis facilitated by precise immunofluorescence microscopy. Epithelial cell markers and focal adhesion kinase signaling were traced in the BC cells as the metastasis progressed. The development of brain metastasis constitutes altered cytoskeletal-associated signaling pathways of cell invasion, adhesion, and migration [104]. Rac1 protein plays a significant role in mesenchymal-like migration in extravasating BC cells. MLCK has been discovered to be expressed in metastatic cancer cells, where it aids in cytoskeletal remodeling and establishing brain metastasis. FAK signaling is an attractive target in the chemotherapy of solid tumors. Clinical and pre-clinical trials for FAK/PTK2 inhibitors for cancer therapy are currently under investigation [117]. TAE226 is a small molecule tyrosine kinase inhibitor that has been tested in two metastatic BC cell lines, MDA-MB 231 and MCF-7 [118]. FAK signaling is also upregulated in colon cancer stem cells and is associated with chemoresistance [119]. In non-small cell lung carcinoma (NSCLC), FAK is recruited by TAGLN2 via HIF-1α and subsequently activates the PI3K/AKT pathway [120]. In Ewing sarcoma cell lines and animal models, a dual inhibitor of FAK and IGF1R induces apoptotic cell death and inhibits metastatic cell invasion [121].

3.6. ECM–receptor interaction pathway

Signaling pathways involving ECM-receptor interactions are often upregulated and gene-enriched. It is well known that ECM has a function in various malignancies and involves tumor shedding, adhesion, degradation, motility, and hyperplasia. During BCBM, BC cells lose epithelial cell markers and gain markers of mesenchymal origin during invasion into the brain parenchyma [104]. BC cells invading brain parenchyma cells bear vimentin, and the number of vimentin-positive cells increases in number as more and more cancer invasion occurs. However, once they colonize the brain, the mesenchymal cells disappear, and epithelial markers take their place. A recent study identified ZNF827 as a master regulator linking these processes during EMT in brain development and BC metastasis and indicates an unprecedented complexity of interaction between the epigenetic landscape and splicing program in controlling EMT [122].

Recently, molecular mechanisms implicated in brain vascular changes, molecules involved in crosstalk, and colonization by BC cells in the hippocampus of BC mouse models have been identified as the brain metastasis progresses with time. The number of PGDF-β and Ki-67-positive cells in the vicinity of blood vessels in the hippocampus of the brain increased over time [104]. Ki-67 is also a prognostic marker impacting the survival times of HER2+ve and TNBC BC patients. PDGF-β is a growth factor frequently overexpressed in gliomas and an inducer of cell proliferation and dedifferentiation [123]. PDGF- β might be of prognostic value in cases of brain metastasis [124]. PDGF- β is instrumental in the colonization of brain cells in the case of TNBC. When bound to its receptor PDGF-R, PDGF instigates pro-metastatic signals in brain pericytes, secreting proteins of the ECM and insulin-like growth

factor 2. IGF-1R augments cell proliferation of epicardial cells during embryogenesis, moderated by FAK [125].

Wyss et al. identified that PDGFR is a novel attractive chemotherapeutic target amenable to tyrosine kinase targeting in BC brain metastasis. Activation of hypoxia-inducible factor-1 (HIF1), along with the loss of miRNA let-7d, boosts the expression of PDGF platelet-derived growth factor and promotes brain metastasis in mice and human BC cell lines. miRNA let-7d increases the activity of HIF-1α, while silencing of HIF-1α or overexpressing let-7d leads to arrested brain metastasis in BC cell lines [126].

3.7. DNA repair pathway

BC cells commonly metastasize to the brain, creating a neuroinflammatory microenvironment. The molecular processes that facilitate colonization are still unknown. One study identified RAD51, a key protein in single-strand and double-strand DNA repair by way of homologous recombination, as the early molecular predictor of brain metastasis in advanced HER2+ve BC patients [127]. Aberrant RAD51 expression is also associated with chemoresistance [128]. Researchers investigated the homologous recombination deficiency scores in BC primary tumors and compared them with their paired brain metastatic counterparts. They found out that those patients with HRD (homology directed repair) mutations showed increased brain metastasis, and BC brain metastasis patients might benefit from PARP-1 inhibitory chemotherapy [129]. The PARP-1 enzyme senses DNA damage in cells and is crucial for DNA repair. PARP-1 inhibitors are lethal to tumor cells and have therapeutic applications [130]. Using a genome-wide CRISPR screen, a study identified a critical inhibitor of DNA double-strand break repair, LRRC31, acting as a tumor suppressor gene in the MDA-MB-231 BCE cell line. Also, they showed that LRR31 disrupts the ATR-MSH2-PKC signaling axis and can be administered as nanoparticles to sensitize breast-to-brain metastatic mice models to radiation [131]. According to the publicly available TGCA dataset of BC brain metastasis, H2AX, a DNA repair marker, has higher mRNA expression in brain metastatic lesions than in primary breast tumors and is associated with poor patient survival [132].

3.8. Wnt and notch pathways

The Wnt and Notch signaling pathways are evolutionarily conserved cell signaling pathways that are important in cancer development, including CSC maintenance, angiogenesis, and tumor immunity, and aberrant activation can lead to tumor formation [133,134]. Members of the Wnt signaling pathway are overrepresented in the basal-like subtype of BC as well as primary breast tumors that metastasize to the brain. WNT signaling that is not catenin-dependent, most likely via ROR1-2, is important in the brain metastases of basal-like and other BC subtypes. Also, high ROR2 expression is a prognostic indicator of early metastasis [135]. Expression of Notch ligands on the cell surface, including delta-like ligands and jagged ligands (DLL1, DLL3, DLL4, JAG1, and JAG2), can also activate Notch signaling in neighboring cells, resulting in NICD (notch intracellular domain) release. NICD can interact with transcriptional regulators to produce a Notch gene expression profile, which then controls key cell fate decisions such as differentiation, cell cycle progression, and survival [136]. Notch1 inhibition could reduce the CD44+/CD24-population and brain metastases in BC [137]. Furthermore, Compound E (a blood-brain barrier-permeable Notch inhibitor) can significantly inhibit brain metastasis. Accumulating evidence suggests that Notch signaling is important in the regulation of BCBM, but clinical experience with Notch pathway inhibitors is still limited. Intriguingly, in vivo Notch1 gene knockout results in increased BC cell proliferation via the AKT signaling pathway, higher serum levels of Il-3 and IL-4, and the conversion of TAMs to M2-type macrophages [138].

3.9. Involvement of other various factors in BCBM

Extensive remodeling of the extracellular matrix is essential for cancer cells to successfully cross the blood-brain barrier. Many proteases have been reported in various malignancies and help achieve this feat by actuating the tumor-promoting microenvironment [139]. Cathepsin S (CTSS) is an evolutionary conserved lysosomal cysteine protease that degrades ECM proteins like laminin 5 and collagen to produce pro-angiogenic peptides [140]. Stromal expression of CTSS is associated with worse outcomes in TNBC patients using immunohistochemistry [141]. Moreover, CTSS induces pro-inflammation attributes within the tumor microenvironment by upregulating CCL2 expression [142]. BC methylation data from TCGA (the cancer genome atlas) is utilized to identify recurrently methylated key candidate genes, GALNT9 and BNC1, that may be involved in BC brain metastasis. Knockdown of these genes by RNAi resulted in a significant increase in the migratory and invasive potential of BC cell lines [143]. GALNT9 (an initiator of O-glycosylation), CCDC8 (a regulator of microtubule dynamics), and BNC1 (a transcription factor with a broad range of targets) may play a role in the progression of primary breast tumors to brain metastases. The products of these genes may generate novel therapeutic targets. The epigenetic landscape in the case of brain metastasis is a great resource for predicting patient outcomes [144]. Researchers a brain metastasis-specific epigenetic signature using supervised machine learning and DNA methylomes and fabricated a DNA methylation-based classifier [145].

As a form of vitamin B3, nicotinamide riboside (NR) has shown clinical potential as a treatment for a number of metabolic disorders and age-related diseases. Bioluminescent NR uptake probes (BiNR) have been developed and validated for non-invasive imaging of NR uptake in vitro and in vivo. NR supplementation increases cancer prevalence and brain metastases of TNBC by a significant amount in TNBC animal models. Nutraceuticals like NR have important roles to play in cancer metabolism, and their use should be customized to the needs of different groups of patients [146].

Higher levels of miR-211, a circulating non-coding micro-RNA, are a potential predictive biomarker for metastatic brain cancer, and knocking it down prevents brain metastasis. It is also linked to poor survival in TNBC mice with metastatic tumors. The micro-RNA miR-211 enhances cancer cell colonization via BBB *trans*-migration by boosting cell attachment capacity to BBB endothelial cells and thereby penetration into the BBB via the SOX/NGN2 axis [147]. Moreover, elevated levels of PART1, a long non-coding RNA (lncRNA), are observed in BC tissues and cell lines, and its silencing inhibits BC cell invasion and migration in vitro. Silencing PART1 also decreased chemoresistance and improved the efficacy of the apoptosis-inducing medication cisplatin in cancer treatment [148]. LncRNAs are part of a complex network that includes chromatin-remodeling proteins, various transcription factors, and other non-coding RNAs, and they exert dominance over a variety of downstream molecules important for cancer cell survival [38]. RNA-Seq was utilized in a study to develop a leading and fundamental BCBM circRNA (circular RNA) profile, which can be used to search for new biomarkers and therapeutic targets of clinical interest. It was revealed that circBCBM1/circ_0001944 is a novel circRNA that is significantly upregulated in BC brain metastases and enhances the cancer cell migration and proliferation of the MDA-MB 231 cell line. CircBCBM1 acts like a sponge for miR-125a, upregulating the sonic hedgehog signaling pathway [149]. Another endothelial protein, plasmalemma vesicle-associated protein (PLVAP), was found in many tumors during angiogenesis. PLVAP is highly expressed in cholangiocarcinomas. PLVAP is a druggable target in HCC and pancreatic cancer [150]. For the first time, PLVAP is identified in BC cells and well-rooted brain metastatic niches near blood vessels [104]. However, these findings need to be validated in cell lines and in vivo experimental models of BC that acquire some of the endothelial properties to colonize brain tissue.

The resting membrane potential of cancer cells is depolarized (−50

to −10 mV) as compared to that of normal cells (−70 mV). Membrane potential in MDA-MB-231 cells shows massive fluctuations (named "blinks" and "waves"). The membrane potential of MCF-10A cells, on the other hand, remains normal but begins to fluctuate after TGFβ1 treatment. Also, MCF-10A cells begin to show hybrid-EMT-like properties like cancer cells and high Vm fluctuations. It is known that cell adhesion molecules are downregulated when cells are in a hybrid-EMT state. The of the membrane potential of BC cells can be the next breakthrough in treating metastatic BC and should be investigated in depth [151].

4. Therapeutic targeting of BC brain metastasis

Multiple modes of local and systemic therapies have been proven effective in ameliorating the lives of brain metastasis patients worldwide. Risk stratification and the choice of chemotherapeutic agents for treating BC patients solely depend on the subtype heterogeneity (hormone receptor status) at initial diagnosis. A summary of various ongoing and completed clinical trials in BCBM is given in Table 1.

4.1. Drugs targeting BC subtypes

Pertuzumab and trastuzumab are used as the first line of therapy to treat HER2+ve brain metastatic BC. In a limited cohort of patients, a single-institution study used fractionated stereotactic brain radiation (fSRT) in addition to pertuzumab and trastuzumab and observed a 68.7% overall response rate. Another study used human neural stem cells to secrete anti-HER2 antibodies in combination with tucatinib and found that mice had a longer median survival time. Whole exome sequencing in PDX (patient derived xenografts) models showed that copy number alterations in CDKN2A and CDKN2B genes exist in a major percentage of HER2+ve and ER + ve patients. The mRNA levels of the CDKN2A gene are also significantly lower in these subtypes. Tucatinib, when combined with abemaciclib, a CDK4/CDK6 inhibitor, increased overall survival in the CDKN2A-deficient PDX mice model of BCBM [152].

It is already known that HER2+ve BC frequently metastasizes to the brain. Trastuzumab and TKIs (tyrosine kinase inhibitors) are the first lines of drugs used in treating HER2+ve BC. TKIs often have off-target adverse effects and are not well tolerated by patients. Tucatinib was given in addition to trastuzumab and capecitabine in a randomized double-blind trial with HER2+ve metastatic BC to the brain, and it improved overall survival [153]. Trastuzumab deruxtecan, an antibody-drug conjugate, demonstrated a high intracranial response rate of 73.3% in HER2+ve BC patients with brain metastases in a prospective trial [154]. ABL kinases serve as a therapeutic target for people with HER2+ brain metastasis by regulating the translation of targets for brain metastases through Y-box-binding protein 1 (YB-1) [155].

Pyrotinib administered orally to HER2+ve BC patients with brain metastases shows an ORR (overall response rate) of 47.6% [156]. The intracranial response rate in HER2+ve BC with brain metastasis was measured in a multicentric clinical trial. Pyrotinib, along with capecitabine, was given as part of the treatment regime in patients older than 18 years of age. Capecitabine is an oral antineoplastic drug that has already been proven effective and has increased disease-free survival (DFS) and overall survival (OS) in HER2-ve BC [157]. The 15-year survival rate was also longer (77.6%) vs 73.3% in early-stage ER-ve and triple-negative BC patients belonging to the capecitabine group [158,159]. Pyrotinib also renders HER2+ve cells sensitive to whole-brain radiotherapy [160].

Fam-trastuzumab deruxtecan-nxki (T-DXd) has already been approved for the treatment of metastatic HER2+ve BC patients. The phase III DESTINY-Breast03 trial compared T-DXd with trastuzumab emtansine (T-DM1) in these patients with brain metastatic disease. Progression-free survival (PFS) and overall response rate (ORR) in patients with metastasis to the brain were 72% and 67.4%, respectively, when treated with T-DXd, which is a substantial improvement over treatment with T-DM1 [161].

Angiogenesis-promoting transcription factor truncated glioma-associated oncogene homolog 1 (tGLI1), activates astrocytes and promotes metastasis to the brain [162]. Extracellular vesicles of breast carcinoma cells have high levels of tGLI1 and miR-1290. These extracellular vesicles are enriched in miR-1290 and trigger the FOXA2-CNTF pathway [163]. An antifungal drug called ketoconazole (KCZ) prevents the selective homing of cancer cells to the brain in the tGLI1 overexpressing mouse model of BC by inhibiting tGLI1. This study emphasizes the fact that there is a need to repurpose drugs for treating BCBM [164].

Tucatinib is a kinase inhibitor that blocks PI3K/AKT/mTOR signaling and tumor growth by reducing the phosphorylation of the HER2 receptor. However, Gedatolisib, a PI3K/mTOR inhibitor, eliminates dormant BC cells in organotypic culture but fails to prevent metastasis in mice models of HER2+ and TNBC. These findings raise concerns about using PI3K inhibitors as a second-line treatment for BC metastases. Although small-molecule tyrosine kinase inhibitors such as lapatinib, gefitinib, erlotinib, and neratinib are clinically approved for cancers like NSCLC, pancreatic cancer, and BC. Neratinib binds to the ATP pocket of the EGFR receptor family, impeding cell proliferation and consequently blocking AKT and MEK pathways. In the case of ER + BC, the HER2 receptor is heavily mutated, which promotes drug resistance. In phase II clinical trial, researchers performed combinatorial therapy using neratinib (inhibitor of EGFR and HER2, HER4) and fulvestrant (inhibitor of ER) in patients manifesting metastasis. Primary or local breast tumors undergo molecular subtype switching while transitioning to metastatic tumors in a significant number of patients with brain metastasis. Whether these subtype switches alter a patient's survival, remains controversial in many studies [165]. A recent meta-analysis of 4 previous randomized trials concluded that aromatase inhibitors lower the 5-year BC recurrence rate in pre-menopausal women with ER + ve BC to a greater extent than tamoxifen [166].

TNBC is the most chemo-resistant subtype of BC due to the absence of hormone receptors. Programmed death ligand 1 (PD-L1) is overexpressed up to 20% in TNBC. Immune cells and endothelial cells both have the PD-L1 receptor, which aids in immune evasion. Atezolizumab is a monoclonal antibody against PD-L1, which stimulates T cells.

against cancer cells by inhibiting their interaction with the PD-L1 receptor. A multicentric phase II trial in China delivered pyrotinib plus capecitabine in 2 cohorts of HER2+ve patients with brain metastases and demonstrated an excellent intracranial objective response rate. Pyrotinib arrests autophosphorylation activity in the HER family of receptors, consequently blocking MAPK and PI3K/AKT signaling pathways. Capecitabine is a nucleoside inhibitor that halts DNA synthesis in tumor cells after being converted to fluorouracil. It has been widely used in TNBC and colorectal cancer as adjuvant chemotherapy. Another antibody-drug conjugate, sacituzumabgovitecan, improved the PFS and OS rates in patients who developed TNBC, regardless of their original BC subtype [167].

4.2. Drugs targeting extracellular vesicles

Exosomes are therapeutically potent extracellular vesicles with a diameter ranging from 30 to 100 nm. Exosomes are excellent vehicles that carry drug molecules inside the cancer cell, posing as novel vaccine delivery agents. In addition to this, exosomes also interact with the tumor microenvironment via many stromal cells and are also associated with the degradation of ECM by secreting matrix metalloproteases, angiogenesis, and cancer progression. The tumor microenvironment is often enriched in hypoxic conditions. In order to adapt to the new, cancer cells secrete exosomes enriched in metastatic potential. These secreted exosomes communicate with the target cells or tumor microenvironment, boosting the metastatic potential of cancer. A study in 2020 pointed out that ITGB3, an integrin receptor, triggers the focal

breast cancer with brain metastasis.

erimental Agent	Class of Agent	Study/Phase	Active comparator	Enrolled Population	Median PFS (in months)	Median OS (in months)	ClinicalTrials.gov identifier (NCT number)	Recruitment status	Reference
ituzumab govitecan	Antibody-drug conjugate	Phase III	Eribulin/Vinorelbine/ Capecitabine/ Gemcitabine	468	5.6	12.1	NCT02574455	Completed	[167]
otinib + Capecitabine	Tyrosine kinase inhibitor	Phase II	None, Single arm	78	[a]	[a]	NCT03691051	Active, not recruiting	[170]
stuzumab deruxtecan	Antibody-drug conjugate	Phase II	None, Single arm	15	14	not reached[b]	NCT04752059	Active, not recruiting	[154]
tuzumab + stuzumab + a Taxane	MoAb and taxane	Phase III	Docetaxel/Nab-paclitaxel/Paclitaxel/ Pertuzumab/ Trastuzumab	1436	20.67	65.3	NCT01572038	Completed	[171]
atinib + stuzumab + ecitabine	PI3K inhibitor	Phase II	Placebo + Capecitabine + Trastuzumab	612	7.6	24.7	NCT02614794	Completed	[153]
stuzumab deruxtecan	Antibody-drug conjugate	Phase III	Trastuzumab emtansine	524	25.1	–	NCT03529110	Active, not recruiting	[161]
etaxel + ecitabine + lophosphamide + rubicin + ecitabine	A fluoropyrimidine carbamate	Phase III	Docetaxel, cyclophosphamide and epirubicin + fluorouracil	1500	%[c]	77.6	NCT00114816	Completed	[158]
atinib plus ecitabine	Tyrosine kinase inhibitor	Phase III	lapatinib + capecitabine	101	7.8	16.4	NCT01808573	Completed	[172]
ulin	Microtubule dynamics inhibitor	Prospective observational study	None, observational study	34	10	–	Observational study	Completed	[173]
TR-102 (Etirinotecan ol)	Topoisomerase I inhibitor	Phase III	Eribulin/Ixabepilone/ Vinorelbine/ Gemcitabine/Paclitaxel/ Docetaxel/Nab-paclitaxel	178	3.9	–	NCT02915744	Completed	[174]
umoxytol followed by I-398	Nanoliposomal Topoisomerase I inhibitor	Phase I	None, Single arm	45	3.6	–	NCT01770353	Completed	[175]

rm study is intracranial objective response rate, and not PFS and OS. The intracranial objective response rate was 74·6% in patients with radiotherapy-naive HER2-positive brain

ched, 3 out of 14 patients died during follow-up.

y after a median follow-up of 4.9 years, recurrence-free survival (RFS) was taken into account which did not differ significantly between the groups.

adhesion kinase (FAK) pathway, which is essential for the endocytosis of released vesicles by engaging in intracellular communication with the target cells in TNBC cell lines. Overexpression of FAK is associated with poor prognosis in BC patients. Dynamin and FAK are chief players in the endocytosis of extracellular vesicles. FAK functions as an adapter between Src and Dynamin-2 and phosphorylates Dynamin-2 which is downstream to FAK signaling. Dynamin-2 once phosphorylated promotes endocytosis of exosomes in a clathrin-dependent manner.

4.3. Drug targeting BBB/BTB

The BBB provides a suitable environment for cancer cells with the potential to spread. After invading the brain tissue, metastatic BC cells reach a macroscopic size, and the existing BBB gives rise to the BTB via new blood vessel formation. BTB is more permeable to chemotherapeutic agents as compared to BBB. According to a phase II trial, ANG1005 (a conjugate of angiopep-2 and paclitaxel) is shown to effectively cross the BBB via lipoprotein receptor-related protein (LRP)-1 expressed on the surface of BBB endothelial cells at an overall patient benefit rate of 71%. In addition, ANG1005 is less neurotoxic than paclitaxel, a conventional drug very frequently given to BC patients with metastases. In vivo survival rates are increased by an angiopepsin-2 micellar duo containing paclitaxel and lapatinib [168].In vivo mouse model and intravenous injection of a TNFR1-selective agonist that increases BBB permeability with minimal toxic effects are used in a new study to selectively unfasten the BBB at micrometastatic sites. A recent study utilized microbubble-assisted focused ultrasound and co-delivered taxane and a previously described siRNA against HER in a novel silica nanoconstruct generated from a trastuzumab-conjugated nanoparticle to disrupt the blood-brain barrier, arresting HER2-resistant metastatic tumors in the brain tissue of mice [169]. HER3 is a promising therapeutic target in metastatic TNBC, displaying high receptor expression density on the cell surface of metastatic tumor cells. Furthermore, HER3 overexpression in brain endothelial cells promotes migration across the BBB. HER3-targeted bioparticles within nucleocapsids are fabricated, which are home to metastatic sites in a human BBB chip and mice. For instance, tight junction proteins and astrocyte foot processes enhance capillary endothelia in the BBB in the brain. Chemotherapy agents, mainly trastuzumab and pertuzumab, have significantly improved the survival of BC patients; however, anti-HER2 therapies are ineffective in treating deep-rooted brain metastasis due to their inability to penetrate the central nervous system. Brain metastases in HER2+ve BC did not breach the BBB, which is common in brain metastases from triple-negative and basal-type BC, according to an analysis of resected brain metastases. The pervasion of the BBB has immense clinical significance given that increased permeability of therapeutic medications is necessary to efficiently traverse the intact BBB.

5. Conclusion and future directions

The heterogeneity of breast tumors in primary and metastatic cancers represents challenges in developing effective cancer therapy. The transcriptional landscape of metastatic brain tumors differs from their primary breast tumor due to subtype-switching. This difference is attributed to the varied properties of BC cells according to their parent molecular subtype and site of metastasis. Also, mutations are acquired by the primary tumor as it transits to the brain. These mutations and gene expressions must be targeted by new and re-purposed chemotherapeutic agents that are different from their primary tumor counterparts. Despite substantial studies into how BC evades the anti-tumor response mediated by immune cells, finding potent drugs to treat BCBM remains a major problem. Understanding the molecular mechanisms of BCBM will contribute to the development of novel strategies against metastasis. Machine learning and single-cell RNA sequencing are currently being carried out to better understand this disease. Still, more research is required to validate the candidate genes and molecular pathways for their use in therapeutic settings in the future. Multi-modal drug treatments and personalized genomic medicine are urgently needed to improve the survival rates in BC patients with brain metastasis.

Author contributions

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Declaration of competing interest

SKB is a co-founder of Sanguine Diagnostics and Therapeutics, Inc. Other authors declare no competing interests.

Data availability

Data will be made available on request.

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