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REVIEW

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Biological profile of breast cancer brain metastasis

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Abstract

Breast cancer is one of the leading causes of death worldwide. The aggressive behaviour of breast tumor results from their metastasis. Notably, the brain tissue is one of the common regions of metastasis, thereby reducing the overall survival of patients. Moreover, the metastatic tumors demonstrate poor response or resistance to therapies. In addition, breast cancer brain metastasis provides the poor prognosis of patients. Therefore, it is of importance to understand the mechanisms in breast cancer brain metastasis. Both cell lines and animal models have been developed for the evaluation of breast cancer brain metastasis. Moreover, different tumor microenvironment components and other factors such as lymphocytes and astrocytes can affect brain metastasis. The breast cancer cells can disrupt the blood-brain barrier (BBB) during their metastasis into brain, developing blood-tumor barrier to enhance carcinogenesis. The breast cancer brain metastasis can be increased by the dysregulation of chemokines, STAT3, Wnt, Notch and PI3K/Akt. On the other hand, the effective therapeutics have been developed for the brain metastasis such as introduction of nanoparticles. Moreover, the disruption of BBB by ultrasound can increase the entrance of bioactive compounds to the brain tissue. In order to improve specificity and selectivity, the nanoparticles for the delivery of therapeutics and crossing over BBB have been developed to suppress breast cancer brain metastasis.

Keywords Breast cancer, Brain metastasis, Immune checkpoint inhibitors, Drug delivery, Blood-brain barrier

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Introduction

Breast cancer is one of the most lethal tumors and it is the second leading cause of death after lung tumor [1]. In spite of the introduction of chemotherapy, radiotherapy and immunotherapy for breast cancer, the survival is still poor and unfavorable. Although several factors are responsible for the poor prognosis, metastasis has been considered as the main factor in this case. The metastasis in breast cancer is one of the main reasons of death in patients. Up to 6% of patients demonstrate metastasis upon the diagnosis and 20-50% of patients will demonstrate metastasis at the next steps. Although basic and clinical experiments have emphasized on highlighting the mechanisms involved in the brain metastasis of breast tumor, the therapy relies on the application of cytotoxic compounds reducing the life quality of patients because of the adverse impacts and in some cases, they demonstrate poor long-term success. In case of metastasis, the 5-year survival rate of patients decreases to 26%. As a result, the breast cancer brain metastasis is pointed as a main challenge for the physicians [2]. Hence, addressing mechanisms of breast cancer brain metastasis, highlighting the therapies and directions can provide new insights in the treatment of patients.

In respect to the importance of the metastasis in the prognosis of patients, the present review would focus on the metastasis of breast tumor cells to the brain tissue. This review will cover the various aspects of brain metastasis from the different models for the investigation of metastasis to the therapeutic application. In addition, the biological profile is evaluated to understand the mechanisms involved in this procedure. The two important pathways are followed by the breast cancer cells for reaching to the brain tissue including crossing over blood-brain barrier (BBB) and intracerebral progression. In order to better understand the underlying mechanisms and direct the novel therapeutics, the genetic mutations in the process of brain metastasis are discussed, also summarized in tables. Understanding the breast cancer brain metastasis has valuable clinical importance, since it can worsen prognosis and overall survival of patients. Moreover, the development of therapeutics based on targeting brain metastasis can improve 5-year survival of patients. Advances in the field of liquid biopsy have improved the diagnosis of breast cancer metastasis that these subjects have been covered in the current paper.

Developed models of breast cancer brain metastasis

Cell line

Notably, it is essential to develop the suitable models to accurately mimic the patient condition and improve the knowledge towards the genetic feature of CNS metastasis [3]. Moreover, such insights are beneficial in the development of therapeutics. The complicated nature of brain metastasis requires the various kinds of models and cell lines have been designed for the pre-clinical approach [4]. The human or mouse parental primary tumors have been utilized for the brain metastatic cell line. Moreover, another source for the brain metastatic cells are spontaneous metastatic cells [5]. The brain metastatic cell lines from breast tumor demonstrate three molecular subtypes. Such cells contain major somatic mutations mentioned in humans such as BRCA1, phosphatase and PTEN, HER2, CDK, EGFR and p53. Moreover, there are other factors with less mutations and frequency including BRAF, MYC, KRAS, RB1 and SMAD4. The brain metastasis in breast tumor can result from HER2 upregulation and ER downregulation. Most of cell lines are from HER2 + and TNBC [6–9]. Furthermore, cell line comprised of MDA-MB-231BR-HER2+ (231BR-HER2+) has the efficacy of brain metastasis rapidly and generating large metastatic tumors in BALB/c nude mice [10].

Animal models

The pre-clinical studies can be misled by the animal models lacking the ability to reproduce the patient conditions, resulting in the failure of clinical trials [11]. Designing engineered mouse models of breast tumor has improved the recognition of certain genes participating in the tumorigenesis. However, such animal models demonstrate poor occurrence of brain metastasis and do not reflect the disease in human [11, 12]. The brain metastasis can be developed via thedirect injection of cells into the blood circulation by the tail vein or into the heart. The injection of cells in this wat results in their spread into the body and reaching to the organs such as brain [13]. The ENU1564 (rat model) and MDA-321br (human model) are used for the brain metastasis. Since the size of rat brain is larger than mouse brain, these models are also beneficial for the imaging approaches. For the field of cancer neuroscience, rat brain metastasis is also advantageous [14, 15]. Another species used for the study of brain metastasis is *Drosophila melanogaster*. The RasV12 upregulation and downregulation of Dlg and GFP in Drosophila can result in the tumorigenesis and increased metastasis of surrounding brain tissue. This species is also beneficial for genetic screening through crossing any RNAi fly line [16, 17]. Table 1 demonstrates the different kinds of cell lines and animal models deployed for the evaluation of breast cancer brain metastasis.

Blood-brain barrier and intracerebral progression during breast cancer brain metastasis

The brainmetastasis has been shown in the various tumorssuch as lung tumor, breast cancer and melanoma, significantly decreasing the survival of patients [22, 23]. However, brain tissue is not the only target of breast

Table 1 The different models for the evaluation of breast cancer brain metastasis

Model	Example	Advantageous	Disadvantageous	Remarks
MDA-MB-231	Subline of MDA-MB-231	Highly metastatic Capacity of being used in the in vivo studies for understanding brain tropism	Poor representation of tumor heterogeneity Genetic engineering and does not reflect the primary tumors	Understanding the genes involved in the process of metastasis including COX2 and HBEGF, among others
BT474, SKBR3	HER2-positive	Improving the knowledge towards the breast cancer subtypes participating in brain metastasis	Lack of representation of TNBC brain metastasis	Improving the knowledge towards the HER2-targeted therapy resistance in the brain
4T1-BR5	Murine brain metastasis model	The major advantageous of this model is being immunocompetent providing the insight about immune-tumor interaction	Its murine origin decreases the relevance to human breast cancer Poor translational application	Understanding the immune-related mechanisms in brain metastasis
SUM149, SUM190	Inflammatory breast cancer-derived lines	Unique properties of inflammatory breast cancer	This is related to the rare subtypes Poor brain metastasis	Highlighting the mechanisms involved in the aggressiveness and metastasis of TNBC
Xenograft Models	Injection of MDA-MB-231 or BT474 cells	High carcinogenesis The direct injection to the brain mimics metastasis	Because of immunocompromised hosts, it has poor value in immune system interaction studies	Allowing to understand the BBB-penetrating drugs and certain mechanisms in the metastasis
Patient-Derived Xenografts (PDX)	From human brain metastases	Mimicking the patient tumor and providing the heterogeneity	Low engraftment rates Poor affordability Time-consuming	Valuable for the personalized medicine and understanding the patient response to therapeutics
Genetically Engineered Mouse Models	HER2/Neu transgenic models	Reflecting the HER2-driven breast cancer malignancy	Slow progression It is not suitable for the high-throughput studies	Highlighting the mechanisms of HER2-positrive breast cancer metastasis to brain tissue
Orthotopic Models	Direct injection into mammary fat pad	Mimicking the primary tumor growth and metastasis	It rarely leads to the brain metastasis without brain-tropic cell lines	Understanding the metastatic cascades and providing tumor microenvironment interactions

Further notes: This table highlights a number of cell lines and animal models for understanding the brain metastasis of breast cancer. The advantageous and disadvantageous of each model have bene provided to further improve the knowledge for the selection of suitable model. Each of these models offers significant benefits for understanding the molecular and cellular mechanisms in the brain metastasis. These models have been advantageous in highlighting the tumor biology, therapy resistance, mechanisms of brain metastasis and providing a platform for testing the novel therapeutic approaches. However, there are still a number of limitations including lack of immune system representation in xenograft models and the complexity of accurately mimicking metastatic processes in vivo. Further information about breast cancer brain metastasis can be found in these references [18–21]

cancer cells and they have demonstrated metastasis into other parts of body including bone, liver, lung and distant lymph nodes [8]. The breast cancer has been categorized into various subtypes based on HER2 and ER and PR receptors [24, 25]. Noteworthy, the genetic profile of these breast cancer subtypes is different and according to this, they utilize various pathways in metastasis and therefore, their metastatic preferences are different. The rate of metastasis has been shown to be higher in HER2 + breast cancer and TNBC compared to luminal subtypes (ER + and PR+) of breast tumpr. Notably, the incidence rate of brain metastasis has been demonstrated to be 20% in TNBC and HER2 + breast tumor, while it is less than 10% in luminal breast tumor [8]. In spite of the introduction of various therapeutics for breast tumor, the prognosis is undesirable, but this is heterogeneous and some of the patients obtain better response to therapy [26]. In order to evaluate the survival of patients with brain metastasis, a number of genetic signatures have been developed. In this regard, the graded prognostic assessment (GPA) is suggested to be a new grading score for the patients [26, 27]. The cancer metastasis is according to the local invasion from the primary site, affecting surrounding tissue followed by intravasation of cancer cells and their spread to blood. Then, the extravasation of tumor cells from bloodstream is observed to the distant sites and increase in the colonization to generate micro- and macroscopic metastases [28, 29]. The TME components exert a significant function in the regulation of brain metastasis of breast tumor cells. In this case, tumor-infiltrating lymphocytes (TILs) have been demonstrated to associate with survival in brain metastasis [30], in spite of the low abundancy of lymphocytes in the brain parenchyma. TILs have been displayed to be present in brain metastases [31]. As a result, the primary brain tumors and brain metastases can be suppressed by the application of immunotherapy [32]. Astrocytes are also the stromal component in the brain tumors and brain metastases, but they exert both tumor-suppressor and tumor-promoting function, highlighting their critical and unique functions in the various subtypes [33]. The generation of plasminogen by the astrocytes can increase apoptosis in tumors, while the astrocyte-derived cGAS and miRNAs transported by gap junctions or exosomes to the cancer cells can promote the generation of brain metastases. Neuroinflammation and ischemia can also change the phenotype of astrocytes into A1 and A2 exerting pro-inflammatory and tissue repair functions, respectively [34]. The tumor-associated astrocytes demonstrate A2 phenotype and therefore, their potential in the regulation of brain metastasis requires more investigation.

Upon the entrance of breast tumor cells into the brain tissue, a number of changes occur interacting with the CNS microenvironment [35]. Such interaction improved cell growth and astrocyte survival was encouraged to enhance brain metastasis of prostate cancer [36]. The astrocytes enhance the levels of IL-23 and overexpress MMP2 in promoting brain metastasis of melanoma [37].

Metastasis pathway to the brain

Before the cancer cells reach to the brain tissue, they should be able to separate from the other regions [38]. A number of factors participate in the process of metastasis including alterations in genomic and epigenetic profile, angiogenesis, interactions occurring between tumor and stroma, intravasation, bloodstream circulation and extravsation. The starting point in this stage is the separation of the breast tumor cells from primary site and it has been comprised of sequencial and orchestrated stages. Notably, the tumor cells should invade the basement membrane into adjacent tissues and then, undergo intravasation for the entrance into blood vessels and lymphatic system. Moreover, the tumor cells should be able to survive in bloodstream and then, undergo extravasation through trans-endothelial migration into distant tissues, followed by colonization and generation of distant metastatic lesions [39–41]. The first steps for the generation of metastatic lesions are tissue invasion and intravasation, and these events include induction of epithelial-mesenchymal transition (EMT), remodelling of extracellular matrix and stimulation of angiogenesis. The degradation of endothelial junction proteins is vital for the intravasation of breast cancer cells into bloodstream. The process of intravasation can be facilitated through perivascular macrophages or interactions occurring between cancer cells and endothelial cells [42]. During extravasation, the tumor cells in the bloodstream can penetrate into cell junctions present among the distant endothelial cells and they can be in form of dormancy. Then, those metastatic tumor cells that have survived, can escape the dormant stage in the generation of micrometastatic foci along blood vessels. One of the common sites for the breast tumor cells, especially TNBC for the metastasis is brain tissues. However, these tumor cells face BBB that will be discussed in the next subsections.

Blood-brain barrier and transformation into blood-tumor barrier

The blood components are effectively separated from the brain tissue through a biological structures known as BBB that is between blood and brain parenchyma [43]. The existence of BBB was mentioned for the first time by Ehrlich and colleagues [44] and since then, further studies focused on understanding the structure and formation of BBB. Anatomically, BBB has been comprised of endothelial cells, pericytes, basement membranes, and astrocytes. The blood vessel wall is generated by the endothelial cells that have been surrounded by pericytes in the basement

membrane and then, the vessels are surrounded by the astrocytic endfeet [45]. The tight junctions are formed in the endothelial cells through junction protein complexes that reduce paracellular transport [46], preserving CNS homeostasis through permitting the passage of certain nutrients into the brain tissue, reducing the entrance of toxic xenobiotic molecules and providing the efflux of toxic substances, metabolites and waste products [47, 48]. The presence of BBB is vital for the physiological function of the brain tissue. BBB is also a major hurdle for the tumor cells in their way to the metastasis and colonizing in the brain tissue. Along with the development of primary or metastatic tumors in the brain, a number of related changes also occur. Noteworthy, tumor progression requires the development of new vessels and such aberrant vessels are responsible for the disruption of the BBB and then, changing into blood-tumor barrier (BTB). Notably, the compromised BTB permeability has been demonstrated in 89% of lesions [49]. The knowledge towards BTB is poor and major knowledge comes from the microenvironment of CNS neoplasms resulting from data of rodent models. BTB shows higher heterogeneity compared to BBB and is easier to leak. From the anatomical view, BTB has been comprised of abnormal pericyte distribution, changes in the basement membrane, loss of astrocytic endfeet and neuronal connections. BTB lacks uniform permeability and this leads to the uneven distribution of drugs in CNS metastasis [50].

Unfortunately, the certain ways followed by the cancer cells in crossing over BBB have not been understood completely [51]. It is suggested that most of the tumor cells utilize a paracellular manner similar to the way that breast cancer cells penetrate through BBB. In this way, the tumor cells squeeze through endothelial cells by impairing the intercellular junctions. The elongated metastatic cells generate cytoplasmic protrusions before and during extravasation to extend to surrounding vascular wall. Such event is observed especially in the capillaries and/or post-capillary venules lacking smooth muscle cells [52]. Noteworthy, the impairment in the endothelial cell junctions is demonstrated to be repaired upon paracellular extravasation lacking damage to BBB [53]. When brain endothelial cells are surrounded by breast cancer cells, the BBB is transformed to BTB by remodelling of pre-existing brain vessels such as reduction in the basement collagen membrane part IV and laminin α2. Moreover, an alteration occurs in the pericyte phenotype to change the expression of desmin and CD13 in influencing the permeability of this barrier, allowing the breast cancer cells in crossing over BBB [54, 55].

Intracerebral progression

Breast cancer cells that have crossed the endothelial barrier of the BBB confront astrocytes [56]. The BBB integrity is maintained by the astrocytes and this is beneficial for providing the homeostasis of cerebral and extracellular environments. The proliferation rate of breast tumor cells can be enhanced by the generation of IL-6, and tumor growth factor beta (TGF-β) [57]. The interaction occurring between cancer cells and astrocytes can lead to the upregulation of Akt/MAPK, resulting in the overexpression of anti-apoptotic factors including GSTA5, BCL2L1, and TWIST1 in cancer cells to trigger drug resistance [58]. Moreover, the secretion of IL-1β by breast tumor cells can increase levels of Notch and JAG1 on astrocytes in enhancing Notch levels in breast cancer stem cells [59].

Genetic mutations and biological factors

The dysregulation of molecular and biological factors has been considered as factors in breast cancer brain metastasis. A number of studies have evaluated the potential of Notch in the regulation of breast cancer brain metastasis. Notably, NOTCH3 demonstrates association with breast cancer invasion. The correlation between NOTCH3 expression and metastasis in ERα$^+$ and TNBC models has been determined [60]. Before the brain metastasis, the induction of microglia should occur. The metastatic breast tumor cells have demonstrated the potential in the secretion of ANXA1 to enhance migration of microglia in increasing cancer metastasis. Moreover, ANXA1 silencing can impair migration of microglia and downregulate STAT3 [61]. The upregulation of PTEN in the astrocytes can impair migration, highlighting the fact that PTEN is an ideal candidate in the treatment of breast cancer brain metastasis [62]. Table 2 summarizes the role of molecular pathways in the breast cancer brain metastasis.

Epithelial-mesenchymal transition as a potential therapeutic target

EMT is a highly dynamic and reversible mechanism stimulated by the EMT-activating transcription factors known as EMT-TFs including Snail, Twist and ZEB proteins, among others [71]. The EMT and related proteins play a significant function in the various steps of tumorigenesis from initiation, primary tumor growth to the invasion, dissemination, metastasis and finally, therapy resistance [72]. EMT is suggested to be a critical step for the brain metastasis of breast tumor cells [73]. The WNT3A downregulation by miR-6838-5p can impair EMT and metastasis of TNBC [74]. The de novo generation of TGF-β can be increased by HER2 to enhance Snail levels in EMT induction and metastasis of breast cancer [75]. However, the studies have been more specific and evaluated the potential of EMT in the brain metastasis. The evasion from the metastatic mass and subsequent infiltration into the brain parenchyma of breast cancer cells for brain metastasis are accelerated by EMT [76].

Table 2 Molecular pathways in the regulation of breast cancer brain metastasis

Molecular factor	Highlight	Reference
ProNGF	ProNGF enhances the brain metastasis via upregulation of Src by targeting TrkA/EphA2 axis	[63]
BCRP	The breast tumor cells demonstrate increased vascular mimicry and upregulation of BCRP, causing resistance to doxorubicin and topotecan	[64]
LRRC31	Suppression of DNA repair to enhance the response to radiotherapy	[65]
miR-199b-5p	The secretion of miR-199b-5p from breast tumor celsl to mediate neurometabolic coupling in enhancing brain metastasis	[66]
Cdk5	Induction of Cdk5 by astrocytes to inhibit MHC-I expression in causing immune evasion and enhancing brain metastasis	[67]
circBCBM1	Enhancing the brain metastasis through sponging miR-125a in upregulating BRD4	[68]
YTHDF3	YTHDF3 upregulates ST6GALNAC5, GJA1, and EGFR, increasing brain metastasis	[69]
MMP3	Loss of Kmt2c or Kmt2d increases MMP3 level through KDM6A to promote brain metastasis	[70]

The metastatic brain colonization of breast tumor cells is highly complicated and the recent studies have highlighted a number of related pathways. LEF1 is an EMT-TF with upregulation in the brain-colonizing cancer cells. The upregulation of LEF1 in breast tumor brain colonization model has been performed, decreasing survival rate lacking EMT involvement. This highlights that in addition to EMT, LEF1 can cause resistance against GSH depletion and controls ROS levels [77]. This provides a new insight that EMT-TFs can also manage the metabolic factors in affecting the brain metastasis of breast cancer cells. The induction of EMT is vital for increasing the penetration of breast tumor cells through BBB that is mediated by SNORA71B [78]. Figure 1 demonstrates the genetic factors and biological mechanisms in the breast cancer brain metastasis.

Exosomes regulating breast cancer brain metastasis

Different kinds of cells including breast tumor cells can secrete exosomes as small extracellular vesicles with size of 30–150 nm [79]. The exosomes have a lipid bilayer and they can transfer various kinds of bioactive molecules including lncRNAs, miRNAs, proteins and others controlling cell-to-cell communication [80]. The interactions among the cells are responsible for the cancer metastasis and therapy resistance. There is high secretion of exosomes from cancer cells [81]. The exosomes have been widely utilized for the delivery of bioactive compounds due to their size, transportation to the various cells, biocompatibility, desirable affinity and capacity in crossing over BBB. Moreover, exosoms play a crucial roles in cancers [82–84]. The bilateral communication between tumor cells and normal cells in the breast cancer can be mediated by exosomes. The exosomes enriched with miRNAs and lncRNAs have shown the potential in affecting transcriptome of targeted cells and changing their metastasis [85]. Different studies have evaluated the function of exosomes in the breast cancer progression regulation. The exosomes have been derived from MSCs capable of penetrating into BBB. The exosomes exert a synergistic impact with carboplatin in breast cancer, beneficial for the treatment of breast cancer brain metastasis [86]. Moreover, the exosomes derived from cancer cells can impair BBB via transcytosis and this can be used for transfer of bioactive compounds via BBB [87]. Moreover, the exosomes derived from adipocytes can increase the proliferation of breast cancer through induction of Hippo pathway [88].

A number of genes demonstrate association with the biogenesis of exosomes and regulating breast cancer brain metastasis. The TTLL4 has a significant association with the cerebral metastasis and upregulation of TTLL4 in breast cancer can enhance polyglutamylation of β-tubulin. Furthermore, in the cells expressing TTLL4, there is an increase in trafficking of secretory vesicles and extracellular vesicles. These extracellular vesicles are beneficial in increasing adhesion of breast cancer cells [89]. The exosomes isolated from brain metastasis breast cancer cells can impair the integrity of BBB through the delivery of lncRNA GS1-600G8.5 [90]. In patients with insulin resistance, there is high risk of metastasis of TNBC. The exosomes isolated from breast adipocytes are able to mediate EMT and metastasis in TNBC. Insulin resistance enhances the brain metastasis of breast cancer and mediate EMT through the adipocyte-derived exosomes [91]. Moreover, the content of exosomes can be evaluated in understanding the brain metastasis in breast cancer. An experiment has shown that exosomal miR-576-3p upregulation and exosomal miR-130a-3p downregulation can mediate cerebral metastasis in breast cancer [92]. On the other hand, the exosomes can be utilized for the treatment of brain metastasis. A delivery system has been developed from exosomes derived from CAR-NK cells and micelle. Such combinatory nanoplatform from exosomes and micelles is beneficial in impairment of ferroptosis defense system. This delivery system has been functionalized with transferrin receptor binding peptide (T7) and CAR expression on the surface of exosomes can improve their transfer into BBB and they specifically target HER2 + breast cancer cells. These nanomaterials can increase ROS levels and mediate photodynamic therapy

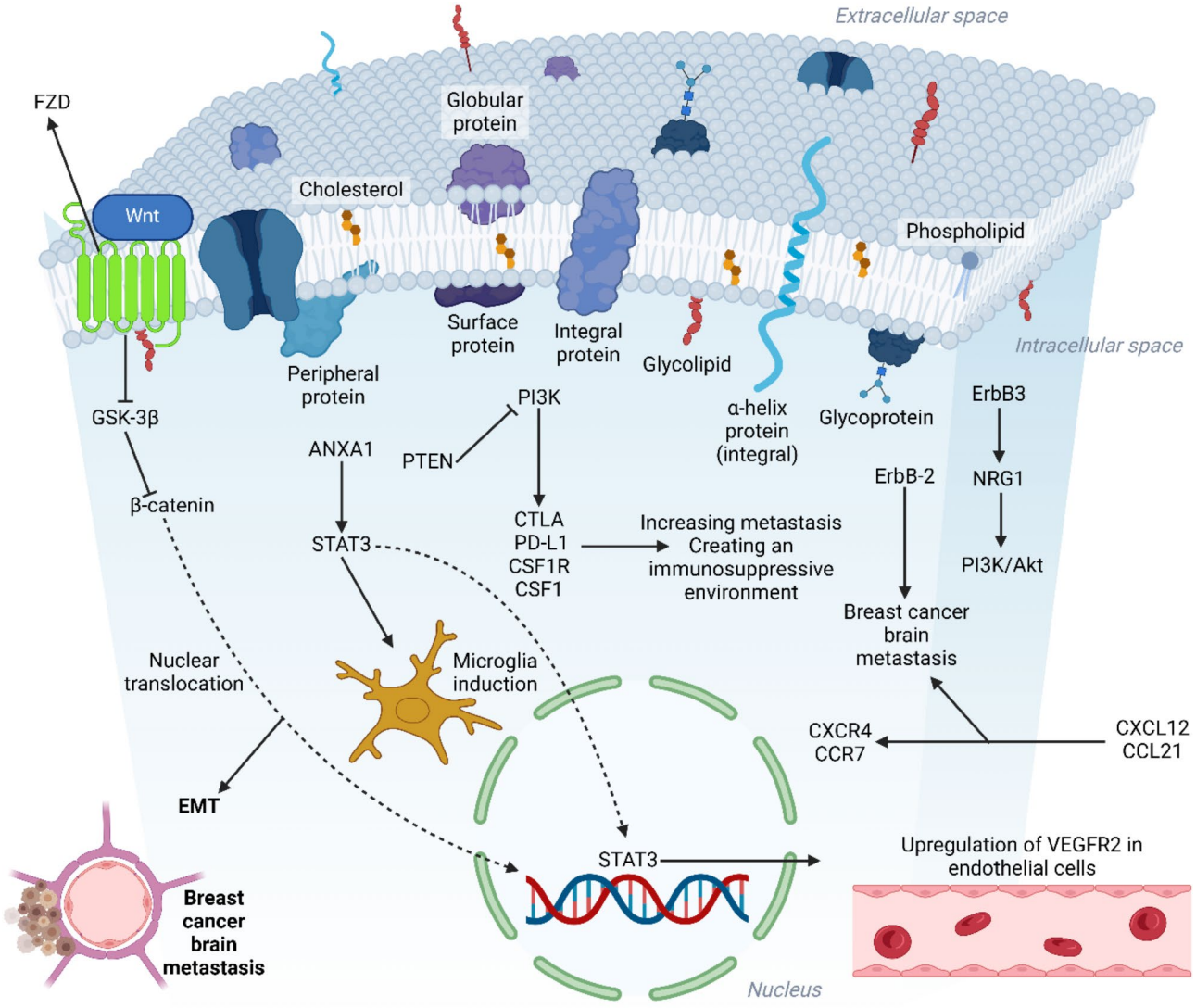

Fig. 1 The role of genetic factors and biological mechanisms in the breast cancer brain metastasis. The different kinds of pathways participate such as Wnt that can mediate nuclear translocation of β-catenin in EMT induction and promoting breast cancer brain metastasis. STAT3 upregulation can occur by ANXA1. On the other hand, STAT3 increases VEGFR2 expression in endothelial cells. PI3K can upregulate CTLA, PD-L1, CSF1R and CSF1 in enhancing cancer metastasis and providing an immunosuppressive microenvironment. CXCL12 and CCL21 can increase the levels of CXCR4 and CCR7 for breast cancer brain metastasis. ErbB3 can increase NRG1 levels in PI3K/Akt upregulation for the survival of breast cancer cells metastasized in the brain tissue. However, one of the limitations of current studies is that the role of receptors has not been understood completely and more focus on this subject should be paid. (Created by Biorender.com)

for the release of cargo in tumor site. These nanoparticles can also exert high anti-cancer activity and are beneficial in the treatment of brain metastasis breast cancer [93]. Figure 2 shows the function of biological factors in the brain metastasis of breast tumor cells. Table 3 highlights the biological factors involved in the breast cancer brain metastasis.

Based on these discussions, the exosomes are promising candidates for understanding the breast cancer brain metastasis because of their potential in intercellular communication and the transfer of biomolecules. The exosomes play a significant function in the diagnosis, prognosis, therapeutic delivery, immunotherapy and highlighting the metastatic potential of breast cancer cells. The isolation of exosomes from the different sources can occur including blood and cerebrospinal fluid, mediating non-invasive liquid biopsies. The content of exosomes including miRNAs, lncRNAs, and some certain proteins can be utilized as biomarkers in the early diagnosis and tumor profiling, highlighting the molecular characteristics of primary and metastatic tumors. The exosomal alterations can provide new insights regarding the disease progression, therapeutic response and resistance features. In addition, the

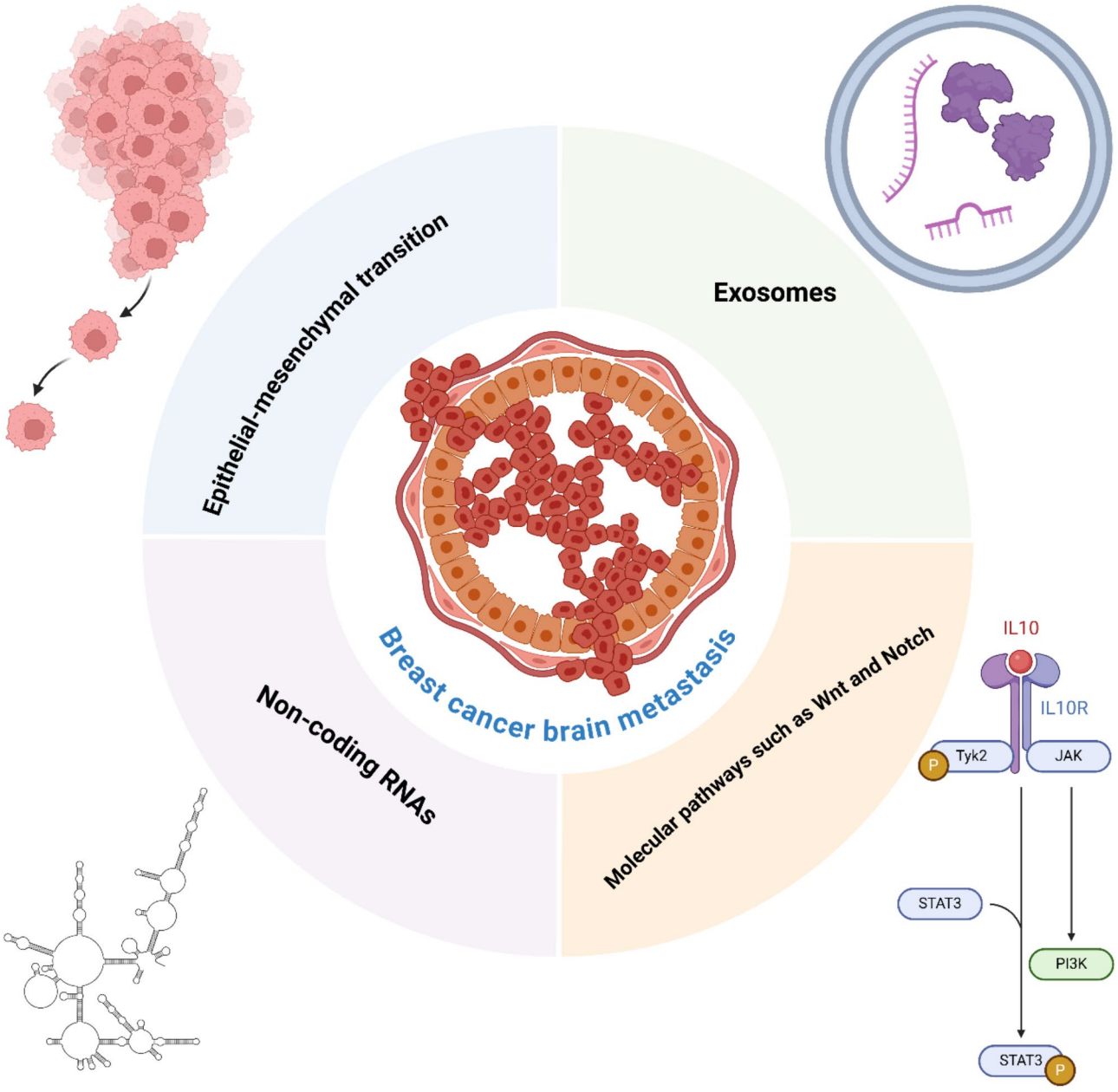

Fig. 2 The function of biological factors in the breast cancer brain metastasis. The induction of EMT in the breast cancer cells can enhance the metastasis into the brain tissue, since it is vital for the tumor cells to be separated from other parts of colony to enter into blood stream for reaching to the brain tissue. The exosomes are also critical regulators of breast cancer brain metastasis, since they can deliver bioactive molecules such as RNA, protein and lipid to affect the progression and metastasis of tumor cells. The non-coding RNAs are also regulators of breast cancer brain metastasis. In addition, the molecular pathways including STAT3, Notch, Wnt, PI3K/Akt/mTOR and ERBB, affecting breast cancer brain metastasis (Created by Biorender.com)

exosomes can provide innovative therapeutic applications such as delivery of chemotherapeutics, siRNA and miRNAs, targeting the certain receptors such as HER2 or integrins on the metastatic lesions as well as controlling the immune reactions. On the other hand, the exosomes can be deployed to transfer tumor-associated natigens in the induction of immunity and suppressing breast cancer brain metastasis. However, this has been ignored, while it has significant clinical importance. The exosomes may accelerate the brain metastasis of breast cancer cells through enhancing pre-metastatic niche formation and the identification of therapeutic targets, providing them valuable factors for personalized medicine. The exosomes can increase vascular permeability and change the astrocyte and microglial behaviour to prepare the brain microenvironment for the metastasis of breast cancer cells. The analysis of exosomes from patients can direct individualized treatment strategies, whereas exosomal

Table 3 The key genetic factors and biological mechanisms in the breast cancer brain metastasis

Genetic factor/biological mechanism	Remark	Reference
Anti-cancer immunity	The absence of microglia can promote metastasis and diminish the responses provided by natural killer cells and T cells Increase in the anti-tumor immunity by microglia Reduction in breast cancer brain metastasis	[94] [69] [68] [66] [67]
PVR/CD155	Brain metastasis-associated fibroblasts are capable of secreting fucosylated PVR/CD155 The secretion of PVR is a result of HIF-1α	[95]
Lipocalin-2	The presence of interactions among innate immune cells and astrocytes can elevate neuroinflammation and brain metastasis through lipocalin-2	[96] [64]

signatures have been integrated to the predictive models for the stratification of high-risk patients. However, the clinical application still requires addressing a number of challenges including standardization of isolation protocols, scalability, and regulatory rules.

Nanoparticles suppressing breast cancer brain metastasis

In the recent years, nanoparticles have been widely utilized for the treatment of breast cancer. The conventional therapeutics such as chemotherapy and radiotherapy have faced a number of challenges such as resistance. Therefore, the nanostructures have been introduced for the treatment of breast cancer brain metastasis. In an attempt, ferritin nanoparticles (HFn) have been utilized to deliver trastuzumab into the brain and increase the selectivity of anti-cancer immunity. The nanoconjugate was developed based on covalent attachment of HFn into trastuzumab and then, modification with HER2 and TfR1 was performed to increase selectivity. Upon intraperitoneal administration, the stable and specific targeting of tumor cells were provided. The exposure of tumor cells to these nanoparticles and docetaxel exerted synergistic impact in reducing growth of cancer cells and engaged macrophages in cancer therapy [97]. Another chemotherapy drug used for the treatment of breast cancer brain metastasis is doxorubicin. Noteworthy, hyperbranched polymers have been functionalized with HER3 bispecific-antibody fragment to increase selectivity towards tumor cells and the release of doxorubicin from the nanostructures was pH-sensitive. The nanostructures release the doxorubicin in a sustained manner and the release was higher in endosomal acidic conditions compared to physiological condition. These nanostructures exerted toxicity against tumor cells and in addition to decrease in growth and size, they improved the survival of mouse model [98]. Upon the metastasis of breast cancer cells to the brain tissue, the challenge is the delivery of drugs to the brain. The benefit of nanostructures is their ability in crossing over or targeting BBB to reach to the tumor site and impair breast cancer brain metastatic cells [99, 100]. In addition to the drug delivery, the nanoplatforms can be utilized to co-deliver drug and gene in cancer therapy. The nanostructures can co-deliver docetaxel and HER2-siRNA with partizle size of 100 nm, capable of selective targeting of HER2 + breast tumor cells. These nanoparticles can impair cancer progression with higher capacity compared to docetaxel and trastuzumab. Moreover, combination with microbubble-assisted focused ultrasound resulted in the impairment of BBB integrity to impair growth of tumor [101]. Such pre-clinical investigations on breast cancer brain metastasis can pave the way for the treatment of patients in the clinical setting. In order to improve the selectivity of nanoparticles, their modification with ligands is performed and one example is the functionalization of iron oxide nanostructures with BRBP1 to increase imaging of breast cancer brain metastasis [102]. Another example is the functionalization of nanostructures with iRGD peptide in increasing specificity towards breast cancer brain metastasis [103]. The advantageous of nanoparticles in the brain metastasis is impairing the adhesion, migration and invasion of breast tumor cells [104], challenging their brain metastasis. Therefore, increasing evidences highlight the desirable efficacy of nanoplatforms in the treatment of breast cancer brain metastasis [105–107].

According to these discussions, the nanoparticles provide valuable insights for the breast cancer brain metastasis therapy and can address the challenges associated with BBB and BTB. The nanostructures have been designed for the delivery of chemotherapy agents, targeted therapies or RNA-based treatments to the metastatic site and reduce the systemic toxicity that both of them are of importance for the clinical application. The surface modification of nanoparticles, especially by ligand conjugation with transferrin or antibodies against BBB receptors can improve the ability to penetrate

through BBB via receptor-mediated transcytosis. The drugs can be loaded in the nanoparticles for the modulation of tumor microenvironment and various kinds of drugs including those disrupting endothelial tight junctions or suppressing astrocyte-related drug resistance pathways can be utilized. The most studied platforms have been suggested to be liposomes, polymeric nanostructures and exosome-mimetic nanocarriers that can deliver doxorubicin, paclitaxel or siRNAs targeting oncogenic mechanisms. The multifunctional nanoparticles capable of combining diagnosis and therapy can be introduced for controlling breast cancer brain metastasis. The clinical efficacy of these nanoparticles should be evaluated in terms of their accumulation at the metastatic site and therapeutic efficacy along with biosafety.

Immune system in breast cancer brain metastasis

After the diagnosis of breast cancer metastasis, multiple strategies should be followed for its treatment. The studies have focused on understanding the immunological profile of breast cancer brain metastasis. These tumors have an immunosuppressive TME comprised of cells including FOXP3 + regulatory T cells, LAMP3 + tolerogenic dendritic cells, CCL18 + M2-like macrophages, RGS5 + cancer-associated fibroblasts, and LGALS1 + microglial cells. Moreover, there is poor expression of PD-1 and PD-L1/2 on CD8 + T cells and cancer/immune/stromal cells, respectively. These tumor cells demonstrate immune evasion resulting from the intractions occurring between checkpoint molecules LAG3-LGALS3 and TIGIT-NECTIN2 with CD8 + T cells and cancer/immune/stromal cells [108]. The single-cell sequencing has been beneficial in understanding the immunological profile of breast cancer brain metastasis. Notably, the factors including CCR5, LYZ, IGKC and MS4A1 can increase immune system function, while SCGB2A2 and CD24 impair the function of immune system in breast cancer brain metastasis [109]. The levels of antigen processing and presenting molecules in breast cancer brain metastasis have been also evaluated [110]. Noteworthy, there is poor expression of β2-microglobulin, transporter associated with antigen processing (TAP) 1, TAP2 and calnexin in the brain lesions. The primary breast lesions in the patients that finally mediate brain metastasis display the poor expression of β2-microglobulin, TAP1 and calnexin. In the lesions lacking metastasis, there was an increase in the levels of CD8 + T cells, possessing a positive association with TAP1 and calnexin. It appears that when breast cancer cells want to mediate brain metastasis, they create an immunosuppressive TME. One of them is the reduction in the levels of CD8 + T cells and M1 macrophages, while there is increase in M2 macrophages [111]. More characterization has demonstrated the reduction in T cell responses correlated with ARG2 expression [112]. Therefore, the immune infiltration shows decrease. On the other hand, anti-cancer immunity can be increased by microglia. The lack of microglia can enhance metastasis and decrease survival and levels of natural killer cells and T cells. Therefore, the presence of microglia is beneficial for enhancing anti-cancer immune responses against breast cancer brain metastasis [96]. The breast cancer brain metastatic senescent cells can be stimulated by doxorubicin and recruit PD1-expressing T cells to the brain tissue. When the senescent is induced by doxorubicin, it can also improve the potential of immunotherapy with anti-PD-1 therapy in the treatment of breast cancer brain metastasis [113]. Another potential in immunotherapy is CD47 blockade that can promote M1 polarization of macrophages and provides 89% reduction in the metastatic brain burden [114]. One of the most common cells in the TME is tumor-associated macrophages (TAMs). Lymphotoxin β demonstrates upregulation in TNBC cells that metastasize to the brain parenchyma and can mediate M2 polarization of macrophages. Therefore, there is a connection between site specificity of metastatic TNBC and metastasis-associated macrophages activation state [115]. Furthermore, other factors in the CNS microenvironment can control brain metastasis. In the brain metastasis derived from TNBC and HER2 + breast tumor cells, there is upregulation of GABA and these cells can proliferate through GABA metabolism that is more prominent in TNBCB brain metastasis [116]. The tumor-infiltrating lymphocytes (TILs) can be utilized as prognostic factors in TNBC and those with high infiltration of TILs demonstrate low rate of distant metastasis. Compared to primary breast cancer, there are lower levels of TILs in brain metastasis and there is also decrease in the levels of CD^{4+} and CD^{8+} cells in the brain [117].

Disruption of blood-brain barrier

Since BBB can decrease the entrance of bioactive compounds to the brain, a number of strategies have been employed to impair the BBB integrity [118]. This process is not invasive and the compliance of patients with this technique has been approved. Upon this disruption, lower levels are drugs are required that diminishes the drug-mediated toxicity. One of the approaches is the application of osmotic pumps, first mentioned on the cerebral cortex of rabbits in 1972 [119]. However, this strategy suffers from a number of challenges such as enhancement in the brain fluid content. Moreover, this strategy needs to be optimized with size of cancer and its mass. Therefore, osmotic opening of BBB and addressing its heterogeneity can be considered in the future [120, 121]. Another method is the application of focused ultrasound combined with microbubbles with intravenous

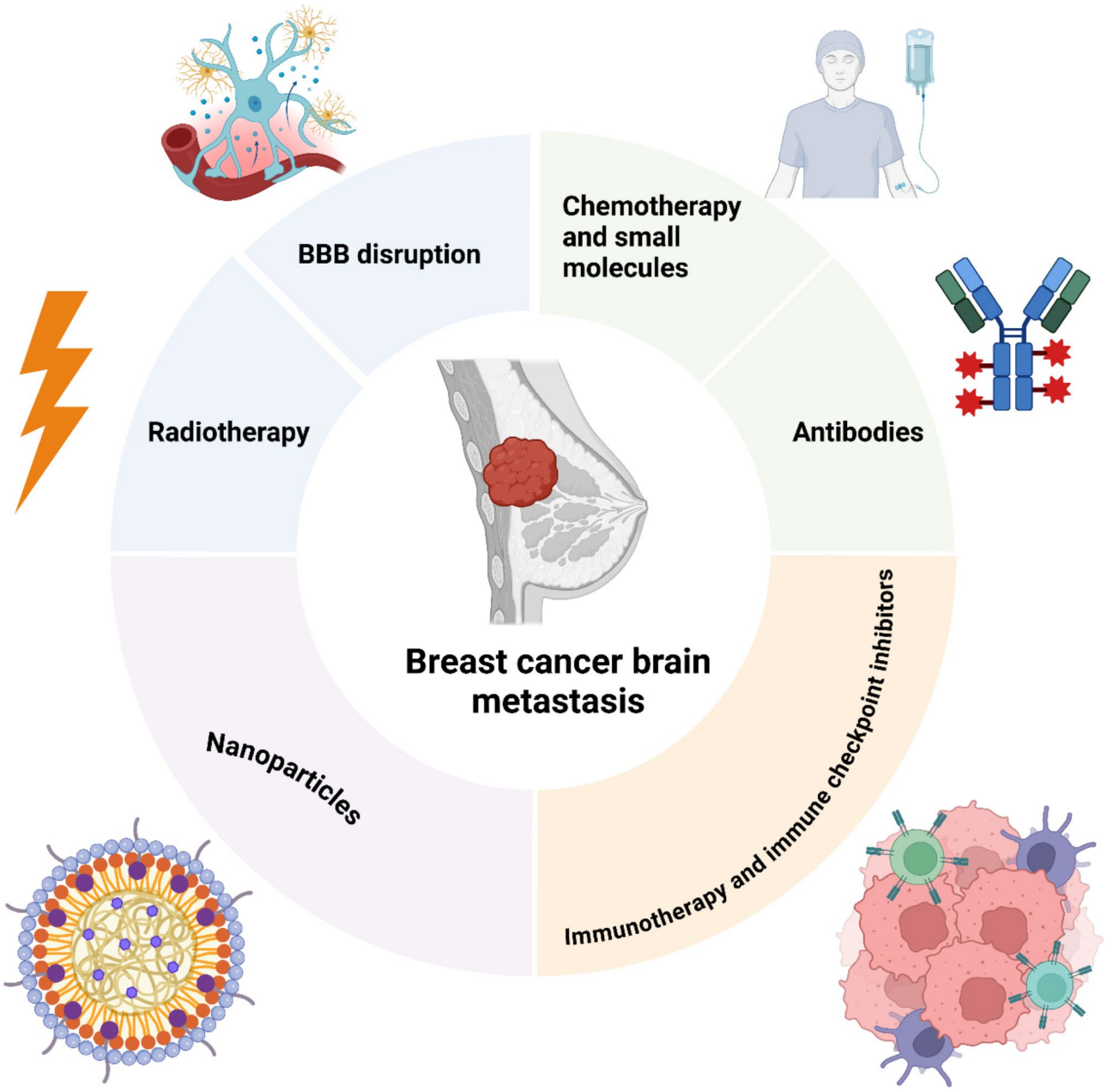

Fig. 3 The treatment strategies for breast cancer brain metastasis. Upon the brain metastasis of breast cancer cells, a number of therapeutic methods are applied to impair tumorigenesis that immunotherapy, application of small molecules and immune checkpoint inhibitors, nanoparticles, radiotherapy and antibodies are among them. In order to improve the entrance of the bioactive molecules into the brain tissue, BBB disruption is followed by microbubble-induced ultrasound (Created by Biorender.com)

administration. The microbubbles are in size of 0.5–10-μm bubbles comprised of protein, lipid or polymer structure. In combination with focused ultrasound, they have been confirmed by FDA for the ultrasound imaging. This strategy has been beneficial in improving the delivery of chemotherapeutics including doxorubicin, bevacizumab, trastuzumab, gene, or nanomedicines across the BBB [122–126] or the application of microbubbles as drug carriers [127]. In order to prevent tissue damage and inflammation, it is encouraged to optimize the energy level of ultrasound. Moreover, repeated and frequent application of focused ultrasound may be needed to preserve the impairment of BBB. More information on BBB heterogeneity and BBB openin can be found here [120, 128]. Figure 3 highlights the therapeutic strategies for the treatment of breast cancer brain metastasis.

Although the procedure of BBB disruption has been considered as an ideal strategy in improving the delivery

to the brain tissue, there are a number of challenges and restrictions to be considered. One of the most prominent issues is related to the safety concerns in which techniques including focused ultrasound can cause damage to the surrounding and healthy tissues if the levels are not optimized and osmotic pumps may increase the fluid content, leading to the cerebral edema. The BBB disruption may also cause inflammation increasing neurological symptoms or mediating long-term side effects. Since BBB has a transient nature, its disruption required repeated interventions to enhance the risk of complications and decrease the patient compliance. Moreover, the efficacy of such techniques may be varied based on the changes in the tumor size, locations and mass with tumor heterogeneity affecting the uniform treatment. The application of BBB disruption not only enhances the delivery of therapeutic compounds, but also increases the entrance of toxic substances including toxins and pathogens to enter to the brain tissue and promote the risk of neurotoxicity or infection. Other limitations related to the application of such technologies is related to the precision required for the focused ultrasound and lack of standardization in protocols. There are a number of issues related to the long-term application of BBB disruption including chronic neuroinflammation, altered brain homeostasis, and cognitive effects that have not been fully understood and addressed. There are also a variety of drug-specific challenges including variable penetration, changes in the size, charge, hydrophobicity and requirement for the dose optimization can complicate the strategy of therapy.

A number of clinical trials have focused on the application of BBB disruption in the cancer patients. In an effort, BBB disruption has been performed by exablate focused ultrasound with doxorubicin (phase I and II). This study has emphasized on the safety, feasibility and preliminary efficacy of the treatment strategy. This study has been based on three locations in USA and the patients receive three treatment cycles including 46 weeks apart (NCT05630209). Another clinical study (NCT05383872) has focused on evaluating the safety of using the Exablate Model 4000 Type 2.0/2.1 for BBB disruption in glioblastoma patients. The questions raised in this study is that BBB disruption can improve the diagnosis of cfDNA in the blood stream to increase the efficacy of liquid biopsy in patients. In this study, low-intensity focused ultrasound has been deployed in combination with intravenously administered microbubble oscillators to non-invasively disrupt the BBB. Such disruption has been performed to enhance the levels of cfDNA in the blood to increase efficacy of liquid biopsy. One of the aims is to obtain at least a two-fold increase in cfDNA levels one-hour post-procedure compared to pre-procedure levels. Another aim is to evaluate the role of cfDNA as biomarker and the differences with tumor tissue biomarkers at the later stages. An open-label, single-arm and phase IIa clinical study has been designed for investigating the efficacy of combining focused ultrasound-driven BBB disruption with bevacizumab in the treatment of glioblastoma patients. Such BBB disruption has been utilized to increase the deliveyr of bevacizumab to the brain tissue. Such combination therapy has been suggested to be feasible and MRI scans demonstrated presence of BBB disruptions up to 2 cm in the targeted regions. In addition, progression-free survival in patients was different from 9 months to 4 months (NCT04446416). Considering these discussions about the clinical application of BBB disruption, if the aforementioned limitations are addressed, a significant improvement can be achieved for the prognosis and treatment of patients. Moreover, the specific studies about the breast cancer brain metastasis and the potential of BBB disruption in accelerating therapy are required.

Liquid biopsy in breast cancer brain metastasis prediction

The early diagnosis of metastasis can be improved by the liquid biopsy [129–131]. It is suggested to determine the receptor status during breast cancer metastasis screening that is based on the National Comprehensive Cancer Network (NCCN) Guidelines in Oncology for Breast Cancer [132] and 5th ESO-ESMO (European School of Oncology-European Society for Medical Oncology) international consensus guidelines for advanced breast cancer [133]. From clinical view, it is beneficial to conduct biopsy or resection according to the variations of the receptor status and genomic profiling. However, it is challenging to conduct biopsy or surgical resection in some cases. Therefore, liquid biopsy is advantageous for the phenotyping or genomic profiling of breast cancer brain metastasis. According to an experiment, ctDNA can be recognized in more than 75% of advanced breast cancer patients [134]. Moreover, the circulating tumor cells are essential for determining the response of patients to chemotherapy [135]. An investigation has demonstrated that there is higher abundancy of ctDNA of CSF from CNS tumors such as breast cancer brain metastasis compared to ctDNA of plasma [136]. The management strategy for HER2+-breast cancer brain metastasis can be determined based on evaluating the ctDNA levels from CSF [137]. As a result, liquid biopsy can be utilized for the phenotypic or genomic profiling [138–140].

A study has focused on 88 breast cancer patients and they have been divided into brain metastasis and lack of metastasis. The metabolomics analysis has been performed using GC-MS and LC-qTOF-MS to highlight the function of liquid biopsy in the early diagnosis of patients. This experiment highlighted the different kinds of metabolites and showed changes in the levels of 47 metabolites.

This liquid biopsy approach demonstrated that a number of pathways including aminoacyl tRNA biosynthesis, valine, leucine and isoleucine biosynthesis, alanine, aspartate, and glutamate metabolism, arginine biosynthesis, glycine, serine, and threonine metabolism demonstrate dysregulation in the breast cancer patients with brain metastasis. Moreover, accuracy of this method was evaluated and ROC was suggested to be 96.9% [141]. One of the challenges is the tissue specimens from patients to direct the future diagnostic and therapeutic approaches. One of the potential ways is to investigate and analyse the cell-free tumor DNA in the plasma known as liquid biopsy to improve the identification of extra-cranial tumors. However, this method may possess a number of limitations including low quantity of cell-free tumor DNA in the plasma of patients and may not provide a proper view of the genomic changes in the brain cancers. In line with this, cell-free tumour DNA from cerebrospinal fluid has been investigated for the identification of brain tumor and in order to improve accuracy, the cell-free tumor DNA can be utilized, enhancing diagnosis and showing heterogeneity in the patients with metastasis [142]. In addition, the droplet digital PCR (ddPCR) and next generation whole exome sequencing (WES) analysis were utilized to investigate HER2-positive patients with breast cancer metastasis. Such analysis demonstrated that there are *TP53* and *PIK3CA* mutations and *ERBB2* and c*MYC* amplification. In addition, post-treatment cDNA analysis highlighted the reduction in the marker levels in plasma along with extra-CNS disease control, whereas there was enhancement in the CSF, mentioning the poor outcome in the CNS [137]. The liquid biopsy of cerebrospinal fluid has been suggested to be appropriate for the diagnosis of metastasis. The overall sensitivity, specificity and AUC of liquid biopsy for the diagnosis was suggested to be 0.65, 0.7 and 0.69, respectively. The results demonstrated that liquid biopsy of cerebrospinal fluid is prioritized to cytology in metastasis diagnosis [143].

In respect to these discussions, liquid biopsy has been emerged as a minimally invasive method for the evaluation of circulating tumor-derived components including circulating tumor cells (CTCs), ctDNA, exosomes and cancer-associated proteins from body fluids such as blood or cerebrospinal fluid. Liquid biopsy has several values for diagnosis of brain metastasis in breast cancer. The diagnosis of breast cancer brain metastasis is mainly based on the application of invasive procedures including brain biopsies. However, one of the advantageous of liquid biopsy is related to the obtaining samples from blood or cerebrospinal fluid to reduce risk on patients, improving longitudinal monitoring. The presence of BBB prevents the tumor cell dissemination and their identification in the bloodstream. However, BBB is compromised in the breast cancer brain metastasis, providing the presence of tumor-associated components including ctDNA or exosomes for entrance into the blood circulation or the CSF. Moreover, the emerging liquid biopsy technologies have been demonstrated to possess high sensitivity and specificity for the identification of ctDNA or CTCs. The liquid biopsy can be beneficial for the purpose of molecular profiling and personalized medicine by ctDNA sequencing or CTC analysis to detect the mutations, amplifications or epigenetic alterations related to the brain metastasis including HER2 and PIK3CA. The insights obtained from liquid biopsy have been beneficial in guiding the targeted therapies or immunotherapy. One of the problems of traditional approaches is capturing a small portion of cancer cells lacking a proper representation of molecular diversity of metastatic lesions. On the other hand, liquid biopsy has been beneficial for providing a systemic overview by the identification of tumor-associated materials from metastatic sites, improving the insight towards the molecular profile. The liquid biopsy can provide a dynamic tracking of the tumor burden through analysing ctDNA or CTCs, monitoring cancer progression and spread.

Conclusion

One of the challenges is the breast cancer brain metastasis allowing the tumor cells for invading another organ. This is a dynamic and complicated process involving EMT, intravasation, circulation and extravasaion in which breast tumor cells disrupt BBB to generate BTB supporting their growth. The molecular factors including STAT3, Wnt, Notch, ERBB, and chemokines can affect breast cancer brain metastasis, requiring the application of therapeutics such as immunotherapy, antibody, small molecules and nanoparticles. The early diagnosis and targeted therapies can be improved by highlighting the key factors such as EMT-related proteins and inflammatory cytokines that are vital for the development of biomarkers and non-invasive monitoring tools including CTCs and ctDNA. MRI and PET scans with targeted contrast agents are imaging techniques in which improve lesion visualization. Modulating early metastatic factors, utilizing BBB-penetrating agents, and focusing on tumor-astrocyte interactions can reduce proliferation and resistance. The downregulation of Akt/MAPK or Notch can suppress drug resistance, whereas combination of immune checkpoint inhibitors (ICIs) with other therapeutics can increase immune cell infiltration in preclinical research. Addressing the BTB heterogeneity and affecting genomic and epigenetic factors can improve therapeutic outcomes in the personalized medicine.

In spite of the improvements in the treatment of breast cancer brain metastasis, there are still a number of challenges in its therapy. BBB provides a big challenge to the

entrance of bioactive compounds and water-soluble substances into the brain. In fact, BBB provides a challenge for the delivery of compounds to the brain [144]. An instance is trastuzumab as a monoclonal antibody that can bind to HER2 protein expressed on the surface of breast tumor cells, suppressing cancer proliferation [145]. However, trastuzumab lacks the efficacy in crossing over BBB, similar to other monoclonal antibodies [146]. As a result, radiotherapy or surgery has been applied to impair BBB integrity to increase the cross over BBB [147–149]. The transport of bioactive compounds across BBB is controlled by the tight junctions present in endothelial cells [150], preventing the entrance of small molecules, since they are considered as the substrates for the efflux transporters, decreasing penetration. Therefore, three pathways can be followed to reach to the brain interstitial fluids including receptor-accelerated transfer, lipid-induced free diffusion and carrier-triggered transport systems [151, 152]. In tumor, there are alterations in the BBB to generate BTB [153]. Although the nature of BTB is considered to be leaky, it also has heterogeneity [154]. As a result, more specific approaches have been developed for crossing over BBB that one of them is application of nanoparticles. However, the biocompatibility and biosafety of nanostructures are an issue and therefore, their clinical application requires highlighting their long-term safety. Moreover, preserving the physico-chemical features of nanoparticles, keeping their effectiveness and large-scale production should be considered.

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