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Heterogeneity and Vascular Permeability of Breast Cancer Brain Metastases

Maria V. Babak^a, Michael R. Zalutsky^b, Irina V. Balyasnikova^c

^aDepartment of Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Hong Kong SAR 999077, People's Republic of China

^bDepartment of Radiology, Duke University Medical Center; 311 Research Drive, Box 3808, Durham, NC 27710

^cDepartment of Neurological Surgery, The Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA.

Abstract

Improvements in the diagnosis and treatment of systemic breast cancer have led to a prolongation in patient survival. Unfortunately, these advances are also associated with an increased incidence of brain metastases (BM), with the result that many patients succumb due to BM treatment failure. Intracranial delivery of many chemotherapeutic agents and other therapeutics is hindered by the presence of an impermeable blood-brain barrier (BBB) designed to protect the brain from harmful substances. The formation of BM compromises the integrity of the BBB, resulting in a highly heterogeneous blood-tumor barrier (BTB) with varying degrees of vascular permeability. Here, we discuss how blood vessels play an important role in the formation of brain micrometastases as well as in the transformation from poorly permeable BM to highly permeable BM. We then review the role of BTB vascular permeability in the diagnostics and the choice of treatment regimens for breast cancer brain metastases (BCBM) and discuss whether the vasculature of primary breast cancers can serve as a biomarker for BM. Specifically, we examine the association between the vascular permeability of BCBM and their accumulation of large molecules such as antibodies, which remains largely unexplored.

Keywords

brain metastasis; breast cancer; vascular permeability; blood-brain barrier; therapeutics

irinabal@northwestern.edu.

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Breast cancer brain metastases and poor prognosis

Despite the significant advancements in the treatment of breast cancer, 10-16% of patients develop BM during the late stages of the disease. The unfortunate event of cancer cell spread to the brain from a primary tumor site is associated with poor prognosis and high morbidity. [1] A typical range of survival of breast cancer patients with BM s is only 2-25 months. Treatment options are limited and frequently include surgical resection of the tumor mass and whole-brain radiotherapy (WBRT), a non-specific treatment modality given to the whole brain, which has been the primary treatment option for patients with BM. In addition, stereotactic radiosurgery (SRS), a non-surgical radiation technique that delivers precisely-targeted narrow beams of radiation to a tumor, and chemotherapy also are commonly used. [2] However, BCBM respond poorly to these treatments, and the therapies serve a mostly palliative function.

The formation of BM is a complex process and occurs in multiple steps involving the invasion of primary breast cancer cells into surrounding tissues, intravasation into the bloodstream, followed by extravasation into brain tissue.[3] This process takes a median of 32 months from the diagnosis of the primary breast tumor,[4] indicating that the primary cancer cells need a longer time to colonize within the brain due to the presence of an impermeable blood-brain barrier (BBB). The BBB is a physicochemical barrier between blood vessels and the brain that maintains brain homeostasis by limiting the access of potentially toxic substances to the central nervous system. With intracranial malignancies, the BBB is eventually transformed into a poorly permeable blood-tumor barrier (BTB).

Breast cancers are highly heterogeneous, and the incidence of BM, as well as patient survival, strongly depend on the breast cancer subtype, indicating the need for a personalized approach to BCBM treatment.[5, 6] In addition to high intertumoral heterogeneity, BM demonstrate a high degree of intratumoral variability, reflected by the heterogeneous distribution of chemotherapeutic agents in metastatic lesions.[7] This poses several fundamental questions: are there correlations between the heterogeneity of BCBM and their vascular permeability? If the answer is yes, is the permeability of BM in a patient predisposed by the vascular organization of the primary breast cancer? This review will focus on the role of blood vessels in the heterogeneous formation of metastatic lesions, and the implications of vascular permeability in the diagnosis and treatment of BM.

Heterogeneity of breast cancer metastases

Breast cancers can be classified into several distinct molecular subtypes, namely luminal A, luminal B, HER2-enriched, and triple-negative, or as basal-like tumors with further classification into additional subtypes.[8] The subtypes can be identified based on a panel of immunohistochemical markers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), the cell proliferation marker Ki- 67, and basal cytokeratins. For example, rare claudin-low breast tumors - characterized by the lack of ER, PR and HER2 receptors - were initially clustered with basal-like breast cancers; however, they subsequently were

demonstrated to possess unique phenotypic features associated with epithelial-to-mesenchymal transition as well as the expression of mammary cancer stem cells.[9]

Different breast cancer subtypes are characterized by different propensities for developing metastasis.[5, 6, 10] Luminal tumors, which constitute more than 75% of breast cancers and are characterized by dominant ER expression, commonly give rise to bone metastasis, and are rarely associated with metastatic progression to the brain. In contrast, the incidence of brain metastasis originating from HER2-enriched and basal-like tumors is significantly higher than for luminal A tumors.[6, 11, 12] The preference of basal-like breast cancers for metastatic sites in the brain was unclear until it recently was discovered that breast cancer cells could reprogram neighboring neurons to promote their metastatic growth inside the brain.[13] Patients with BM originating from triple-negative breast cancer (TNBC) usually have markedly shorter overall survival compared with patients with BM from luminal or HER2-enriched cancers.[11, 14] This can be explained in part by the later occurrence of BM for tumors with positive hormone receptor status.[15]

Several molecular profiling studies have demonstrated that BM retain similar molecular features and gene alterations as their parent breast cancer subtype.[16, 17] However, it has been reported that one in five patients underwent receptor change upon metastatic formation in the brain, leading to significant discrepancies between receptor status in the primary breast tumor and subsequent BM.[18-21] This observation is supported by epigenetic profiling and whole-exome sequencing of metastatic brain tissue, which demonstrated substantial differences in the genetic and epigenetic landscapes of primary and metastatic tumors.[22]

Breast cancer brain metastases in the context of vascular permeability

A systematic review of the breast cancer literature revealed a strong correlation between poor prognosis and high microvessel density for breast cancer patients.[24] It has been suggested that the vascular organization presented in and around the tumor might determine the predisposition of various breast cancer subtypes to form metastases.[23] The analysis of gene and RNA signatures of more than 3,000 human breast and murine mammary tumors revealed that basal-like and claudin-low tumors are characterized by markedly higher vasculature expression than other breast tumor subtypes (Fig. 1).[23] A subsequent comparative gene expression analysis of human breast cancer cells, endothelial cells and organs harboring breast metastasis, including the brain, bone marrow, lymph nodes, lung, and liver revealed specific “vascular content” and “activated endothelium” gene signatures, which were also highly expressed in basal-like and claudin-low tumors, respectively. The formation of distant metastases was also associated with a VEGF/hypoxia signature in basal-like and claudin-low tumors.[23, 25] The highest expression of the “vascular” and “endothelium” gene signatures was observed in claudin-low tumors. This likely reflects their ability to express endothelial genes and demonstrate characteristics of endothelial cells such as endothelium-like morphology in three-dimensional matrices (Fig. 2). Additionally, claudin-low and basal-like tumors showed high vascular permeability *in vivo*. Xenograft experiments showed extensive perfusion of contrast agent through non-vascular tumor-lined channels and paracellular spaces, which was not observed in luminal cancer models.[23, 26]

In agreement with these findings, immunohistochemical analysis of 431 breast cancers revealed that basal-like and triple-negative tumors had the highest vascular proliferation.[27, 28]

A valuable experimental mouse model of breast tumor heterogeneity was developed via a retroviral barcoding strategy and was used to demonstrate that various clonal populations even within the same tumor showed different behavior during metastatic progression.[29] The clones that were able to enter the bloodstream and colonize secondary sites were characterized by a high degree of vascular leakiness and the ability to mimic endothelial-like cells, resulting in direct contact of tumors cells with the blood. Several clones were able to differentiate into endothelial-like cells and generate vascular-like structures, which serve the purpose of supplying nutrients and oxygen to the tumor independent of angiogenesis. This vascular mimicry was characterized by increased expression of SERPINE2 and SLPI. Moreover, these two proteins were found to program vascular mimicry in basal-like and claudin-low cell lines but not luminal cancers,[29] and were linked to the metastatic spread of primary cancer cells to the brain.[30] Taken together, these results suggest that the endothelium-like characteristics of basal-like (claudin-low) tumors programmed by SERPINE2 and SLPI genes facilitate interaction of the tumor with the surrounding endothelium, initiating metastatic progression as well as facilitating BBB penetration, leading to the formation of distant BM.

The role of blood vessels in the brain metastatic processes

The mechanisms regulating the invasiveness of metastatic cells into the brain are poorly understood. It is known that the development of metastases depends on the disruption of tight junctions reflected by decreased expression of occludin, claudin-5 and ZO-1.[31-33] Additionally, the decreased expression of junctional adhesion molecule A (JAM-A) has been observed during BBB breakdown, demonstrating the effects of junctional adhesion molecules on BBB integrity during BM development.[34, 35] The initial transformation of an impermeable BBB into a poorly or highly permeable BTB involves CD31⁺ endothelial capillaries, which are widely distributed in the brain in the absence of metastases but become much larger and less dense in metastatic lesions, indicative of blood vessel dilation. [7, 33] Additionally, the transformation of a healthy BBB to a BTB is associated with the increased expression of the proliferation-inducing molecule, vascular endothelial growth factor (VEGF).[33, 36] The subsequent transformation of metastases from a low permeability state into highly permeable metastatic lesions involves the vascular remodeling of pre-existing blood vessels rather than *de novo* angiogenesis. This process is associated with decreased expression levels of basement collagen membrane part IV and laminin α_2 , which constitute endothelial and astrocytic basement membranes, and are important BTB components (Fig. 3).[7, 33, 37] The most noticeable differences between permeable and non-permeable metastases were related to pericytes and characterized by an increased expression of the desmin⁺ subpopulation[32,7] and a decrease in the CD13⁺ pericyte subpopulation. Changes in pericyte coverage contribute to brain abnormalities and metastasis progression.[38, 39]

Transcriptome profile comparisons between poorly and highly permeable experimental metastases revealed significant differences in gene expression patterns. The major source of differential expression was in neuroinflammatory response as reflected by activation of astrocytes surrounding the metastatic lesions.[40] Astrocytes, the most abundant cell population in the central nervous system, are star-shaped cells with extending end-feet that attach to the vascular membrane surrounding endothelial cells and pericytes, thereby contributing to maintenance of the BBB. The population of reactive astrocytes (RAs) is highly heterogeneous and characterized by the high expression of glial fibrillary acidic protein (GFAP).[41] The presence of GFAP⁺ astrocytes is crucial for formation of the BTB but not for the transformation of poorly permeable metastases into highly permeable ones.[33] Notably, altered sphingosine 1-phosphate 3 (S1P3) signaling was found in 40-89% of astrocytes surrounding the most permeable metastases. In addition, progression to the advanced stages of brain metastasis correlated with the ability of metastatic cells to reprogram the naïve tumor microenvironment into a pro-metastatic microenvironment by activation of a STAT3 signaling pathway in a subpopulation of RAs.[42]

Assessment of brain vascular permeability and drug uptake in preclinical models

Without an opening of the BTB, only a few drugs can pass the tight endothelial junctions to reach tumor cells; therefore, assessment of vascular permeability during treatment is important. The evaluation of alterations in BBB integrity upon the formation of BM has been studied in various *in vitro*, *in vivo* and microfluidic models.[45-47] The classical method to measure vascular permeability is known as the Miles Assay or Evans blue dye method. This method is based on the assumption that vasculature in and around metastatic brain tumors varies in permeability from essentially non- permeable undisrupted BBB with normal blood capillaries to a leaky tumor vasculature that allows free passage of macromolecules such as albumin.[48] Evans blue dye specifically binds to albumin and under physiological conditions, is restricted from crossing the BBB. A leak of the dye across the barrier, associated with the disintegration of the barrier, can be quantified spectrophotometrically. It has been demonstrated that BCBM turn blue when mice are injected with Evans blue dye, thus indicating BTB permeability.[49]

Alternatively, vascular permeability can be assessed by fluorescence microscopy and quantitative autoradiography using fluorescent dyes and radiolabelled tracers conjugated to macromolecules, respectively. A comparison of the passive permeability of the BTB in five experimental mouse models of BM using Texas Red-conjugated dextran (TRD) and [¹⁴C]aminoisobutyric acid (AIB) revealed that most metastases were more permeable than normal brain to both tracers.[3, 22] Subsequently, it was shown that normal brain tissue surrounding an intracranial tumor also was characterized by an increased permeability, leading to enhanced accumulation of chemotherapeutic agents.[50]

Chemotherapeutic agents can be also conjugated to fluorescent markers or labeled with radioisotopes to investigate the efficiency of drug penetration through the BBB or BTB. The uptake of radiolabeled paclitaxel and doxorubicin into BM was studied using innovative

animal models involving the breast- colonizing subline of the highly invasive triple-negative breast cancer, MDA-MB-231 (MDA-MB- 231Br), and the murine 4T1 mammary breast cancer cell line.[7] It was shown that paclitaxel accumulated in greater than 85% of metastatic lesions; however, only 10% of metastases demonstrated more than a 50-fold concentration increase to the level corresponding to a cytotoxic paclitaxel concentration. Similar results were obtained with radiolabeled doxorubicin. Importantly, accumulation of the drugs was heterogeneous not only among metastatic lesions but also within the same metastasis.[7] The high degree of intermetastatic and intrametastatic heterogeneity of BCBM poses a serious therapeutic challenge; however, it is still not clear what determines the spread of multiple lesions in a given patient, *e.g.* the size or location of the lesion. Whereas the size of the lesion determined the uptake efficiency of fluorescent Texas Red dye, no correlation was found between the size of the lesion and paclitaxel uptake.

Winkler *et al.* developed an elegant approach for evaluating the heterogeneous uptake of anticancer drugs into metastatic brain lesions.[51] In this study, two PI3K/mTOR inhibitors with different brain permeability were evaluated in an animal model of BM originating from melanoma cells. The highly permeable metastases responded equally well to brain-permeable and brain-impermeable inhibitors, whereas metastases with an intact BBB were responsive exclusively to the brain-permeable inhibitor.

These data indicate that even though BM are more permeable than normal brain, in agreement with enhanced dye penetration through the BTB, there are not enough regions with sufficient permeability to achieve efficient drug uptake. Similar to PI3K/mTOR inhibitors, the efficacy of lapatinib and capecitabine against breast cancer BM was dependent on BTB permeability, which varied significantly among tumors.[52, 53] Therefore, even if the drug has effective antimetastatic activity, BTB permeability markedly affects the activity of chemotherapeutic agents against intracranial metastases.[7, 52]

Heterogeneous vascular characteristics of breast cancer brain metastases - clinical implications

The treatment options for breast cancer BM depend on the subtype of the primary breast cancer and its molecular characteristics. However, the relationship between the vascular permeability of a BM and its response to different treatments is not clear.

Surgery

Surgical resection can be an effective treatment across all breast cancer subtypes for a single metastasis but is relatively ineffective for multiple metastases.[54] However, little is known about the association between the efficacy of BM surgical removal and breast cancer subtype. It has been shown that invasive surgery might cause dissemination of cancer cells by triggering extensive vascularization[55] and the growth of distant, otherwise-dormant, metastases.[56] Therefore, metastatic recurrence might not only be associated with the natural progression of the disease but also with postoperative triggers. It has been shown that blood vessel invasion is a strong predictor of postsurgical recurrence in endometrial cancers.[57] This leads to a question: because BCBM differ in vascular organization, which is

dictated by the primary breast cancer subtype, can their vascular characteristics have predictive value for postoperative recurrence? Recently, the incidence of postoperative recurrence for intracranial metastases was reported to be significantly higher in basal and HER2-positive breast cancer subtypes with similar correlations observed for the postsurgical recurrence of primary breast cancers. [58, 59] These findings suggest that a potential negative impact of invasive surgical procedures is the acceleration of dissemination and growth for BM originating from breast cancer subtypes with well-defined vascular signatures.

Radiation

Radiation treatments that are routinely used clinically for BCBM include WBRT, SRS and their combination. WBRT can be a highly neurotoxic treatment; however, it is often the treatment utilized for patients with poor prognosis and multiple lesions. SRS is a less debilitating technique and is preferred for the treatment of patients with better prognosis and/or limited metastases. Radiation treatment options are commonly used for all breast cancer subtypes and the first-line therapy for patients with TNBC due to the lack of systemic treatment options available for these patients.[54] It is important to note that besides its cytotoxic effects, radiation therapy also has been shown to induce BTB intracranial tumor opening in patients and in animal models,[60, 61] providing a strong rationale for combining radiation treatments and systemic therapies.

Because the choice of radiation treatment is dictated by the number and size of the metastatic lesions that are present, the measurement of the vascular BTB permeability of metastases to a contrast agent provides important information that can be used for accurate determination of treatment options, evaluation of the penetration of cancer therapeutics and patient response to cancer treatment. Monitoring and assessment of BM characteristics usually is performed using positron-emission tomography (PET), magnetic resonance imaging (MRI) and computed tomography (CT). The sensitivity of MRI and CT can be enhanced by paramagnetic gadolinium contrast agents such as gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA). Gd-DTPA has a size that is similar to many chemotherapeutic agents and does not pass through a healthy BBB; in contrast, after progression of intracranial disease, Gd-DTPA passes through the BBB and reaches brain tissue, resulting in the enhancement of signal intensities in postcontrast T1-weighted MRI. [62]

The analysis of metastatic brain lesions by contrast-enhanced MRI (CE-MRI) has demonstrated a high degree of heterogeneity of BCBM in animal models.[63] Similarly, dynamic CE-MRI (DCE- MRI), a diagnostic imaging technique providing information about the kinetics of gadolinium-based contrast agent accumulation in tissues, showed that before radiation treatment, BTB permeability was highly heterogeneous between patients and even between the lesions in the same patients.[61] Although no correlations with a histological grade or patient age were found, some correlations between BTB permeability and tumor size were noted. These data are in agreement with studies in animal models demonstrating that tumors with diameters of more than 0.5 mm were more permeable to sodium fluorescein, whereas more diffusely infiltrating tumors did not show significant permeability.

[64] As expected, radiation treatment had different effects on lesions depending on their initial permeability characteristics. Lesions with high permeability did not display any significant changes in BTB opening during the course of the study; in contrast, low-permeability lesions exhibited an up to 4.7-fold increase in permeability 1-2 weeks after radiation treatment and an up to 8.2-fold increase 1 month after radiation treatment.[64] Based on the combined evidence from patients with primary brain tumors and BM, we speculate that performing systemically administered therapies 1-4 weeks after the completion of radiation therapy might benefit low permeability metastatic lesions but offer no further advantage in lesions that are already highly permeable. These studies have important implications for understanding the effects of radiotherapy on opening the BTB. Moreover, although commonly believed, radiation does not always induce BTB disruption.

Targeted treatment

The discovery of HER2-targeted drugs, such as trastuzumab, pertuzumab, ado-trastuzumab emtansine (T-DM1) as well as multiple tyrosine kinase inhibitors significantly increased the survival of patients with HER2-positive breast tumors; however, a large proportion of these patients (app. 25-30%) eventually develop BM as the first site of recurrence.[54, 65] This can be explained by the increased invasiveness of HER2-enriched tumors in comparison with luminal tumors, but also the inability of HER2-targeted therapies to penetrate the BBB and BTB.[65] Although the BTB is considered to be more permeable than the BBB based on the experiments with fluorescent dyes and Gd contrast agents, the results obtained with these small molecules are not representative of the BTB penetration that would occur with larger molecules such as antibodies. Trastuzumab and pertuzumab are humanized monoclonal antibodies that bind to different extracellular domains of the human HER2 receptor. The BBB is only permeable to lipophilic molecules of up to about 400-500 Da, whereas conventional antibodies and drug-antibody conjugates of >150 kDa do not freely cross even the more permeable BTB barrier.[66] Nonetheless, it has been shown that the intracerebral concentration of trastuzumab was at least 400 times lower than in serum before radiotherapy but 70 times lower after radiotherapy.[67] In contrast, only 5% of the injected dose of radiolabeled trastuzumab could cross the BTB in a BCBM animal model;[68] moreover, no improvement was observed when trastuzumab was given after SRS. [69] The distribution of trastuzumab among metastatic lesions was highly heterogeneous and did not correlate with BM size, permeability or vessel density as well as the expression of tight junctions. [70] Comparison of tumor tissue from mice with BCBM and treated with a single equal dose of trastuzumab or T-DM1 revealed no differences in perivascular penetration, vessel density or vascular fraction for the two proteins.[71] However, after 14 days of treatment, T-DM1 decreased vascular fractions and reduced vessel diameter consistent with its improved anticancer efficacy.[71] An alternative to conventional antibodies is the use of single-domain anti-HER2 antibody fragments (also known as VHH molecules), which have an about 10-fold lower molecular weight than trastuzumab. Fluorine 18-labeled HER2-targeted single-domain antibody fragments were shown by PET imaging to penetrate the BTB and bind to HER2-expressing BCBM in a murine model.[72]

The effectiveness of targeted treatment of HER2-positive BCBM can be improved by using the combination of trastuzumab with other drug molecules;[54, 73] however, the design of

combination therapies does not consider the effects of these drug molecules on the permeability of trastuzumab explicitly. Recently, it was demonstrated that the accumulation of trastuzumab in brain lesions was significantly attenuated by co-treatment with the antiangiogenic agent bevacizumab, an observation which could reflect drug-induced changes in vascular structure.[70] These findings might explain the poor response to trastuzumab and antiangiogenic agent combinations in clinical trials[74] and emphasizes the importance of considering vascular permeability as a factor to guide in the selection of effective drug combinations.

Immunotherapy

Although breast cancer typically is not considered an immunogenic cancer, it has been demonstrated that breast cancer patients, particularly those with a HER2-enriched phenotype, can benefit from immune checkpoint blockade with anti-PD-1 and anti-CTLA-4 antibodies.[75] BCBM are characterized by an immune microenvironment with significantly lower levels of tumor-infiltrating lymphocytes than present in primary breast tumors, and BCBM frequently express PD-L1 and PD-L2 receptors.[76, 77] The therapeutic potential of immune checkpoint blockade in patients with BCBM remains to be ascertained because clinical data are not yet available for anti-PD and anti-CTLA therapies in patients with BCBM. This approach seems worth exploring because promising results were obtained when the immune blockade was evaluated for the treatment of BM originating from non-small lung cancer,[78, 79] suggesting that immune checkpoint inhibitors might be useful for the treatment of other brain malignancies. The permeability of the BTB to immune checkpoint antibodies remains largely unexplored but is an exciting question because it might provide valuable information if the anticancer effects of immunotherapy in BM are related to their impact on T cells inside the tumor or within the peripheral compartment.

Concluding remarks:

The formation of distant BM is associated with poor prognosis in breast cancer patients and is related to poor permeability of the BTB, which is a major factor in treatment failure. Therefore, increasing BTB permeability can be highly desirable for therapy because it facilitates increased passage of chemotherapeutic and other types of drugs through the barrier, which should improve treatment outcomes. The assessment of vascular permeability of BM in preclinical models and in the clinical setting provides valuable information about the heterogeneity of metastatic lesions, allows for monitoring disease progression and the response of patients to treatment. In addition, recently identified relationships between the permeability of metastatic lesions and the vascular organization of their primary breast cancers suggest that vascular characteristics might be useful for stratification of breast cancer patients with BM to select those that will most likely benefit from specific therapies, resulting in improved prognosis.

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Outstanding questions:

- Can the vascular characteristics of breast cancers serve as an indicator for identifying cohorts of patients with BM that will respond to antiangiogenic drugs?
- If BCBM undergo molecular changes, how does this affect their vascular characteristics?
- Is the permeability of “highly permeable” BCBM sufficient for effective drug uptake?
- What drug characteristics (molecular size, lipophilicity) would be suitable for treating “highly permeable” BCBM?
- How can we increase BTB permeability for improved chemotherapeutic and macromolecular delivery without compromising normal brain?
- Can the BTB be selectively disrupted without affecting the BBB?
- Do primary breast tumors and secondary BM exhibit the same barriers to delivery of antibodies and other macromolecular therapeutics?
- What are the reasons for the strikingly high heterogeneity of trastuzumab accumulation in metastatic brain lesions?
- Can the changes of *in vivo* permeability of trastuzumab and other macromolecules upon co-treatment with other drugs be used to rationalize the design and regimens of combination therapies?
- Are breast cancer BM permeable to immune checkpoint inhibitors?
- What types of preclinical models best recapitulate the hemodynamic characteristics of BM in patients, thereby providing useful tools for developing improved therapies for these patients?

Highlights:

- Blood vessels play an essential role in the formation of brain micrometastases as well as in the transformation from poorly permeable to highly permeable BM.
- BCBM are highly heterogeneous among different metastatic foci and even within the same tumor.
- The vascular organization of primary breast cancers facilitates blood-brain barrier penetration, leading to the formation of distant BM.
- The vascular permeability of brain lesions is an important parameter for evaluating disease progression, the course of treatment, and the efficacy of cancer therapies.
- The association between vascular permeability of BCBM and their accumulation of large molecules such as antibodies remains mostly unknown.

Fig. 1. Vascular signatures within different breast cancer subtypes.

Gene expression signatures from human breast tumors (left) and murine mammary tumor models (right) demonstrated an association between a breast cancer subtype and its vascular properties. The picture is adapted from reference [23] with permission from the publisher (Springer).

THE ROLE OF BREAST CANCER SUBTYPE IN BRAIN METASTASES

Fig. 2. The role of breast cancer subtype in brain metastases.

The incidence and characteristics of secondary BM are predisposed by the subtype of breast cancer and its vascular organization. The part of the figure demonstrating morphological characteristics of basal-like breast cancer and endothelial cells in 3D Matrigel culture is taken from reference [23] with permission from the publisher (Springer).

Brain metastases: BBB vs. BTB

Fig. 3. Brain metastases: BBB vs. BTB.

Upon the invasion of brain tissue by metastatic cancer cells, the BBB becomes disrupted, resulting in the formation of a more permeable BTB. This transformation occurs through a series of molecular transformations involving adjacent blood vessels. Surprisingly, only a few types of cancer cells can breach the BBB, while most of them are prevented from entering the brain. As a consequence of the impermeability of the BBB, most anticancer drugs cannot accumulate in the brain at the concentrations required to exert the desired therapeutic effect. [43, 44]