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# Advances in Decoding Breast Cancer Brain Metastasis

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## Abstract

The past decade has witnessed impressive advances in cancer treatment ushered in by targeted and immunotherapies. However, with significantly prolonged survival, upon recurrence, more patients become inflicted by brain metastasis, which is mostly refractory to all currently available therapeutic regimens. Historically, brain metastasis is an understudied area in cancer research, partly due to the dearth of appropriate experimental models that closely simulate the special biological features of metastasis in the unique brain environment; and to the sophistication of techniques required to perform in-depth studies of the extremely complex and challenging brain metastasis. Yet, with increasing clinical demand for more effective treatment options, brain metastasis research has rapidly advanced in recent years. The present review spotlights the recent major progresses in basic and translational studies of brain metastasis with focuses on new animal models, novel imaging technologies, omics “big data” resources, and some new and exciting biological insights on brain metastasis.

### Keywords

Breast cancer; Brain metastasis; Animal models; Genomics; Neuroimaging

## I. Introduction

Brain metastasis is the most common central nervous system (CNS) malignant disease, outnumbering primary gliomas by 10:1 [1,2]. Major solid cancers, such as melanoma, lung and breast cancers, produce high incidence of CNS metastasis [3,4]; though sporadic cases of brain metastasis have been reported for a wide variety of malignant cancers [5–8]. Despite dramatic advances in cancer treatment and prolonged patient survival brought upon by targeted and immunotherapies, a growing number of patients manifest brain metastasis in the clinic upon recurrence [9,10], for which the only treatment options remain palliative [11]. For example, the brain metastasis incidence upon recurrence has apparently accelerated since the early 2000s after widespread prescription of trastuzumab in HER2+ breast cancer patients [12,13]. Thus, more effective treatment options for brain metastasis is an emerging unmet need.

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Historically, brain metastasis is an understudied area in cancer research (Fig. 1B), partly due to the dearth of appropriate experimental models and to the sophistication of techniques for in-depth studies. However, history taught us that any revolutionary cancer therapies must come from solid and groundbreaking biological insights of the disease. The research of brain metastasis began to gain attention in recent years. A simple PubMed search for “brain metastasis” or “brain metastases” indicates an exponentially accelerating growth of publication in the topic since around 2010 (Fig. 1). Thus, this review will focus on the progresses that help us gain new insights on the disease; particularly, the new tools for scientific studies of brain metastasis. We will highlight several novel biological discoveries that shed new light on the mechanism of brain metastasis and may translate into outcome-improving clinical practices. For earlier developments of this field, the readers are referred to previous comprehensive reviews [14–17].

## II. New models and tools to study brain metastasis

### A. New brain metastasis models

Metastasis is a complex biological phenomenon resulting from the successful completion of multiple step of molecular and cellular processes, including leaving the primary tumor, entering the circulation (intravasation), surviving in the circulation, exiting from the circulation (extravasation). Colonizing at the secondary organ site, and finally outgrowing to symptomatic metastatic tumor [18]. Therefore, proper in vivo animal models are indispensable for scientific investigation of brain metastasis [19]. Novel nude rat breast cancer brain metastasis models seem to be good additions in the toolbox for brain metastasis studies [20]. A handful of in vivo brain metastasis models (Table 1) covering distinctive subtypes of breast cancer had been previously reported [21], yet no inflammatory breast cancer (IBC) brain metastasis models has been reported previously. Thus, recent report of new experimental brain metastasis models by tail-vein injection for several inflammatory breast cancer (IBC) lines is a good addition to the repertoire of in vivo breast cancer brain metastasis models [22]. Recently, breast cancer patient-derived xenograft (PDX) models have been successfully used to induce brain metastatic lesions for testing efficacy of dual inhibition of PI3K and mTORC1 in treating HER2+ human breast cancer brain metastasis in mice [23].

Of interesting note, a recent phase 2 clinical trial of pembrolizumab (anti-PD1) immunotherapy in melanoma and non-small-cell lung cancer (NSCLC) patients showed unprecedented durable activity against brain metastasis, suggesting that the host immune system may play critical roles in metastatic cancer outgrowth in the “immune-privileged” CNS microenvironment [24]. This counterintuitive clinical observation certainly warrants further in-depth mechanistic investigations. However, most reported in vivo brain metastasis models have been established using human cancer cells growing in immune-compromised mice. The use of xenograft models of human cancer cell lines or PDXs grown in mice has enabled many important discoveries about brain metastasis. However, xenografts in immunocompromised animals discount the impact of the adaptive immune system on preventing or promoting brain metastasis. To address this issue, the field needs to develop

partially humanized animal models to allow for evaluation of human cancer xenografts in an immunocompetent host, which is highly challenging.

To study the impact of immune system on brain metastasis, murine tumor-derived allograft in syngeneic mouse background can be an alternative approach, although mouse tumor models may not perfectly recapitulate human disease. To this end, our group has performed some pilot studies to expand the experimental brain metastasis models into immune-competent mouse strains. In addition to the previously reported brain metastatic 4T1 mammary tumor line [25,26], we have successfully established an experimental brain metastasis model using dissociated MMTV-Neu/PTEN-loss mammary tumor cells in the FVB female mice (unpublished data). Recently, we also showed that EO771, a spontaneous mammary tumor line from the C57BL/6 mice, is able to produce aggressive brain metastasis in the syngeneic host after intracarotid artery injection (unpublished data). Using these brain metastasis models in immuno-competent mice will shed more light on the roles of the host immune system in modulating brain metastasis, and enable development of strategies that potentially enhance the efficacy of powerful immunotherapy treatments against brain metastasis.

### B. Novel imaging techniques

Neuroimaging remains the mainstay modality for brain metastasis diagnosis [27,28]. There have been major advances in neuroimaging. For example, technological advances in multiphoton laser scanning microscopy have improved our ability to survey single steps of the CNS metastatic cascade via live imaging through a chronic cranial window in vivo [29]. A modified MRI modality has been applied to evaluate the integrity of the blood-brain barrier in mouse model bearing the MDA-MB-213 breast cancer brain metastasis [30]. Intriguingly, the glial cells are activated and have upregulated translocator protein (TSPO) in response to metastasis; thus, agents targeting the upregulated translocator protein (TSPO) on activated glia have been developed for SPECT/PET imaging [31]. Because glial activation begins at the very early stage of cancer cell metastatic colonization in the CNS, this method could potentially enable detection of brain metastases substantially earlier than the current clinical approach of MRI [31]. In the meantime, radio-immunotherapy has made significant strides forward in treating and monitoring brain metastasis progression using armed antibodies [32]. Confocal imaging of brain slices also prove to be a highly useful technique for the studies of brain metastasis [33]. Another interesting report described a bioluminescence-based imaging approach using the 5HRE-ODD-Luc reporter gene to assess the hypoxia conditions in intracranial lesions [34]. More recently, development of SMART 3D (Spatial filtering-based background removal and Multi-chAnnel forest classifiers-based 3D ReconsTruction), an integrative imaging platform/pipeline for 3D quantitative analysis of heterogeneous metastasis has been reported [35].

From a seemingly unrelated field, new tissue clearing technologies (termed CLARITY, for Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/Immunostaining/in situ-hybridization-compatible Tissue hYdrogel), developed in the neuroscience field, have dramatically facilitated researcher’s ability to see deep into the tissue, especially the brain [36]; it is reasonable to anticipate that the investigation of brain metastasis can borrow such

revolutionary neuroscience technologies to enable detailed delineation of cancer cell behavior deep in the brain. Collectively, it can be envisioned that complementary applications of various new advanced imaging technologies (Table 2) will greatly enhance our ability to visually depict the biological interactions taking place in CNS metastasis at the molecular and cellular levels, which will provide structural insight on functional implications.

**Omics technologies for deep understanding of brain metastasis—**Taking advantage of the rapid progress in technologies enabling comprehensive interrogation of biological systems at the molecular levels, investigators have studied brain metastasis at the genome [37–39], epigenome [40–42]; transcriptome [43], proteome [44–46], and metabolome [47] levels and made interesting findings [48,49]. Several existing datasets (Table 3) have provided great value and insights to the studies of brain metastasis [50,51]. For example, large-scale next-generation sequencing (NGS) was performed for comprehensive genomic profiling of advanced squamous cell lung cancer cells from 79 patients with stage IV disease and revealed PI3K aberrations and clonal heterogeneity in brain metastasis [52]. Built on an 86-patient case series containing multiple tumor types (including lung, breast and renal cell carcinomas), whole-exome sequencing (WES) of matched clinical examples were used to delineate the evolutionary progression from primary cancer to brain metastasis [53]. Intriguingly, the epigenomic landscape of melanoma brain metastasis was also explored for potential new therapeutic targets, which revealed that DNA hypermethylation of the HOX family genes, particularly HOXD9, suppresses its transcriptional activity and promotes brain metastasis. [41]. Interestingly, a 2D-DIGE (Difference in Gel Electrophoresis) proteomic analysis was performed to identify potential regulatory networks in breast cancer brain metastasis [54], and similar integrated genomic profiling of breast cancer brain metastasis sheds important new light by identifying common molecular alterations of the disease, such as enriched overexpression of cell cycle and G2/M transition pathway genes and increased overall methylation [42].

Many of these comprehensive profiling studies utilize patient specimens that add significant clinical relevance for the data from such projects; yet there is a general lack of concordance for the conclusions of these studies, because a variety of reasons may complicate the interpretation, such as the heterogeneity nature of source materials. A general consensus framework still waits to be developed for the molecular alternations that promote brain metastasis. Still, the gradual buildup of these “big data” resources is a great leap forward for the field that will undoubtedly facilitate generation of new hypothesis or verification of existing hypothesis with precious patient-derived information.

## III. New biological insights on brain metastasis

### A. Epigenetic regulations and microRNA in brain metastasis

Epigenetic dysregulation is an integral part of the tumorigenic and metastatic processes, and simultaneous modulation of multiple genetic programs through microRNA is an important epigenetic regulatory mechanism [40]. For example, our team found that PTEN in metastatic breast cancer cells was epigenetically and reversibly down-regulated by astrocyte-released

microRNA19-a [50]. MicroRNA profiling that compares the primary and brain metastatic breast cancer specimens identified miR-509 as a critical determinant for brain metastasis [55]. In another study comparing breast cancer patients with or without brain metastasis, up-regulation of microRNA-10b was identified to be strongly associated with the development of brain metastasis [56]. Several microRNAs were able to suppress brain metastasis in breast cancer cells: 1) microRNA-7 down-regulates KLF4 in the cancer stem-like cell (CSC) population of MDA-MB-231 brain-seeking cells [57]; 2) microRNA-146a targets beta-catenin and hnRNPC in the MDA-MB-435 experimental brain metastasis model [58]; 3) microRNA-1258 targets heparanase to suppress brain metastasis in the MDA-MB-231 experimental brain metastasis model [59]. In contrast, miR-141 was shown to promote brain metastasis in several breast cancer cell lines [22].

Similar studies in lung cancer also identified miR-145-5p [60], microRNA-95-3p [61], and microRNA-378 [62] as important determinants in brain metastasis. In lung-to-brain metastasis initiating cells, STAT3 was found to regulate target miR-21 that promotes the brain metastasis phenotype [63].

Notably, because of the multi-target nature of microRNA regulation, they may serve as better classifier for brain metastasis prediction than gene expressions. To that end, studies have aimed to identify microRNA signatures as predictive biomarker for the development of brain metastasis in melanoma [64] and lung cancer [65]. In addition to microRNA, long noncoding RNA (lncRNA) is also an important epigenetic regulatory mechanism [66]. Indeed, it was demonstrated that lncRNA MALAT1 promotes brain metastasis by inducing epithelial-mesenchymal transition in lung cancer [67]. The exploration of epigenetic alterations in brain metastasis may lead to novel and alternative targets for therapeutic intervention.

### B. Interactions between metastatic cancer cells and CNS microenvironment

An essential feature of metastatic cancer cells is its intimate interaction with the stromal cells in the host organ sites, which could provide a permissible and even supportive microenvironment for metastasis to thrive. Having a highly heterogeneous cell populations and complex cellular structure, the brain stromal cells interact intimately with the invading metastatic cancer cells; however, the mechanistic insights remain elusive. In the past several years, major progresses have been made in this realm with several landmark publications. Our group characterized an intricate relationship among breast cancer cells, astrocytes and microglial cells where astrocyte-released exosomes transfer PTEN-targeting microRNA into cancer cells to mediate PTEN down-regulation in the cancer cells, resulting in CCL2 up-regulation and recruitment of brain metastasis-promoting microglia cells [50]. Interestingly, a reciprocal interaction of mutual promotion of cancer cell proliferation and astrocyte cell survival was also found to be an early event of prostate cancer brain metastasis [68]. Faithful to its essential role in mediating CNS homeostasis, astrocytes were found to facilitate melanoma brain metastasis via secretion of IL-23 and reciprocal up-regulation of MMP2 and metastatic invasion of the cancer cells [69]. Intriguingly, there is another new-found reciprocal interaction where cancer cells promote the assembly of carcinoma-astrocyte gap junctions and utilize these communication complexes to up-regulate secretion of

inflammatory cytokines from astrocytes, which ultimately supports tumor growth and survival of cancer cells via the STAT1 and NF-kappaB pathways [51]. Additionally, the CNS stroma-derived plasmin and the plasminogen activator (PA) inhibitory serpins from cancer cells represent another interaction that enhances metastatic outgrowth by promoting cancer cell survival and vascular co-option [70]. All these in-depth mechanistic discoveries are exciting and much hope has been placed on translating these novel findings into groundbreaking therapeutic strategies against brain metastasis.

### C. Blood-brain barrier and brain metastasis

Blood-brain barrier (BBB) refers to specialized blood vessel structures in the CNS that are lined by endothelial cells fortified with tight junction complexes, which effectively shield the brain parenchyma from cells and macromolecules in the general circulation; the brain-facing side of the BBB is surrounded by a thick basement membrane, which is supported by pericytes; and then astrocytic end-feet make the outer layer of the BBB. BBB provides a sanctuary organ environment for neuronal functions [71,72]. Apparently, BBB plays crucial roles in 1) affecting metastatic cell colonization in the brain, and 2) modulating treatment efficacy of brain metastasis by serving as a formidable barrier for the majority of therapeutic agents [73–77]. To establish a metastatic lesion, cancer cells have to first pass the BBB for successful extravasation into the CNS parenchyma [77,78]. Many systemically efficacious anti-cancer therapeutics cannot penetrate the BBB and become ineffective as brain metastasis treatment regimen. Therefore, there have been extensive efforts recently aiming to understand the biology of tumor-BBB interaction and to find novel ways that facilitate drug delivery through the BBB. To this end, the permeability of blood-brain barrier is highly heterogeneous at sites of metastasis [79,80,30]. Furthermore, pericytes were shown to have significant contribution to the permeability of blood-tumor barrier in multiple experimental breast cancer brain metastasis models [81]. Src kinase was found to promote the breast cancer cell extravasation through the BBB [82]. Ex vivo assessment of the blood brain barrier with Evans blue also proved to be a useful technique in studies of breast cancer brain metastasis models [82,83]. And new efforts that take advantage of nanotechnology to overcome the BBB demonstrated promising results [84,85]. Intriguingly, focused ultrasound was shown to disrupt the BBB and thus facilitated antibody delivery to the brain metastasis lesions resulting in growth inhibition [86]. Overall, there have overwhelming evidence to support the notion that BBB is highly heterogeneous and selective in the cases of brain metastasis; and new delivery technology such as nanomedicine may finally bring into reality that BBB be disrupted to allow efficient delivery of therapeutics into the brain for brain metastasis treatment.

## IV. Conclusion

Scientific and clinical investigations of brain metastasis have boomed in the past decade. Ongoing efforts are aimed for deeper mechanistic understanding and better treatment options of brain metastasis. The right approach is to tackle the challenging issues of brain metastasis from multiple new angles, such as epigenetic alterations in the cancer cells, reciprocal communications between the CNS stromal cells and metastatic cancer cells, different ways to modulate the BBB to improve treatment efficacy, and others. At the same

time, a continuous development of new and better experimental technologies (better animal models, higher resolution of tissue imaging, comprehensive and thorough molecular characterization of disease specimens, nanotechnology-enhanced delivery, etc.) certainly expedite the speed of mechanistic and functional discoveries. It is hopeful that such concentrated attacks on multiple fronts of this dreadful disease may lead to major breakthroughs that will significantly and meaningfully improve the therapeutic efficacy and clinical outcome for brain metastasis patients in dire needs.

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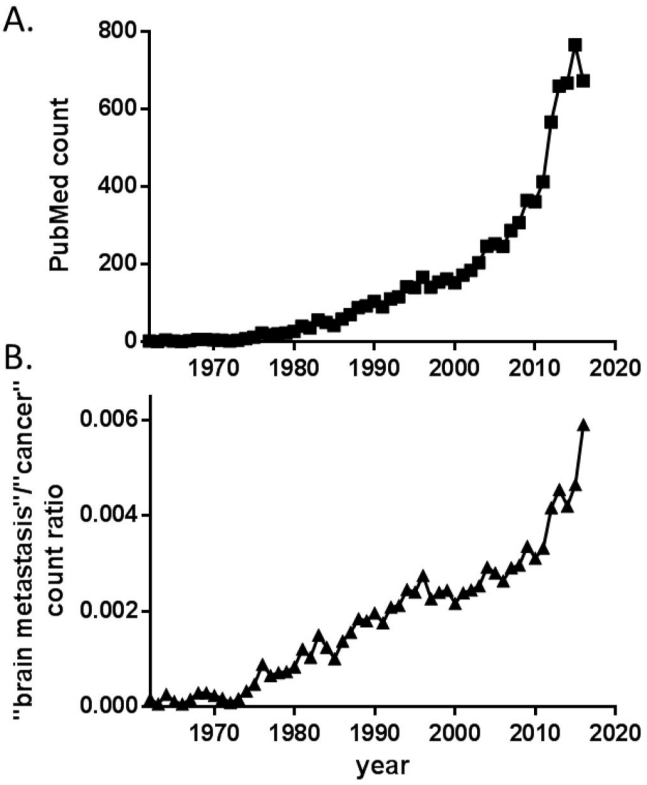


**Figure 1.**

Acceleration of brain metastasis research since 2010. (A) Yearly publication counts using “brain metastasis” or “brain metastases” as search keyword in PubMed. (B) Paper counts featuring “brain metastasis” normalized to publication counts using general search term “cancer”.

**Table 1**

In vivo breast cancer brain metastasis models

| Subtype | Human (xenograft) | Rodent (syngeneic) |
|---|---|---|
| **TNBC** | MDA-MB-231<br>MDA-MB-435<br>HCC70 | 4T1 (BALB/c)<br>EO771 (C57BL/6) |
| **HER2+** | MDA-MB-361<br>BT474<br>HCC1954<br>DF-BM354 (PDX)<br>DF-BM355 (PDX) | MMTV-Neu/PTEN$^{-/-}$ (FVB) |
| **ER+** | MCF-7<br>T-47D | – |
| **IBC** | SUM149<br>MDA-IBC3 | – |

Note: TNBC, triple-negative breast cancer; IBC, inflammatory breast cancer.

**Table 2**

Several new research-facilitating imaging technologies for brain metastasis

| Name | Description | Ref |
|---|---|---|
| MPLSM | Multiphoton laser-scanning microscopy through a chronic cranial window to enable long-term in vivo microscopy. | [29] |
| Gd-MRI | Gadolinium-enhanced MRI used to evaluate the blood-brain barrier (BBB) integrity in brain metastasis mouse model. | [30] |
| TSPO-targeted SPECT/PET | Non-invasive SPECT/PET imaging based on induction of translocator protein (TSPO) expression following glial activation. | [31] |
| 5HRE-ODD-luc | HIF-1α reporter construct, 5HRE-ODD-luc, enables non-invasive imaging of hypoxia dynamics in brain metastasis mouse model. | [34] |
| SMART 3D | An integrative imaging platform that enables multiplexed quantitative 3D analysis of metastasis in situ from the molecular level to the whole organ scale. | [35] |
| CLARITY | Transformation of intact tissue into a nanoporous hydrogel-hybridized form for transparent visualization. | [36] |

Note: SMART 3D, Spatial filtering-based background removal and Multi-chAnnel forest classifiers-based 3D ReconsTruction; CLARITY, Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/Immunostaining/in situ-hybridization-compatible Tissue hYdrogel.

## Table 3

Public datasets related to brain metastasis

| Accession | Description |
|---|---|
| **GSE14020** | Affymetrix microarray profiling of distinct organ-specific metastatic tumors (brain, bone, lung and liver) of breast cancer patients. |
| **GSE12276** | Affymetrix microarray profiling of primary breast cancer tumors with clinical follow-up of brain or other metastatic relapse and survival. |
| **GSE2603** | Affymetrix microarray profiling of MDA-MB-231 sublines of different metastatic tropisms (bone and lung) and primary breast cancer tumors with metastasis follow-up. |
| **GSE2034** | Affymetrix microarray profiling of primary human breast cancer tumors with brain metastasis follow-up information. |
| **GSE3141** | Affymetrix microarray profiling of primary lung cancer specimens with clinical follow-up data of brain metastasis-free survival time. |
| **GSE14690** | Illumina DASL (the c**D**NA-mediated **A**nnealing, **S**election, extension and **L**igation) cancer panel profiling of matched and unmatched breast cancer primary tumor and brain metastasis. |
| **GSE20016** | NHGRI (National Human Genome Research Institute) cDNA array profiling of laser-captured epithelial cells from resected human brain metastases and unmatched primary breast tumors. |
| **GSE38057** | Illumina DASL cancer panel of primary HER2+ breast tumors with or without brain metastasis relapse. |
| **GSE19184** | Illumina human and mouse beadchip profiling of 4 different types of cancer (MDA-MB-231Br3, PC14Br4, KM12M, A375SM) experimental brain metastases and orthotopic xenografts in mice. |