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Review article

Experimental models for cancer brain metastasis

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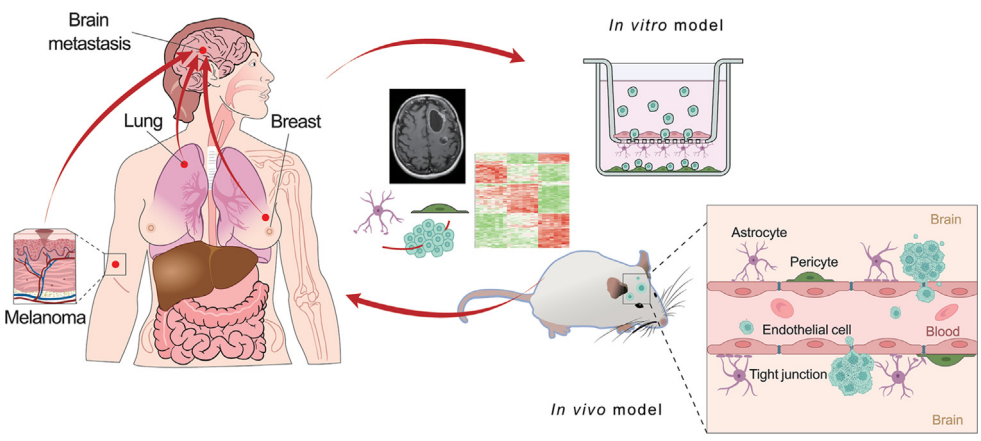
HIGHLIGHTS

- The lack of an acceptable and reliable guide for selecting *in vitro* or *in vivo* brain metastasis models hinders the development of brain metastasis therapies.
- There is an urgent need to employ accurate *in vitro* and *in vivo* models to recapitulate the complexities of brain tumor metastasis and to unravel the intricate cellular and physiological processes involved.
- Precise *in vitro* and *in vivo* brain metastasis models are crucial for investigating cellular and molecular mechanisms and serve as preclinical platforms to assess novel treatments.
- An array of emerging techniques, such as bio-three-dimensional (3D) printing, novel real-time imaging, artificial intelligence, and precise gene editing, holds promise for redefining the landscape of cancer brain metastasis model development.

GRAPHICAL ABSTRACT



Due to increasing incidence and limited treatments, brain metastases are an emerging unmet need in modern oncology. With the majority of brain metastases occurred in patients with lung cancer, breast cancer, and melanoma. Unraveling the multifaceted cellular and physiological processes associated with metastasis is best achieved by using *in vivo* and *in vitro* models that recapitulate the requisite tumor cell and microenvironment mechanisms at the organismal level. Analyses based on suitable model of metastasis will lead to clinic doctors better stratification of cancer patients and discover of new therapeutic opportunities.

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ABSTRACT

Brain metastases are a leading cause of cancer-related mortality. However, progress in their treatment has been limited over the past decade, due to an incomplete understanding of the underlying biological mechanisms. Employing accurate *in vitro* and *in vivo* models to recapitulate the complexities of brain metastasis offers the most promising approach to unravel the intricate cellular and physiological processes involved. Here, we present a comprehensive review of the currently accessible models for studying brain metastasis. We introduce a diverse

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Animal model
In vitro model

array of *in vitro* and *in vivo* models, including cultured cells using the Transwell system, organoids, microfluidic models, syngeneic models, xenograft models, and genetically engineered models. We have also provided a concise summary of the merits and limitations inherent to each model while identifying the optimal contexts for their effective utilization. This review serves as a comprehensive resource, aiding researchers in making well-informed decisions regarding model selection that align with specific research questions.

Introduction

Cancer remains a substantial global health challenge, with over 19 million new cases reported each year.[1] Despite the overall progress in cancer treatment, options for managing metastatic cancers, particularly brain metastases, remain constrained.[2–4] The distinct characteristics of the brain, including the blood–brain barrier (BBB), intricate microenvironment, and immune privilege, all pose significant obstacles to devising treatments for cancer cells that metastasize to the brain. Developing innovative strategies that significantly enhance therapeutic efficacy in the context of metastatic brain tumors remains the "holy grail" of cancer research.

Metastasis is a complex process in which cancer cells arise from primary tumors and tend to metastasize to specific organs.[5] For metastatic cells, these steps include the separation of malignant cells from the primary neoplasm, intravasation, and invasion through the bloodstream and lymphatic system, subsequent entry into the circulatory system, circulating tumor cell (CTC) extravasation into specific organs, and adaptation to foreign organ microenvironments.[6] However, in brain metastasis, the crossing of cancer cells across the BBB is a pivotal event. Researchers have studied this process for many years and gained insight into the mechanisms by which cancer cells transmigrate through the BBB. This involves adhesion molecule-mediated translocation, disruption of the basement membrane, and modulation of BBB permeability.[7] Upon successful crossing of the BBB, cancer cells colonize the brain parenchyma and reshape the surrounding niche. During this period, various molecules and cytokines play crucial roles in the colonization and growth of cancer cells by promoting cell proliferation, inhibiting apoptosis, and reciprocal interactions.[8]

Precise brain metastasis models are crucial for thorough investigations into the cellular and molecular mechanisms underlying the development of this disease. They also serve as preclinical platforms for assessing the effectiveness of novel treatment approaches. Current models come in a variety of forms, including *in vitro* models such as cell cultures, three-dimensional (3D) organoids, and multicellular microfluidic setups, and *in vivo* models such as syngeneic, xenograft, patient-derived, and genetically engineered mouse models (GEMMs). Each model has distinct advantages; however, each faces considerable limitations, which underscores the complexity of replicating multifaceted processes and conditions integral to brain metastasis. Echoing the renowned adage that "every model is flawed, yet some are useful," it holds true that a singular model often can only recapitulate a specific facet of the intricate processes and conditions involved in brain metastasis. For instance, *in vitro* models allow controlled manipulation and observation of isolated cellular responses but lack the dynamic complexity of interactions observed in living organisms. In contrast, *in vivo* models, including patient-derived xenografts (PDXs) and genetically engineered mice, offer a closer approximation of the human physiological context. However, they may not fully recapitulate the metastatic journey because of variations in immune response, genetic background, and species-inherent differences. The careful selection of models that align with scientific questions serves as the cornerstone for rigorous and credible research. This enhanced the validity and clinical relevance of our conclusions.

To address the need for accurate model selection in the study of brain metastasis, we offered an extensive review of currently available model options. We introduce the establishment, advantages, practical applications, and limitations of each model [Table 1]. In addition, we discuss further development of these models using emerging techniques. This review offers a comprehensive perspective and valuable insights to guide the appropriate utilization of models for studying brain metastasis.

In vitro models of brain metastasis

Blood–brain barrier transwell model

In brain cancer metastasis, tumor cells must penetrate the BBB to infiltrate the brain. The BBB is a selective safeguard barrier for the brain, permitting the entry of essential nutrients while restricting hazardous substances.[9,10] It consists of endothelial cells lining the blood vessels, along with astrocytes/pericytes enveloping the vessels to maintain BBB integrity and permeability.[11,12] The Transwell devices are widely used to model the BBB *in vitro*. This system entails a semipermeable porous membrane with brain endothelial cells/astrocytes grown on it, creating two separate compartments representing the bloodstream and brain side[13] [Figure 1A].

The BBB-Transwell model is a valuable tool, especially for studying the process of cancer cells traversing the BBB.[14] In this scenario, cancer cells are seeded into the upper compartment, a tight monolayer of brain endothelial cells grows on the membrane, and astrocytes/pericytes are added to the lower compartment to create a brain-like

Table 1
Summary of current models of brain metastasis.

General type of modeling	Model	Advantage	Disadvantage
In vitro	BBB-Transwell model	• Low cost • Short time • High-throughput screening	• Static system • Lack of adaptation to microenvironmental cells
In vitro	Organoid	• Three-dimensional • Self-organized	• High cost • Lack ECM proteins
In vitro	Microfluidic BBB model	• Dynamic observation • High compacity with other techniques • High bionic hemodynamics	• Time-consuming • Specified expertise • Not suitable for high-through screening
In vivo	Allograft models	• High stability • Facilitate observation	• Artificial tumor microenvironment • High mortality
In vivo	Genetically engineered models	• Mimicking the process of cancer cells metastasizing from the primary site to the brain • High fidelity in reflecting tumor and microenvironmental reciprocal interaction	• Rarely generate metastasis • Extracranial metastases

BBB: Blood–brain barrier; ECM: Extracellular matrix.

microenvironment.[15]

This model offers a powerful means to investigate the interactions between metastatic cancer cells and BBB cell types. Utilizing this model, Fujimoto et al. discovered that pericytes in the BBB can suppress the penetration of metastatic cancer cells into the brain. In addition, conditioned medium from pericytes inhibits the proliferation of cancer cells.[16] Interestingly, the other major non-endothelial cell type in the BBB, astrocytes, were found to play an opposing role to that of pericytes. Using the BBB-Transwell model, Chen et al. determined that metastatic lung cancer cells and BBB astrocytes could assemble a junction consisting of protocadherin 7 and connexin 43. This engagement stimulates cytokine production, such as interferon-α (IFNα) and tumor necrosis factor (TNF) from astrocytes, which supports tumor growth and resistance.[17]

The BBB-Transwell system also serves as a convenient platform for introducing experimental interventions. For example, one can modify the culture conditions or manipulate the genetics or protein expression of lung cancer cells, endothelial cells, and astrocytes/pericytes to investigate their impact on cancer cell migration and metastasis.

Using a BBB-Transwell model comprising human brain endothelial cells, astrocytes, and lung cancer cell lines, Zhu et al. found that the CXC-chemokine receptor 4 (CXCR4) antagonist (AMD3100) inhibits lung cancer cell proliferation and migration.[18] Yin et al. used a BBB-Transwell model, in which brain capillary endothelial cells (BCECs) were seeded in the upper chamber and H1975 non-small cell lung cancer (NSCLC) cells were adhered to the lower chamber, to evaluate the penetration efficiency of the epidermal growth factor receptor (EGFR) -tyrosine kinase inhibitors (TKIs) or programmed cell death-ligand 1 (PD-L1) antibody across the BBB and its efficacy in inhibiting lung cancer cell proliferation.[19]

In summary, the BBB-Transwell model provides a controlled and reproducible system for studying brain metastasis. The use of Transwell devices is relatively simple and does not require complex equipment or techniques, allowing convenient large-scale cell migration experiments. Using the Transwell device, the number of cells that pass through the semipermeable membrane can be quantitatively analyzed to evaluate cell migration ability and invasiveness. The microenvironment of the upper and lower chambers can be easily manipulated by adding various factors and cytokines, enabling studies on how chemotactic signals, cell adhesion molecules, and therapeutics affect the ability of tumor cells to penetrate the brain. However, it is important to note that although the BBB-Transwell model is powerful, it has considerable limitations. The 3D structure of brain tissue and complex cell–cell interactions cannot be recapitulated. Moreover, because the cancer cells in the upper compartment of Transwell devices are static, the influence of the bloodstream and hemodynamics on cell migration cannot be explored. Further development of *in vitro* models for brain metastasis is required to address these scenarios.

Organoid

Organoids are 3D structures generated from stem cells or dissociated tissues that mimic the architecture, cellular composition, and

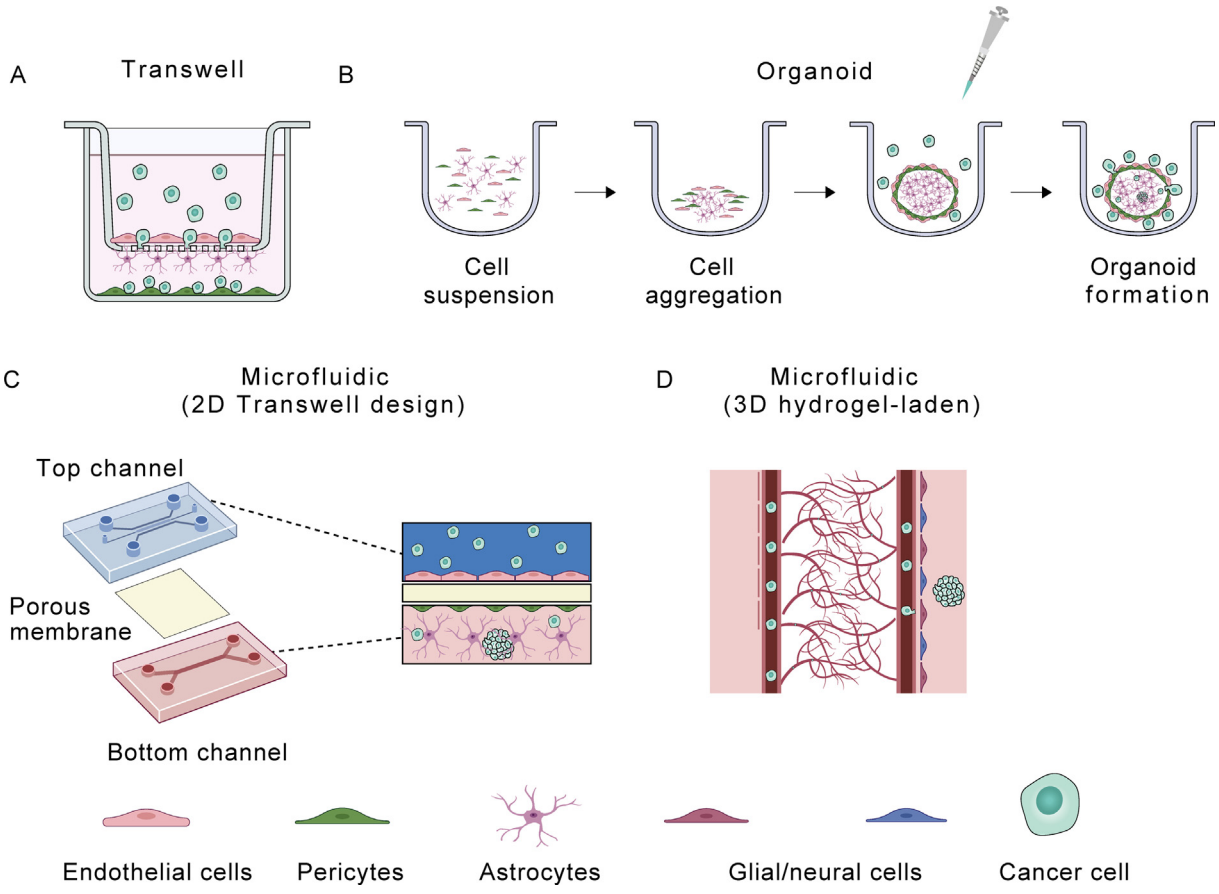

Figure 1. *In vitro* brain metastasis models. (A) Transwell models are simple and commonly used. It consists of a porous membrane where endothelial cells are cultured, situated between the top and bottom or opposite sides of the chamber where other cell types are cultured. (B) Brain metastatic organoids were generated by the spontaneous assembly of endothelial cells, pericytes, and astrocytes in a low-attachment culture vessel after the addition of cancer cells. (C) Microfluidic model with a 2D Transwell-like design, consisting of a porous membrane sandwiched between two or more channels, where the top channel is typically cultured with cancer cells and endothelial cells, and the bottom channel contains other cell types. (D) 3D hydrogel-laden microfluidic platform in which cancer cells and glial/neural cells in co-culture were seeded in one channel and allowed to self-assemble to form a microvascular network. 2D: Two-dimensional; 3D: Three-dimensional.

function of specific organs or tissues.[20,21] Brain organoids provide a more faithful representation of the intricate 3D structure of the brain tissue and the dynamic cell–cell interactions that occur within it, circumventing one of the limitations of the Transwell system. When cancer cells were co-cultured with brain organoids, they adhered to the surface, proliferated, and migrated into the brain organoids, mimicking the cancer brain metastasis process[22] [Figure 1B].

Quaranta et al. established a platform for small-cell lung cancer (SCLC) brain metastasis using brain organoids co-cultured with SCLC cell lines. By labeling cancer cells with the mKate 2 fluorescent tag, they found that cancer cells could invade the organoids within 24 h of co-culture, and they continued to migrate and proliferate, resulting in diffuse infiltration of the entire organoid in less than a week.[23] Using a brain organoid model, Choe et al. demonstrated that the lung-specific X protein (LUNX) promotes lung cancer cell proliferation and invasion into the brain. The researchers have also reported a notable trend in astrocyte accumulation around invading tumor cells.[24]

In the context of lung cancer brain metastasis, brain organoids provide a powerful platform for studying the complex interactions between tumor cells and the brain microenvironment. This platform is amenable to genetic manipulation, drug perturbations, temporal longitudinal tracing, and screening using high-content imaging, and high-throughput omics. However, brain organoids have certain limitations. Although these models exhibit some features of the human brain, they do not fully replicate the complexity and functionality of an entire organ. Moreover, this *in vitro* model lacks the immunological components that are often involved in cancer metastasis. With further advancements in organoid technology, including the integration of immune cells, these platforms should gain increased physiological relevance and offer deeper insights into the mechanisms underlying cancer brain metastasis.

Microfluidic blood–brain barrier model

Both Transwell and organoid models share a common limitation: the absence of blood flow, which is a critical parameter in the context of brain metastasis. A microfluidic platform overcomes this limitation by providing a highly controlled fluid flow that mimics complex biological fluid environments, including blood circulation and hemodynamic conditions. Microfluidic platforms have evolved from an orthodox Transwell design, which comprises porous membranes placed between the upper and lower channels to create a sandwich-like assembly. In this sandwich design, endothelial cells are cultured in the upper channel, whereas the lower channel is seeded with other brain cells such as pericytes and astrocytes. The involvement of the two microchannels stimulates the passage of the culture medium, representing the dynamic nature of circulating blood and the extracellular matrix[25] [Figure 1C]. This platform also allows precise quantitative analysis of cancer cell migration by offering parameters such as cell numbers, velocities, and distances.

Liu et al. developed a multiorgan microfluidic model for studying lung cancer brain metastasis. This chip consists of two biomimetic organ units – an upstream "lung" and a downstream "brain," characterized by a functional BBB structure. It allows real-time visualization and monitoring of the entire process of lung cancer brain metastasis, from the growth of the primary tumor to breaching the BBB and eventually reaching the brain parenchyma. Using this platform, the authors demonstrated that aldo-keto reductase family 1 B10 (AKR1B10) was significantly elevated in lung cancer cells that successfully reached the brain.[26] Microfluidic platforms are powerful tools for studying drug resistance. The antimetastatic effects of drugs can be measured by the transmigration of lung cancer cells across the BBB. Xu et al. combined a microfluidic chip model with proteomics to explore the mechanisms of acquired drug resistance in lung cancer-derived brain metastasis. They found that cancer cells with acquired resistance to chemotherapeutic agents (cisplatin, carboplatin, and pemetrexed) and TKIs showed a substantially altered spectrum of protein expression. The hyperactive glutathione metabolism pathway and aldehyde dehydrogenases (ALDH1A1 and ALDH3A1) were significantly overexpressed in drug-resistant brain metastases, offering new insights into acquired drug resistance in lung cancer brain metastases.[27]

The microfluidic BBB model has gained significant popularity as a valuable tool for studying lung cancer brain metastasis, owing to its ability to replicate blood flow, incorporate multiple cell types in human brains, manipulate culture conditions or genetics, and provide a quantitative readout of cell migration. The recent development of this platform through the introduction of 3D printing technology has further enhanced its strength. Using 3D printing, a hydrogel matrix with intricate microchannels and spatial orientation is introduced into the microfluidic system, mimicking the 3D analogy of the native microenvironment during lung cancer cell penetration into the brain tissue[28] [Figure 1D]. This analogy allows for improved tumor cell and BBB communication and provides cell attachment sites for the bioactivation of the cellular factors required for cell proliferation, differentiation, and migration in *in vitro* cultures. Overall, microfluidic devices offer a physiologically relevant and controlled platform for investigating various aspects of lung cancer brain metastasis.

Although microfluidic platforms offer a plethora of advantages in research applications, particularly in the study of brain metastases, they come with their own set of limitations. A primary limitation is the inherent complexity associated with the design and fabrication of microfluidic devices. Specialized equipment and a certain level of expertise are often required to achieve the desired precision and functionality. As the field of microfluidics grows, there is an observable lack of standard protocols and device designs. This absence makes drawing comparisons between studies and replicating results a significant challenge. Polydimethylsiloxane (PDMS) is the predominant material used in the construction of microfluidic devices, owing to its notable attributes such as optical clarity and biocompatibility. However, PDMS tends to absorb small hydrophobic molecules, potentially introducing variables that may skew experimental results.

In conclusion, although microfluidic platforms have revolutionized many aspects of biomedical research, researchers must remain cognizant of their limitations. Addressing these challenges will pave the way for more refined and universally applicable research outcomes.

***In vivo* models of brain metastasis**

Syngeneic allograft models

Syngeneic models are transplantation models obtained by injecting a recipient mouse of a specific genetic background with cell lines previously established through the isolation of tumor cells from a mouse with the same genetic background[29] [Figure 2A]. The advantage of syngeneic models lies in the fact that the transplanted cells, tumor microenvironment, and host are from the same strain, making this model particularly relevant for studying the process of metastasis.[30] Different cell lines have been developed as syngeneic metastasis models, primarily in C57BL/6 and BALB/c mice. These cells have different features that must be considered when choosing your syngeneic model of interest, including tumor origin (melanoma, lung, breast, etc.). The first mouse metastasis model was the B16 melanoma model, which was developed from a spontaneous mouse tumor that metastasized to the lung and brain when reintroduced into syngeneic mice.[31] The B16 model laid the foundation for the seminal work of Fidler et al., which established many fundamental concepts of metastasis progression, including organ tropism, clonal diversity, and tumor heterogeneity.[32] This model remains the gold standard for studying metastasis and has revealed many aspects of metastatic progression over the years, from elucidating essential immune system components that have paved the way for advances in current immunotherapies to more recent genome-wide screening studies identifying microenvironmental regulators of metastasis.[33,34]

Another syngeneic model is the *KRAS-G12D* lung adenocarcinomas (LUADs) mouse model. Inactivation of *Trp 53* in the *Kras*-driven LUAD

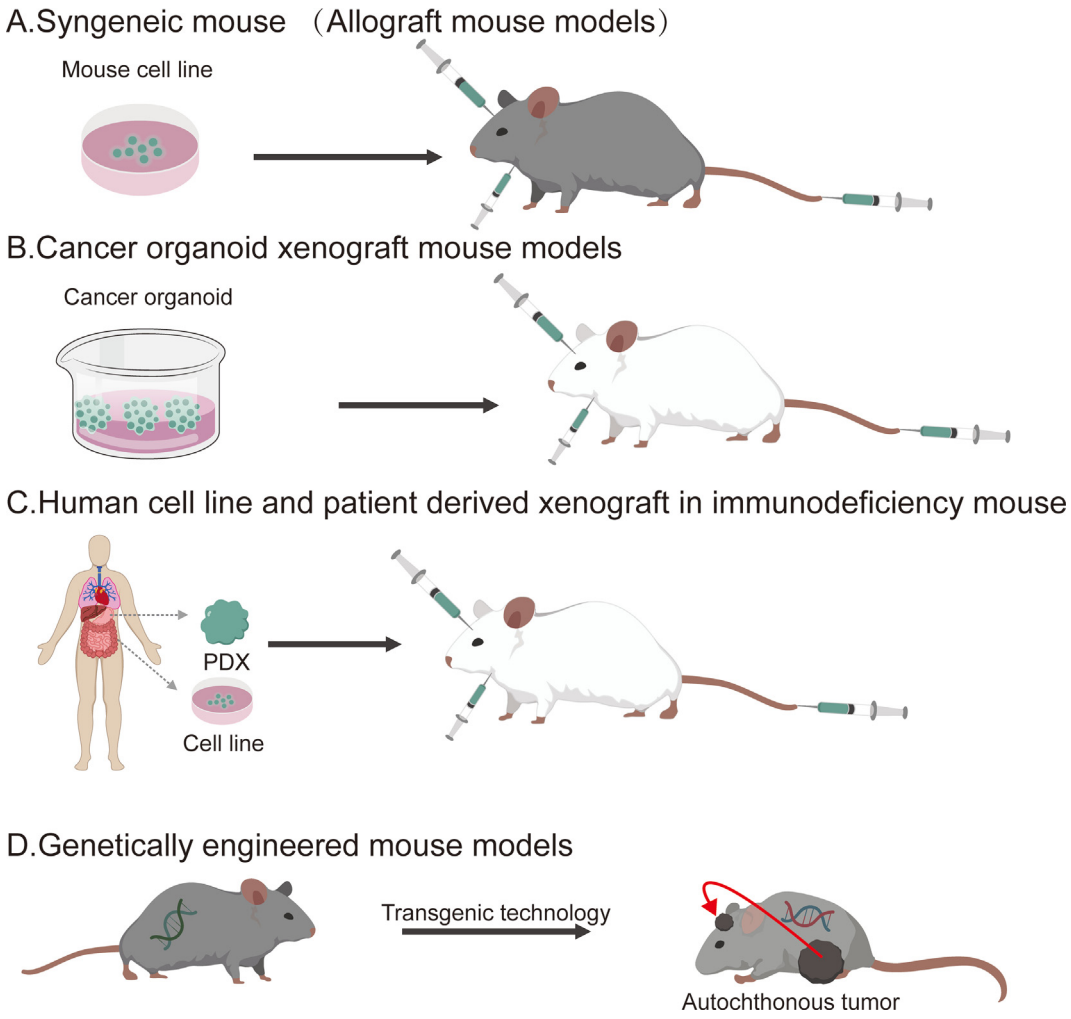

Figure 2. *In vivo* brain metastasis mouse models. (A) Syngeneic mouse model: mouse tumor cells were implanted into immune-competent mice. (B) Cancer organoid xenograft mouse model: tumor organoids were transplanted into immunodeficient mice. (C) Human cell line and patient-derived xenograft in immunodeficient mouse models: A human tumor cell line or the given graft from surgical resection or biopsy was implanted in immunodeficient mice. (D) Genetically engineered mouse models: tumor suppressor gene deletion or oncogene activation was established in mice. PDX: Patient-derived xenograft.

model promotes local and distal metastasis, accompanied by more rapid tumor progression, highlighting the importance of this tumor suppressor pathway in constraining metastasis formation *in vivo*.[35] Jing et al. developed a mouse model of lung cancer brain metastasis by initiating LUAD tumors in *KrasLSL-G12D/+*; *Trp53flox/flox* (KP) mice through intranasal delivery of Cre recombinase. They derived a cell culture population from an early-stage LUAD lesion, which they named KPad1 cells. When injected into syngeneic immunocompetent C57BL/6 mice or C57BL/6-derived B6-albino mice, KPad1 cells show an indolent metastatic phenotype compared with cells derived from aggressive KP LUAD tumors. When injected into syngeneic immunocompetent C57BL/6 mice, KPad1 cells show an indolent metastatic phenotype. They found that genetic screenings of tumor intrinsic immune regulators identified the stimulator of interferon genes (STING) pathway as a suppressor of the metastatic outbreak.[36]

The 4T1 mammary model is another syngeneic model originally established from a spontaneous mouse mammary tumor.[37] When 4T1 cells are implanted orthotopically into the fat pads of syngeneic mice, they migrate to the lungs, liver, brain, and bone, which are common sites of metastasis in humans. Other syngeneic models of breast cancer include transgenic models based on oncogene expression in the mammary gland under the control of mammary tumor viruses.[38]

Overall, syngeneic mouse tumors are immunologically compatible with their hosts, and tumor transplantation does not provoke an immune response. Instead, tumors grow within an intact immune system. Different types of tumor cell lines can be used in this model, including spontaneous, transgenic, and carcinogen-induced tumor cell lines. Syngeneic mouse models are best suited for screening novel immuno-oncological agents or for gaining insight into antitumor responses in the context of an intact immune system. Given the rapid growth of tumors in syngeneic mice, these models are less suited for studying early events in tumor growth associated with cancer stem cells or understanding the contributions of heterogeneous tumor microenvironments. Additionally, typically do not recapitulate the mutational heterogeneity observed in human tumors.

Xenograft models

Xenograft models are based on the implantation of human tumor cells into immunocompromised animals to avoid reactions between graft and host.[39] Four types of tumor xenograft animal models have been developed for metastasis research by implanting cancer cell lines, organoids, or patient tumors into immunodeficient animals, namely orthotopic, intravenous, intracardiac, and intracarotid injection models [Figure 3]. Xenograft models offer distinct advantages, notably their enhanced replicability and genetic stability in tumor propagation. This enables the study of human tumors without significant genetic alterations in tumor cells over successive generations in animal models. Mimic realistic tumor

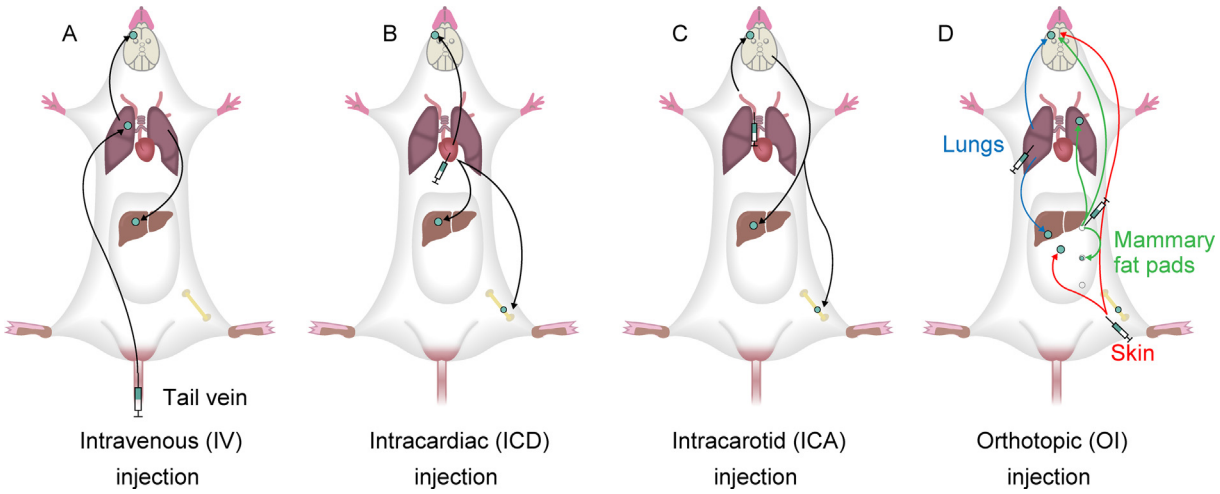

Figure 3. Injection routes used in brain metastasis models. (A) When ectopic intravenous (IV) injection was performed, the primary route of tumor cell dissemination was to the lungs, followed by secondary spread to the brain and other organs of the body. (B) When an ectopic intracardiac (ICD) injection route is chosen, the tumor cells primarily spread to the brain and abdominal organs, including the bone, followed by secondary spread to the lungs. (C) When an ectopic, intracarotid (ICA) injection is performed, the tumor cells first disseminate to the brain, followed by their spread to abdominal organs, including the bone, and finally the lungs. (D) Orthotopic injection (OI), organ-specific injection of tumor cells results in tumor growth at the primary site, followed by the metastatic spread of tumor cells to the abdominal organs and brain. Orthotopic brain metastasis models were developed for lung carcinoma (blue arrows), melanoma (red arrows), and breast carcinoma (green arrows).

microenvironments: within xenograft models, human tumors grow in physiologically relevant tumor microenvironments that mimic the immunity, nutrients, and hormone levels found in the human primary tumor site. Accelerated therapeutic impact with higher predictive ability: numerous studies have found that xenograft models exhibit responses to anticancer agents similar to those of actual therapy.[40]

The main aim of xenograft metastasis model research is to link basic and clinical research and complement the use of *in vitro* model systems.[41] Animal models of tumor xenografts provide a sophisticated platform for studying the process of tumor metastasis *in vivo*. By revealing the related signaling pathways and disease mechanisms of certain oncogenes or tumor suppressor genes, this platform will enable us to better understand their roles in the occurrence and development of tumor metastasis. In addition, these models provide a research tool for preclinical drug response evaluations that can determine antitumor efficacy beyond drug toxicity, pharmacokinetics, and pharmacodynamics.[42]

Zebrafish is emerging as a valuable model organism to investigate metastasis *in vivo* due to its capability of high-resolution imaging and ease of genetic manipulation.[43,44] Fan et al. reported that over-expressed microRNA-330-p (miR-330-p) significantly enhances the brain metastasis potential of A549 cells, while knockdown miR-330-p reduces the brain metastasis ability of the H1975 cells. Interestingly, osimertinib and gefitinib exhibited different inhibitory effects in a zebrafish brain metastasis model.[45]

Mice are the most commonly used xenograft models. Different mouse strains with unique immunodeficiency backgrounds have been used in cancer research, including athymic nude mice, severe combined immunodeficiency (SCID) mice, and non-obese diabetes (NOD)/SCID mice (SCID mice with additional levels of immunodeficiency).[46] Among these strains, NOD/SCID mice exhibited the best immunodeficiency, owing to the absence or defect of almost all types of immune cells, B cells, T cells, dendritic cells, macrophages, and natural killer cells. This is followed by SCID mice, which lack B and T cells, and nude mice, which lack T cells.

Grafting of cancer cell lines

Cell line-derived xenograft (CDX) models are based on a variety of tumor cell lines that grow easily in culture and rapidly form tumors after inoculation into immunodeficient mice[47] [Figure 2C]. These cells may be implanted subcutaneously, intravenously, or orthotopically to induce tumors in different microenvironments. The advantage of CDX mouse models is that they cover a broad range of human tumor cell lines commonly used for high-efficiency xenotransplantation.

Although many human cancer cell lines can form tumors upon implantation in mice, metastasis is relatively rare. However, the analysis of CDX models of metastasis has provided fundamental insights, including the functional validation of metastatic genes, preclinical studies of potential therapies, and organ tropism studies.[48–50] Human cancer cell lines have been used to study metastasis *in vivo*, including the MDA-MB-231, A375, and PC9 cell lines, to model breast cancer, malignant melanoma, and lung cancer, respectively. Some of the subvariants with enhanced metastatic capacity were generated through multiple rounds of *in vivo* selection.[51] For example, Manuel et al. identified plasmin from the reactive brain stroma as a defense against metastatic invasion and plasminogen activator inhibitory serpins in PC9 cells as a shield against this defense.[52] Rodrigues et al. investigated how exosome affects brain metastasis *in vivo*, and also evaluated whether pre-treatment with 10 μg of cell migration-inducing and hyaluronan-binding protein (CEMIP) could promote the early stages of colonization of MDA-MB-231 cells in intracardiac injection model.[53] Different injection methods can be used to study the trend of metastasis in different organs. Intravenous injection is used for lung metastasis, whereas intracardiac and intracarotid injections are suitable for brain metastasis studies.[54] In general, intracarotid injection models of lung cancer brain metastases are more common than other models and have a higher probability of brain metastases than orthotopic injections in the lung. In fact, intracarotid injection models of lung cancer brain metastasis better mimic the process of tumor cells crossing the BBB.[55]

In summary, the analysis of CDX models has revealed biological processes associated with metastatic colonization, molecular mechanisms associated with genomic and metabolic heterogeneity of tumors and metastases, and the evolution of metastasis in response to therapy.[56]

Grafting of cancer organoid

Recently, emerging organoid culture technology has allowed cancer cells to grow in a 3D matrix, leading to critical progress in cancer research. The establishment of living tumor organoid biobanks offers a platform for high-throughput drug screens.[57] Although organoids represent a powerful resource for finding effective therapeutic strategies directed toward specific tumor subtypes, they do not account for the interplay between tumor cells and the surrounding tissue

microenvironment because this interplay cannot be recapitulated in a dish.[58,59] Many studies have highlighted the importance of the tumor microenvironment in influencing tumor cell identity and behavior, emphasizing the necessity of validating the results obtained *in vitro* using animal model systems.[60,61] To overcome these limitations, researchers have developed a method to transplant organoids into immunodeficient mice to obtain a xenograft model, named the organoid-based xenograft model[62] [Figure 2B].

A tumor organoid, also known as a "cancer surrogate," uses the patient's tumor tissue for *in vitro* 3D cultures to simulate the biological characteristics of the tumor. Organoids cultured from cancer lesions are mainly used to simulate the occurrence and development of tumors and to analyze changes in tumor-related omics.[63] Patient-derived organoids bridge the conventional gaps in PDX models and have potential applications in clinical cancer research, particularly in the modeling of cancer, individualized therapy, tumor drug screening, tumor immunotherapy, and translational medicine.[64–66] He et al. generated a pair of organoids derived from primary tumors and metastases in the same colorectal cancer (CRC) patients to model CRC metastasis. Their results illustrated that SRY related high-mobility-group box protein 2 (SOX2) is associated with CRC metastasis and may serve as a potential prognostic biomarker and therapeutic target for CRC.[67]

Grafting of patient-derived tumor fragments

The tumor fragments harvested from the patient can be transplanted into the flanks or particular organs of immunocompromised mice maintained under controlled laboratory conditions and carefully monitored[68] [Figure 2C].

PDX models are generated by directly implanting patient tumors into host mice, thus avoiding potential selective pressures associated with growth in culture.[69,70] Therefore, the PDX model is more likely than the CDX model to reproduce the structure, heterogeneity, and histopathology of a patient's tumor. However, the rate of metastasis is low. One key factor is the choice of implantation site, which can influence the metastatic capacity of the PDX model. Orthotopic implantation appears to improve the metastatic behavior of the PDX model, possibly by enabling the tumor to interact with the relevant microenvironment, closely mimicking the physiological conditions that mimic spontaneous metastasis.[71] Sharma et al. established PDXs from breast cancer patients to examine the efficacy of the specific frequencies 27.12 MHz in suppressing breast cancer brain metastasis.[72] In a murine intracardiac injection model, Bernatz et al. found that treatment with the taxane docetaxel (a drug used for breast cancer treatment) increased the risk of brain metastasis.[73]

Genetically engineered mouse models

GEMMs, created through genetic engineering techniques, allow for the manipulation of specific genes to study their function and regulatory mechanisms in tumor development and distance metastasis [Figure 2D]. GEMMs of cancer brain metastasis can more accurately simulate the process of cancer cell invasion, migration, and the formation of metastatic lesions in the brain, thus facilitating the investigation of the mechanisms and treatment strategies for cancer brain metastasis. In some GEMM models, genetic modifications led to the formation of tumors with secondary spread to the brain. However, a major problem with GEMM-induced tumors is the low incidence of metastatic spread, which may, in part, be explained by the rapid development of primary lesions.[74]

The concomitant loss of *Trp 53* and *Rb1* in mice causes tumor formation in multiple organs, including the lungs. Meuwissen et al. established a SCLC mouse model through the conditional inactivation of *Trp53* and *Rb1* in lung epithelial cells.[75] This model demonstrated a certain incidence (14 of 33 mice) of metastasis to distant organs, including the bone, brain, adrenal glands, ovaries, and liver. However, it does not meet the criteria for a reliable model of lung cancer brain metastasis because of its relatively low occurrence of brain metastasis. The *KrasG12D; p53−/−* (KP) mouse model, developed by Jack et al., is another widely used model for studying lung cancer.[74] In this model, lung epithelial cells experience concurrent activation of oncogenic *Kras (KrasG12D)* and loss of the *p53* tumor suppressor protein, leading to tumor formation. The tumor progression in this model faithfully replicated the histopathological characteristics observed during human cancer development. However, it should be noted that metastatic events are infrequent in this model, despite *Kras* and *P53* mutations being highly prevalent in lung cancer brain metastasis in humans.[76] To enhance the metastatic potential, Ji et al. introduced a loss-of-function mutation in serine/threonine kinase 11 (LKB1) into the KP model. This modification resulted in an increased incidence of metastasis to 60% (27 out of 44 mice), primarily affecting the lymph nodes and skeletal system but not the brain.[77]

Despite tremendous efforts, a robust mouse model of lung cancer with brain metastasis remains lacking. This could be attributed to several reasons: (1) lung cancer, especially NSCLC, has a relatively low propensity to metastasize to the brain compared to other cancers, such as breast cancer or melanoma. For example, the mouse mammary tumor virus-polyoma virus middle T antigen (MMTV-PyMT) breast cancer model shows prevalent metastasis to both the brain and lung[78]; the *Tyr-BRAF^{V600E} Pten−/−* melanoma model also exhibits a high incidence of brain metastasis.[32] Its inherent low metastatic potential makes it challenging to develop a mouse model that accurately recapitulates brain metastasis observed in human lung cancer patients. (2) Species differences between mouse and human biology, particularly in terms of brain anatomy, immune responses, and molecular pathways involved in metastasis, could limit the success of establishing mouse models of brain metastasis.

In the future, a more comprehensive understanding of the complex human lung cancer brain metastasis process may provide directions to reconstitute the human metastatic environment in mice, creating a "humanized" GEMM that can robustly replicate brain metastasis from the lungs. This model should significantly facilitate research on lung cancer brain metastasis and warrant widespread use in the lung cancer research community.

Conclusion

The quest for suitable models that replicate the intricate journey of brain metastasis remains pivotal, enabling a deeper understanding of biological and molecular intricacies and facilitating the development of new therapies and chemical drugs. Progress has been made notably, the pioneering work from Hatherell et al. established the feasible Transwell models for metastasis studies.[13] Later, Bang et al. utilized microfluidic BBB models to emulate cancer cells traversing the barrier.[79] These *in vitro* models help us understand how tumor cells penetrate the BBB. To further investigate how tumor cells colonize the brain, an array of mouse models was established, including syngeneic, xenograft, and GEMMs models, to mimic an expansive spectrum of metastatic phenotypes spanning diverse cancer types. The systemic brain metastasis model has greatly empowered us to unravel the complexity of brain metastasis.

In the future, an array of emerging techniques – bio-3D printing, real-time imaging, artificial intelligence (AI), precise gene editing, and more—hold promise for refining the landscape of cancer brain metastasis models. Bio-3D printing, for instance, can introduce intricate cellular interactions and microenvironmental elements into *in vitro* models, emulating the complex interplay between tumors and brain cells, along with extracellular components. The advent of advanced optical imaging, exemplified by light-sheet imaging with fish models, offers the potential for the real-time visualization of metastatic processes. This could be used to evaluate dynamic alterations in cell heterogeneity and plasticity during tumor growth, even in response to treatments. Moreover, the integration of AI and machine learning into imaging data analysis holds the key to unlocking diagnoses at an early stage of brain metastasis. By observing imaging over a single day, we can anticipate brain tumor metastasis by detecting alterations in the brain environment that facilitate tumor cell colonization.

Considering the complexity of brain metastasis, interdisciplinary integration of technology could significantly enhance our ability to develop precise therapies to treat this lethal disease.

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Authors contribution

Maojin Yao, Jianhao Zeng, and Zihao Liu conceived and designed the study. Zihao Liu and Shanshan Dong designed, analyzed, and wrote the manuscript. Mengjie Liu designed and produced illustrative materials. Jianhao Zeng and Maojin Yao supervised and revised the manuscript. Yuqiang Liu and Zhiming Ye participated in data organization and discussion. All the authors have read and approved the final version of the manuscript.

Ethics statement

None.

Data availability statement

The data sets used in this study are available from the corresponding author upon request.

Conflict of interest

None.

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None.

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