The biology of brain metastases—translation to new therapies

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Abstract

Brain metastases are a serious obstacle in the treatment of patients with solid tumors and contribute to the morbidity and mortality of these cancers. It is speculated that the frequency of brain metastasis is increasing for several reasons, including improved systemic therapy and survival, and detection of metastases in asymptomatic patients. The lack of preclinical models that recapitulate the clinical setting and the exclusion of patients with brain metastases from most clinical trials have slowed progress. Molecular factors contributing to brain metastases are being elucidated, such as genes involved in cell adhesion, extravasation, metabolism, and cellular signaling. Furthermore, the role of the unique brain microenvironment is beginning to be explored. Although the presence and function of the blood–brain barrier in metastatic tumors is still poorly understood, it is likely that some tumor cells are protected from therapeutics by the blood–tumor barrier, creating a sanctuary site. This Review discusses what is known about the biology of brain metastases, what preclinical models are available to study the disease, and which novel therapeutic strategies are being studied in patients.

Introduction

Metastatic brain tumors are the most frequently occurring intracranial neoplasms in adults with the annual incidence in the USA estimated to be 200,000 cases.[1] Furthermore, an estimated 8–10% of adults with cancer develop symptomatic brain metastases.[2,3] The majority of brain metastases originate from primary cancers in the lung (40–50%) or breast (15–25%), or from melanoma (5–20%).[2,3] The frequency of diagnosis of metastatic brain tumors seems to be increasing as a result of improved imaging modalities and earlier detection as well as longer survival after primary cancer diagnosis because of more-effective treatment of systemic disease.

The distribution of brain metastases correlates with blood flow and tissue volume, with 80% detected in the cerebral hemispheres, 15% in the cerebellum, and 5% in the brainstem.[4] The majority of patients exhibit multiple tumors at the time of brain-metastasis diagnosis. Clinical features include headache, neurological deficit, and seizures. Neuropsychological testing demonstrates cognitive impairment in 65% of patients with brain metastases,[5,6]

which might be a result of destruction or displacement of brain tissue by the expanding tumor, peritumoral edema leading to further disruption of surrounding white matter tracts, increased intracranial pressure, and/or vascular compromise.

Therapeutic approaches for brain metastases include surgery, whole-brain radiation therapy (WBRT), stereotactic radiosurgery (SRS), chemotherapy, growth factor inhibitors, or a combination of these therapies. Survival for patients with brain metastases treated with WBRT typically ranges from 4–6 months, but can be as long as 12–24 months for selected patients.[6] Strong positive prognostic factors include good functional status, age <65 years, no sites of metastasis outside of the central nervous system (CNS), controlled primary tumor,[7] the presence of a single metastasis in the brain, long interval from primary diagnosis to brain relapse, and certain cancer subtypes such as HER2-positive breast cancer and *EGFR*-mutant non-small-cell lung cancer (NSCLC).[8–10] In up to half of patients the cause of death is attributed to CNS progression.[11] Randomized clinical trials have shown that surgery or SRS combined with WBRT improves overall survival compared with WBRT alone in patients with a single metastasis in the brain. In patients with four or fewer brain metastases, SRS results in equivalent overall survival but worse intracranial disease control compared with SRS plus WBRT.[11–15] The combination of radiotherapy and chemotherapy improves response rate and/or progression-free survival in some studies, but not overall survival.[16–18]

We review our current understanding of the biology of brain metastasis, present preclinical approaches that are available to model the disease, including emerging imaging techniques, and discuss novel therapeutic strategies to target the unique features of CNS metastases.

Brain metastasis formation

The metastatic cascade, whereby cancer cells escape from the primary tumor site, invade surrounding tissue, intravasate into the bloodstream or lymphatics, and arrest, extravasate, survive and proliferate within a secondary site (Figure 1), is an inherently inefficient process.[19] Certain tumor types demonstrate an organ-specific pattern of spread; for example, prostate cancer frequently metastasizes to bone and melanoma frequently metastasizes to the lung, liver and brain.[20,21] Stephen Paget was the first to hypothesize that the propensity of cancer cells to spread to specific sites was dependent upon two factors: the cancer cell (the 'seed') and the receiving organ environment (the 'soil').[22] An alternative hypothesis attributed to pathologist James Ewing proposes that circulatory patterns between the primary tumor and specific secondary organs are sufficient to explain the majority of organ-specific metastatic spread.[23] A newer hypothesis is that cancer cells can bring their own soil—stromal components from the primary site including activated fibroblasts—to secondary sites.[24] Researchers have sought to understand how molecular and genetic features of the primary cancer cell, the brain microenvironment, and the angiogenic pathways influence spread of cancer to the brain and its growth there (Table 1).

Brain microenvironment

Once metastatic cancer cells enter the brain circulation, they might arrest in sites of slow flow within the capillary bed at vascular branch points,[25] which is then followed by early changes in the brain microenvironment.[26] The arrested cancer cells encounter brain vascular endothelial cells, which seem to promote metastatic tumor cell growth and invasion.[25,27] In addition, stromal cells such as fibroblasts associated with the primary tumor are involved in metastatic nodules in the brain (Figure 1).[24] These co-disseminating stromal cells provide survival and proliferative advantages to the tumor cells and facilitate early colonization of metastatic foci. The brain also provides an environment that differs from most other organs, and the factors that either promote or suppress colonization and proliferation are poorly

understood (Table 1). Thus, successful treatment of CNS metastases may require targeting of both tumor and host responses.

Response of host stromal cells—After infiltration into the brain tissue, cancer cells encounter a number of host cell types, including microglia and astrocytes. Microglia displaying stellate morphology with thick cellular processes, characteristic of an activated state, have been observed around extravastated cancer cells within 7 days of intracarotid injection of breast cancer cell lines into mice.[26] Activated astrocytes with thick processes and upregulated expression of glial fibrillary acidic protein can be seen even earlier, when cancer cells are still in the intravascular space, and they remain closely associated with cancer cells throughout their growth into macrometastases.[26] In a xenograft model, reactive astrocytes and microglia were in direct contact with tumor cells along the border of the tumors as well as infiltrating the inner tumor mass. Similar findings were reported in human specimens. In addition, *in vitro* co-cultures demonstrated that glia induced a fivefold increase in metastatic cell proliferation, leading the researchers to hypothesize that reactive glia creates an altered brain microenvironment that is more permissive to tumor growth and invasion.[28] Consistent with these findings, expression of endothelin 1, a regulator of numerous transforming processes, was observed in peritumoral astrocytes in 85% of hematogeneous metastases of the human brain.[29] Furthermore, additional studies suggest that microglia can enhance the invasion and colonization in the brain tissue by breast cancer cells similar to other tissue-specific stromal cells such as osteoblasts and osteoclasts in the bone.[30,31] Most likely, during the early stages of brain metastasis formation, there is a balance between the protective or trophic, and cytotoxic functions of microglia, dependent on the release of a variety of factors and ultimately determined by signals from tumor cells.[32]

Protective roles of astrocytes—Astrocytes within the brain microenvironment might serve to protect brain metastases from cytotoxicity induced by chemotherapeutic drugs.[33] In co-culture experiments, the presence of astrocytes but not fibroblasts dramatically reduced 5-fluorouracil-induced and cisplatin-induced apoptosis in human tumor cells.[34] The protective effect was lost when cells were separated with a trans-well membrane, suggesting that direct contact of astrocytes with tumor cells, rather than secreted factors, was mediating the chemoprotective effect. Similar effects have also been demonstrated in human melanoma cell lines,[35] breast cancer cells, and lung cancer cells.[36]

Soluble factors also have an important role in tumor cell–astrocyte interaction. Activated astrocytes surround brain metastatic lesions from lung cancer, both in an experimental mouse model and in the human brain.[37] Tumor cell-derived factors (including migration inhibitory factor, interleukin [IL]-8, and plasminogen activator inhibitor 1) induced astrocytic activation and release of molecules (IL-6, TNF-α, and IL-1β) that promoted tumor-cell proliferation *in vitro*. Furthermore, neurotrophins and their receptors have a role in the invasion and colonization of brain-prone melanoma cells.[38,39]

Angiogenesis

The growth and proliferation of primary and metastatic tumors is dependent on the establishment of an adequate blood supply.[40–42] A tumor can recruit blood vessels via different mechanisms: angiogenesis (sprouting from existing blood vessels), vasculogenesis (formation of a *de novo* vascular system from endothelial precursor cells), co-option (growth of cancer cells along existing blood vessels), intussusception (vessel remodeling and expansion by the insertion of interstitial tissue columns into the lumen of pre-existing vessels), vasculogenic mimicry (cancer cells lining blood vessels), cancer cells that

transdifferentiate into endothelial cells,[43] and cancer stem-like cells that form an inner lining of blood vessels in the brain.[44–47]

Changes in brain vasculature—Kienast *et al.*[25] used multiphoton laser scanning microscopy and a mouse cranial window model to follow in real time brain metastasis formation from both lung cancer and melanoma cell lines. After extravasation, there was a persistent correlation between tumor cells with micro-vessels and either vessel co-option (with melanoma) or angiogenesis (with lung carcinoma). Previous studies showed a similar association between metastasizing tumor cells and blood vessels.[27,28,48,49] Kusters *et al.*[48] showed that a melanoma brain metastasis could grow up to 3 mm through co-opting pre-existing blood vessels. Similar observations in murine experimental brain metastases models from breast cancer and melanoma cells indicate that an active adhesion mechanism exists between tumor cells and the vascular basement membrane.[27]

Metastatic brain tumor vessels in preclinical models and human surgical specimens have significantly larger diameters and thicker basement membranes when compared with the vessels of normal brain.[50] Brain metastases from murine melanoma, murine fibrosarcoma, human lung carcinoma, and human colon carcinoma have a lower microvascular density than the surrounding normal brain parenchyma, and they all contain dilated blood vessels with large lumens.[50] The mechanism for this enlargement seems to involve endothelial cell proliferation.

Angiogenic factors in brain metastasis—The progressive growth of the majority of metastatic brain tumors is critically dependent on the expression of VEGF.[51] In a brain metastatic variant of MDA-MB-231 cells, there was a significant increase in VEGF-A production, and inhibition of VEGFR activity significantly reduced brain tumor burden after intracarotid injection in mice.[52] Moreover, when human lung cancer cells transfected with antisense *VEGF165* were injected into the carotid artery of nude mice, the formation of brain metastases was substantially decreased compared with untransfected cells.[51] However, in cells transfected with *VEGF121* or *VEGF165,* no increase in metastasis was observed, indicating that VEGF expression is necessary but not sufficient for the production of brain metastases. In another study, VEGFR inhibition with cediranib did not impede tumor cell extravasation in a murine model of brain metastasis from a prostate cancer primary.[53] These data suggest heterogeneity between different tumor cells and types.

Activation of integrin $\alpha_v\beta_3$ on tumor cells strongly promotes metastatic breast tumor growth in the brain by enabling tumor cells to attract blood vessels independent of hypoxia, an effect mediated by post-transcriptional control of VEGF expression.[54] Other growth factors including angiopoietin 1 and 2, bFGF, PlGF, SDF1α, PDGF and IL-8 have been implicated in the angiogenesis pathway in primary tumors and gliomas, but their role remains to be established in metastatic brain tumors.[43,55]

Hostile microenvironment impairs drug delivery—In cancer, the balance between proangiogenic and anti-angiogenic factors tips towards angiogenesis owing to the overexpression of proangiogenic growth factors. This overexpression leads to blood vessels that are structurally and functionally abnormal, exhibiting heterogeneous and often sluggish blood flow and hyperpermeability.[50,56–59] A heterogeneous and inefficient blood vessel network creates regions of hypoperfusion or no perfusion where reduced nutrients and oxygen, and impaired waste removal, creates an abnormal metabolic environment, characterized by hypoxia and acidosis. Furthermore, vascular hyperpermeability induces tumor interstitial hypertension, which hinders drug penetration owing to the lack of convective transport.[60,61]

Clearly, host–tumor interactions including angiogenesis have an important role in the formation and expansion of brain metastases. However, the knowledge of the molecular mechanisms required during the formation of meta-static lesions is still lacking. What is certain is that the molecular and cellular changes within metastatic brain tumor vasculature and the microenvironment should be considered when designing strategies to improve the delivery and efficacy of therapeutic agents.

Modeling and imaging brain metastases

Substantial progress has been made over the past two decades in modeling human cancer in the mouse and, more recently, model systems have emerged that recapitulate many aspects of metastatic disease to the CNS. An important barrier to the development of reliable CNS mouse models is that in most orthotopically transplanted primary tumors that give rise to metastatic foci, the animals succumb to systemic disease before the brain can be reliably studied. Therefore, current models often rely on either direct implantation of tumors into the brain or iterative selection of brain-seeking clones resulting from experimental hematogeneous dissemination to the brain to enrich metastatic disease in the CNS. These experimental metastasis models inevitably miss the initial steps of the metastatic cascade and thus may not reflect the full clinical manifestation of the disease.

Animal models of brain metastasis

Various preclinical animal models for the study of brain metastases and their advantages and limitations are summarized in Table 2. Rodent model systems for brain metastasis have been reported for multiple cancer types, including melanoma,[62,63] lung carcinoma,[64] and breast carcinoma.[65–71] The emergence of new animal models of brain metastasis that more closely mimic human disease provides the tools necessary to understand all of the steps involved in the process. Unfortunately, the field is still limited by the few spontaneous models available and the complexity of more clinically relevant models.

Melanoma—Melanoma mouse models have been established using a variety of murine and human melanoma cell lines injected via an intracardiac, intracarotid or, less successfully, intravenous route. Most models show a combination of parenchymal and leptomeningeal deposits at the time of sacrifice, with occasional dural deposits.[72]

Breast cancer—Yoneda *et al.*[70] established a brain-seeking clone (MDA-MB-231BR) of the human breast cancer cell line MDA-MB-231 by serial *in vivo* and *in vitro* passages. In a mouse xenograft, when the MDA-MB-231BR cell line was transfected with HER2, the HER2-overexpressing clones demonstrated a threefold increase in the number of large brain metastases compared with untransfected MDA-MB-231BR cells; however, the number of micro-metastases was unaffected.[73] This model has also been used to probe the effect of anti-HER2 therapies on the development and propagation of brain metastases.[74] Alternatively, human breast cancer cells implanted near the surface of the brain provide a model to directly observe tumor and vessel growth using a cranial window and intravital microscopy (Figure 2a–c).[75,76]

Spontaneous metastasis—Few reports describe cell lines capable of reproducibly spontaneously metastasizing to the CNS from a primary tumor. These include a variant of the murine B16 melanoma cell line (named G3.5), generated through successive rounds of selection of brain metastases following intravenous injection, which leads to spontaneous metastasis to the CNS from subcutaneous primary tumors in 80% of mice.[77] Using a similar method, a brain metastatic variant of the triple-negative breast cancer cell line CN34 (CN34-BrM2) capable of metastasizing to the CNS from a primary mammary tumor in about 42%

of mice was generated.[78] A procedure that might more accurately replicate the selection mechanisms of clinical brain metastasis formation was established from long-term, low-dose metronomic chemotherapy treatment of mice injected subdermally with a highly metastatic variant of the WM239A human melanoma cell line.[63] Among long-term surviving mice, 20% had spontaneous brain metastases, and cell lines generated from these metastases spontaneously metastasized to the brain parenchyma after orthotopic transplantation and removal of the primary tumor.[63]

Preclinical imaging techniques

Visualization and quantification of metastatic disease burden in the brain is an indispensible component of any metastasis model system. Several novel methods of *in vivo* imaging have been developed that allow for serial measurements over time before animal sacrifice, including window models (Figure 2a–c,f,g). For instance, the MDA-MB-231 human breast cancer cell line has been engineered to express a naturally secreted Gaussia luciferase (Gluc) and in an orthotopic primary and brain metastatic mouse model, quantitative serum and urine Gluc levels correlated well with overall tumor burden.[76] Importantly, Gluc activity in the blood revealed early growth of metastatic brain tumors before they could be detected by bioluminescence imaging (Figure 2e).

An alternative adaptation of quantitative fluorescent and phosphorescent imaging techniques has recently been developed that interrogates blood–tumor barrier (BTB) permeability within and surrounding metastatic brain tumors in mice, using tumor cells expressing enhanced green fluorescent protein (eGFP) and Texas red dextran as a marker of passive permeability.[79] This model also allows simultaneous quantification of BTB permeability using radiolabeled chemotherapeutic drugs. Combining these techniques with the MDA-MB-231BR HER2-overexpressing mouse model previously described,[70] Lockman *et al.*[79] analyzed over 2,000 brain metastases and demonstrated that BTB permeability was compromised in over 89% of lesions.[79] However, ^{14}C-paclitaxel and ^{14}C-doxorubicin only reached cytotoxic concentrations in about 10% of metastases.[79]

Other powerful imaging technologies include MRI, PET and near-infrared optical imaging.[80,81] In the past, the majority of MRI studies of brain tumors in mice were performed using high-field-strength MRI (≥7 Tesla) because of its high spatial resolution and signal-to-noise ratio (Figure 2d).[82,83] However, several techniques enhance the sensitivity of clinical-strength MRI scanners (1.5–3 Tesla) for the detection of small brain metastases in rodents. When MDA-BR-231-eGFP cells are labeled with fluorescent μm-sized superparamagnetic iron oxide (MPIO) particles, single labeled breast cancer cells can be detected in the rat brain parenchyma as small dark or hypointense voxels on T2★-weighted MRI that then develop into metastases after 4 weeks.[84] The same cells labeled with ferumoxides-protamine sulfate (FEPro) could be detected and quantified within 2 weeks of intracardiac injection using T2★-weighted 3-Tesla MRI with better sensitivity than bioluminescence imaging, providing a powerful tool for both spatial and temporal monitoring of metastatic tumor growth and progression.[85,86]

Novel therapeutic strategies

The role of chemotherapy in the treatment of brain metastasis has been limited owing to multiple factors: poor blood–brain barrier (BBB) penetrability of many systemically active chemotherapeutic drugs; the tendency for many patients to have had multiple rounds of chemotherapy prior the development of CNS metastatic disease; and the historical exclusion of patients with brain metastases from clinical trials testing new agents. New therapies and strategies are increasingly being explored that aim to enhance drug delivery to the brain, complement the effects of radiation therapy, substitute the role of radiation therapy, or act as

preventative agents against the development of new metastases. Furthermore, with the development of new models and imaging techniques (Figure 2h,i), preclinical treatment strategies can become more focused on established brain metastases, necessary for translational potential.

Crossing the blood–brain barrier

The BBB is a selective barrier between the systemic circulation and cerebrospinal fluid that is formed by specialized endothelial cells lining the cerebral microvasculature, together with pericytes and astrocytic perivascular endfeet (Figure 3).[87] Tight junctions between adjacent cells force most molecules to pass through, rather than around, endothelial cells, creating a physical barrier. Transport systems on the luminal and abluminal membranes regulate the passage of small hydrophilic molecules. Large hydrophilic molecules, including many chemotherapeutic and molecular-targeted drugs, are excluded from the CNS unless they can be actively transported by receptor-mediated transcytosis. In addition, the BBB expresses high levels of drug efflux pumps such as the P-glycoprotein (PgP)/multi-drug resistance proteins,[88] which actively remove some chemotherapeutic drugs from the brain.

When metastatic tumors grow beyond 1–2 mm in diameter within the brain parenchyma, the BBB becomes structurally and functionally compromised.[56,58,75,89–92] In an experimental brain metastasis model of eight human tumor lines, lesions smaller than 0.2 mm^2 contained an intact BBB; however, larger tumor-cell clusters resulted in leakage of sodium fluorescein indicating that the function of the BBB is directly related to the size of the lesion, and a growing tumor mass may disrupt the interaction of astrocytes and endothelial cells.[93] In addition to changes in blood vessel permeability, there was a significant reduction in PgP expression to 5% and 40% of normal levels in brain metastases from melanoma and lung carcinoma, respectively.[94] The disruption of the BBB might not be homogeneous, and the BBB might remain intact at least in parts of tumors. Thus, barriers to drug delivery to such lesions might still need to be overcome.

It is widely thought that macroscopic metastatic brain tumors have a disrupted BBB, as evidenced by homogeneous contrast enhancement on MRI. Brain metastases, distinct from infiltrative gliomas, have sharp borders and can usually be completely resected if their location allows. However, not all single metastases are amenable to surgical resection, and even those tumors that are assumed to be totally resected at the time of surgery have a recurrence rate of up to 50% when postoperative radiation therapy is not employed.[95] In addition, there are likely micrometastatic deposits in many patients at the time of diagnosis that are not well visualized by MRI, have an intact BBB, and contribute to CNS recurrence after initial local therapy, and some macroscopic tumors have a relatively intact BBB.[79] Therefore, strategies to circumvent the challenges of an intact BBB are likely to be needed in some patients for improved intracranial disease control (Table 3).

An alternative approach to physical and local disruption techniques is to design drugs that can be shuttled across the BBB using receptors that are naturally expressed on the endothelial cells of the BBB (Figure 3). Preclinical studies have demonstrated feasibility using antibodies against transferrin and insulin receptors, as well as peptides targeting the receptor lipoprotein receptor-related protein (LRP-1).[96–98] LRP-1 is highly expressed at the BBB, is involved in the transport of proteins and peptides such as β-amyloid, tissue plasminogen activator, melanotransferrin and receptor-associated peptide, and is upregulated in brain tumors.[96,97,99–101] GRN1005 (Angiochem, Montreal, QC, Canada), is paclitaxel linked to angiopep2 (a ligand for LRP-1) and is in phase I clinical trials in patients with metastatic brain tumors or malignant gliomas.[102,103] GRN1005 has antitumor activity in subcutaneously implanted glioblastoma and lung tumors and extends the survival of mice with intracerebral tumors.[104] In addition, in the MDA-MB-231BR mouse model, there was a

fourfold to 54-fold increase in *in vivo* uptake of GRN1005 into brain and brain metastases compared with paclitaxel.[105] Another mechanism to increase the concentration of anticancer agent in the CNS is to inhibit their efflux by targeting the efflux transporters that comprise the BBB (Figure 3a–c).

Sensitizing tumor cells to radiation

Although WBRT leads to stabilization or shrinkage of tumors in at least half of patients, many patients have tumor recurrence either at sites of original disease or in new sites, some of which may have been present at the time of initial treatment but below the threshold of detection. There is, therefore, continued interest in developing drugs that act to sensitize tumor cells to radiation therapy, with the goal of improving local and distant control rates over radiation alone while sparing toxicity to normal tissue. Multiple agents with preclinical radiosensitizing properties have failed to show benefit in randomized controlled trials, including lonidamine, metronidazole, misonidazole, motexafin gadolinium, bromodeoxyuridine, and efiproxiral.[6,106–111] Several chemotherapeutic agents with preclinical evidence of both radiosensitizing properties and BBB penetration have been tested in trials in combination with WBRT, including temozolomide and topotecan, but none has emerged as clearly superior to WBRT alone.[16,18,112] The histone deacetylase inhibitor vorinostat has shown promise as a radiosensitizer in multiple cells lines as well as in brain using the MDA-MB-231BR mouse model, where an improvement in both tumor growth delay and overall survival was seen compared with radiation alone.[113] In a separate study using the MDA-MB-231BR mouse model, vorinostat as a single agent reduced the size and number of brain metastases formed compared with vehicle-treated controls, raising the possibility that this agent might be useful as a prophylactic agent in patients who are at high-risk of tumor relapse in the brain.[114] The mechanism of the prevention of metastasis by vorinostat seems to be the induction of DNA double-strand breaks associated with the downregulation of the DNA repair gene *RAD52*, indicating that vorinostat sensitizes the tumor to DNA-targeting therapies such as radiation.[114] Vorinostat in combination with WBRT is being tested in a phase I clinical trial in patients with brain metastases from multiple solid tumor types and in a phase I trial in combination with SRS in patients with brain metastases from NSCLC.[115,116]

Targeting angiogenesis

There are conflicting preclinical data as to whether inhibition of angiogenesis results in tumor growth delay and a reduction in metastatic potential, or promotes altered growth patterns via vessel co-option and increased metastatic potential.[48,49,52] There are limited clinical data to support the phenomenon of vessel co-option and infiltrative growth of metastatic brain tumors under the influence of antiangiogenic therapy,[46] but angiogenesis inhibitors have not been systematically studied in brain metastases mainly owing to concerns about the potential for intracranial hemorrhage. Such concerns have been substantially allayed with the publication of reviews of large clinical trial datasets and two prospective clinical trials. These studies show that the risk of CNS hemorrhage in patients with solid tumors that have not spread to the CNS, as well as in patients with stable brain metastases at the time of initiation of anti-angiogenic therapy, is low (0.8–3.3%) and not above rates that would be expected independent of antiangiogenic agent exposure.[117–120] Therefore, clinical trials have been launched to determine the safety and efficacy of various antiangiogenic agents in combination with either radiation therapy or single-agent chemotherapy for the treatment of new or progressive brain metastases from solid tumors.[121]

Other targeted therapies

NSCLC—Brain metastases from NSCLC have been shown to respond to the EGFR inhibitors gefitinib and erlotinib. In patients with unselected NSCLC brain metastasis, there was a response rate of 10–38% to gefitinib (complete and partial), with a median duration of response of 9–13.5 months.[122,123] Similar findings have been documented with erlotinib.[124–126] As for extracranial disease, response is highly dependent on the presence of an activating *EGFR* mutation.[122,123] Patients who were treatment naive were particularly responsive: in 23 Asian never-smokers with brain metastases from a NSCLC primary treated with first-line erlotinib or gefitinib, a 70% CNS response rate was observed,[127] and all seven patients with brain metastases enrolled on a phase II study of erlotinib for chemotherapy-naive advanced-stage NSCLC achieved an objective CNS response.[128] Retrospective data indicate that patients with brain metastases from *EGFR*-mutant NSCLC might have improved overall survival compared with *EGFR* wild-type cancers, particularly when they receive treatment with an EGFR inhibitor.[10] As new genetic subpopulations of NSCLC are identified that can be targeted with small-molecule inhibitors, such as cancers harboring an *ALK* rearrangement that are responsive to crizotinib,[129] it will be important to assess whether there is a differential response to targeted therapies in the CNS compared with extracranial sites of disease. The CNS responses seen with the first generation of small-molecule EGFR inhibitors in properly selected patients suggest that the use of drugs that are highly effective is at least as important as drug delivery for treating patients with brain metastases.

HER2-amplified breast cancer—Patients with HER2-positive breast tumors are at high risk of developing brain metastases, with a frequency as high as 35% in patients with advanced-stage disease.[130,131] A combination of factors likely explains the increased incidence of CNS disease in these patients, including the ability of HER2 to increase brain colonization[73] via its downstream molecules including heparanase—a target of microRNA-1258[132]—and improved control of systemic disease with the anti-HER2 monoclonal antibody, trastuzumab, which has poor BBB penetration.[130] Lapatinib, a small-molecule tyrosine kinase inhibitor of EGFR and HER2, has limited activity as a single agent for patients with refractory brain metastases,[133] but slightly higher activity in combination with capecitabine.[134] In a phase III randomized study of capecitabine plus lapatinib versus capecitabine alone for advanced-stage, trastuzumab-refractory breast cancer, fewer patients in the combination arm had symptomatic CNS progression as part of the first progression event compared with those not receiving lapatinib.[135] Furthermore, in a preclinical study using an experimental model of HER2-positive breast cancer brain metastases, lapatinib inhibited the formation of large brain metastases by 54%, suggesting preventative activity and supporting the clinical findings.[74] It should be noted that trastuzumab not only directly affects HER2-expressing tumor cells but also acts as an antiangiogenic cocktail by downregulating multiple proangiogenic factors and inducing endogenous antiangiogenic factors, and normalizes blood vessels of breast cancer grown in the leptomeningeal space.[75] Unfortunately, the decrease in VEGF expression in cancer cells is compensated by an increase in VEGF expression in host cells. These findings suggest that an anti-VEGF agent (for example bevacizumab) might be beneficial when combined with trastuzumab or lapatinib.[75] Ongoing clinical trials of combined anti-VEGF and anti-HER2 therapies in patients with metastatic breast cancer will likely shed on whether this hypothesis is supported by the data.[136]

Melanoma—Melanoma accounts for 5–20% of all brain metastases, and 40–60% of melanomas carry an activating mutation in the gene encoding BRAF, a serine–threonine protein kinase.[137] In a phase I study, administration of an inhibitor of mutated BRAF, PLX4032 (Plexxikon, Berkeley, CA, USA), to patients with metastatic melanoma harboring

this mutation resulted in complete or partial tumor regression in the majority of patients. However, patients with active brain metastases were excluded from this study.[137] In a separate study using the BRAF inhibitor GSK2118436 (GlaxoSmithKline, Brentford, UK), all seven evaluable patients with previously untreated brain metastases showed CNS tumor shrinkage, including three complete responses, and parallel extracranial responses were noted in most patients.[138]

Conclusions

Many genes whose products are necessary for the steps of brain metastases formation—in particular extravasation and colonization—have been identified. New developments of more clinically relevant models and advanced imaging techniques over the past couple of years have begun to facilitate understanding of the intricacies of brain metastases. It is increasingly evident that the brain microenvironment has a key role in the metastatic growth process, as well as in resistance to antitumor therapies. Delivery of drugs across the BBB is a challenge, albeit more so in smaller lesions. Although the BTB is not as tight as the BBB, concentrations of drugs in brain lesions are usually lower compared with extracranial sites. The grand challenge now is to integrate this knowledge and develop novel strategies to target the unique microenvironment of brain metastasis.

Acknowledgments

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Key points

- Longer survival of patients and more-sensitive detection of metastatic disease by improved imaging modalities might contribute to the increased incidence of detected brain metastases

- Novel preclinical models that more accurately represent clinical brain metastases and imaging techniques that allow study of the formation of brain metastases and their response to treatments are emerging

- Brain metastases grow by co-opting existing blood vessels and/or by forming new blood vessels; the brain microenvironment promotes tumor cell survival, tumor growth and resistance to therapy

- Although a lesser problem in large metastases, the blood–brain barrier (BBB) could prevent therapeutic access to micrometastases; strategies to enhance drug delivery across the BBB are under investigation

- Differential expression of genes involved in intravasation and extravasation, metabolism, cell adhesion, and cellular signaling in brain-specific metastatic clones have been identified

- Targeted therapies, including inhibitors of EGFR, HER2, PI3K and BRAF, have shown promise in the treatment of brain metastases, but require testing in randomized trials

Review criteria

Information for this Review was compiled by searching the PubMed database for articles published before 1 March 2011. Search terms included "brain metastasis animal model", "brain metastasis blood–brain barrier", "brain metastasis genes", "brain metastasis microenvironment", "brain metastasis therapy", "brain tumor angiogenesis", "central nervous system metastasis animal model", "intravital microscopy", and "small animal imaging". Full articles were checked for additional material when appropriate, and articles that cite key publications were also checked.
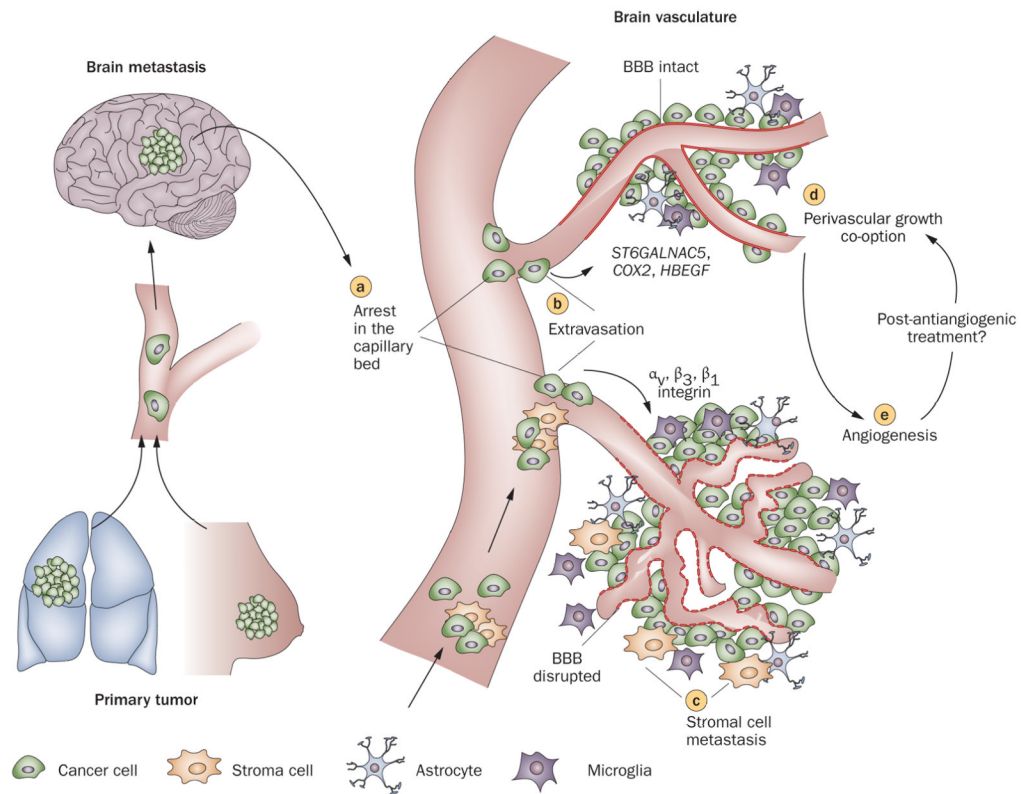
Figure 1.
Steps in the formation of hematogenous metastasis to the brain. Once tumor cells shed from primary tumors arrive in brain vasculature, **a** | they arrest in the capillary bed primarily owing to size restriction, and **b** | subsequently extravasate across the BBB and enter the brain parenchyma. Three genes mediate cancer cell trans-BBB migration: *HBEGF*, *COX2*, and *ST6GALNAC5*.[78] Also, activation of integrins, such as $\alpha_v\beta_3$[54,139] and β_1,[27] is suggested to control tumor-cell arrest and adhesion to the vasculature. **c** | Metastasizing tumor cells (seeds) may bring their own host cells (soil) in metastasis.[24] After extravasation, tumor cells either **d** | grow along pre-existing blood vessels (perivascular growth),[49] or **e** | recruit new blood vessels (angiogenesis) to obtain sufficient nutrients to support their proliferation. Abbreviation: BBB, blood–brain barrier.
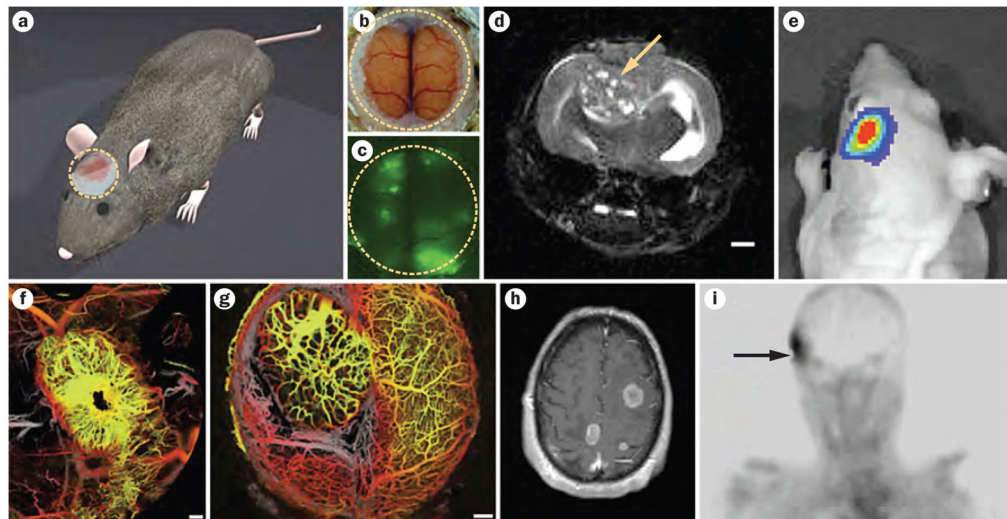
Figure 2.
Imaging brain metastases in preclinical models and patients. **a** | Chronic cranial window (yellow dotted line) for intravital brain imaging (courtesy of Lance Munn). Intravital imaging through cranial window showing **b** | normal brain (bright field image) and **c** | metastatic brain tumors (green fluorescent protein-expressing breast cancer cells). **d** | T2-weighted MRI image of a metastatic brain tumor (arrow) after direct implantation of breast cancer cells (courtesy of Christian Farrar). Scale bar: 1 mm. **e** | Whole-body bioluminescence imaging of brain metastasis using Gaussia luciferase (Chung, E. *et al.* unpublished data). Optical frequency domain imaging of murine mammary carcinoma grown in **f** | breast (primary) and **g** | brain (metastasis). Scale bars: 0.5 mm. **h** | T1-weighted post-contrast MRI showing multiple enhancing mass lesions consistent with brain metastases (courtesy of A. F. Eichler). **i** | 99mTc-folate image of human brain metastasis (arrow) from a primary breast cancer (Courtesy of Phillip S. Low and Endocyte Inc.). Permission for parts f and g obtained from Nature Publishing Group © Vakoc, B. J. *et al.* *Nat. Med.* **15**, 1219–1223 (2009).
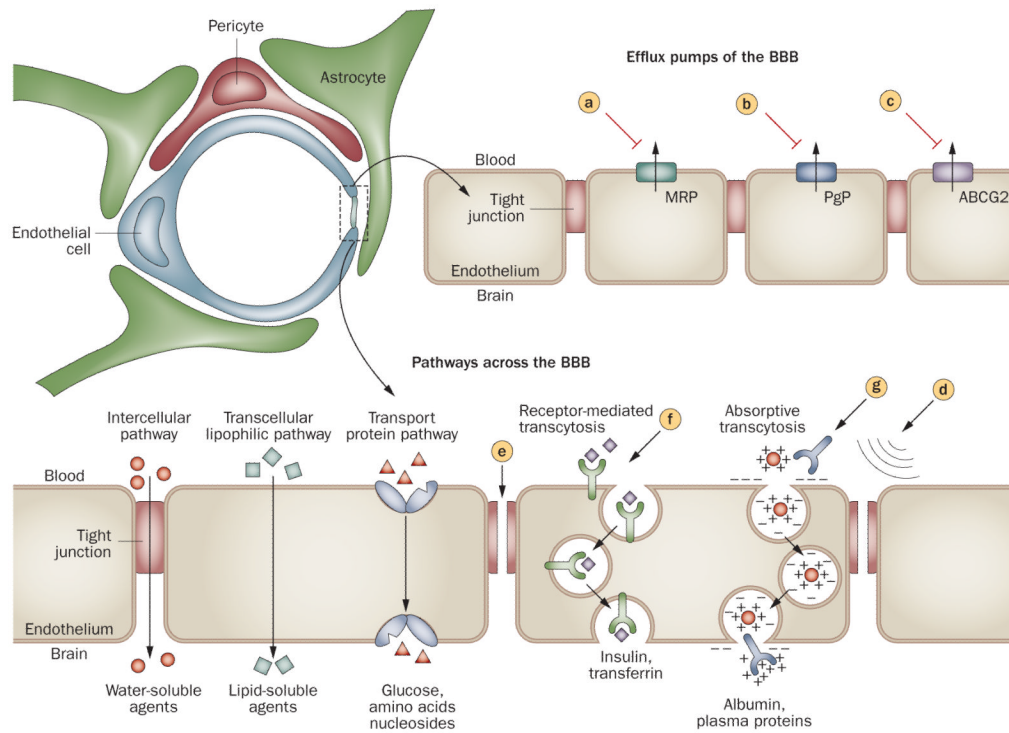
Figure 3.
Approaches to enhance drug delivery to the brain. Schematic diagram of the BBB with an enhanced illustration of the brain capillary endothelial cell. The main drug efflux transporters of brain capillary endothelial cells include MRPs, PgP, and ABCG2. All of these transport proteins have been targeted for pharmacological inhibition. **a** | probenecid, sulfinpyrazone and MK-571; **b** | verapamil, cyclosporin A, quinidine, valspodar, elacridar, biricodar, zosuquidar, and tariquidar; and **c** | GF120918, elacridar, and fumitremorgin C. Tight junctions normally restrict penetration of water-soluble compounds across the BBB, but they can be disrupted by mechanical and pharmacological methods, via **d** | ultrasound and **e** | bradykinin analogs, respectively. **f** | Receptor-mediated transcytosis of transferrin or insulin has been used to increase transport of drugs across the BBB, and **g** | cationization (that is, antibodies) can increase uptake of molecules by absorptive transcytosis. Abbreviations: ABCG2, breast cancer resistant protein; BBB, blood–brain barrier; MRPs, multidrug resistant proteins; PgP, P-glycoprotein.

Table 1

Genes involved in the formation of brain metastasis

Genes	Function	Primary tumor type	Comments
COX2	Important in prostaglandin production, possibly leading to increased permeability of BBB	Breast	Inhibition suppresses penetration of an artificial BBB, and enhances brain-metastasis-free survival[78]
HBEGF	EGFR ligand—increases cell growth, motility, and invasiveness	Breast	Inhibition suppresses penetration of an artificial BBB, and enhances brain-metastasis-free survival[78]
ST6GALNAC5	Sialyltransferases catalyze the addition of sialic acid to gangliosides and glycoproteins, and cell-surface sialylation has been implicated in cell–cell interactions	Breast	Inhibition suppresses penetration of an artificial BBB, and enhances brain-metastasis-free survival[78]
HK2	Important in glucose metabolism, oxidative phosphorylation, and antiapoptosis	Breast	High HK2 expression is associated with poor patient survival after craniotomy[140]
FOXC1	Transcription factor essential for mesoderm development; involved in brain development and brain tumorigenesis	Breast	Predicts poor overall survival in basal-like breast cancer, a higher incidence of brain metastasis and a shorter brain-metastasis-free survival in lymph-node-negative patients[141]
HER2	Receptor tyrosine kinase of the EGFR family	Breast	Overexpression increased the incidence of large brain metastases ($>50\ \mu m^2$)[73]
VEGFA	Angiogenic growth factor	Breast	Increased in brain-metastatic clones, and VEGFR inhibition decreased brain tumor burden via a reduced number of blood vessels, decreased proliferation and increased apoptosis[52]
		Melanoma	Overexpression accelerated growth, accompanied by dilation of co-opted tumor vessels with concomitant induction of vascular permeability[48]
		Lung and colon	Decreased expression significantly decreased the incidence of brain metastases[51]
LEF1	A transcriptional effector of the canonical WNT pathway	Lung	Part of a signature that predicts lung metastasis to the brain; knockdown inhibited brain metastasis, and decreased colony formation and invasion in vitro[142]
HOXB9	Belongs to the homeobox transcription factor gene family, which is critical for embryonic segmentation and limb patterning—a TCF4 target	Lung	Part of a signature that predicts lung metastasis to the brain; knockdown inhibited brain metastasis, and decreased colony formation and invasion in vitro[142]
CDH2, KIFC1, and FALZ3	N-cadherin is a calcium-dependent cell–cell adhesion molecule	Lung	Highly predictive of brain metastasis in early-stage and advanced-stage lung cancers—causal role is not clear[143]
STAT3	Important transcription factor in cellular signaling pathways	Melanoma	Reduction suppressed brain metastases—affected angiogenesis in vivo and cell invasion in vitro[144]
$\alpha_v\beta_3$	Important for sprouting endothelial cells, contributes to angiogenesis, supports invasion and metastasis	MDA-MB-453	Activated $\alpha_v\beta_3$ enhances brain metastatic tumor growth through continuous upregulation of VEGF, leading to increased angiogenesis and decreased hypoxia[54]

Genes	Function	Primary tumor type	Comments
HDAC3, JAG2, NUMB, APH1B, HES4, and PSEN1	Notch signaling pathway genes that determine cell fates through communication with their environment	MDA-MB-453	Inactivation of Notch significantly inhibited migration and invasion[145]

Abbreviation: BBB, blood–brain barrier.

Table 2

Preclinical rodent animal models of brain metastasis

Tumor dissemination routes	Methods	Advantages	Disadvantages
Direct implantation model			
Leptomeningeal metastasis[75,146,147]	Inoculation of tumor cells or fragments into the subarachnoid space, or subarachnoid catheter for delivery to the cerebrospinal fluid space	Relatively simple procedure; convenient for longitudinal imaging study with molecular or intravital microscopic imaging	Bypasses precolonization steps of metastasis; does not model cerebral brain metastases
Intraparenchymal implantation[27,148,149]	Inoculation of tumor cells or fragments directly into the brain parenchyma by hand or stereotactic guidance	Simple way to study metastatic tumor growth inside the brain; the procedure has been extended by placement of a cranial window or burr hole (at the site of inoculation)	Bypasses precolonization steps of metastasis
Hematogeneous metastasis model			
Intracardiac injection[25,78]	Injection of single-cell suspension of tumor into the left ventricle of an animal	Relatively simple procedure and can bypass pulmonary arrest and retention	Dissemination of tumor cells to sites other than cerebral target; decreased reproducibility due to the blind nature of the inoculation compared with direct implantation
Intracarotid artery injection[72,150,151]	Injection of metastatic cells into the internal carotid artery followed by permanent ligation	Produces predominately cerebral tumors and minimal noncerebral metastasis	Murine carotid is very small and significant microsurgical skill is required
Spontaneous metastasis model			
Spontaneous formation[63,77,152]	Spontaneous brain metastases subsequent to removal of primary tumor from either parental or metastatic variants	Recapitulates complete metastatic steps; generated from other simpler model systems; useful for confirming hypotheses	Longer duration requiring resection of primary tumors or multiple preselection processes; most complicated method to run a controlled experiment

Table 3

Strategies to circumvent the blood–brain barrier

Strategy	Methods	Potential applications
Physical		
Convection-enhanced delivery[153]	Catheters placed around the resection cavity at the time of surgery, left in place for infusion of drug for 1–2 days	Large brain metastases that cannot be totally resected; to improve local control rates after gross total resection
Osmotic BBB disruption[154]	Intra-arterial infusion of hyperosmotic agent before infusion of chemotherapeutic, antibody, or nanoparticle drug	Multiple brain metastases from a chemosensitive primary tumor
Targeted ultrasound BBB disruption[153]	Intravenous injection of preformed gas bubbles before pulsed ultrasound treatment	Single or limited number of brain metastases from a chemosensitive primary; single refractory or recurrent brain metastasis
Pharmacological		
Bradykinin analogs[155–157]	Intravenous delivery to transiently increase the permeability of the BBB	In combination with chemotherapy (for example, RMP-7 increased the delivery of carboplatinum into intracranial brain tumors)
Exploiting RMT:[158] TfR, IR, IGF-1R, LRP-1	To achieve RMT, chemotherapy of choice linked to an antibody that targets the TfR, IR, IGF-1R, or LRP-1	Broad applicability for single or multiple brain metastases
PgP inhibitors[159–161]	Inhibiting the drug efflux pump (for example, HM30181A, cyclosporine A, valspodar, elacridir, zosuquidar)	Administration concurrently with chemotherapy for broad applications

Abbreviations: BBB, blood–brain barrier; IGF-1R, insulin-like growth factor-1 receptor; IR, insulin receptor; LRP-1, low density lipoprotein receptor-related protein 1; PgP, P-glycoprotein; RMT, receptor-mediated transcytosis; TfR, transferrin receptor.