Cancer Research

Review

A Blazing Landscape: Neuroinflammation Shapes Brain Metastasis

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Abstract

Brain metastases are more common than primary CNS tumors and confer grave prognosis on patients, as existing treatments have very limited efficacy. The tumor microenvironment has a central role in facilitating tumorigenesis and metastasis. In recent years, there has been much progress in our understanding of the functional role of the brain metastatic microenvironment. In this review, we discuss the latest advances in brain metastasis research, with special emphasis on the role of the brain microenvironment and neuroinflammation, integrating insights from comparable findings in neuropathologies and primary CNS tumors. In addition, we overview findings on the formation of a hospitable metastatic niche and point out the major gaps in knowledge toward developing new therapeutics that will cotarget the stromal compartment in an effort to improve the treatment and prevention of brain metastases.

Introduction

Brain metastases are one of the deadliest forms of tumor metastasis. Arising in 10%–30% of adult patients with systemic malignancies (1), brain metastases confer dismal prognosis, with a median survival of less than one year (Fig. 1A; refs. 2, 3). The main cancer types that frequently metastasize to the brain are lung, breast, melanoma, renal, and colorectal cancers (2). Different studies suggest that the incidence of brain metastasis is twice to 10 times higher than primary central nervous system (CNS) malignancies (e.g., glioma; refs. 4, 5). Interestingly, postmortem studies suggest higher incidence of brain metastases compared with clinically diagnosed incidence (6). Moreover, the incidence of brain metastasis appears to be on the rise (7). Possible explanations for this apparent increase include better diagnosis of smaller, asymptomatic brain metastasis by MRI, and improved control of extracranial disease by systemic therapy, enabling the emergence of otherwise not clinically manifested metastasis (1, 6).

When discussing brain metastases, it is important to keep in mind that they are not a single clinical entity: major differences in the diagnosis, treatment, and prognosis depend on various parameters, including the primary tumor from which metastases developed, suitability for targeted therapies, number of metastases, stage of extracranial disease, etc. Brain metastasis from different primary tumors can occur early in the clinical course of the disease, at the time of initial diagnosis (synchronous), or sometimes months or years after surgical removal of the primary tumor (metachronous). The currently used diagnosis-specific graded prognostic assessment (DS-GPA) of patients with brain metastases are associated with tumor-specific parameters: The DS-GPA for non–small cell lung carcinoma (NSCLC), breast cancer, and malignant melanoma includes molecular predictive markers, such as EGFR, Her2, and B-Raf, respectively, to identify subgroups with a significantly improved overall survival (OS; refs. 8–10). For example, patients with the best melanoma molecular markers (mol-GPA) score have an estimated OS of 34.1 months compared with 7.1 months in the past (8). In addition to molecular subgroups, the OS of patients with brain metastasis also correlates with the infiltration pattern of macrometastases at the brain parenchyma/metastatic interface: while metastases of renal cell cancer are mainly noninfiltrative and are additionally protected by a highly vascularized collagen capsule, the majority of NSCLC brain metastases infiltrate into the adjacent brain parenchyma with tumor cell cohorts, and malignant melanoma cells favor an angio-cooptive infiltration (11). In general, an infiltrative phenotype is associated with a poor prognostic outcome (11). However, the underlying mechanisms that differentiate patterns of brain metastatic infiltration are poorly understood.

In brain metastasis, earlier studies identified gene signatures in primary breast cancer cells that were associated with brain tropism (12), while a more recent study demonstrated branched evolution that distinguishes the mutation landscape in the primary tumor from its brain metastases (13). Thus, additional coevolution with the brain microenvironment may be required to enable brain colonization by disseminated metastatic cells. There is a growing understanding that the metastatic microenvironment plays a crucial role in enabling brain tropism and colonization of disseminated tumor cells. Herein, we summarize the main findings of recent preclinical studies focused on the biology of the brain metastatic microenvironment, with emphasis on the role of neuroinflammation.

The Brain Microenvironment

Reciprocal interactions between cancer cells and the microenvironment were shown to contribute to tumor progression and to organ-specific metastasis (14–17). The brain harbors a unique

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AACR 423

microenvironment: the macroglia cell population, which is composed of 75.6% oligodendrocytes and 17.3% astrocytes and the microglia that represent 6.5% of the nonneuronal cells (18). The brain microenvironment also includes endothelial cells, pericytes, ependymal cells, resident and infiltrating immune cells, and other cell types, which play a crucial role in maintaining physiologic homeostasis by communications with neurons (Box 1; Fig. 1B; ref. 18).

Similarly to other organs of the body, cells in the microenvironment of the brain can sense and respond to danger signals induced by pathogen invasion or tissue damage, resulting in instigation of neuroinflammation (19). This physiologic response in the brain microenvironment is reminiscent to changes that occur in the brain metastatic niche, as will be discussed herein. Notably, preclinical studies of brain metastasis and of the interactions of metastatic tumor cells with the microenvironment mostly rely on murine models. While all models entail certain limitations (Box 2), they nevertheless provide important insights on metastatic extravasation, invasion into the brain parenchyma, growth in the brain, interactions with the microenvironment, and therapeutic responses.

Neuroinflammation in Brain Metastasis

Neuroinflammation in the CNS is instigated in response to damage signals. Cells in the brain parenchyma can sense and respond to danger signals resulting from pathogen invasion (pathogen-associated molecular patterns, PAMP) or tissue damage (damage-associated molecular pattern, DAMP). In the first line of response, DAMP/PAMP signals rapidly activate the adjacent astrocytes and microglia (45). This locally circumscribed first line of glial response is very potent in the defense from pathogenic intruders or in repairing tissue damage without recruiting other cell types. Thus, astrocytes and microglia are central in regulating the CNS homeostasis and tissue repair. However, if this first line of defense is not effective or the damage/affected area is too large, then full blown neuroinflammation develops. This is characterized by enhanced secretion of cytokines and chemokines, persistent activation of astrocytes and microglia, increased blood vessel permeability, and recruitment of immune cells (46, 47). Knowledge on the instigation and regulation of neuroinflammation mainly originated in studies of neurologic diseases and CNS injuries (19, 48). Dysregulation of these pathways leads to uncontrolled damage response, which contributes to the pathology of neurodegenerative diseases (49), exacerbates vascular injury following stroke (50), drives autoimmune diseases (51), and facilitates primary CNS tumors (52).

Many of the changes described in the brain metastatic microenvironment are reminiscent of these dysregulated tissue damage responses, including permeability of the BBB (53), instigation of astrogliosis and neuroinflammation (54), and recruitment of leukocytes (55), which in turn collectively facilitate metastatic growth of invading cancer cells (25, 56). Therefore, findings from these fields may help us understand the roles of cellular brain components at various phases of brain metastasis. These parallels suggest that the physiologic tissue damage response in the brain is hijacked during colonization by metastatic cells, and neuroinflammation is recently emerging as a promising direction to uncover processes associated with the development of brain metastases (57–59).

Box 1: Physiologic roles of the different brain cells

- Neurons are the excitable component of the CNS, their axons conduct electrical current. The development and specialization of neurons during embryogenesis is a very complex and temporally regulated process (20).
- Oligodendrocytes produce myelin, which is essential for proper electric conduction of neuronal signaling (21, 22).
- Astrocytes have many functional roles in maintaining brain homeostasis: they regulate potassium and pH levels, modulate synaptic transmission by glutamate and GABA uptake, and control the vascular tone and cerebral blood flow (23). Two main classes of astrocytes include protoplasmic astrocytes residing within the gray matter and fibrous astrocytes located in the white matter (21).
- Microglia are specialized resident macrophages in the brain, which originate in the yolk sac. Microglia secrete growth factors and inflammatory mediators, scavenge cell debris, and is involved in the CNS homeostasis (24).
- Endothelial cells and their extracellular matrix form the blood vessels of the brain. Endothelial cells express efflux pumps and establish adjacent tight junctions, both of which are necessary for selective permeability of the blood–brain barrier (BBB; ref. 25).
- Pericytes are mesenchymal cells, which constitute an integral part of the neovascular unit of the BBB. Pericytes occur throughout the vasculature of the body, but are most dense in brain capillaries. Pericytes regulate cerebral blood flow (26) and produce proteins of the basal lamina (27).
- Ependymal cells line the ventricles of the brain and are part of the blood–cerebrospinal fluid (CSF) barrier (BCSFB; refs. 28, 29).
- Choroid plexus is a specialized organ within the brain that produces the CSF and is also part of the BCSFB (28).
- Post-capillary venules: an important part of the BBB. Composed of at least seven layers: 1, endothelial cells connected by tight junctions; 2, inner basement membrane; 3, media, 4, outer basement membrane; 5, Virchow–Robin space with pericytes, macrophages, and other cell types; 6, a third basement membrane; 7, the glia limitans, consistent of astrocyte end-feet (See Fig. 1B).
- Blood–leptomeningeal barrier (BLMB): an important immunologic barrier, typically activated during meningitis. Activation switches the cellular and molecular composition of the CSF from an immunosuppressive body fluid to a highly immune active one.
- Infiltrating immune cells can be recruited to the brain via the BCSFB to the CSF or via the blood capillaries, mainly via post-capillary venules (29, 30). T cells and monocyte-derived macrophages contribute to neuronal functions and are central to neuronal repair (31, 32) and during the brain response to pathogen invasion and inflammation (33).

Astrocytes in neuroinflammation

Activation of astrocytes in response to tissue damage is termed "astrogliosis." It is characterized by upregulation of the

Box 2: Murine models of brain metastasis to study the brain microenvironment and neuroinflammation

- **Models of spontaneous brain metastasis**

  By definition, a spontaneous model of brain metastasis is a model in which cancerous cells from the primary tumor are allowed to detach, invade, circulate, extravasate, and colonize the brain. The main advantage of utilizing such models is that they allow investigation of the different metastatic stages, as well as studying early stages of brain metastasis in a clinically relevant setting. However, most spontaneous models are time consuming and do not always provide high percentages of brain metastasis, making large cohorts of mice necessary.
  - The primary tumors in spontaneous models are either orthotopically inoculated or arise in a genetically engineered mouse model (GEMM) of a certain cancer type. Brain metastasis in GEMMs was reviewed elsewhere (34, 35).
  - Resection of the primary tumor, when feasible, allows better modeling of the clinical settings, and usually entails a period of latency before metastatic relapse, thus enabling a time window for preclinical preventative studies (36).
  - Spontaneous models of brain metastasis can be established in immunocompetent or in immunodeficient mice, depending on the source of the cells to be studied.

  Summary of murine models of spontaneous brain metastasis

  - **Melanoma:**
    - RMS: orthotopic (subdermal), immunocompetent, primary tumor removal. Micrometastases, 50%; macrometastases, 23% (37).
    - YDFR.CB3: orthotopic (subdermal), immunodeficient, micrometastases 75% (38).
    - 131/4-5B2: orthotopic (subdermal), immunodeficient, primary tumor removal. Micrometastases, 66%; macrometastases, 20% (39).
  - **Lung**
    - A549: orthotopic (intrathoracic), immunodeficient. Macrometastases, 61% (40).
  - **Breast**
    - 4T1: orthotopic (intraductal), immunocompetent, primary tumor removal. Micrometastasis, 67% (41).
    - Fgf4-MCF7: orthotopic (intraductal), immunodeficient. Macrometastases, 35% (42).
    - MDA-MB-231 and CN34BrM: orthotopic (bilateral intraductal), immunodeficient, NSG mice. Macrometastases: MDA-MB-231, 100%; CN34BrM, 90% (43).

- **Models of experimental brain metastasis**

  Experimental metastasis models include the direct inoculation of tumor cells into the circulation and provide a model for hematogenous dissemination of cancer cells to generate brain metastases.

- Intracardiac injection: inoculation of tumor cells into the left ventricle of the heart.
- Intracarotid injection: inoculation of tumor cells directly into the cerebral circulation.

  Experimental models are widely used, as they achieve a high penetrance of brain metastasis in relatively small cohorts of mice and are a valuable tool for studying macrometastases, especially in preclinical testing of therapeutic intervention.
  Disadvantages of these models include the technically challenging nature of injection techniques, and the fact that the high load of injected cells may not accurately mimic the clinical setting in which metastasis occurs.

- **Models of intracranial tumor cell injections**

  Tumor cell growth in the brain can be achieved by intracranial injection: the direct inoculation of tumor cells from various cancer types directly into the brain tissue. Intracranial models of brain metastasis can be established in immunocompetent or in immunodeficient mice (including patient-derived xenografts; ref. 44), depending on the source of the cells to be studied.
  Intracranial injections yield rapidly growing brain lesions and are an important tool to assess the growth pattern of tumor cells in brain, the various infiltration types and dissemination into the brain parenchyma, and to test the efficacy of novel therapeutics in a preclinical setting of full blown macrometastasis. However, the injection of cells into the parenchyma may initially cause trauma to the brain, thus invoking astrogliosis and neuroinflammation. Therefore, when employing such models, it is important to design suitable controls.

cytoskeletal glial fibrillary acidic protein (GFAP), upregulation of proinflammatory genes (60), and identified by the formation of a "gliotic scar": reactive astrocytes with elongated processes that form a physical and functional wall around demarcated brain lesions (61). Astrogliosis has neuroprotective functions that support neuronal recovery during the acute phase, but may entail detrimental inflammatory responses that induce further tissue damage (62). Astrogliosis is also evident in brain metastases: astrocytes surround and infiltrate brain metastases in mice, and are recruited to the brain–metastases interface in human brain metastases (57, 59, 63), and therefore the complex interactions between astrocytes and tumor cells are a topic of intense research.

Proinflammatory signaling in astrocytes was shown to be activated by lung cancer cell–derived factors such as macrophage migration inhibitory factor (MIF), IL8, and plasminogen activator

Doron et al.
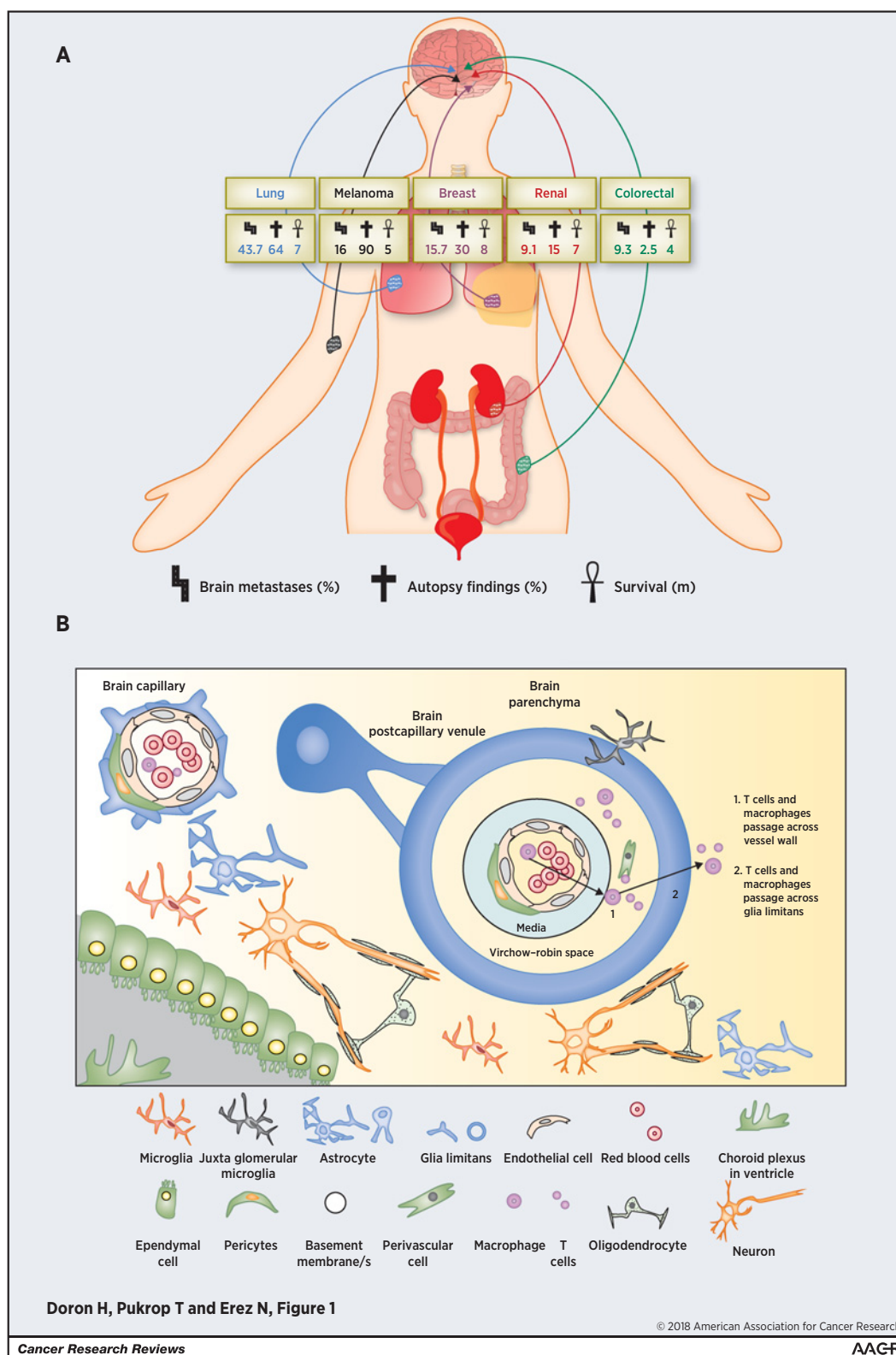
Figure 1.
A, Epidemiology of brain metastases. Summary of main epidemiologic findings from various primary tumor types that metastasize to brain (percentage of brain metastasis cases diagnosed; m, months; refs. 1, 2, 171–173). **B,** The brain microenvironment. Illustration of different cell types in normal brain. The physiologic roles of different brain cells are detailed in Box 1.

inhibitor-1 (PAI-1) in coculture experiments (64). Once activated, astrocytes secrete TNFα, IL6, and IL1β. Such proinflammatory activation of astrocytes stimulated *in vitro* proliferation of lung cancer cells (64). While the molecular players may vary, inflammatory activation of astrocytes seems to be a general feature of brain metastasis: melanoma cell–secreted factors induced IL23 expression in astrocytes, leading to enhanced transendothelial migration of melanoma cells *in vitro* (65). Notably, astrocytes also express IL23 during autoimmune diseases, such as multiple sclerosis (MS; ref. 66). In breast cancer, tumor cell–derived IL1β activated JAG1 signaling in astrocytes, which in turn promoted cancer stem cell (CSC) self-renewal *in vitro* and experimental brain metastasis *in vivo*. Brain metastasis-free survival in patients with breast cancer correlated with lower expression of IL1β, suggesting clinical relevance of these findings (67). Similarly, secretion of MMP-1 and COX-2 by brain-metastasizing breast cancer cells induced expression of CCL7 by activated astrocytes, which in turn promoted BBB permeability and experimental brain metastasis *in vivo* (68). These findings implicate the importance of proinflammatory signaling by astrocytes to promote multiple aspects of brain metastasis. However, the functional role of specific cytokines and chemokines that were identified in coculture studies should be further evaluated *in vivo*.

Induction of neuroinflammation was shown to precede macrometastases: brains bearing spontaneous melanoma micrometastases already express higher levels of the inflammatory mediators CCL17, CCL2, and CXCL10 (37). Notably, CXCL10 was also shown to be expressed by astrocytes in Alzheimer's disease in association with senile plaques (69), and in response to LPS exposure and stroke (60). These findings implicate stromal cell–derived CXCL10 in neuroinflammation, as well as in the formation of brain metastasis. Similarly, CCL17, shown to be expressed by microglia in Alzheimer's disease (70), is upregulated in brains of mice inoculated with vemurafenib-resistant melanoma cells (71). This may implicate stromal cell–derived CCL17 in supporting chemoresistance to BRAF inhibitors. The parallels of inflammatory mediators in brain pathologies and in brain metastasis suggest that canonical neuroinflammatory pathways are hijacked by tumor cells to promote metastases formation and growth.

The mechanisms by which astrocytes are activated by tumor cells are still largely unresolved. The close proximity between astrocytes and tumor cells, resulting from astrogliosis, implicates reciprocal paracrine signaling as a main communication route (37, 65, 67, 72–75). Assembly of carcinoma–astrocyte gap junctions composed of connexin 43 (Cx43), resulting in proinflammatory activation of astrocytes and enhanced resistance of tumor cells to chemotherapy *in vitro* and *in vivo* (76–78). This interaction may represent another aspect of cancer-induced astrogliosis and gliotic scar formation, physically "bridging" between astrocytes and tumor cells. Once recruited and activated by brain-metastasizing cells, astrocytes induce growth-promoting signaling in tumor cells (76, 79).

The reciprocal interactions of tumor cells and astrocytes were also shown to contribute to chemoresistance and tumor cell survival due to upregulation of GASTA5, BCL2L1, and TWIST1 expression in cancer cells *in vitro* (77). Other studies demonstrated that bidirectional interactions between astrocytes and breast cancer cells support chemoresistance via activation of the endothelin axis (80). Blocking this pathway with an antagonist of the endothelin receptor was beneficial in the treatment of breast

and lung cancer experimental brain metastases (81). Interestingly, the initial response of astrocytes to brain invasion by tumor cells was suggested to be antitumorigenic: astrocytes were shown to induce tumor cell apoptosis via plasmin activator (PA) secretion. Secretion of Serpins (PA inhibitors) by tumor cells prevented apoptosis and increased vascular cooption in experimental brain metastasis (73). Thus, while the early response of astrocytes to brain invasion by tumor cells may be antitumorigenic, growth-promoting mechanisms eventually prevail and enable metastatic growth.

Microglia in neuroinflammation

Microglia are important mediators of the brain response to tissue damage (82), and are similarly activated at the tumor–brain interface in brain metastasis (83, 84). IHC analysis of microglia in human brain metastases of NSCLC, breast cancer, and melanoma indicated intense microglia activation with evident peritumoral accumulation and intratumoral infiltration (85). Thus, the localization of microglia is reminiscent of the gliosis response to traumatic brain injury sites, at the interface between normal and damaged brain tissue (86).

Proinflammatory signaling in microglia was shown to be instigated following lipopolysaccharide (LPS) injection, including expression of TNFα, iNOS, and IL6 (87). Moreover, targeting of activated microglia/macrophages was shown to reduce tumor growth in a model of intracranial injection of breast cancer cells (84). Microglia were also shown to facilitate invasion of metastatic breast carcinoma cells in an organotypic *ex vivo* brain slices model (88). This proinvasive function of microglia could be inhibited by microglia depletion, by the WNT inhibitor Dickkopf-2, by LPS treatment, or by CXCR4 inhibition, indicating the molecular pathways involved (82, 88, 89). Moreover, similar to the role of the PI3Kγ pathway in mediating immune suppression by tumor-associated macrophages (TAM) in primary breast tumors (90), a recent study demonstrated the activation of PI3K in patients with breast cancer brain metastases, and identifies its function as a master regulator of the metastasis-promoting function of microglia (91).

Findings from primary brain malignancies and from brain metastasis indicate that the metastasis-promoting functions of microglia phenotype are induced by tumor cells (92–94). Interestingly, astrocytes were also shown to modulate the recruitment of microglia to brain metastasis *in vivo* (95), adding another dimension to the complex neuroinflammatory networks that facilitate metastasis. However, while in neurodegenerative pathologies, astrocytes and microglia were demonstrate to interact reciprocally in mediating inflammatory responses that contribute to disease progression (96, 97), surprisingly little is known about the molecular factors that underlie the role of microglia in brain metastasis, and their crosstalk with astrocytes.

Recruited immune cells

Recruited macrophages and T cells are a prominent feature of neuroinflammation and brain metastasis. However, in most studies, there is no clear distinction between resident microglia and recruited macrophages regarding identification and function. A study utilizing multitransgenic mouse models that enable ontology tracing, demonstrated that bone marrow–derived macrophages are recruited into primary brain tumors (glioma) and to experimental brain metastases, where they are reprogrammed to express distinct inflammatory gene signatures

(92). These findings are supported by a study that demonstrated *in vitro* functional differences between microglia and monocyte-derived macrophages in facilitating tumor cell invasion, evident by their response to CSF-1R blockade (89).

Macrophages were shown to infiltrate into experimental breast cancer brain metastases, and to enhance tumor cell invasion and colonization via secretion of Cathepsin S (98). Intracranially implanted breast cancer cells were demonstrated to express high levels of lymphotoxin β, which propelled the polarization of infiltrating macrophages toward a tumor-promoting M2-like phenotype (99), suggesting that metastases-recruited macrophages may function in promoting an immunosuppressive brain microenvironment. Macrophages were also shown to induce breast cancer cell invasion into the brain via CSF-1 secretion (89). Interestingly, targeting macrophages in a model of primary CNS malignancy (glioma) resulted in blockade of tumor progression (93).

T cells were also implicated in brain colonization. In intracranial injections of melanoma, breast, and colon cancer cells into mice, regulatory T cells (Tregs) were shown to infiltrate cancerous lesions and to mediate immunosuppression (100). In human brain metastasis, T cells are found mainly at the interface of the brain parenchyma/brain metastases tissue and in the metastatic stroma. Several studies suggested that CD8$^+$ T cells may play a functional role in the microenvironment of brain metastasis. Moreover, while the density of regulatory T cells is not correlated with prognosis, high density of CD3$^+$ or CD8$^+$ T cells was shown to be correlated with better OS (57, 101). A study of experimental melanoma brain metastasis in mice reported a similar infiltration pattern, where most of the CD3$^+$ T cells were CD4$^+$ rather than CD8$^+$ lymphocytes (85). Interestingly, the infiltration of the effector T cells was suggested to be facilitated by the presence of extracerebral tumor lesions, which activated the endothelial cells of the BBB to enhance leukocyte trafficking into brain metastases (102). Taken together, these findings implicate the adaptive immune system in brain metastasis and suggest that immunotherapy could be a promising therapeutic avenue for the treatment of brain metastasis, as discussed below. However, the recruitment, trafficking, functions, and activation of T cells in the microenvironment of brain metastases are still largely unresolved.

Thus, activation of brain stromal cells and recruited immune cells orchestrates neuroinflammation and facilitates metastatic growth. These observations support cotargeting of both tumor cells and the inflammatory stroma in brain metastases.

The BBB and Neuroinflammation in Brain Metastasis

Brain-metastasizing cancer cells initially encounter the BBB, a highly specialized vascular structure that selectively controls the blood flow and its contents into the brain (53). The BBB is composed of endothelial cells connected by tight junctions, pericytes and the end-feet of astrocytes, which surround the basement membrane of blood vessels, effectively limiting passive paracellular permeability. In addition to this physical barrier, the BBB contains active molecular transport systems (103), which impair CNS drug delivery. Importantly, the BBB is also central in regulating the recruitment and trafficking of peripheral immune cells into the brain. For example, brain endothelial cells express abluminaly CXCL12, the ligand for CXCR4, to prevent CXCR4-

expressing leukocytes from infiltrating the brain parenchyma under normal conditions (104). Similarly, the astrocyte end-feet express the death ligand CD95L, which, under physiologic conditions, leads to T-cell apoptosis (105). Therefore, under normal conditions, the BBB also functions as an immunologic barrier. During active neuroinflammation, T cells and myeloid cells enter the brain mainly via post-capillary venules and their infiltration requires not only transmigrating the endothelial cells, but also overcoming the glia limitans (see Box 1; Fig. 1B). This is enabled during neuroinflammation by activation of brain endothelial cells and downregulation of immunologic barrier molecules on perivascular cells, to permit the entry of peripheral immune cells to the brain parenchyma. However, very little is known about the regulation of immune cell infiltration via the BBB in the context of brain metastases.

Following extravasation in the brain, tumor cells may outgrow, or remain dormant. Findings from mouse models of experimental brain metastasis of breast cancer (106) and melanoma (107) demonstrated that tumor cells were predominately positioned along the abluminal surfaces of microvessels in the perivascular space, suggesting that cooption of blood vessels in the brain and initial growth on the inner basement membrane is a central mechanism during the early growth of brain disseminated cells. Notably, the initial interactions with the brain perivascular niche may also affect dormancy, as suggested by preclinical imaging studies in mice, that detected brain-metastasizing cancer cells (from lung, breast cancer, and melanoma) in a dormant state at the perivascular niche in the brain (108–110). The specific mechanisms of vessel interactions and vascular cooption depend on various molecules, and may be fostered via adhesion with integrin beta-1 (106), and expression of L1CAM by brain-metastasizing cancer cells (73, 107). Clinical evidence from patients with melanoma brain metastasis suggested that initial metastatic growth along blood vessels is operative also in human brain metastasis (106). Interestingly, astrocytes and perivascular macrophages, which are part of the BBB, may be involved in this process: a study using various breast cancer cell lines showed that astrocyte-derived HGF induced c-MET activation in tumor cells, which in turn enhanced adhesion to endothelial cells (111). Taken together, these findings link astrocytes and microglia as part of the BBB, with tumor cell extravasation, dormancy, and immune cell trafficking (Fig. 1B).

The brain–tumor barrier

The brain–tumor barrier (BTB) is the vascular system around and within CNS tumors and brain metastases. Permeability of the BTB is a subject of debate; some studies suggested that impaired BBB and permeable BTB are hallmarks of brain metastasis (53). Conversely, other studies in various mouse models of brain metastasis (including breast cancer and melanoma), reported that the permeability of the BTB is vastly heterogeneous, and is not well correlated with metastatic growth rate (112–115). Interestingly, quantitative fluorescence microscopy in preclinical tumor models of glioma compared with brain metastasis of breast cancer, reported that glioma-associated vasculature is more permeable than the vasculature in brain metastases (116).

Increased BBB permeability was shown to be an early event, associated with the formation of spontaneous brain micrometastases in mice (37). Increased permeability may also be part of the instigated neuroinflammation in the metastatic microenvironment. CCL2 upregulation in cancer cells resulted

in increased transendothelial migration of small-cell lung carcinoma (117).

Moreover, endothelial cells are active mediators of proinflammatory signaling that facilitates increased BBB permeability and enhanced invasion capacity of tumor cells: secretion of TNFα by brain endothelial cells induced by tumor cell–secreted substance P, augmented breakdown of the BBB and colonization of breast cancer cells in brain by modifying the localization and distribution of tight junctions on brain endothelial cells (118). A study on breast cancer brain metastasis showed that VEGF secretion from breast cancer cells induced activation of inflammatory STAT3 signaling in endothelial cells. STAT3 inhibition resulted in decreased invasion of breast cancer cells to brain, and suppression of angiogenesis and metastasis (119). These studies suggest that proinflammatory signaling contributes to increased BBB permeability thus enhancing tumor cell invasion into the brain.

In this context, it is important to ask whether modifying the brain vessel permeability has therapeutic potential. While at early stages, enhanced permeability may contribute to metastatic invasion, selectively increasing the BTB permeability in overt brain metastasis was suggested to improve efficacy of therapeutic targeting in a mouse model of brain metastasis (120). In clinical studies, whole-brain radiotherapy (WBRT) for brain metastasis was reported to result in increased BBB permeability in patients with brain metastases from NSCLC (121). However, future studies are required to better elucidate the interplay between neuroinflammation, BTB permeability, and improved therapeutic delivery and efficacy of drugs to treat brain metastases.

The Brain Microenvironment and Targeted Therapeutics

Most chemotherapies, targeted therapeutics, and immunotherapy that are routinely used systemically to treat metastatic disease are often less effective in treating brain metastases from solid tumors. The brain microenvironment is an important determinant in the response to systemic therapeutics, and was shown to actively mediate resistance to chemotherapy in multiple *in vitro* and *in vivo* mouse studies (77, 80, 122).

Preclinical testing of microenvironment-targeted therapeutics resulted in some promising outcomes. For example, targeting of Cathepsin S, secreted by both tumor cells and macrophages reduced experimental brain metastasis of breast cancer (97) and cotargeting of tumor cells and VEGF receptor-2 inhibited growth of breast cancer xenografts in brain (123). In clinical trials, targeting VEGF-A with bevacizumab was tested in combination with WBRT in patients with brain metastases from various solid tumors, but did not prove to be effective (124), and a first-in-human clinical trial with BLZ945, a drug targeting CSF-1R, is ongoing for metastatic solid tumors (ClinicalTrials.gov). Preclinical studies also showed that cotargeting astrocytes using macitentan, an antagonist of the endothelin receptors, was beneficial in experimental brain metastasis of lung and breast cancers (81, 125). The results from preclinical studies emphasize the potential advantages of therapeutic combinations that include targeting of the brain microenvironment (Table 1). Nevertheless, data on the benefits of microenvironment-targeted therapeutics in brain metastases is very limited, as most clinical trials of targeted therapeutics are focused on molecules expressed by cancer cells (126, 127).

Immunotherapy for treatment of brain metastasis

The approval of immunotherapeutic strategies and immune checkpoint blockade dramatically changed the landscape of treatment strategies for several cancer types, in particular melanoma, NSCLC, and renal cell carcinoma (128), which have a high rate of brain metastasis. Brain metastases originating in tumors with a high mutagenic load (such as melanoma and lung cancer) are likely to be better candidates for immune checkpoint therapy. Indeed, immune checkpoint inhibition with mAbs targeting the CTLA4 (Ipilimumab, anti-CTLA-4) and antibodies that target the programmed cell death protein 1 (nivolumab, pembrolizumab, anti-PD-1) have revolutionized the treatment of metastatic melanoma (129).

Findings of T-cell composition and infiltration in patients with brain metastasis suggest that these considerations may be relevant also to brain metastatic relapse: while some patients have very little TILs in brain, high density of CD3$^+$ or CD8$^+$ T cells correlated with better OS (101, 130). Notably, expression of the PD-1 ligand (PD-L1) was demonstrated in human specimens of brain metastases from melanoma (131) and breast cancer (130), and correlated with higher density of tumor-infiltrating lymphocytes expressing PD-1, suggesting that upregulation of immune checkpoints may be important for the ability of brain metastases to evade the immune system and enhance immunosuppression.

In this context, it is also important to consider the role of myeloid derived suppressor cells (MDSC), immature myeloid cells with suppressive activity on T cells via immune checkpoint signaling. While not much is known about MDSCs in brain metastasis, they were implicated in immune suppression in neuroinflammatory diseases (132) and their infiltration to human glioblastoma tumors was associated with reduced TILs in brain lesions (133). Moreover, blockade of MDSCs was suggested to be beneficial in combination with immune checkpoint treatment in mouse models of glioblastoma (134) and in prevention of brain metastasis (135), suggesting that MDSC blockade should also be considered in immunotherapy of human brain metastasis.

Patients with brain metastasis were previously excluded from clinical trials with immune checkpoint inhibitors, limiting the available knowledge on the efficacy of immunotherapeutics for brain metastasis. However, several recent clinical studies performed in patients with melanoma or in NSCLC with active brain metastases that were treated in monotherapy or in combination of both checkpoint inhibitors, demonstrated benefit on OS of double checkpoint blockade (136–141), and multiple other studies are ongoing (142). Notably, the beneficial effects of anti-CTLA-4 and anti-PD1 on brain metastasis may be mediated, in part, by sustained systemic effects on activated T cells that infiltrate the brain and engage in antitumor responses. This is supported by a recent preclinical study demonstrating that efficient immune checkpoint inhibition for the treatment of intracranial melanoma depended on systemic activation of CD8$^+$ T cells and enhancement of their recruitment to the brain (102). Additional clinical findings, as well as preclinical mechanistic studies, are required to investigate the efficacy, and provide the rationale for immune checkpoint blockade in the treatment of brain metastasis.

Radiotherapy, a standard therapy approach for CNS tumors and brain metastasis, was shown to induce immunostimulatory effects that may be therapeutically beneficial, including downregulation of immunosuppressive cytokines, enhanced immune cell recruitment, and increased CTL efficacy (143). These effects on activation of the immune response result, at least partially, from

Doron et al.

Table 1. Concise summary of main findings and targetable molecules in neuroinflammation instigated in brain metastasis

Neuroinflammatory pathways or function found in brain metastasis	System	Targeted?	References	Neuroinflammatory pathways or function in CNS pathology	References
Proliferation of metastatic cells is mediated by: Tumor cell secreted: MIF, IL8, and PAI-1 Astrocyte secreted: IL1β, TNFα, and IL6	In vitro	No	(64)	C/EBP-β NFκB	(148) (149, 150)
Tumor cell invasion to the brain is regulated by: Astrocyte-derived:	In vitro				
IL23		Anti-IL23 Ab	(65)	Antigen presentation	(66)
CCL7		shCCL7	(68)	MAPK, NFκB	(151)
MMP2		Anti-MMP2 Ab,	(153)		
MMP9		Anti-MMP9 Ab, ONO-4817[a], Marimastat[a], Batimastat[a]		Leukocyte transmigration	(152)
Tumor-derived:	In vivo				
MMP2		NO	(65)		
COX-2		shCOX2,	(68)		
MMP1		shMMP1, GM6001[b]			
CSC self-renewal is mediated by:					
JAG1–Notch–HES5 signaling in astrocytes	In vitro	shHES5, shJAG1	(67)	Notch	(154)
IL1β secreted from tumor cells	In vivo	shIL1β, Compound E[c]			
Activated astrocytes express gliosis related gene signature: Cxcl10, Lcn2, Timp1, Serpine1	In vitro, In vivo	NO	(37)	STAT3, NFκB	(155)
Astrocytes induce Gsta5, Bcl2l1, Twist1 expression in tumor cells → chemoresistance	In vitro	siRNA of survival genes	(77)	Gap-junction intercellular communication	(156)
Astrocytes activate the Endothelin axis in tumor cells, providing chemoprotection	In vitro	Carbenoxolone (CBX)[d], BQ123[e], BQ788[e]	(80, 81)		
	In vivo	Macitentan[e]+Paclitaxel	(125)	ET-1 is a potent vasoconstrictor	(157)
Gap junctions allow communication between tumor cells and astrocytes	In vitro, In vivo	CBX, shcGAS	(76)	Gap-junction intercellular communication	(156)
Initial anti-tumorigenic responses of astrocytes to metastasizing cells mediated via:			(73)		
FasL, plasminogen activator from astrocytes	In vitro	anti-FasL		Tissue damage, Apoptosis	(158)
Serpins from reactive stroma		α2-antiplasmin			
Tumor cells expressing L1CAM	In vivo	shL1CAM, shSerpins			
Microglia facilitate transport of cancer cells into the brain	In vitro	WNT-inhibitor Dickkopf-2, Clodronate[f]	(88)	Wnt/βcatenin	(159)
Microglia defense response is hijacked by carcinoma cells	In vitro	Anti-CSF-1 Ab 5A1, Anti-CD34 Ab	(89)	Microglia survival and growth	(160)
Depletion of the anti-inflammatory microglia/ macrophage cell population attenuate metastatic colonization	In vivo	Clodronate[f]	(84)	M1 and M2 phenotypes of microglia/macrophage	(161)
Different phenotypes of metastasis-associated macrophages in dural vs. parenchymal metastasis	In vivo	NO	(99)	Macrophages are involved in neuroinflammation	(162)
NK cells and CD8+ T cells are required for efficient anti-PD-1/anti-CTLA-4 intracranial tumor response	In vivo	PD-1/CTLA-4 combined blockade	(102)	Immune checkpoints are possible therapeutic targets in AD	(163)
Adhesion and angiogenesis are mediated via: Tumor cell secreted IL1b, CXCL1, IL18 Astrocyte-derived HGF Endothelial cells expressing CXCR1	In vitro, In vivo	Pterostilbene[g]	(111)	HGF attenuates autoimmunity in experimental autoimmune encephalitis (EAE)	(164)
STAT3 activation induces cancer cell-directed VEGF secretion → STAT3 activation in endothelial cells → upregulated VEGFR2 in endothelial cells	In vitro, In vivo	WP1066[h]	(119)	STAT3	(165)

NOTE: Findings from preclinical studies in which targeting of neuroinflammation showed efficacy in the treatment of brain metastases, as well as targets that may be promising.
[a]MMP inhibitors.
[b]BBB-permeable metalloproteinase inhibitor.
[c]A potent BBB-permeable g-secretase inhibitor.
[d]Gap junction inhibitor.
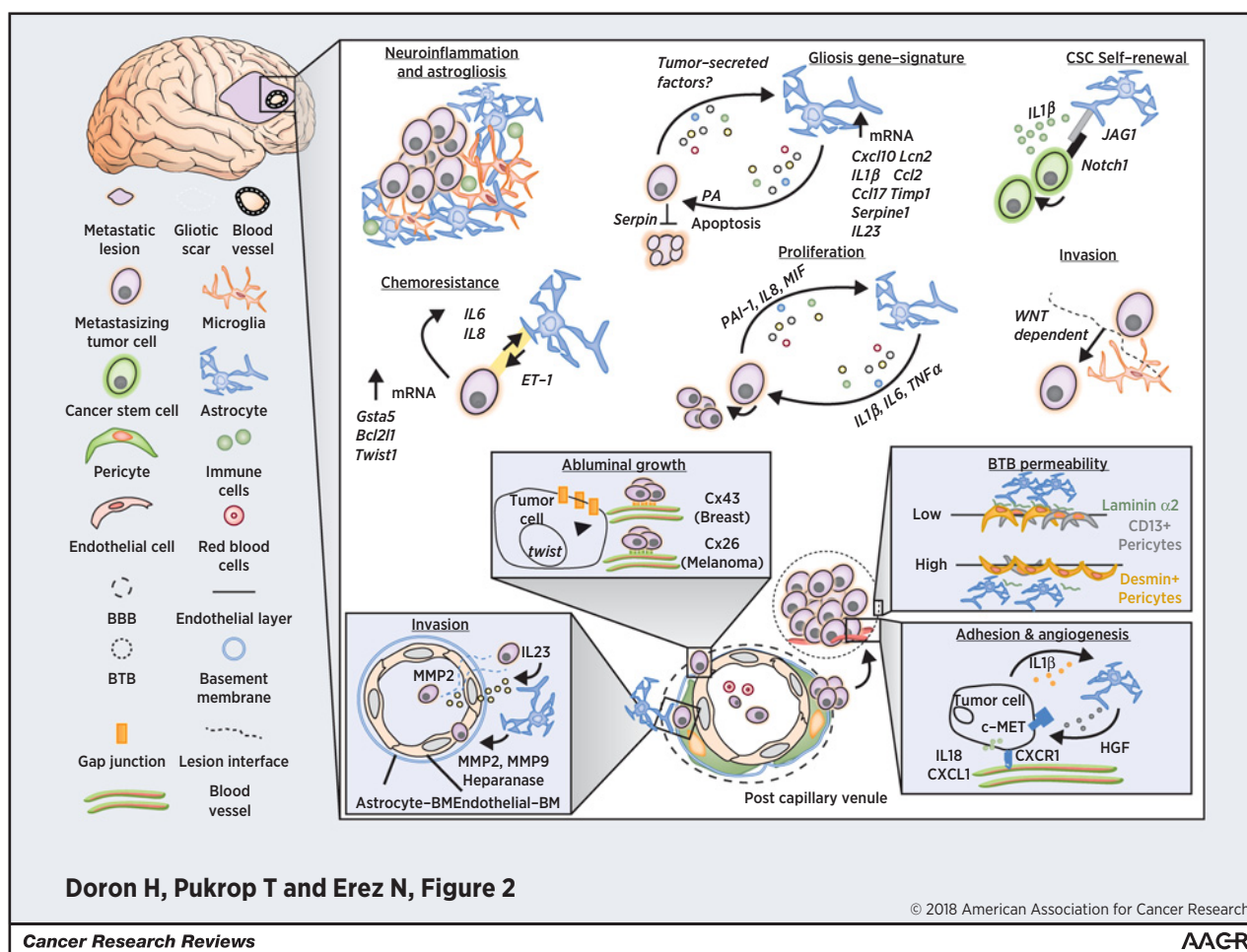[e]Antagonists of ETAR and ETBR.
[f]Bisphosphonate.
[g]c-MET inhibitor.
[h]STAT3 inhibitor.

radiation-induced tissue damage and necrosis. However, radiotherapy was also demonstrated to enhance the expression of PD-L1 in tumor cells and in antigen-presenting cells in a mouse model of breast cancer, suggesting that it may also have immunosuppressive effects (144). Importantly, recent preclinical and clinical studies, which assessed the combination of radiotherapy with

Doron H, Pukrop T and Erez N, Figure 2

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Figure 2.
Reciprocal interactions between brain-metastasizing tumor cells and the brain microenvironment facilitate metastatic growth. Main findings of neuroinflammatory networks operative in brain metastasis.

immunotherapy, found potential synergism between these two treatment modalities (143, 145). While these results still need to be validated in larger studies, the data suggest that combining radiotherapy with immune checkpoint blockade may prove to be beneficial in targeting the metastatic microenvironment to combat brain metastases.

Conclusions and Future Perspectives

The incidence of brain metastasis is rising and survival remains very poor. Therefore, there is an acute need to define mechanisms and test novel therapeutic approaches for brain metastasis. The accumulated knowledge gained in past years from the studies discussed here clearly indicate that reciprocal interactions between tumor cells and brain stromal cells are a driving force of brain metastasis. This is mediated partially by hijacking of physiologic tissue damage response and immune cell trafficking pathways in the brain microenvironment, and subsequent induction of local neuroinflammation around the metastatic lesion (Fig. 2).

Studying the metastatic niche and mechanisms that sustain dormancy are promising approaches toward uncovering the ear-liest stages of metastasis. Understanding the early events that precede the formation of brain macrometastases will greatly advance our ability to design more efficient therapeutics. Future studies aimed to promote this characterization should focus on elucidating the tumor-derived and stromal cell–derived factors that govern the instigation of neuroinflammation and vascular changes at the premetastatic niche. To facilitate this research direction, development of novel preclinical models of brain metastasis that will include genetic tools for specific targeting of candidate factors is required (some available experimental and genetic tools are summarized in Tables 1 and 2, respectively). Moreover, because recent studies suggest that the operative mechanisms in micrometastases may be different than the ones operative in full-blown brain macrometastases (146), experimental tools that enable this distinction are also needed.

Clinical targeting of brain metastases formation at early stages will only be feasible if diagnostic tools for patient stratification are available. This may include identification of predictive biomarkers that will reflect changes in the brain microenvironment (e.g., circulating inflammatory proteins, circulating DNA, and tumor-derived extracellular vesicles), or the presence of disseminated

Table 2. Genetic tools to study the brain microenvironment and neuroinflammation in murine models

Available strains	Enables the study of	Potential applications
GFAP-Cre/ER (166)	Astrocytes	Knockdown of astrocyte-specific genes
GFAP-GFP (167)		
ALDH1L1-GFP (60)		Isolating metastasis-associated astrocytes by FACS
CX3CR1-CRE (168)	Microglia	Knockdown of microglia-specific genes
CX3CR1-CRE/ER (168)		
CX3CR1-GFP (168)		Isolating metastasis-associated microglia by FACS
PDGFRb-Cre/ER (169)	Pericytes	Knockdown of pericyte-specific genes
Cre-LoxP System to Measure EV Uptake (170)	Tumor-derived exosomes	Studying exosomes in premetastatic niche

NOTE: Genetic tools are valuable in studying the reciprocal interactions between cancer cells and cells of the microenvironment. Combining these tools to create unique orthotopic spontaneous brain metastasis models of brain metastasis will facilitate the attainment of innovative functional experiments on mechanisms of premetastatic niche, screening of potential drugs, validation of diagnostic techniques, etc.

tumor cells. Such markers could potentially be identified in liquid biopsies of blood and/or CSF and allow personalized design of treatment approaches, following removal of primary tumors, to monitor progression to systemic disease.

Another major limitation of developing more efficient tools for early, preventive targeting of brain metastasis is the efficiency of imaging modalities (147). Insights from preclinical models of the early stages can be applicable to human disease only if available imaging methods will enable earlier diagnosis, in patients with yet asymptomatic brain metastatic lesions. Further studies are also needed to thoroughly characterize cancer type–specific responses of the brain metastatic microenvironment, as well as unifying mechanisms that are common to the brain response during brain metastasis originating from different tumors, to test their applicability as therapeutic targets.

While knowledge from preclinical studies on the brain metastatic microenvironment is emerging, data from clinical studies on microenvironment targeting in the treatment of brain metastasis therapy is still limited. Hopefully, in the coming years, we will see integration of the preclinical findings described in this review into the design of novel clinical strategies aimed at prevention and better treatment of brain metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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