Brain Metastasis Organotropism

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Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Brain metastases are associated with poor prognosis irrespective of the primary tumor they originate from. Current treatments for brain metastases are palliative, and patients with symptomatic brain metastasis have a one-year survival of <20%. Lung cancer, breast cancer, and melanoma have higher incidences of brain metastases compared with other types of cancers. However, it is not very clear why some cancers metastasize to the brain more frequently than others. Studies thus far suggest that brain-specific tropism of certain types of cancers is defined by a winning combination of the following factors: unique genetic subtypes of primary tumors or its subclones enabling detachment, dissemination, blood–brain barrier penetration, plus proliferation and survival in hypoxic low-glucose microenvironment; specific transcriptomic and epigenetic changes of colony-forming metastatic cells, allowing their outgrowth; favorable metastasis-permissive microenvironment of the brain created by interactions of cancer cells and cells in the brain through triggering inflammation, recruiting myeloid-derived suppressor cells, and promoting metabolic adaptation; immunosuppression resulting in the failure of adaptive immune response to recognize or kill cancer cells in the brain. Here, we briefly review recent advances in understanding brain metastasis organotropism and outline directions for future research.

Distant metastasis is responsible for >90% of cancer-related deaths (Chaffer and Weinberg 2011). Tumors of different origins display unique patterns of dissemination with preferential colonization of a particular set of organs. For example, colon cancer most commonly metastasizes to the liver whereas prostate adenocarcinoma predominantly disseminates to the bone (Nguyen et al. 2009). Breast cancer can metastasize to multiple organs with high success rates in the lungs, bones, brain, liver, and other organ sites (Lowery and Yu 2017). Although this organotropism can be partly explained by anatomical proximity between organs (e.g., the liver collects the venous drainage of the colon system via the portal vein), the past two decades of research provided a strong evidence for the existence of cellular and molecular programs guiding tumor cells to particular organs. Specifically, the concept of premetastatic niche (PMN) postulates that soluble factors and extracellular vesicles (EVs) produced by cells at the primary tumor sites can modify the microenvironment of distant organs in various ways to accommodate traveling cancer cells and facilitate their outgrowth (Kaplan et al. 2005; Hiratsuka et al. 2006; Costa-Silva et al. 2015; Liu and Cao 2016).

Editors: Jeffrey W. Pollard and Yibin Kang
Additional Perspectives on Metastasis: Mechanism to Therapy available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a037242
Cite this article as *Cold Spring Harb Perspect Med* 2020;10:a037242

The dilemma of site-specific metastasis emerged as early as 1889, when English surgeon Stephen Paget coined his prominent "seed and soil" theory, in which metastasizing cancer cells are the "seeds" and the metastasis-favoring organ microenvironment is the "soil" (Paget 1889). Current research using high-throughput technologies suggests that the relationship between "seeds" and "soil" is complex and bidirectional, with reciprocal interactions between tumor cells and distant organs helping each other to meet. For example, cancer-derived EVs uptaken by host cells, such as resident macrophages, at distant organs can trigger the profibrotic and proinflammatory responses ultimately supporting future metastasis (Costa-Silva et al. 2015). At the same time, certain organs display a milieu intrinsically favoring metastasis; for example, highly fenestrated sinusoidal endothelia of the liver show high permeability to soluble factors and extravasating cells (DeLeve 2007; Brunt et al. 2014; Juza and Pauli 2014). This environment is in striking contrast with brain's highly selective blood–brain barrier (BBB), which is extremely difficult to penetrate by traveling cancer cells because of its tightly adjoined endothelial cells (Wilhelm et al. 2013). This contradiction is puzzling because many patients present with metastases in the brain but not the liver (Budczies et al. 2015; Obenauf and Massagué 2015). Clearly, tumor cells can exploit a specialized set of mechanisms to penetrate the BBB and preferentially seed into the brain.

Brain metastasis is a serious consequence of cancer with no available curative care at the moment (Achrol et al. 2019). The dynamic, complex interplay between the brain microenvironment and disseminating metastatic cells has yet to be fully explored for the purpose of developing new effective targeted therapies for this disease. Understanding tumor cell tropism to the brain may provide an insight on how to tackle metastatic disease to the brain from various primary cancer sites. In this review, we summarize and critically analyze recent discoveries in the field of brain metastases, specifically addressing questions of early metastatic seeding and outgrowth in this organ. Additionally, we discuss the role of host adaptive immunity in a brain metastasis-specific context, including the basic biology behind this process as well as recent clinical trials examining immune checkpoint inhibitors for treatment of brain metastatic disease.

BRAIN METASTASIS REPRESENTS AN IMPOSING CLINICAL CHALLENGE

A diagnosis of brain metastatic disease indicates extremely poor prognosis, and most patients with this diagnosis face a very slim chance of survival. General population-based epidemiology studies reported brain metastasis incidence rates of 2.8 to 14.3 per 100,000 people (Table 1); the width of this range probably reflects difficulties in pathological verification of autopsies and improvement of neuroimaging techniques over time. In the United States, every year 21,000 to 43,000 patients are diagnosed with brain metastases (Fox et al. 2011). Cancer registry data indicate that 5.3% to 9.6% of all newly diagnosed cancer patients will develop brain metastasis (Table 2); however, this could have been underestimated because of the infrequency of autopsies in patients who die from metastatic disease. For example, an autopsy study by Tsukada and colleagues revealed that 309 of 1044 autopsy cases (29.6%) of breast carcinoma showed intracranial metastases (Tsukada et al. 1983). In another study, 2375 autopsies of patients with different cancers revealed brain metastases in 15% of subjects (Posner and Chernik 1978). Another reason to believe that the real incidence of brain metastasis is substantially higher is that

Table 1. Rates of brain metastasis incidence in general population

Study	Country	Incidence per 100,000 population
Guõmundsson (1970)	Iceland	2.8
Fogelholm et al. (1984)	Finland	3.4
Walker et al. (1985)	United States	8.3
Materljan et al. (2004)	Croatia	9.9
Counsell et al. (1996)	Great Britain	14.3

Table 2. Prevalence of brain metastasis in patients diagnosed with primary cancer

Study	%	Country
Davis et al. (2012)	6.0	United States
Schouten et al. (2002)	8.5	Netherlands
Barnholtz-Sloan et al. (2004)	9.6	United States
Nayak et al. (2012)	8.5–9.6	Global
Matsuda et al. (2018)	5.3	Japan

established clinical practices do not recommend routine brain magnetic resonance imaging (MRI) in patients without neurological symptoms. Interestingly, metastases to the brain are generally much more prevalent than primary brain malignancies such as gliomas (Gavrilovic and Posner 2005; Davis et al. 2012), which could be explained by the relatively low number of dividing cells in the brain.

Brain metastasis generally affects elderly people 50–80 yr old (Fox et al. 2011). Nonetheless, some pediatric cancers, most commonly sarcomas, can also give raise to brain metastases (Graus et al. 1983; Kebudi et al. 2005). Among the primary tumors that most frequently metastasize to the brain are lung cancer, melanoma, breast carcinoma, renal cancer, and colorectal adenocarcinoma (Table 3). Breast cancer is the primary tumor that most commonly metastasizes to the brain in women. Other cancer types rarely metastasize to the brain.

Treatment of brain metastasis is generally done with palliative intent. It includes treatments such as chemotherapy using BBB-penetrating drugs (Freilich et al. 1995; Lesser 1996; Korfel and Thiel 1999; Tawbi et al. 2018), surgical resection when the number of brain lesions is limited (Alvarez-Breckenridge et al. 2019; Olesrud et al. 2019), whole-brain radiotherapy (Brown et al. 2017; Sun et al. 2018; Or et al. 2019), and stereotactic radiosurgery (Badiyan et al. 2016). These treatments may lead to a partial response or short-term stable disease, but are unable to extend patient survival substantially (Kotecha et al. 2018). Individuals with a single cranial metastasis have more treatment options available such as surgery, yet their median survival is only ~60% longer (35.6 vs 22.6 wk) (Patchell 2003). Untreated brain metastases lead to death in ~2 mo after diagnosis (Markesbery et al. 1978).

Thus, metastases to the brain are a global public health burden, and unfortunately, many

Table 3. Ranking of primary tumors by frequency of associated brain metastasis

Study	Barnholtz-Sloan et al. (2004)		Cagney et al. (2017)		Schouten et al. (2002)		Smedby et al. (2009)	
Rank	Type of cancer	%	Type of cancer	%	Type of cancer	%	Type of cancer	%[a]
1	Lung cancer (all)	19.9	Melanoma	28.2	Lung cancer (all)	16.3	Lung cancer (all)	44.1 (33.5)
2	Melanoma	6.9	Lung Adenocarcinoma	26.8	Renal cancer	9.8	Melanoma	12.3 (5.8)
3	Renal cancer	6.5	Non-small-cell lung cancer	25.6	Melanoma	7.4	Colorectal cancer	9.0 (7.4)
4	Breast carcinoma	5.1	Small cell lung cancer	23.5	Breast carcinoma	5.0	Prostate cancer	8.6
5	Colorectal cancer	1.8	Squamous cell carcinoma of the lung	15.9	Colorectal cancer	1.2	Renal cancer	7.8 (4.7)
6			Bronchioloalveolar carcinoma	15.5			Other solid tumors	18.2 (10.5)
7			Renal cancer	10.8			Breast cancer	(32)

[a]Data shown for men (no parentheses) and women (parentheses).

decades of cancer research have offered little, if any, therapeutic benefit to patients with this disease.

BRAIN-SPECIFIC METASTASES DISPLAY A UNIQUE BIOLOGICAL PHENOTYPE

Early metastatic colonization of the brain requires cancer cells to trigger the expression of a specific gene set and communicate with host cells to succeed in penetrating the BBB and propagating under conditions of hypoxia and glucose shortage (Peters et al. 2004; Winkler 2015; Lowery and Yu 2017). The gene alterations unique of brain metastatic cancer cells were elegantly shown in a recent study by the Massagué group (Basnet et al. 2019), who used a novel method for in situ transcriptomic profiling of rare cell populations. In mice, early micrometastases in the brain and lungs derived from intracardiacally injected MDA-MB-231 cells showed a remarkable difference in gene expression patterns, also displaying a substantial divergence with the transcriptome of cells injected orthotopically into the mammary fat pad inducing primary tumor (Fig. 1). Compared with those of lung colonies and mammary tumors, the transcriptome of brain-seeking cancer cells had lower levels of oxidative stress and antioxidative response, suggesting adaptation of metastasizing cells to peculiar metabolic conditions of the brain (Flavahan et al. 2013). These in vivo findings are consistent with findings from patients, whose brain metastatic lesions harbor mutations undetectable in matched primary tumors, regional lymph nodes, or extracranial metastases (Brastianos et al. 2015). Interestingly, many such mutations represent clinically actionable targets such as ERBB2, BRAF, MYC, and BRCA2 (Brastianos et al. 2015), potentially opening new avenues for targeted therapies.

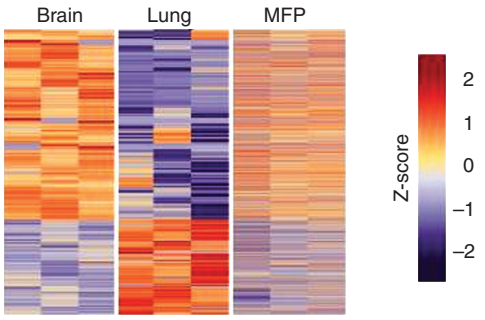

Figure 1. Heatmap representation of differentially expressed genes identified by Flura-seq in MDA231 cells. Cells residing in the brain, lung, or mammary fat pad (MFP), compared with the expression of these genes in the third organ. (Reproduced from Figure 4 in Basnet et al. 2019, under the Creative Commons Attribution 4.0 International Public License [CC BY 4.0; see creativecommons.org/licenses/by/4.0/].)

In line with general mRNA changes occurring in early metastatic colonies, disseminating tumor cells were also shown to produce a specific set of cancer-derived microRNAs (miRNAs) that can shape the surrounding milieu to facilitate metastasis (Zhou et al. 2014). It was found that EVs from metastatic MDA-MB-231 breast cancer cells are highly enriched in miR-105, an miRNA targeting a migration-related protein zonula occludens 1 (ZO-1). Via microvesicular transport, cancer cell-derived miR-105 transferred into endothelial cells, regulating their migration properties. By targeting ZO-1, miRNA-producing cancer cells diminished tight junctions and destroyed the barrier function of endothelial monolayers; this led to increased vascular permeability and promoted metastasis in this experimental model (Zhou et al. 2014).

In addition to differences at a transcriptional level, brain metastases show a unique epigenetic phenotype. To this end, analysis of 425 tumor-specific, differentially methylated loci showed higher overall genome methylation in 35 breast cancer brain metastases compared with 50 nonmatched primary mammary carcinoma samples (Salhia et al. 2014). This finding highlights the fundamental epigenome alterations in brain metastatic cells.

It is also important to note that following BBB penetration, many disseminated cancer cells can undergo dormancy by adopting a slow-cycling state and gaining stem-like characteristics through expression of SOX2 and SOX9 (Malladi et al. 2016). The key determinants of cancer cells fate, specifically, whether they should proliferate or stay latent, are yet to be fully recognized. Although the quiescent state may last for years or even decades, the consensus

is that dormant cells will eventually awake under influence of unidentified factors and give rise to outgrowing metastatic foci (Sosa et al. 2014). Collectively, these studies point at a distinctly altered genetic and epigenetic program activated in cancer cells on their dissemination into the brain, which largely determines cancer cells homing to this organ and facilitates their subsequent outgrowth.

Altered transcription programs of metastatic cancer cells that facilitate organotropism to the brain are significantly dependent on genetic subtypes of primary tumors. For example, ~50% of non-small-cell lung cancer patients with epidermal growth factor receptor (*EGFR)* mutation or anaplastic lymphoma kinase (*ALK*) rearrangement develop metastases in the central nervous system, strongly implicating these two genes in determining brain organotropism (Rangachari et al. 2015). Additionally, brain metastases derived from triple-negative or basal-type breast cancers were reported to effectively disrupt the BBB and therefore subsequently colonize the brain, whereas HER2/neu-positive breast cancer cells failed to alter BBB permeability (Yonemori et al. 2010). Counterintuitively, patients with HER2-positive primary breast cancer and brain metastasis have a 30.8% probability of having metastases in other organs, such as the liver and lung (Kim et al. 2018). Interestingly, spatial distribution of metastases within the brain is also affected by the molecular subtype of primary tumor (Takano et al. 2016). Evidence from these studies indicates that molecular subtypes of primary tumors not only define overall survival, risks of relapse, and response to therapies but also predetermine the tropism of disseminating cells to seek particular organs, including the brain.

MICROENVIRONMENT IN SUPPORT OF BRAIN METASTASIS

Organotropism to the brain is a highly complicated phenomenon and is also regulated by interactions of metastatic cancer cells with the brain tumor microenvironment. The brain microenvironment has a unique composition and also consists of multiple cell populations with a diverse array of functions (Table 4). In the light of the "seed and soil" hypothesis, some of these cell populations could, under certain conditions, be supportive of metastasizing cancer cells and facilitate their seeding and propagation. Some of these cells could elicit their prometastatic action long before the arrival of tumor cells (brain PMN concept), whereas another may create a growth- and proliferation-permissive microenvironment for the newly extravasated cancer cells or their clusters.

Extracellular Vesicles and Exosomes

Recently, tumor-derived EVs were found to be in control of shaping the PMN of different organs (Costa-Silva et al. 2015; Hoshino et al. 2015; Plebanek et al. 2017). Under physiological conditions, EVs serve as means of intercellular communication by transporting various biomolecules (Isola and Chen 2017). However, Lyden's group showed that EVs isolated from the brain-seeking subline of breast cancer cells traveled exclusively to the brain when injected retro-orbitally into tumor-free mice (Hoshino et al. 2015). Similarly, breast cancer sublines metastasizing to other organs secrete EVs that traveled exclusively to the corresponding organs. Importantly, 98% of mouse EV-harboring cells were brain endothelial cells, suggesting endothelium as a major determinant of BBB penetration and subsequent brain metastasis (Hoshino et al. 2015). These EVs may awaken dormant disseminated tumor cells, which are localized in tight proximity with brain microvascular endothelium (Carbonell et al. 2009; Ghajar et al. 2013; Bentolila et al. 2016). Alternatively, tumor-derived EVs may promote microvascular hyperpermeability, which has been described as a feature of brain micrometastases (Schwartz et al. 2016), thus enhancing extravasation. Studies of human disease and experimental mouse models in our laboratory revealed a loss of tumor suppressor "phosphatase and tensin homolog deleted on chromosome ten" (PTEN) in brain metastases from PTEN-positive primary breast tumors. Intriguingly, such PTEN down-regulation was reversed after in vitro culture of cancer cells isolated from brain metastasis lesions in

Table 4. Major cell populations in the brain

Cell type	Main function	Approximate percentage (Pelvig et al. 2008; von Bartheld et al. 2016)
Neurons	Receive, conduct and transmit electrical signals	33% of all brain cells
Glial cells	Supporting neurons	66% of all brain cells
Oligodendrocyte (macroglia)	Creating the myelin sheath (Bradl and Lassmann 2010)	75.6% macroglia
Astrocyte (macroglia)	Nutrient supplementation of the nervous tissue and tissue repair	17.3% of macroglia
Glioblast (macroglia)	Undifferentiated macroglial cells	Unknown
Microglia	Resident immune populations of the brain	6.5% of glial cells
Endothelial cells and pericytes	Blood supply	10%–30% of nonneuronal cells
Infiltrating (nonresident) immune cells	Elimination of pathogens, PAMPs and DAMPs	Varies, unknown

Abbreviations: DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns.

mice, suggesting a brain microenvironment-induced PTEN loss. We discovered that brain metastatic tumor cells uptake the astrocyte-derived EVs carrying PTEN-targeting miR-19a that down-regulates PTEN in tumor cells, leading to NF-κB activation and up-regulation of CCL2 to facilitate metastasis through recruitment of myeloid-derived suppressor cells (Zhang et al. 2015). Taken together, these findings suggest that both tumor- and stroma-derived EVs may help disseminating cancer cells to form distant metastases in the brain.

Neuroinflammation

There is accumulating evidence that brain neuroinflammation orchestrated under physiological conditions by response to pathogen-associated molecular patterns or tissue damage (damage-associated molecular pattern) can promote metastatic outgrowth in the brain (Doron et al. 2019). In a model of spontaneous melanoma brain metastasis, early micrometastatic brain lesions recruited activated Glial fibrillary acidic protein (GFAP)-positive astrocytes, which in turn produced proinflammatory molecules such as CCL2, IL-1β, and IL-6, thus enhancing tumor growth (Schwartz et al. 2016). Interestingly, the activation of astrocytes was at least in part mediated by disseminating tumor cells, which up-regulated the expression of an astrogliosis-related wound-healing gene signature in these cells through unidentified secreted factors. Intracranial coinjection of melanoma cells with astrocytes resulted in a substantial growth advantage (ninefold) of tumor cells as compared with tumor cells injected alone, implicating that astrocytes could modulate cancer cell proliferation in the tumor microenvironment (Schwartz et al. 2016). Consistently, astrocyte-driven cancer cell proliferation through release of proinflammatory cytokines was also documented by several other groups (Sierra et al. 1997; Seike et al. 2011).

Like astrocytes, microglial cells are actively recruited to metastatic brain lesions (Bowman et al. 2016; Qiao et al. 2019), and their elimination by targeting colony stimulating factor-1 receptor (CSF-1R) resulted in a reduction of both the size and number of melanoma-derived brain metastases in mice injected with tumor cells via internal carotid artery (Qiao et al. 2019). Several attempts have been made to explore the underlying mechanisms of these reductions. Metastasis-promoting effects of microglia, such as tumor cell invasion, were negated by pharmacological inhibition of PI3K in vitro and in vivo. On the other hand, small-molecule PI3K inhibitor administration reduced PDL-1 and CTLA-4 levels in mouse brain lesions

induced by stereotactical intracortical injection of 4T1 breast cancer cells (Blazquez et al. 2018). Another study provided evidence that activated microglia may control invasion and colonization of the brain by metastasizing cancer cells via the Wnt pathway (Pukrop et al. 2010). However, these findings have an observational nature, and the in-depth mechanism underlying the function of activated microglia on brain metastasis development remains to be elucidated.

Altogether, these studies indicate that activated glial cells (macroglia and microglia) actively infiltrate the brain metastasis lesions (possibly because of cancer-derived chemoattractants) and further stimulate tumor outgrowth via release of proinflammatory molecules that trigger neuroinflammation.

Extracellular Matrix in Control of Metastatic Outgrowth

In recent years, multiple lines of evidence strongly implicated the noncellular component of tumor microenvironment, the extracellular matrix (ECM), in promotion of metastatic seeding and outgrowth (Yuzhalin et al. 2018a,b; Zhang et al. 2019). Genes encoding major ECM proteins are associated with epithelial-to-mesenchymal transition and poor prognosis across many cancer types (Yuzhalin et al. 2018c). Furthermore, physical stiffness of tumor ECM directly correlates with metastasis (Wei et al. 2015), and orientation of collagen fibers in relation to the tumor is an independent prognostic indicator in breast carcinoma (Conklin et al. 2011). However, the role of ECM in brain metastasis is not well understood, primarily because the brain lacks structurally well-defined stromal space (Bonneh-Barkay and Wiley 2009). Unlike ECM in other parenchymal organs, brain ECM predominantly localizes near vascular basement membranes and therefore is likely to have a different composition of collagens, proteoglycans, and glycoproteins (Quail and Joyce 2017). Because of these structural differences, the functional role of brain ECM could be unique. In-depth comprehensive proteomic or functional analyses of brain ECM would provide critical insights in this direction.

High expression of collagens, proteoglycans, and hyaluronic acid binding proteins is associated with poor prognosis in lung cancer (Stevens et al. 2017). Importantly, multiple cell surface receptors that bind to ECM molecules are also overexpressed in these tumors, indicating a reciprocal interaction between cancer cells and the tumor microenvironment (Stevens et al. 2017). One such receptor, hyaluronan-mediated motility receptor (HMMR), was shown to correlate with lung cancer brain metastasis and mediate colonization of the brain in vivo, specifically affecting early survival and outgrowth of metastatic lesions. Remarkably, genetic knockdown of HMMR reduced the activation of mitogen-activated protein kinase/ERK and AKT in H2030 lung cancer cells cultured as ECM-embedded organoids but not in cells cultured in an ECM-free environment. The expression of HMMR in cancer cells was negatively regulated by miR-34a (Stevens et al. 2017), an miR linked to ECM deposition in cardiac fibroblasts (Zhang et al. 2018). Overall, this study showed that tumor initiation and propagation of early metastasis can be mechanistically connected through activation of the ECM receptor HMMR, and thus therapeutic inhibition of HMMR-hyaluronic acid interactions in the ECM may lead to clinical advances in treating brain metastases from lung cancer.

In an experimental brain metastasis model, the in-depth characterization of tumor-derived and stromal proteolytic networks has uncovered a role of cathepsin S (a member of a large family of proteases involved in cleavage of multiple targets, including the ECM) in metastasis formation (Sevenich et al. 2014). Highlighting the clinical relevance of this molecule, these investigators documented a negative association between cathepsin S levels and metastasis-free survival in a cohort of patients with brain metastases. In the same study, genetic depletion of cathepsin S in cancer and stromal cells diminished brain metastatic burden in mice. Follow-up mechanistic studies showed that cathepsin S promoted the transmigration of disseminating tumor cells through the BBB by inducing a cleavage of intercellular tight junctions (Sevenich et al. 2014). It is unclear, however, what

upstream signals induce cathepsin S expression in metastasizing tumors and whether these signals are attributable to activity of host cell populations in the brain.

Interestingly, there seem to be a correlation between reelin (a major brain ECM glycoprotein) and HER2 expression in human brain metastatic lesions derived from mammary carcinoma (Jandial et al. 2017). Reelin was localized near tumor-associated astrocytes, which induced reelin expression in breast cancer cells on their coculture (Jandial et al. 2017). Importantly, astrocyte-derived factors promoted proliferation of HER2-positive breast cancer cells through enforcing HER2 phosphorylation, and knockdown of the reelin gene, *RELN*, in breast cancer cells reduced astrocyte-driven HER2-dependent cell proliferation.

Collectively, these studies point to the specific contribution of ECM to brain PMN development; in particular, extracellular signaling in the brain can promote growth of disseminated cancer cells as well as enable their entry into the brain parenchyma through destruction of BBB integrity. More systematic research along this line would identify the true significance of extracellular cues in the context of brain metastasis.

ADAPTIVE IMMUNE RESPONSE IN BRAIN METASTASES

The microenvironment of brain metastases is to a large extent shaped by infiltrating lymphocytes exerting the adaptive immune response program. $CD3^+$ and $CD8^+$ cytotoxic T-cells can infiltrate brain metastasis lesions and be found in both stromal and epithelial compartments, with higher abundance in the former (Harter et al. 2015; Duchnowska et al. 2016). Interestingly, distribution of T cells in brain lesions may be observed in three distinct histological patterns: perivascular, stromal, and diffuse (Harter et al. 2015). However, it remains unclear what signals control such different patterns of infiltration. The highest numbers of $CD3^+$ and $CD8^+$ T cells were found in brain metastases from renal cell carcinoma and melanoma, whereas brain lesions from the lung, breast, and colon cancers displayed considerably fewer of these T cells (Harter et al. 2015). Higher counts of tumor-infiltrating T cells correlated with smaller brain metastasis size without affecting overall survival (Harter et al. 2015), whereas subjects whose tumor-infiltrating T cells expressed higher levels of PD-1 had longer overall survival after resection of metastases (Duchnowska et al. 2016). Along similar lines, another study confirmed that elevated peritumoral $CD3^+$ T-cell density positively correlated with brain metastasis-associated survival, and 52% of peritumoral T cells expressed PD-1 on their surface (Zakaria et al. 2018). It is important to note that infiltration of T cells into brain lesions can be restricted by certain ECM components, such as tenascin C (Huang et al. 2010), suggesting a link between the noncellular component of tumor stroma with T-cell–mediated killing of cancer cells.

Immune checkpoint blockade can promote $CD8^+$ T-cell numbers in mouse experimental brain metastasis; however, such an increase was shown to be caused by $CD8^+$ cell trafficking from outside of the brain rather than their proliferation in situ (Taggart et al. 2018). Notably, preclinical immune checkpoint blockade treatment of brain metastasis-bearing mice did not alter the activation/exhaustion status of T cells, suggesting that this therapy regimen can attract lymphocytes to the metastatic site but is unable to unleash their cytotoxic potential (Taggart et al. 2018).

Despite the higher mutation burden of human brain metastases, they seem to contain substantially fewer T cell clones compared with paired primary lung tumors (Mansfield et al. 2018). Various mechanisms may contribute to the observed lower diversity of T cell clones. For example, it could be because of the difficulty of T cells to penetrate the BBB, thus only few T-cell clones infiltrating within the brain lesions. In this respect, T-cell migration through the BBB is not a passive process and can be controlled by soluble factors such as IFN-γ (Sonar et al. 2017). However, some studies suggest that T-cell trafficking into the brain may occur directly via migration through choroid plexus epithelial cells of the brain–cerebrospinal fluid barrier (Strazielle et al. 2016), rather than the BBB. How immune

cells respond to brain–tropic cancer cells and through what means these cancer cells manage to escape the immune surveillance is not well known. Better understanding of these important questions could bring new insights on how to mobilize immune response to control brain metastasis.

IMMUNOTHERAPY FOR TREATMENT OF BRAIN METASTASIS

Despite the knowledge gap described above, there have been remarkable efforts to therapeutically stimulate the immune response to control brain metastasis. Recent advances in immune checkpoint blockade therapies have led to a breakthrough in enhancing the survival outcome and quality of life of patients with melanoma (Carreau and Pavlick 2019), advanced renal cell carcinoma (Motzer et al. 2018), bladder cancer (Konala et al. 2019), non-small-cell lung cancer (Nadal et al. 2019), and other cancer types (Wei et al. 2018). However, clinical trials of immune checkpoint blockade therapies frequently exclude patients with brain metastatic disease. Thus, there is only a handful of studies that explored the effect of immune checkpoint blockade therapies in patients with brain metastases.

In a retrospective analysis of 146 melanoma patients treated with ipilimumab (an anti-CTLA4 antibody) for their brain metastases, a modest global immune-related overall response rate of 11% was achieved, and four patients (3%) showed immune-related complete responses (Queirolo et al. 2014). Most patients (73%) experienced progressive disease. In another study, melanoma patients with brain metastases benefited from ipilimumab therapy if they had small and asymptomatic lesions and were not taking corticosteroids (Margolin et al. 2012), showing a median overall survival of 7.0 mo compared with 3.7 mo for those who had symptomatic large brain metastases and were taking corticosteroids. These findings were corroborated by recent experimental studies in mice that revealed an involvement of corticosteroids in metastasis seeding and outgrowth (Obradović et al. 2019).

The combination of ipilimumab with nivolumab (an anti-PD1 antibody) showed a much more promising clinical benefit, with 24 patients (26%) having a complete response and 28 patients (30%) having a partial response in brain metastases from melanoma, thus resulting in a 56% intracranial objective response (Tawbi et al. 2018). A better clinical benefit from this therapy was achieved in patients with tumor PD-L1 expression (at least 5%), which is similar to findings obtained for extracranial disease (Larkin et al. 2015). These results are consistent with those of another study in which a combination of ipilimumab and nivolumab yielded a 46% intracranial response in patients with melanoma brain metastases (Long et al. 2018). In this study, patients receiving nivolumab alone had a significantly lower rate of intracranial progression-free survival after 6 mo of treatment compared with patients receiving dual therapy (20% vs. 53%) (Long et al. 2018). Interestingly, the intracranial response and survival rates in this study were lower in patients who previously had BRAF and MEK inhibitors therapy (Long et al. 2018); suggesting that evolution of BRAF and MEK inhibitors-resistant clones toward an immune resistance phenotype (Hugo et al. 2015).

In a trial of pembrolizumab (an anti-PD1 antibody) monotherapy in patients with brain metastases from melanoma, four of 18 patients had a partial intracranial response ranging from 4 to 10 mo (Goldberg et al. 2016). In the same study, six of 18 patients with brain metastatic disease from non-small-cell lung cancer responded to pembrolizumab, including partial and complete intracranial responses in two and four patients, respectively (Goldberg et al. 2016).

Findings from these clinical efforts show that current immune checkpoint blockade treatments have a modest effect on survival of patients with brain metastases, largely for a lack of response in a significant proportion of patients. Therefore, future studies should focus on understanding principal genetic and phenotypic differences between responders and nonresponders to immune therapies to gain novel insights on how brain–tropic cancer cells succeed in immune escape, which could guide the devel-

opment of new strategies to optimize and amend current immunotherapy regimens to more effectively control brain metastasis.

CONCLUDING REMARKS

Patients with metastases to the brain have a poor prognosis. The key to combating this disease is understanding its etiology and pathogenesis at a molecular level. The emergence of novel high-throughput technologies and their applications in biology and medicine have substantially advanced the "seed and soil" concept put forward more than a century ago. Currently, organotropism and subsequent development of brain metastasis can be defined as a successful combination of a multitude of factors, which can be conditionally pooled into several groups (Fig. 2):

1. The distinctive genetic profile of primary tumors enabling detachment, metastatic dissemination (as a prerequisite for metastasis in general), followed by destruction of the BBB and subsequent seeding within the brain followed by propagation under low-glucose and hypoxic conditions. Accumulation of certain genetic alterations as a result of clonal evolution (McGranahan and Swanton 2017) that is influenced by the mutation rate and doubling time of individual tumors.

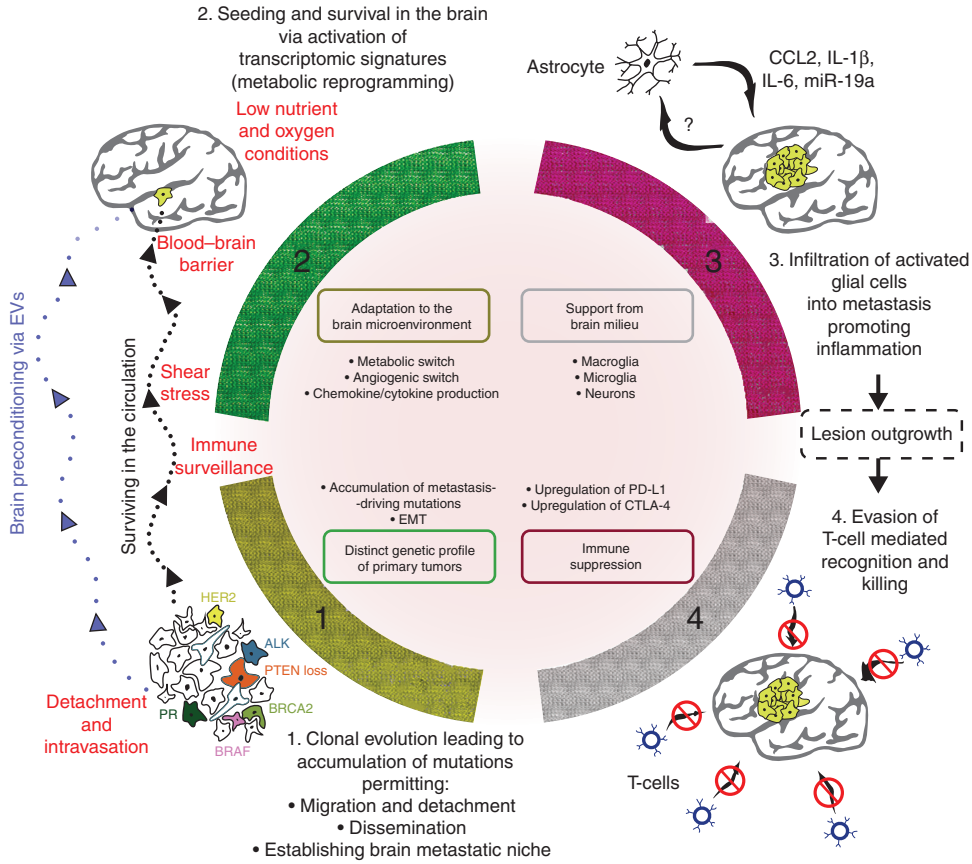

Figure 2. Schematic representation of key phases enabling brain organotropism of cancer cells and leading to establishment of metastatic colonies. Factors limiting brain organotropism are in red color. Abbreviations: EVs, extracellular vesicles; EMT, epithelial–mesenchymal transition; CCL2, C-C motif chemokine ligand 2; IL, interleukin; miR, micro RNA; CTLA4, cytotoxic T-lymphocyte-associated protein 4; PD-L1, programmed death-ligand 1.

2. The emergence of transcriptomic and epigenetic changes in the newly established colonies, enabling successful growth of micrometastases through metabolic adaptation (Fischer et al. 2019) and activation of an angiogenic switch program (Baeriswyl and Christofori 2009). These changes include production of chemokines and cytokines to disarm both resident and recruited immune cells with their subsequent shift into immune-suppressive phenotype (Condamine et al. 2015).
3. The brain microenvironment accommodates cancer cells, and the brain positively responds to biochemical and biomechanical signals from cancer cells, enforces activation of proinflammatory response (Quail and Joyce 2013) followed by ECM remodeling (Yuzhalin et al. 2018b), and attracts myeloid cells with their subsequent transformation into myeloid-derived suppressor cells (Ouzounova et al. 2017). These events can be influenced by susceptibility of host cells to obey cancer signals as well as the biological phenotype (i.e., aggressiveness) of tumor cells themselves.
4. The inability of tumor-infiltrating lymphocytes to recognize and successfully eliminate brain–tropic metastatic cancer cells, resulting from failure of patrolling immune cells (Vinay et al. 2015) to kill disseminating tumor cells in the circulation; failed T-cell infiltration through the dense tumor ECM (Caruana et al. 2015); T-cell dysfunction/exhaustion caused by the up-regulation of PD-L1, CTLA-4, and other immune inhibitory receptors (Wherry and Kurachi 2015); or reprogramming by myeloid-derived suppressor cells (Ouzounova et al. 2017).

The requirement for a combination of many different events limits the efficiency of the metastatic process, with <0.01% of tumor cells injected into the circulation forming metastatic foci (Fidler 1970). Importantly, the individual contribution of each event to the onset and progression of metastasis is obscure; it is likely that some of these events are substantially more important than others, and dissecting individual roles of every element will be a key to fully understanding brain organotropism. Future research in the field should encompass the investigation of these key features of brain organotropism: (1) a unique ability of brain-seeking cancer cells to penetrate the BBB; (2) peculiarities of cancer cell metabolic adaptation in the brain; (3) interaction of cancer cells with resident cells in the brain; and (4) brain-specific immune suppression. Importantly, to boost antitumor immunity in the brain, it is needed to develop novel strategies, including innovative immune checkpoint blockade regimens as well as more effective chimeric antigen receptor T cells. In addition, future investigations should use high-throughput technologies such as mass spectrometry, mass cytometry, next-generation sequencing, etc. to unbiasedly search for new molecular targets for brain metastasis therapies. More in-depth studies of brain metastasis organotropism are required to warrant successful treatment of this dreadful disease and open new horizons in improving patients' quality of life.

ACKNOWLEDGMENTS

We thank members of the Yu laboratory for helpful discussions. We thank Bryan Tutt in the Department of Scientific Publications of MD Anderson Cancer Center for editing the manuscript. This work was supported by National Institutes of Health grants R01-CA112567-06 (D.Y.), R01CA184836 (D.Y.), R01 CA208213 (D.Y.), R21CA223102 (D.Y.), and the China Medical University Research Fund (D.Y.). D.Y. is the Hubert L. & Olive Stringer Distinguished Chair in Basic Science of MD Anderson Cancer Center. We apologize for not being able to cite all the relevant original research and review articles because of space limitation.

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