Published in final edited form as:

Clin Exp Metastasis. 2014 February ; 31(2): 257–267. doi:10.1007/s10585-013-9623-4.

Breaking and entering into the CNS: clues from solid tumor and nonmalignant models with relevance to hematopoietic malignancies

Soumit K. Basu,

Alexander B. Osborn Hematopoietic Malignancy and Transplantation Program, Mary Babb Randolph Cancer Center, West Virginia University School of Medicine, Morgantown, WV, USA

Mary Babb Randolph Cancer Center and Section of Hematology-Oncology, Department of Medicine, West Virginia University School of Medicine, Morgantown, WV, USA

Scot C. Remick,

Alexander B. Osborn Hematopoietic Malignancy and Transplantation Program, Mary Babb Randolph Cancer Center, West Virginia University School of Medicine, Morgantown, WV, USA

Mary Babb Randolph Cancer Center and Section of Hematology-Oncology, Department of Medicine, West Virginia University School of Medicine, Morgantown, WV, USA

Manish Monga, and

Mary Babb Randolph Cancer Center and Section of Hematology-Oncology, Department of Medicine, West Virginia University School of Medicine, Morgantown, WV, USA

Sara Crile Allen and James Frederick Allen Lung Cancer Program, Mary Babb Randolph Cancer Center, West Virginia University School of Medicine, Morgantown, WV, USA

Laura F. Gibson

Alexander B. Osborn Hematopoietic Malignancy and Transplantation Program, Mary Babb Randolph Cancer Center, West Virginia University School of Medicine, Morgantown, WV, USA

Department of Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV, USA

Soumit K. Basu: skbasu@hsc.wvu.edu

Abstract

Various malignancies invade the CNS sanctuary site, accounting for the vast majority of CNS neoplastic foci and contributing to significant morbidity as well as mortality. The blood–brain barrier (BBB) exhibits considerable impermeability to chemotherapeutic agents, severely limiting therapeutic options available for patients developing metastatic CNS involvement, accounting for poor outcomes. The mechanisms by which malignant cells breach the highly exclusive BBB and subsequently survive in this unique anatomical site remain poorly understood, with most of the current knowledge stemming from non-malignant and solid malignancy models. While solid and

Correspondence to: Soumit K. Basu, skbasu@hsc.wvu.edu.

Conflict of interest The authors declare no relevant conflicts of interest.

hematologic malignancies may face different challenges once within the CNS (e.g., solid tumor parenchymal metastasis compared to masses/nodules/leptomeningeal disease in hematologic malignancies), commonality exists in the process of migrating across the BBB from the circulation. Specifically considering this last point, this review aims to survey the current mechanistic knowledge regarding malignant migration across the BBB, necessarily emphasizing the better studied solid tumor and nonmalignant models with the intention of highlighting both the current knowledge gap and additional work required to effectively consider how hematopoietic malignancies breach the CNS.

Keywords

Cancer; Metastasis; Blood–brain barrier

Introduction

While locally arising malignancies of the CNS number roughly 20,000 per year, most malignant CNS foci involve cancers of non-CNS origin, accounting for 170,000–200,000 cases annually [1]. Autopsy data suggests that as many as one-quarter of cancer patients may demonstrate malignant CNS involvement [2], spanning a wide variety of both solid and hematopoietic malignancies [1, 3–6]. Among solid tumors, the most common include melanomas and carcinomas of breast, lung, renal, and colorectal origin [2, 3]. Within hematologic malignancies, lymphomas commonly breach the CNS, with incidence varying with type from 5 to 50 %. [7] Such variation has led to analyzing risk factors for lymphomatous CNS breach which include older age, elevated serum LDH, greater extranodal or retroperitoneal involvement, anatomically contiguous lesions, hypoalbuminemia, and higher grade [8]. Lymphoid leukemias, particularly acute lymphoblastic leukemia (ALL), also involve the CNS, with 5–10 % of adult ALL patients demonstrating CNS involvement at presentation [9] and 26 % demonstrating CNS involvement in an autopsy series spanning 20 years [10]. The same series noted CNS involvement in 21 % of nonlymphocytic acute leukemia cases.

CNS involvement adversely affects survival in both solid and hematologic malignancies [4, 7, 11, 12], with ~25 % of patients succumbing to cancer demonstrating CNS involvement in some autopsy series [2]. Therapies for such patients remain limited, consisting primarily of irradiation, systemic chemotherapy, surgery, and introduction of antineoplastic agents directly into cerebrospinal fluid (CSF). This last approach suffers from the availability of few agents capable of safe administration into CSF with the prognosis for patients with malignant CNS involvement remaining months at best [4, 7, 11–15]. Furthermore, significant morbidities may ensue from these modalities especially when considering children in whom radiation therapy may lead to developmental and cognitive issues [16] while chemotherapy such as methotrexate may result in acute and long term toxicities [17]. Despite the significant disease burden and poor outcomes, mechanisms underlying malignant CNS infiltration remain poorly understood.

Establishing a metastatic CNS focus

The BBB insulates the CNS microenvironment from the circulation, representing a highly exclusive barrier against transit of small molecules, macromolecules, and cells [18–20], an observation dating back to 1909 when Goldmann noticed that intravenously injected trypan blue did not stain the CNS [21]. A large part of both its anatomic and physiologic exclusivity resides in the brain microvascular capillary endothelial cell (BMVEC) layer whose features include a lack of fenestrations and a relatively impermeable tight junction network. Such features restrict the diffusion and paracellular transit to primarily lipid soluble drugs <400 Daltons, excluding ~98 % of small molecule and virtually all large molecule drugs [22]. Such features pose an even more daunting barrier to cells, and in fact the BBB efficiently excludes circulating cells, even those capable of routinely transmigrating across endothelial layers including leukocytes, except under specific circumstances [19, 23–25].

Data analyzing the anatomic distribution of brain metastases shows greater frequency in anatomic watershed areas, suggesting a significant role of blood flow and thereby hematogenous seeding [26]. This suggests that non-CNS malignancies must breach the BBB in order to establish CNS foci, in so doing overcome an anatomic and physiologic barrier more stringent/exclusive than other capillary beds with regard to both small molecule permeability and cellular trafficking. Successful establishment of metastatic foci via hematogenous invasion of the CNS necessitates a multistep process involving malignant cells successfully binding to the blood-side of the BBB, migrating across the physical components of the BBB, and surviving in the CNS microenvironment (Fig. 1). Mechanisms for establishing CNS foci vary with the type of malignancy and its growth pattern. Malignancies forming parenchymal mass/nodular lesions may face different microenvironmental challenges compared to those causing leptomeningeal disease or surviving suspended as single cells in the CSF (e.g., nutrient diffusion, blood supply, nature of cell–cell interactions). While solid tumors primarily manifest as parenchymal metastases, leptomeningeal disease represents a frequent manifestation of hematologic malignancies [12].

Adhesion

Macroscopic considerations: anatomic and physical factors

As noted, the anatomic distribution of brain metastases suggests hematogenous seeding [26]. To establish CNS foci malignant cells must exit from a primary site, enter the circulation, and interact with BMVECs on the blood side of the BBB. Subsequent migration across the BBB and its tight junctions requires circulating malignant cell adhesion to BMVECs of sufficient strength to overcome the effects of blood flow and allow for sufficient steady-state interactions for migration. Imaging studies demonstrate that circulating malignant cells lodge at vascular branch points in the microcirculation [27]. This phenomenon could enhance malignant cell adhesion to BMVECs and would synergize with other factors increasing the potential number of adhesion interactions. For example, identified risk factors for CNS involvement by lymphoid malignancies include increased malignant cell burden, high proliferative index, and widespread distribution (extranodal/extramedullary parenchymal involvement) [7, 8, 10, 28], which could increase the frequency of malignant

adhesion events with BMVECs via a higher number/probability of malignant cell–BMVEC interactions, and consequent probability of a successful transmigration.

Considerations at the cellular level

Expression of specific receptors and integrins affects the localization and chemoresistance of malignant cells [29–37]. Some correlative data exists regarding adhesion receptor expression and malignant CNS breach (e.g., CD56 expression in ALL [38]), but specific molecules and mechanisms involved in malignant cell adhesion to BMVECs remain poorly characterized.

The solid tumor literature implicates β_1 integrins in adhesion of melanoma as well as breast and lung carcinomas to the vascular basement membrane of CNS capillaries [39], not surprising given the ubiquitous general involvement of β_1 integrins in adhesion [30, 37]. However, this study neither addressed luminal adhesion on BMVECs, nor specificity given that the β_1 integrin subunit partners with various α -integrins. Some evidence suggests a role of the α_v integrin as one such potential α -integrin partner. In an experimental rat model of HER2-positive breast cancer brain metastasis, intetumumab directed against the α_v integrin reduced brain metastasis burden and increased overall survival [40]. The mechanism by which α_v integrin blocking resulted in this remains unclear as does the role of the α_v integrin in models beyond HER2-positive breast cancer. Another breast cancer model implicates intercellular adhesion molecule 1 (ICAM-1) via the vasoactive peptide angiotensin II in brain and other metastases [41]. Using a mouse model and the BMVEC cell line hCMEC/D3 [42], Rodrigues-Ferreira et al. demonstrated that treatment with angiotensin II increased both the frequency and the incidence of metastases by MDA-MB-231 breast carcinoma cells at multiple organ sites, including brain, and increased MDA-MB-231 adhesion to BMVECs by ~1.5 fold, proportional to the upregulation of both ICAM-1 mRNA and protein expression on the carcinoma cells. Such upregulation may confer increased tumor cell avidity for endothelial cells, supporting increased adhesion interaction time and the probability of CNS breach.

The generation of invadopodia by certain malignant cells represents another potential determinant of enhanced adhesion. First described in 1989, Chen noted by interference reflection microscopy that Rous Sarcoma Virus transformed chick embryonic fibroblasts demonstrated rosette contact sites via membrane protrusions made up of a microfilament meshwork containing the cytoskeletal elements, actin, α -actinin, vinculin, and the pp60^{src} oncogene product. Naming these protrusions “invadopodia,” he noted them as absent in normal cells [43]. An emerging literature has since demonstrated positive correlations between invadopodia and metastatic potential [47]. In the adhesion context, invadopodia offer greater surface area for contact between potential adhesion determinants on malignant cells with those on BMVECs. Adhesion determinants such as β_1 integrin localize to invadopodia, enhancing the stability of adhesion interactions [44]. Factors increasing invadopodia, including increased activity of the epidermal growth factor (EGF) receptor [45], would likely increase the probability of brain metastases. In breast cancer, this positive correlation between EGF signaling and brain metastases holds up with clinical epidemiology showing increased CNS metastatic risk in Her2-positive breast cancer, [46, 47] with

invadopodia possibly contributing. Evidence also implicates invadopodia in the invasiveness of ALL as well via the activities of RhoA, Cdc42, and possibly the *abl* tyrosine kinase [48].

Further insights regarding hematopoietic malignancies may emerge by considering leukocyte adhesion and migration into the CNS. Leukocyte adhesion to vascular endothelial cells involves interactions between leukocyte LFA-1 with endothelial ICAM-1. As noted, ICAM-1 may enhance adhesion by breast carcinomas to BMVECs [41], and hematopoietic malignancies may likewise plausibly exploit an adhesion system well known to their nonmalignant counterparts. The expression of CD56 in ALL predicts increased CNS involvement [38] and may affect other hematopoietic malignancies as well. Interestingly, CD56 expression occurs in many small cell carcinomas [49, 50], which have a propensity for brain metastasis.

Migration

Sufficient disruption of the BBB to allow cellular migration relies on physiologic changes following adhesion which may vary with the particular malignancy. For example, expression of certain molecules (CD56 in ALL [38], ICAM-1 and EGFR in breast carcinomas [41, 45–47]) may mark certain biological features of malignant cells relevant to CNS breach. The anatomic location of the BBB lies at the tight junctions between BMVECs (Fig. 1, inset B). A large part of BBB tight junction integrity owes to members of the claudin and the occludin families of proteins. Loss of function of these proteins can result in loss of tight junction integrity and consequent loss of BBB exclusivity. Other physiologic factors, including the influence of astrocytes and soluble mediators including VEGF and MMP-2 also modulate the BBB functionality [19, 24, 51].

Breach of the luminal barrier after adhesion

The first steps towards malignant CNS breach after adhesion to BMVECs involve disruption of the luminal tight junction barrier system. In principle, breach may involve physical barrier destruction, undermining of homeostatic mechanisms maintaining it with a consequent loss of its physical or functional integrity, or both. The physical destruction of the luminal barrier appears a common initial assault by both solid and hematologic malignancies. In ALL, matrix metalloproteases (MMP) 2 and 9 activity in leukemic cells directly correlated with their BBB migration capability [52]. In this model, MMPs 2 and 9 digested the claudin and occludin interactions required to maintain the integrity of the BBB's luminal aspect. Diverse malignancies demonstrate correlation of MMP activity with metastatic potential [53]. While definitive evidence for a direct role of MMP activity in transluminal BBB breach remains elusive, the ALL model provides a plausible mechanism contributing to CNS breach in many metastatic malignancies. Given the wide variety of malignancies demonstrating invasiveness related to MMP activity, targeting this common “brute force” proteolytic system may prove amenable to therapeutic intervention in certain settings.

Less direct mechanisms to breach the BBB also have precedent. Lee et al. [54] implicated MMP2 as enhancing malignant transmigration, but found the MMP2 source in their studies as BMVECs demonstrating increased COX-2 expression after association with malignant cells. Fan et al. [55] noted breast cancer adhesion to BMVECs via the β_4 integrin as

enhancing ErbB2-dependent vascular endothelial growth factor (VEGF) production by malignant cells and consequent disruption of tight junction integrity, an observation consistent with an autoimmune encephalomyelitis model where loss of BBB integrity resulted from claudin 5 and occludin downregulation in response to increased VEGF [51]. Ample evidence supports a pathogenic role of VEGF production in ALL [56–58] which potentially superimposes with MMP production to disrupt BBB integrity. Other mechanisms may also apply—in an animal study of breast cancer metastasis to the brain, McGowan et al. [59] noted >70 % reduction in brain metastasis formation after small homologous RNA mediated Notch-1 inhibition in malignant cells.

Emerging data from genetic profiling of CNS metastases, while limited, have also yielded interesting observations. Microarray analysis of breast cancer clinical samples by one group identified 17 potential genes as positively correlated with brain metastases including those for COX2, the epidermal growth factor receptor ligand HBEGF, and the $\alpha(2,6)$ sialyltransferase ST6GALNAC5 [60]. Primary versus metastatic focus profiling of matched clinical samples additionally identified increased expression of VEGF, HER2, and the basal- and claudin-low subtype signatures as positively correlated with brain metastases [61]. Microarray identification of COX2, VEGF, HER2, and EGFR pathway components recapitulate some of the above cited experimental data, further supporting potential roles in malignant CNS breach. Taken together, these studies demonstrate that distinct malignancies may demonstrate overlapping mechanisms of BBB breach while a given malignancy may also have unique gene expression profiles that underlie propensity for BBB transmigration.

Migration following breach of the luminal tight junction network

Upon disrupting the integrity of the BMVEC luminal tight junction, malignant cells must still migrate across the endothelial layer. The migration process likely requires a stimulus driving transmigration, with a variety of chemokines/chemokine receptors potentially modulating this process. In the case of T cell ALL, CCR7, a receptor for the chemokines CCL19 and CCL21, appears important for CNS infiltration. In a mouse model the CCR7-negative T-ALL line DND41 did not cause CNS disease, while the same cell line retrovirally transduced to express CCR7 (DND41/CCR7⁺) did [62]. Nonmalignant models such as those exploring how the HIV virus enters the CNS also provide potential insights into stimuli for BBB transmigration. In this context, CCL2 appears to offer a specific driving stimulus in response to which CCR2-expressing HIV-infected leukocytes migrate across the BBB, with the study initially making this observation also showing that such transmigration did not occur in response to a number of other stimuli/cytokines including CXCL10, CCR3/MIP-1 α , and CCR5/RANTES [63]. Interestingly, increased CCL2 levels in ALL patients' CSF appear to correlate with increased CNS involvement; [64] additional cytokines implicated in stimulating ALL migration include CXCL12 and other soluble factors elaborated by astrocytes, meningeal cells, and choroid plexus epithelial cells [65, 66]. Closer examination of circulating tumor cells and other CSF chemokines may better elucidate potential chemokine/receptor systems involved in supporting malignant BBB transmigration.

After responding to a migratory stimulus, cells must traverse the BMVEC layer and its basement membrane by traveling through or between BMVECs and their associated extracellular matrix to enter the CNS. This process poses many topologic and logistic problems, and it would seem reasonable that cells aberrantly transmigrating across the BBB would readily take advantage of pre-existing pathways and systems utilized by cells which ordinarily traverse the BBB. Diapedesis of leukocytes across the BBB represents such an established process, offering insights into mechanisms that malignant cells may utilize to transmigrate across the BBB.

Among the various leukocytes, those of the monocyte-macrophage lineage frequently transmigrate across the BBB, found in the CNS under “normal” circumstances [23]. Monocyte diapedesis across the BBB involves interactions with several adhesion molecules including activated leukocyte cell adhesion molecule (ALCAM/CD166), junctional adhesion molecule-A (JAM-A), platelet endothelial cell adhesion molecule/CD31 (PECAM-1), and CD99. Blocking antibodies against ALCAM and JAM-A significantly decrease monocytic transmigration across the BBB. Expressed by both monocytes and BMVECs, current evidence suggests diapedesis involves homotypic interactions between these molecules expressed on both [25]. Quite plausibly, hematopoietic malignancies could exploit some or all of these factors in BBB transmigration and thus the positive correlation between PECAM-1 expression by ALL cells with their adhesion and transmigration capability across human BMVECs would not prove surprising [66]. Even less surprising in this context, acute myeloid leukemias (AML) of monocytic origin have a higher frequency of CNS involvement compared with nonmonocytic AMLs. Considering that activated T-cells transmigrate across the BBB [23], the CD6 expressed by them may facilitate this process as a ligand for ALCAM [67]. Other adhesion determinants may also prove relevant for BBB transmigration as an earlier study [66] also demonstrated that VE-cadherin expression by ALL cells positively correlated with adhesion and migration capability across human BMVECs, which also express VE-cadherin.

After breaking through the BMVEC luminal tight junction network and traversing the BMVEC layer, a malignant cell has only to penetrate the BMVEC basement membrane for CNS breach. Here, invasion through the basement membrane would likely proceed as at non-CNS metastatic sites, utilizing MMPs both secreted and concentrated on invadopodia [53, 68–70]. However, entering the CNS is followed by the challenge of whether the metastatic malignant cell has the capability of surviving in its new microenvironment.

Survival and persistence within the CNS

While the role of the tumor microenvironment’s contribution to the survival, persistence, and even chemoresistance of many malignancies stands generally well established [37], specifics relating to contributions of the CNS microenvironment to the survival of infiltrating malignant cells remain incompletely understood. After malignant cells transmigrate across the BBB, they come into contact with stromal and parenchymal elements of the CNS, with some of these interactions essential for tumor cell survival and persistence. Akers et al. [65] demonstrated that ALL cell lines migrated toward soluble factors elaborated by CNS microenvironment elements including meningeal cells,

astrocytes, choroid plexus epithelial cells, and demonstrated that adhesion to these CNS stromal elements resulted in enhanced chemoresistance. Such chemoresistance also occurs with the association of astrocytes with lung cancer, breast cancer, and melanoma [71, 72] as well as with adhesion of malignancies in other non-CNS microenvironmental milieus [37]. Interactions between CNS stromal elements and malignant cells may contribute to disease persistence and relapse by sheltering malignant cells behind the BBB and thereby from the effects of systemic chemotherapy. Microglia may also mediate protection as suggested in an animal model study of two breast cancer cell lines. Microglial association with the breast cancer cell line MCF-7 tripled its invasiveness into CNS parenchyma while inhibition/depletion of microglial cells via the bisphosphonate clodronate decreased not only invasiveness but also the overall tumor burden in the CNS parenchyma. This same study implicated the role of the Wnt and *c-jun* pathways in this phenomenon, demonstrating that the corresponding synthetic inhibitors inhibited this microglia-induced invasiveness [73].

In solid tumor metastases additional interactions required for persistence and survival include those required for angiogenesis, with VEGF having an established role [72, 74]. VEGF also appears to play an important role with regard to CNS breach by hematologic malignancies, but its role in this milieu appears more complicated. As noted, hematologic malignancies often reside in the leptomeninges and CSF as opposed to parenchymal masses [10, 12], but may also may form nodular lesions (e.g., granulocytic sarcomas, lymphomatous masses/nodules). However, review of autopsy pathology suggests some potential differences based on certain distributions of CNS involvement. CNS breach by acute nonlymphocytic leukemias demonstrated more focal parenchymal infiltration and hemorrhage compared with ALL [10]. Such varying microenvironmental milieus may impose considerably different survival stresses. Many hematologic malignancies elaborate significant amounts of VEGF with mounting evidence of a role in CNS breach [57, 58, 65, 75–78]. Angiogenesis in this context may impose microenvironmental effects—increased microvessel density can correlate with worse prognosis/outcome in both lymphomatous mass/nodule and leukemic contexts [75, 77]. In some scenarios, evidence suggests an autocrine role for VEGF in hematologic malignancies in addition to other non-angiogenic microenvironmental modifications including modulation of local barrier integrity and cytokine elaboration [51, 56–58, 75, 77]. As more preclinical models become available [4], a better and more complete appreciation of malignant cell interactions with the CNS microenvironment will likely emerge.

Discussion and therapeutic implications

Therapeutic modalities for treating malignant CNS involvement remain limited with associated outcomes poor overall. While intrathecal chemotherapy remains a mainstay for patients with CNS involvement by hematologic malignancies, in the primary prevention setting the best evidence for intrathecal chemoprophylaxis lies in ALL and Burkitt's lymphoma where it currently represents standard of care [7]. Prophylactic intrathecal chemotherapy administration via lumbar puncture or Ommaya reservoir in ALL has reduced the burden of CNS involvement from >50 % to less than 10 % overall [7, 9, 79, 80], yet CNS relapse still accounts for 30–40 % of relapse in this disease [81]. In Burkitt's lymphoma intrathecal chemoprophylaxis has reduced the burden of CNS disease from

nearly 50–80 % to a range between 4 and 27 % depending on the systemic chemotherapy regimen and use of radiation [7, 82]. The role of intrathecal chemoprophylaxis in other lymphomas remains less defined, but some subsets of the common diffuse large B-cell type demonstrate notable degrees of CNS involvement without it (primary mediastinal 17 %, breast 11–21 %, testicular 7–31 %, intraocular 50–90 %, mantle cell 4–23 %) [7, 83–87]. Patients afflicted with such subtypes frequently receive intrathecal chemoprophylaxis as a result. Despite the absence of prospective placebo-controlled randomized trials regarding chemoprevention of secondary lymphomatous CNS involvement [82, 88], this epidemiology may still guide administration of intrathecal chemoprophylaxis or other agents though CNS relapse in the higher risk groups remains an issue [82, 88].

In contrast, prevention of CNS involvement in the solid tumor realm consists of modalities such as prophylactic cranial irradiation (PCI) in small cell lung cancer (SCLC) but little else overall to address primary prevention of malignant CNS involvement. PCI for SCLC offers limited benefit, with some reduction in the incidence of brain metastases but with questionable effects on quality of life and overall survival [89–91]. Otherwise effective strategies for primary prevention remain lacking despite the considerable morbidity and mortality of malignant CNS involvement. However, a recent phase 2 study of the EGFR inhibitor erlotinib and radiation therapy for treatment of brain metastases in non small cell lung cancer (NSCLC) found 9 of 17 otherwise unselected patients to have EGFR mutations, a frequency considerably higher than the expected 10–15 % frequency [92], suggesting a potential role for EGFR mutations in brain metastasis. The trial noted an 86 % overall response rate, with a much better median survival in mutation-positive patients compared with mutation-negative ones (19.1 vs 9.3 months) while a second phase 2 trial noted a 93 % overall response rate in EGFR mutation-positive NSCLC [93]. This efficacy raises the question as to whether erlotinib or other tyrosine kinase inhibitors could work in a primary prevention setting for select cases. Hematologic malignancies also express tyrosine kinases (e.g. CD117/c-kit in >80 % AML [94]), but effects on CNS breach remain unclear. Taking all this into account, effective primary prevention strategies against malignant CNS breach could improve overall outcomes—the noted models of adhesion and transmigration offer the possibility of targeting novel pathways, singly or in combination with other pathways in order to minimize the burden of malignant CNS disease.

Initial steps of the CNS breach process, at least in principle, represent reasonable targets for therapeutic intervention. For example, agents effectively interfering with the initial adhesion step of circulating malignant cells to BMVECs (Fig. 1, inset A) would potentially deny a sufficient interaction for subsequent malignant transmigration. The adhesion molecules involved in the transmigration process represent potential targets as well (Table 1) with monoclonal antibodies having the benefit of relatively long circulating half-life resulting in a more constant steady state adhesion blockade allowing for less frequent and more convenient dosing. If targeted to molecules on malignant cells, monoclonal antibodies may additionally exert anti-tumor activity. Furthermore, intrathecal administration of monoclonal antibodies has precedent, offering the potential for superimposed CNS prophylaxis.

While the need for developing new agents to advance outcomes in malignant CNS involvement remains, benefit may also result from use of a number of already available

agents targeting some pathways implicated in BBB breach (Table 1). For example, bevacizumab, an anti-VEGF monoclonal antibody, may have such potential [95] when considering the role of VEGF in compromising BBB integrity. Data regarding the effect of bevacizumab on preventing brain metastases remains lacking and represents a timely topic for exploration. The suggested role of angiotensin II in the mouse model of breast cancer [41] raises the question of whether using agents blocking the renin-angiotensin system, in addition to current standard therapies, may offer increased benefit. Breast cancer models implicating COX-2 upregulation in the development brain metastases [54, 60] raise the question of whether available COX-2 inhibitors such as celecoxib may reduce the burden of metastatic CNS involvement. Tyrosine kinase inhibitors may also have a role in both primary prevention and treatment settings as well [92, 93]. Clearly, more robust mechanistic data must emerge in order to optimally benefit patients.

Conclusions

The current understanding of how various malignancies breach the BBB has proven inadequate to combat the significant population burden and the individual morbidity/mortality associated with malignant CNS involvement. The currently available therapeutic options only marginally impact afflicted patients, with the prognosis of most patients still measured in months. Understanding the mechanisms by which malignant cells breach the BBB offers the possibility of thwarting malignant cell adhesion or migration, possibly preventing the establishment of metastatic malignant CNS foci. Mechanisms of malignant CNS breach may parallel nonmalignant models describing relatively common events such as leukocyte transmigration into the CNS as well as metastatic breach into non-CNS organs. With regard to hematologic malignancies, nonmalignant and solid tumor models provide a foundation for initiating further mechanistic investigation of CNS breach while appreciating the disease specific differences that are likely to exist. Investigation into such mechanisms is required to accelerate the development of therapeutic targets and strategies aimed at reducing the incidence of metastatic malignant CNS involvement.

Acknowledgments

We thank Jeff Welsh of FolioType Creative, LLC, Morgantown, WV for generation of Fig. 1.

Funding National Institutes of Health (NHLBI) R01 HL056888 (LFG), National Cancer Institute (NCI) R01 CA134573NIH (LFG), P30 GM103488 (LFG), WV CTR-IDEA NIH 1U54 GM104942, the Alexander B. Osborn Hematopoietic Malignancy and Transplantation Program, and the WV Research Trust Fund. The funders had no role in conceptualization decision to publish, or preparation of the manuscript.

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Fig. 1. Steps involved in breach of the CNS by a malignant cell. A circulating cancer cell in a brain capillary interacts with a BMVEC initially via a receptor-ligand mediated adhesion prior to undergoing transmigration (*inset A*). *Inset B* illustrates the tight junction between BMVECs, highlighting the various types of molecules involved in maintaining its integrity which represent both potential barriers as well as interacting partners in the transmigration process. Pericytes (*inset C*) and astrocyte foot processes contribute to the BBB integrity while factors such as VEGF and MMPs (*lightning symbol*) contribute to loss of BBB integrity. Many of

these interactions may be shared among various tumor cells that breach the CNS with clinically significant consequences

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Table 1**Potential Targets Implicated in Malignant CNS Breach**

Target/pathway	Malignancies	References	Available therapeutic agents
VEGF	Many including ALL, lymphomas, breast	[51, 55–58, 74, 78, 96, 97]	Bevacizumab
Angiotensin II	Breast	[41]	ACE inhibitors, ARBs
COX-2	Breast, lung	[54, 60, 98]	Celecoxib
Adhesion molecules (α_v , β_1 , β_4 , CD6, CD56, others)	Multiple solid and hematologic cancers	[38–40, 44, 55, 99]	A few monoclonal antibodies (e.g., intetumumab, natalizumab)
Matrix metalloproteases (MMPs 2, 9)	Multiple solid and hematologic cancers	[52–54, 68–70]	–
Chemokines (CCR7, MCP1/CCL2, CXCL12)	T-ALL, B-ALL	[62, 64–66]	Plerixafor targeting CXCR4/CXCL12
Tyrosine kinases (EGFR)	Lung, breast, others	[45, 92, 93, 100]	Erlotinib