Role of the Neural Niche in Brain Metastatic Cancer

John Termini1, Josh Neman2, and Rahul Jandial2,3

Abstract
Metastasis is the relentless pursuit of cancer to escape its primary site and colonize distant organs. This malignant evolutionary process is biologically heterogeneous, yet one unifying element is the critical role of the microenvironment for arriving metastatic cells. Historically, brain metastases were rarely investigated because patients with advanced cancer were considered terminal. Fortunately, advances in molecular therapies have led to patients living longer with metastatic cancer. However, one site remains recalcitrant to our treatment efforts, the brain. The central nervous system is the most complex biologic system, which poses unique obstacles but also harbors opportunities for discovery. Much of what we know about the brain microenvironment comes from neuroscience. We suggest that the interrelated cellular responses in traumatic brain injury may guide us toward new perspectives in understanding brain metastases. In this view, brain metastases may be conceptualized as progressive oncologic injury to the nervous system. This review discusses our evolving understanding of bidirectional interactions between the brain milieu and metastatic cancer. Cancer Res; 74(15): 4011–5. ©2014 AACR.

Introduction
Metastases are responsible for the majority of deaths from cancer. Dissemination of primary cancers to secondary organ sites is most ominous when tumors spread to the brain. Brain metastases indicate poor prognosis, likely exclusion from clinical trials, and cause neurologic deficits that impair patient’s lives in fundamental ways. Existing therapies are restricted to palliative radiation and neurologic surgery, with limited chemotherapeutic options (1).

As the global incidence of cancer has increased, brain metastases now appear in 8% to 10% of all cases (2, 3). Several factors underlie this epidemiologic shift. Major advances in neuroimaging have led to increased detection of brain metastases in patients (4). Although targeted therapies have improved the management of systemic disease, poor bioavailability to the brain increases its potential as a sanctuary site for metastatic disease. Fortunately patients with cancer are living longer, but extended survival from primary disease has unmasked brain metastases as one of the major challenges limiting outcomes for those with advanced disease (5).

Metastasis is not the biologic narrative of an autonomous cell. More likely, it is a dynamic interplay between neoplastic cells and newly encountered microenvironments. Traditionally metastatic spread was conceived as a late event occurring long after primary tumors progressed locally (6). This may still be true for some tumors; however, recent evidence also suggests parallel progression of primary and metastatic disease. Indeed, circulating tumor cells (CTC) can be detected in newly diagnosed early-stage cancer (6). Despite this, patients may progress to clinically detectable brain metastases years to decades after primary diagnosis.

As CTCs course through the cerebral vasculature, the blood brain barrier (BBB) poses an innate obstacle to brain intravasation (7). Although formidable, the BBB is penetrable. CTCs can effectively (although inefficiently) cross the intact BBB and form micrometastases (8). The ability of cells to traverse the BBB, as well as the clinical latency between the appearance of early circulating cells and development of brain metastases raises an intriguing biologic question. Could the rate-limiting event in the formation of brain metastases be the last step of the metastatic cascade – colonization of the neural niche?

After crossing the BBB, disseminated primary tumor cells arrive in a dynamic cellular and molecular landscape that presents unique selection pressures. There is a paucity of research on brain metastases from this perspective, which offers an opportunity for new discoveries to extend the quality and duration of patient’s lives. This review will focus on the reciprocal interactions between brain metastases and the neural niche.

Defining the Brain Milieu
Unique cell types comprise the brain milieu. Neurons are the principal components of the central nervous system (CNS), and are electrically excitable cells that use neurotransmitters in synaptic connections for communication. Five classical neurotransmitters [glutamate, γ-aminobutyric acid (GABA), seroton- nin, acetylcholine, and dopamine] are released from presynaptic vesicles and activate respective receptors on postsynaptic...
neurons (9). Viability of neurons is maintained by endogenous growth factors called neurotrophins [nerve growth factor, brain—derived neurotrophic factor (BDNF), NT-3, NT-4], which regulate cell fate, growth, and plasticity (10). Neurotrophins also modulate germinial zones that are repositories of neural stem cell (NSC) niches in the adult brain (the subgranular zone of the dentate gyrus in the temporal lobes and the subventricular zone of the frontal lobes; refs. 11, 12). As neurons acquire the ability to generate action potentials and communicate by synaptic transmission, they lose many essential metabolic pathways (13). As a consequence, the adult brain relies on microenvironmental metabolic support from surrounding glial cells.

Homeostasis in the brain microenvironment is maintained by supportive glial cells called astrocytes (14). Branching astrocytic processes cloak most cellular components throughout the CNS, including the BBB. In the uninjured CNS, astrocytes play an essential role in the maintenance of extracellular ionic environment and pH, clearance and release of extracellular glutamate, and provision of metabolic substrates for neurons (15). Purinergic signaling is the most important pathway by which astrocytes communicate with other cells (16). This includes ATP as the main transmitter for communication among neighboring astrocytes. ATP is also important in paracrine signaling to neurons, blood vessels, and microglial cells. The other prominent glial cells in the CNS are the myelin producing oligodendrocytes. A single oligodendrocyte can extend its processes to 50 axons, wrapping each with approximately 1 μm of myelin sheath. In addition, oligodendrocytes can receive synaptic inputs from neurons and participate in metabolic homeostasis of the brain milieu (17, 18).

Insights from Neuroscience

Our understanding of how the brain responds to injury has grown dramatically in recent years. This will likely yield new ideas about the relationship between metastatic cells and the neural niche. Traumatic brain injury (TBI) disrupts normal circuitry by causing neuronal and glial cell death, inducing a cascade of microenvironmental perturbations (19). TBI damages the biosynthetic capacity of neurons to make GABA, and can lead to a toxic accumulation of the glutamate precursor (20). This disruption in the balance of GABA and glutamate is associated with increased epileptic brain activity increasing the potential for additional damage.

The brain can limit the spread of injury by forming a glial scar to seal off damaged areas. In the adult brain, astrocytes are typically quiescent and not proliferative unless activated by disease (21). These "reactive" astrocytes respond with hypertrophy and increased expression of intermediate filament glial fibrillary acidic protein (GFAP) in a process termed gliosis. Experimental ablation of proliferating reactive astrocytes disrupts scar formation causing increased neuronal loss and demyelination (15). This exacerbates clinical deficits and impairs functional recovery, supporting the essential role of glial scar forming astrocytes in neural protection and repair. The astrocytic response to injury is supplemented by resident oligodendrocyte progenitor cells, which participate in gliosis and regeneration. Oligodendrocyte progenitor cells locally secrete chondroitin sulphate proteoglycan (NG2), which diminishes axonal regrowth (22).

Neoplastic Adaptations to the Neural Niche

Cancer cells arrive in the neural niche to execute the last step in the metastatic cascade—colonization (23). It is unknown whether cells already possess latent traits that facilitate brain colonization or if advantageous mutations are acquired under neural niche selection pressures (24, 25). It may be possible that primary tumors evolve and release cells with brain-trophic mutations at an appreciable frequency to succeed despite the inefficient process of metastasis. Alternatively, premetastatic cells may evolve under new microenvironmental pressures after their arrival in the brain that drives fate determination.

It is more plausible that some combination of these strategies takes place. Tumor cells may survive by exploiting a period of dormancy allowing for the development of bidirectional interactions that transform the local brain milieu into a hospitable niche. Once this favorable environment is achieved, they could escape dormancy and progress to clinically relevant macrometastases. Given the heterogeneous patterns observed in the clinical progression of brain metastases, tumors most likely use varied approaches that are specific to a patient’s biology and history of medical intervention. In all scenarios, the brain's microenvironment provides the critical selection pressure.

The role of astrocytes in brain metastases has been defined by seminal contributions from several groups (8, 26–33). Early work demonstrated the role of paracrine signaling via cytokine release by astrocytes in facilitation of brain metastases. In addition to releasing various interleukins, reactive astrocytes upregulate survival genes (GSTA5, BCL2L1, TWIST1) in breast cancer cells, resulting in resistance to chemotherapeutic agents (34). Furthermore, with direct relevance to mechanisms of treatment efficacy, inhibition of platelet-derived growth factor receptor β+ astrocyte subgroups with Pazopanib prevented the outgrowth of Her2+ brain metastases in animal models (35). The indispensable contribution of astrocytes to metastatic colonization is well established and continues to be robustly investigated.

The ability of metastatic cells to alter the physiologic microenvironment of the brain toward a tumor-favorable microenvironment was recently described by our group (36). Because NSCs can differentiate into neurons or glial cells based on temporal and molecular signals arising during development or brain injury, their role in metastasis was investigated. Histologic interrogation of neurosurgical specimens identified NSCs in breast metastases and adjacent brain. Reactive astrocytes were also seen, and interestingly they resembled those found in glial scars of patients with TBI. Moreover, metastatic cells overexpressed BMP-2, which is also known to be a strong molecular cue for NSC differentiation into astrocytes. Coculture experiments with metastatic cells from surgical specimens and NSCs showed that tumor cell growth is inhibited by NSCs initially. However, over time, metastatic cells were able to escape NSC inhibition by steering them to an astrocytic lineage through paracrine BMP-2 signaling.
The effect of neurotransmitters on brain metastases has also been explored (37). Comparison of primary and brain metastatic tissue and cells revealed upregulation of both cell surface GABA transporters and receptors. Although it was anticipated that GABA would potentiate growth through receptor signaling, experiments revealed an energetic role as a metabolic substrate following cytosolic shuttling through GABA transporters. A proliferative advantage was conferred by the ability of metastatic cells to take up and catabolize GABA with the resultant formation of NADH through the GABA-shunt. Together these findings suggest that cancers may survive in the brain microenvironment by co-opting neurotransmitters as oncometabolites.

The brain's extracellular space is rife with growth factors that maintain cellular homeostasis (38). These growth factors could serve as powerful substrates for metastatic tumor cells that possess or evolve the ability to utilize the associated signaling pathways (39, 40). This would be particularly advantageous in the context of metastatic dormancy or micrometastasis where angiogenesis is sparse. Our preliminary findings suggest that tumor cells express neurotrophin receptor TrkB and are selectively activated by BDNF from the brain microenvironment. Recognizing that breast cancer metastasizes to the brain in approximately 40% of Her2\(^+\) patients, we are investigating a possible interaction between TrkB and Her2\(^+\) (41). Our preliminary results show that BDNF induces TrkB-Her2 dimerization in newly derived metastatic cells (unpublished). Cooperation of neurotrophin and Her2 receptors could result in amplified oncogenic signaling from transactivation of both receptors by extracellular BDNF in the brain. Given the abundance of neurotrophins in the neural niche, this could partly explain the proclivity of breast cancer to spread to the brain in Her2\(^+\) patients.

The brain milieu most likely selects for metastatic cells that are best able to overcome natural barriers to infiltration, as well as utilize the nutrient resources and signaling pathways of the brain (Fig. 1). Metastatic cells that cross the BBB and over-express BMP-2 could modify the local cellular milieu by differentiating NSCs into astrocytes. After this niche construction, the innate and amplified astrocytic response provides cytokine stimulation to tumor cells that could allow early survival as dormant or micrometastases. With time, some cells may acquire a more neural phenotype with expression of classical neuronal receptors and signaling pathways allowing further growth advantage. Ultimately the progressive selection and evolution of metastatic cells could allow for extensive colonization of the brain and subsequent neurologic injury.

Central Nervous Ecosystem

For nearly a century, the "no new neuron" theory was held as dogma, suggesting the brain is unable to regenerate with little plasticity remaining after developmental maturity (42, 43). With the discovery of germinal niches in the brain, the notion of the brain as immutable has been shed and a renaissance in neuroscience has occurred. New methods such as "optogenetics" are allowing neuroscientists to control the activity of...
neuronal groups with extraordinary precision (44, 45). New insights about the anatomical and functional architecture of the human prenatal brain are revealing transcriptional land-
scapes that may eventually guide neural repair in the context of oncologic injury (46).

In parallel the importance of the tumor microenvironment has been established in cancer biology. Despite originating in disparate germ line lineages, tumor cells are able to inhabit the neural niche. This ability to adapt to and possibly co-opt the brain’s natural resources demonstrates the resilience and adaptability of cancer. Borrowing from ecological principles, metastasis could be an adaptive radiation of neoplastic cells from the primary tumor (47, 48). Each malignant scout having the opportunity to undergo its own divergent evolution and create distinct metastatic species (49).

The proverbial "soil" of the brain is a unique and mostly unchartered environment. It is likely that technological and creative advancements in neuroscience will reveal unanticipated biologic complexities and clinical opportunities in metastatic brain tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Grant Support
J. Termini was supported by NIH grant R01CA176611-01. J. Neman was supported by California Institute for Regenerative Medicine (CIRM) grant TG2-01130. R. Jandial was supported by NIH grant 2K12CA001727-10A1.

Received April 21, 2014; revised May 21, 2014; accepted May 22, 2014; published OnlineFirst July 17, 2014.

References
11. Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subven-
17. Morrison BM, Lee Y, Rothstein JD. Oligodendroglia: metabolic support-
21. Anderson MA, Ao Y, Sofroniew MV. Heterogeneity of reactive astro-
29. Fitzgerald DP, Palmieri D, Hua E, Hargrave E, Herring JM, Qian Y, et al. Reactive glia are recruited by highly proliferative brain metastases of breast cancer and promote tumor cell colonization. Clin Exp Metas-
35. Grill B, Palmieri D, Qian Y, Anwar T, Liewehr DJ, Steinberg SM, et al. Pazopanib inhibits the activation of PDGFRbeta-expressing astro-

Published OnlineFirst July 17, 2014; DOI: 10.1158/0008-5472.CAN-14-1226
Correction: Role of the Neural Niche in Brain Metastatic Cancer

In this article (Cancer Res 2014;74:4011–5), which appeared in the August 1, 2014 issue of Cancer Research (1), the sentence beginning “After this nice construction” on page 4013 was missing a letter. The corrected sentence is below. The publisher regrets this error.

The online version has been corrected and no longer matches the print.

After this niche construction, the innate and amplified astrocytic response provides cytokine stimulation to tumor cells that could allow early survival as dormant or micrometastases.

Reference

Published OnlineFirst September 16, 2014.
doi: 10.1158/0008-5472.CAN-14-2344
©2014 American Association for Cancer Research.