Basic Biology of Brain Metastasis

2

Monika Vishnoi, Robert A. Scranton,
Samuel K. Asante, and Robert C. Rostomily

Introduction and Epidemiology

Brain and other nervous system cancers are extremely lethal and represent 1.4% of all new cancer cases in the United States (SEER 2018, 2008–2014). In 2018, there were an estimated 23,380 new cases of brain and other nervous system cancers and 16,380 estimated cancer deaths. Brain cancer (BC) is divided into two different types depending on origin site: (1) primary cancer, confined to the brain; (2) secondary cancer, metastasized to the brain from a different primary site. Secondary brain tumors are extremely aggressive and about 30–40% of cancer patients with primary tumor (melanoma, breast, lung, etc.) have been diagnosed with brain metastasis (BM) at some stage after initial cancer diagnosis (Table 2.1). Lung and breast cancer are the most frequent cancers that metastasize to the brain in men and women respectively [1]. Certain molecular subtypes such as HER2 amplification in breast cancer and anaplastic lymphoma kinase (ALK) positivity in non-small-cell lung cancer (NSCLC) carry a higher rate of brain metastasis [2, 3]. Other factors associated with incidence of brain metastasis include age, ethnicity, and geographic location [1].

Table 2.1 SEER-based incidence of brain metastases determined by primary tumor site

Primary cancer site	Estimated new cases, 2018	Estimated deaths, 2018
Breast cancer (female)	266,120	40,920
Lung and bronchus cancer	234,030	154,050
Prostate cancer	164,690	29,430
Colorectal cancer	140,250	50,630
Melanoma of the skin	91,270	9320
Bladder cancer	81,190	17,240
Non-Hodgkin lymphoma	74,680	19,910
Kidney and renal pelvis cancer	65,340	14,970
Uterine cancer	63,230	11,350
Leukemia	60,300	24,370

Among all primary cancer types, the overall 2-year and 5-year survival rates for brain metastatic patients are 8.1% and 2.4%, respectively [1, 4]. The therapeutic management of brain metastasis depends on the number and location of metastatic tumors, and can include whole brain radiation therapy (WBRT), surgical resection, stereotactic radiosurgery, systematic chemotherapy, targeted therapy, and immunotherapy. Poor blood-brain barrier (BBB) permeability can

M. Vishnoi · R. C. Rostomily (✉)
Department of Neurosurgery, Houston Methodist Research Institute, Houston, TX, USA
e-mail: rrostomily@houstonmethodist.org

R. A. Scranton
Department of Neurosurgery, Goodman Campbell Brain and Spine, Indiana University, Indianapolis, IN, USA

S. K. Asante
Department of Neurosurgery, Houston Methodist Hospital, Houston, TX, USA

R. Ramakrishna et al. (eds.), *Central Nervous System Metastases*,
https://doi.org/10.1007/978-3-030-42958-4_2

limit the effectiveness of systemic chemotherapy to effectively treat brain metastasis [5]. Combined targeted and immunotherapeutic approaches can produce shrinkage of brain metastasis, can slow tumor growth, and can prevent or/delay neurologic symptoms [1]. In brain metastatic clinical trials, multimodal combination therapies provide more survival benefit to patients than individual treatments; however, posttreatment toxicity may adversely affect patients' quality of life (QOL) [1, 6]. Therefore, it is imperative to understand the complexity of the brain metastatic cascade and translate these findings in clinical settings to develop effective therapies with the ultimate goal of increasing patients' QOL and survival. In this review, we will discuss the molecular and genetic properties of the tumor progenitor cells responsible for brain metastatic seeding and colonization, as well as their modulation through tumor-host niche interactions, the neuro-inflammatory cascade, and neovascularization.

Seed and Soil

The realization that the profile of metastatic sites did not reflect a stochastic distribution of the seed based on passive blood flow and target organ mass suggested that specific cellular and molecular mechanisms were actively involved in regulating metastasis. Stephen Pagett first proposed that this phenomenon is governed by the unique match of the metastatic cancer seed with a conducive soil—the "seed and soil" hypothesis [7]. To achieve successful metastasis, cancer cells must shed from their primary site, survive and self-renew in the circulation (blood/lymph), intravasate, and colonize distant organs where they must survive and grow (Fig. 2.1) [8–10].

The heterogeneous populations of tumor cells that comprise the primary tumor possess distinct molecular and cellular phenotypes evidenced by their differential proliferative, invasive, angiogenic, and metastatic abilities [11, 12]. The metastatic cascade exerts further selection pressure

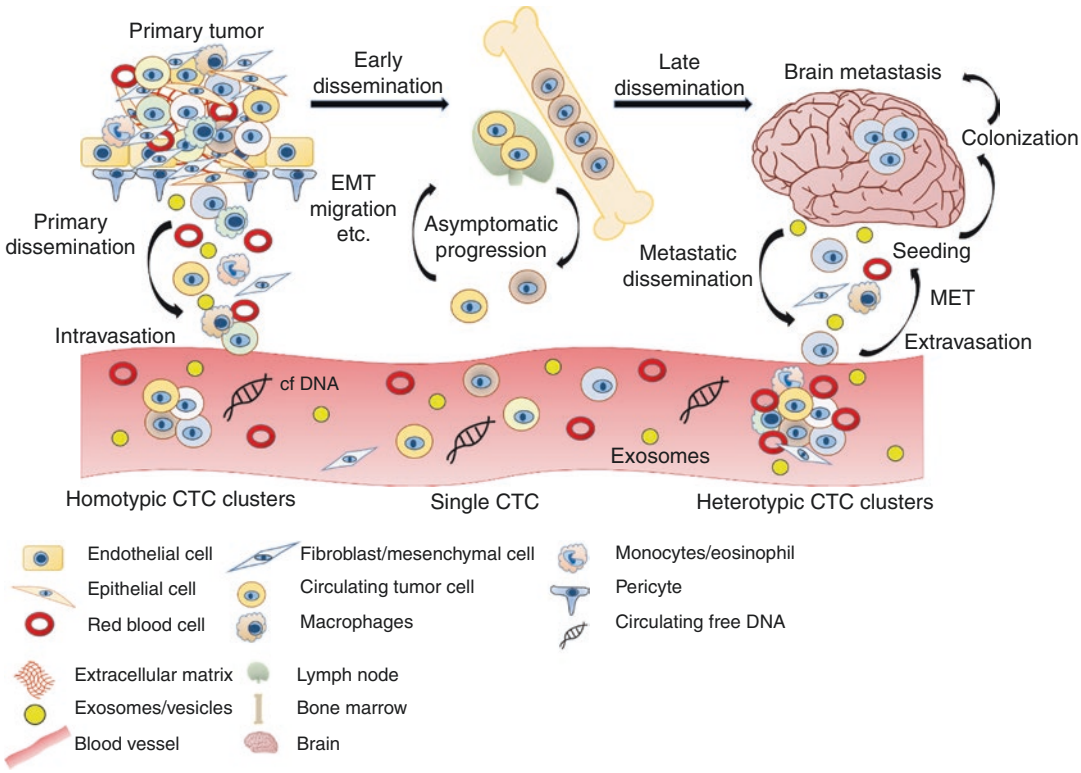


Fig. 2.1 Steps of brain metastatic cascade

that influences cell proliferation, quiescence, adhesion, invasiveness, plasticity, cell-surface (growth and hormone) receptors, and immunogenicity that ultimately defines the metastatic potential [8, 9]. The metastatic "seeds" mobilize and invade the lymphatic or vasculature system where they disseminate as single cells or cell clusters (tumor emboli) [9, 13, 14]. These cells then are often home to, and interact with, conducive microenvironments at distant organs (soil) where stromal and host factors govern their colonization, survival, and growth. Therefore, organ-specific colonization and macro-metastases formation are highly complex processes that depend upon specific "homeostatic mechanisms" and interactions with extracellular matrix (ECM) proteins and cells (immune, stromal, fibroblasts cells, etc.) that comprise the target organ microenvironmental niche [8, 9, 15, 16]. The unique properties of the brain environment (BBB and neural niches) and how they impact BM formation and growth are discussed more in detail below.

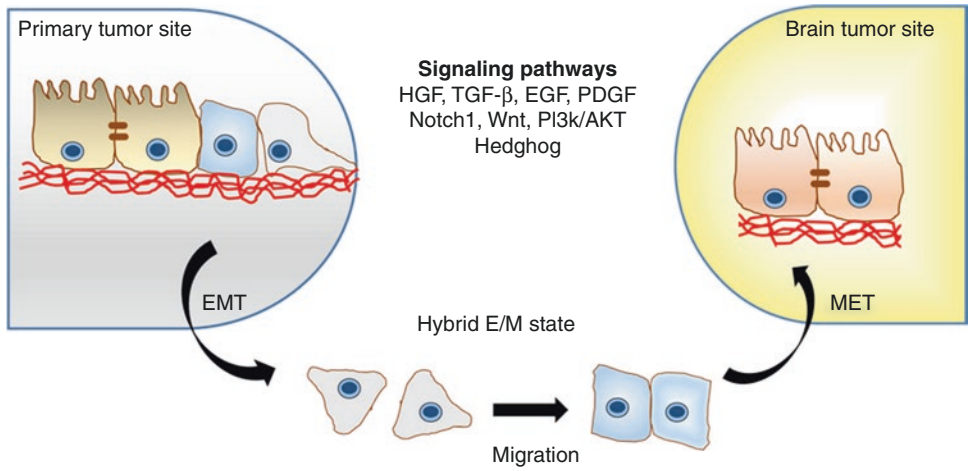
The Early Dissemination Phase of Brain Metastasis

Epithelial-Mesenchymal and Mesenchymal-Epithelial Transitions: EMT/MET

Implied in the "seed and soil" hypothesis and critical to the manifestation of a metastasis is the capacity of a primary cancer cell or aggregate to navigate an imposing biological gauntlet, roughly divided into discrete stages: (i) separation from the primary tumor mass and invasion into and survival in the blood stream (intravasation), (ii) exit from the blood stream to achieve colonization within a distant organ (extravasation), and (iii) survival and growth in a distant organ (Fig. 2.1). A major advance in the conceptualization of metastasis came with the realization that the cellular phenotypes required of the first stage (intravasation) of the metastatic cascade recapitulated the features of a developmentally and morphogenetically recognized phenomenon termed epithelial mesenchymal transition (EMT) first described by Elizabeth Hay in the context of embryogenesis [17].

At the molecular level, EMT is driven by transcription factors such as ZEB1/2, SNAIL, SLUG, and TWIST1 and signaling through HGF, TGF-β, EGF, PDGF, Notch1, Wnt, PI3k/AKT, and Hedgehog pathways that together promote motility, migration, and invasion of tumor cells (Fig. 2.2) [18–25]. For example, TWIST1 is critical for mammary epithelial carcinoma cell extravasation and is implicated in metastatic capacity for numerous cancers [26]. Downregulation of E-cadherin (an epithelial cellular adhesion protein) and upregulation of N-cadherin (the so-called "cadherin switch") accompany EMT allowing a cell typically held in tight apposition to become mobile and correlate with the metastatic potential of cancers metastasizing to the brain [27, 28]. This is followed by degradation of the epithelial basement membrane and invasion through the endothelial basement membrane, and then transit into the blood vessel [29–31]. In addition to promoting invasiveness, EMT promotes malignant phenotypes through effects on immunosuppression, treatment resistance, and cancer stem cells (CSCs) [25]. Therefore, EMT contributes broadly to BM formation through production of metastatic "seeds," activation of malignant cellular properties, and reprogramming of the tumor microenvironment.

To successfully generate metastases, disseminated tumor cells must survive in the blood stream (see below), extravasate from the circulation, colonize, and grow in distant organs. Despite the presumed importance of EMT in the initial dissemination of metastatic cancer cells, metastatic tumors frequently retain epithelial features of the primary tumor [25]. These conflicting observations are reconciled by recognizing that the final phase of the metastatic cascade (extravasation, colonization, and macro-metastatic growth) requires reversal of the mesenchymal to epithelial phenotype; a process termed the mesenchymal-epithelial transition (MET) [32–34]. Activators of EMT signaling are lacking at sites of metastatic colonization, including the brain, which promotes MET and macro-metastatic growth [8, 25, 35]. This cross-talk between



Epithelial markers	Transcription factors	miRNA	Stem-cell markers	Mesenchymal markers
E-cadherin	Twist1/2	mir-21	Nanog	N-cadherin
Claudin	Snail	mir-200	Sox-2	Fibronectin
Occludin	Zeb1/2	mir-205	Oct4	FSP-1
Desmoplakin	Slug	mir-34a	LGR5	Vimentin
Mucin-1	bHLH	mir-145	CD133	Vitronectin
Cytokeratin	β-catenin	Let-7		SMA
				FGFR2
				MMPs

Fig. 2.2 Molecular mechanisms of epithelial (E)-mesenchymal (M) transition during brain metastasis

extravasated cancer cells and the microenvironment of distant organs underscores the importance of the metastatic niche or "soil" for successful generation of metastases. Here, we will discuss the mechanism of cross-talk between metastatic cancer cells and the brain that specifically contributes to BM formation. While the precise mechanisms of EMT and MET that regulate BM formation have yet to be elucidated, it is significant that both processes promote the phenotypes of CSCs, the putative "seeds" for BM formation [7, 25].

Cancer Stem Cells

Many cancers possess a subpopulation of cancer stem cells (CSCs) that play critical roles in tumorigenesis, treatment resistance, and progression and are commonly considered the "seed" for metastasis [11]. Although they comprise a minority population of tumor cells, CSCs are of great clinical importance by virtue of their increased resistance to treatment and putative role in formation and/or growth of BMs (Fig. 2.3) [36]. Cancer stem cells (CSCs) are operationally defined by properties of proliferation, self-renewal, multi-lineage differentiation, and, importantly, the capacity to recapitulate the cancer phenotype in vivo [37]. The correlation of specific molecular markers with CSC phenotypes has facilitated the investigation of the role of CSCs in BMs. For instance, in breast cancer the CD44 hi/CD24 low CSC phenotype is responsible for maintaining self-renewal and proliferation through Notch signaling and drives metastatic progression in the brain [22, 38, 39]. On the other hand, the chemokine CXCR4/12 signaling axis provides microenvironment cues to CSCs for proper homing and brain colonization [40]. Of note, targeting CSC phenotypes CD44 hi/CD24, CD133, and BMI1 or inhibiting CXCR4/12 and the Notch signaling axis effectively eradicates brain metastatic spread and improves therapeutic efficacy [39, 41, 42]. In concert with MET and local angiogenesis, they drive growth of macroscopic brain tumors [43, 44]. Another critical observation is that cancer cells can switch

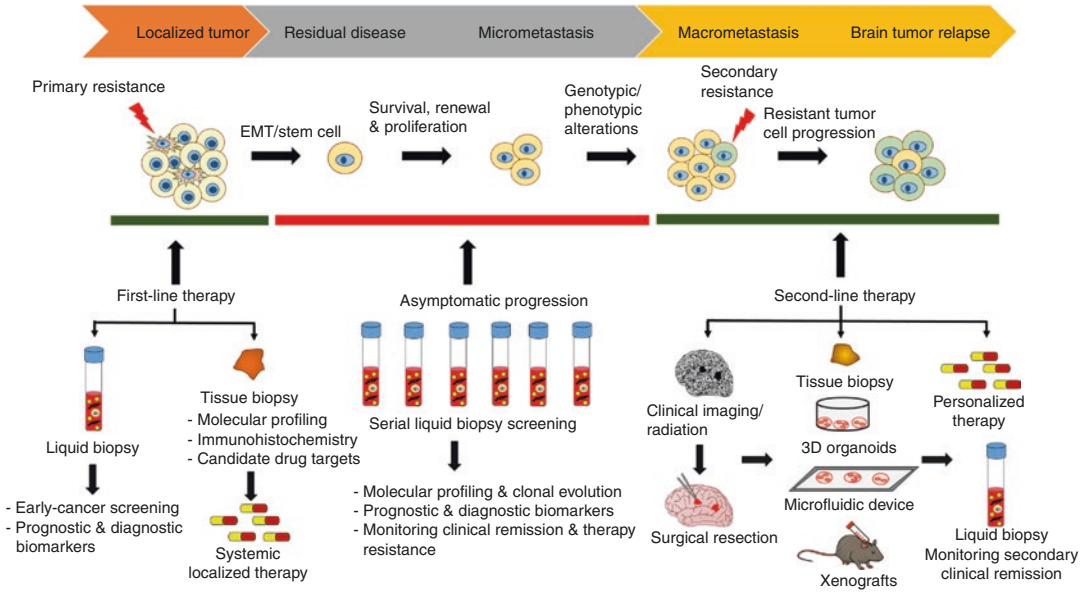


Fig. 2.3 Molecular landscape of brain metastasis and its therapeutic implications

between non-CSC and CSC phenotypes in response to microenvironmental cues such as hypoxia [44–46]. This plasticity has profound implications for context-dependent identification and assessment of CSC burden and development of CSC-targeted therapies. Regardless of how metastatic cells acquire stem-like properties, they must all navigate and survive a journey through the blood stream. The recent refinement of methods to identify and characterize circulating tumor cells (CTC) has shed further light on the phenotypes and mechanisms employed by CSCs to colonize the brain (Fig. 2.3).

The "Liquid" Phase of Brain Metastasis

Circulating Tumor Cells (CTCs) and Dormant Cancer Cells (DCCs)

As putative metastatic "seeds," circulating tumor cells (CTCs) drive metastasis and disease recurrence [47, 48]. CTCs may colonize distant sites and rapidly progress to form macro-metastases or remain as dormant cancer cells (DCCs) in permissive pre-metastatic niches that after months or years are triggered to form macro-metastases. CTCs may also derive not only from the primary tumor site but also from distant macro-metastases—a mechanism termed "self-seeding" (see Fig. 2.1).

CTCs are a minority heterogeneous cancer cell population that can be isolated from patient blood by various techniques such as flow cytometry, magnetic beads, and microfluidic devices and identified based on immune phenotyping, cell size, and deformability [48–51]. CellSearch™, which captures EpCAM-positive epithelial-derived CTCs, is currently the only Food and Drug Administration (FDA)-approved platform for CTC analysis, although it excludes potential EpCAM-negative CTCs that may be a significant contributor to BM formation [39]. Disseminated CTCs intravasate and migrate into the blood circulation and survive as single cell or cluster/emboli. CTC clusters have survival advantages as they more effectively resist anoikis, bloodstream shear forces, environmental or oxidative stresses, and immune surveillance [52–54]. Higher CTC counts in peripheral blood correlate with disease burden and worse patient survival in various malignancies such as melanoma and breast, lung, prostate, and pancreatic cancers [54–56].

Although CTCs are not routinely identified in the majority of BM patients, BM formation presumably requires the existence of CTCs at some point prior to their clinical manifestation. More than two CTCs were detected in only 5.9% of patients with oligo-metastatic NSCLC to the brain; the frequency of more than three CTCs in BM patients with systemic metastases and other tumor types ranges widely from 0% to 25% [55]. These data underscore several important considerations: (i) CTC dissemination and thus detection may be intermittent with periods of dormant residence in other sites, and (ii) in addition to unidirectional production of CTCs from the primary cancer, CTCs may also arise from metastatic deposits including BMs, the so-called "self-seeding" mechanism. The identification of CTCs in cerebrospinal fluid (CSF) from BM patients with concurrent leptomeningeal disease (LMD) may represent a form of "self-seeding" [57].

CTCs are heterogeneous and specific subpopulations may have unique tropism for colonizing the brain [9, 48, 58, 59]. Using an expanded CTC isolation protocol, Boral et al. demonstrated that inclusion of EpCAM-negative CTCs with CSC markers markedly increased CTC yields in breast carcinoma patients [60]. Stratifying these metastatic patients based on the presence of BMs, they identified a 121-gene signature associated with BMs [60]. Other studies have shown that a specific subpopulation of EpCAM-negative CTCs from breast cancer patients has a unique propensity for forming BMs in experimental models [61]. These studies indicate that identifiable subpopulations of CTCs may have specific capacity to generate BMs that could theoretically be targeted systemically to prevent BMs. Clinical and biological relevance of CTCs is an area of ongoing investigation, but one that appears to have great promise to inform prognosis, treatment responses, metastatic risk, and even new therapeutic approaches. The utility of liquid biopsies for BMs extends beyond the detection of CTCs, allowing profiling of exosomes and circulating-tumor DNA (ctDNA) (see Fig. 2.3).

Exosomes

Exosomes are small membrane bound extracellular vesicles secreted by cancer cells that contain DNA, RNA, proteins, and lipids [62]. Exosomes function locally within primary and metastatic tumors as well as remotely through vascular dissemination and cellular uptake at metastatic sites [63]. They are increasingly analyzed in liquid biopsies since they inform tumor growth, evolution, and pathogenesis and in BMs are responsible for inducing a plethora of biological processes, such as EMT, angiogenesis, metastasis, therapy resistance, and epigenetic/stem-cell regulation (Figs. 2.1 and 2.3) [64]. In breast cancer, expressions of mir-122 and mir-210 were associated with brain metastasis [65, 66]. In melanoma, CD46 receptors are responsible for uptake of tumor-associated exosomes in BBB endothelial cells [67].

An important function of exosomes in BM biology is their capacity to generate organotropic pre-metastatic niches conducive to DCC growth or CTC homing, colonization, and proliferation. In experimental studies, the brain preferentially takes up exosomes from neurotropic metastatic cancer cell lines through specific direct interactions with CD31+ BBB endothelial cells [68]. Exosomal organotropism also appears to be related to specific integrin profiles with ITGB3 highly upregulated in brain tropic exosomes [69]. Remarkably, "educating" mouse hosts with exosomes redirects the organotropism of cancer cell lines to reflect patterns of exosomal uptake. These data suggest that targeting brain-specific exosomes may be a useful future strategy to mitigate BM formation.

In addition to roles in organotropism, exosomes are implicated in promoting immunosuppressive "havens" for DCCs, angiogenesis that triggers progression and growth of micrometastases, and disruption of the BBB [62, 63, 70]. Of note, experimental evidence implicates exosomal microRNAs (miRNAs) generated from astrocytes and BM cells during tumor growth through reversible epigenetic downregulation of PTEN and conversion of resident microglia from the

M1 to M2 immunosuppressive phenotype [71, 72]. These observations demonstrate the important roles of both primary tumor-derived and local neural cell-derived exosomes in orchestrating the complex processes of BM tropism and growth. Exosome-based targeted therapies therefore may be a useful strategy to mitigate BM formation and progression [63].

ctDNA

Circulating-tumor DNA (ctDNA) is released into biological fluids by apoptotic or necrotic cancer cells. ctDNA is detected in most systemic cancers with increased levels corresponding with metastasis [73]. In breast and melanoma carcinomas, two cancers with high propensity for BMs, ctDNA is detectable in over 80% of cases [73]. Levels of ctDNA are associated with tumor burden and patient survival [64, 74, 75]. To our knowledge no data exist demonstrating an association between ctDNA and BM incidence or a pathogenic role in BM genesis. However, analysis of ctDNA from cerebrospinal fluid (CSF) is emerging as a useful marker for patients with parenchymal BMs and leptomeningeal disease that may be more sensitive and specific than plasma-derived ctDNA [76, 77]. For instance, in patients with central nervous system (CNS)-restricted metastatic disease, CSF ctDNA was detected in 58% versus 0% from plasma and importantly, changes in CSF ctDNA detection corresponded with clinical treatment responses [77]. In another study, genomic mutations were identified after sequencing of CSF DNA in 63% (20 of 32) of patients with parenchymal CNS metastases, while detection of ctDNA in CSF has been reported for 75–100% of patients with LMD [76, 78]. These studies indicate the potential for ctDNA to serve as a biomarker for tracking tumor progression and treatment response [75, 79, 80]. Multicenter large cohort studies are required to evaluate the evolutionary changes of ctDNA over the course of metastatic cancer treatment and their correlation with disease status and patient survival.

The Final Metastatic Phase: Brain Colonization, Growth, and the Role of the Brain Microenvironment

In the final stage of BM formation, CTCs and/or DCCs, which have colonized permissive pre-metastatic niches, engage the brain microenvironment (BME) and through reciprocal interactions undergo macro-metastatic growth. The complex interactions between BM cells and resident neural cells (astrocytes, neurons, and microglia), infiltrative immune cells, brain microvasculature, extracellular matrix proteins, metabolic changes, cytokine signaling, and even synaptic inputs result in reprogramming of BM cells and BME to facilitate BM survival and growth. An additional important element of the BME is the blood-brain barrier (BBB) and subsequent formation of a blood-tumor barrier (BTB), critical to the process of CTC extravasation, immune cell infiltration, and systemic delivery of therapeutic agents. With selected examples we will address clinically relevant highlights of these interactions.

The Blood-Brain Barrier (BBB) and Blood-Tumor Barrier (BTB)

The blood-brain barrier (BBB) is a highly specialized semipermeable structure consisting of endothelial cells, pericytes, and astrocytes, which form tight junctions that restrict access to the brain from the circulation [1]. The neurovascular unit of the BBB maintains homeostatic environmental conditions for normal neuronal function and provides a barrier to CTC extravasation that must be overcome for BM formation (reviewed in Ref. [81]). Three molecules, cyclooxygenase COX2 (also known as PTGS2), the epidermal growth factor receptor (EGFR) ligand HBEGF, and the α2,6-sialyltransferase ST6GALNAC5 have been identified as mediators of cancer cell extravasation across the BBB [82]. ST6GALNAC5 promotes adhesion of tumor cells to brain endothelial cells, whereas COX2 and HBEGF promote cell migration across the BBB [82]. In addition, matrix metalloproteinases

(MMPs) and vascular endothelial growth factor (VEGF) facilitate extravasation, seeding, and micrometastasis formation through ECM destruction and creation of a vascular niche [1, 83–87].

In the BM peri-tumoral region, the BBB is modified to generate a so-called blood-tumor barrier (BTB) characterized by increased local permeability. Changes in BBB characteristic of the BTB are mediated by alterations in endothelial cell tight junctions and pericyte function, and are associated with neuroinflammation and changes in ECM components [1]. The molecular mechanisms underlying permeability changes in the BTB include upregulation of VEGF and downregulation of zona occludens (ZO) and vascular endothelial cell adhesion molecule (VE-CAM) in endothelial cells, altered expression of desmin and CD13 in pericytes, and elaboration of other molecules including membrane transporters, tumor necrosis factor (TNF) receptors, claudin-5, and angiopoietin-2 [1, 88–92]. Of clinical relevance, these changes in permeability result in heterogeneous uptake that may enhance uptake of drugs and antibodies normally restricted by the intact BBB [1, 54, 93–96].

Immune BM Microenvironment

BMs generate an inflammatory and immunosuppressive microenvironment that promotes tumor growth and treatment resistance [97]. The immune BM microenvironment involves complex interactions between tumor and resident neural cells and infiltrating cells of lymphoid (cytotoxic-CD4+, helper-CD4+, T-regulatory [T-reg] cells, and natural killer) and myeloid (dendritic/antigen presenting cells, macrophages, and myeloid-derived suppressor cells [MDSCs]) lineage [98–101]. Intense interest in tumor-infiltrating lymphocytes (TILs) has been fostered by the success of immune checkpoint inhibitors (ICIs) in treatment of systemic melanoma, and, more recently, other cancers with a propensity for BMs including NSCLC and breast cancer [102–105]. In fact, recent trials have demonstrated variable activity of ICIs against BMs [98, 106]. CTLA4 and PD-L1/PD-1 inhibitors block tumor-mediated immunosuppressive mechanisms that typically decrease cytotoxic T-lymphocyte (CTL) function [105].

Harter et al. profiled the quantity and topography of all TILs (CD3+) and specific subpopulations of T-reg cells (FoxP3+) and CTLs (CD8+) and PD-1/PD-L1 expression in BMs in both mixed tumor and breast carcinoma-restricted cohorts [105]. TILs and their subpopulations were detected in all BM types but with different frequencies (highest in renal cell carcinoma) and patterns of distribution (diffuse in melanoma, stromal in carcinomas). In contrast to other studies, where expansion of cytotoxic T-lymphocytes and infiltration of T-cells correlate with patient survival, none of the TIL or PD-L1/PD-1 metrics were associated with patient survival [107–109]. By contrast, the presence of a peri-tumoral and to a lesser extent stromal mononuclear infiltrate and lower PD-1/PD-L1 expression in lung adenocarcinoma BM patients predicted better survival after resection [110]. In another study of NSCLC patients, disparate responses of the primary and BM lesions to PD-1 blockade mirrored a decrease in BM-specific PD-1 expression in paired primary and BM samples [102]. In paired breast cancer primary and BM samples, TILs are decreased in BMs as was the proportion of "adaptive" immune phenotypes (TIL+/PD-L1+) expected to be responsive to ICIs [103, 104, 111]. In melanoma BMs, increased immune cell infiltration corresponded with increased PD-L1+, survival, and enrichment of oxidative phosphorylation compared with non-CNS metastases [100]. Overall the melanoma BMs had reduced immune cell infiltrates, and gene expression analysis revealed an immunosuppressive phenotype compared with non-CNS metastases. Of note, the metabolic signature positively correlated with patient survival, and preclinical models demonstrated that inhibition of oxidative phosphorylation was a promising therapeutic target of MAPK-resistant melanoma BMs [100].

In addition to TILs, other immune cells including myeloid cells are implicated in BM growth [72]. The association between reduction of peripheral myeloid-derived suppressor cells (MDSCs) and BM incidence in lung cancer

patients treated with combination systemic bevacizumab and TKIs suggests that MDSCs may play a role in the immunosuppressive BM microenvironment. Experimental studies of mouse mammary carcinoma BMs also demonstrated that MDSCs generate a "pre-metastatic niche" for BM formation [90]. T-regulatory (T-reg) cells suppress immune reactions by secretion of factors such as TGF-β and IL-10 and higher T-reg cell burden in tumors and peripheral blood is associated with poor clinical outcomes [112, 113]. In lung adenocarcinoma, FOXP3+ T-reg cells are detected in BMs albeit at lower numbers than in primary tumors [102]. Finally, resident microglia and systemically derived macrophages are implicated in early stages of BM formation and contribute to the immunosuppressive microenvironment [114]. In summary, the complex and immune BM microenvironment generates an immunosuppressive state and plays critical roles in BM formation from the pre-metastatic niches to macro-metastatic growth. Further elucidation of the diversity of immunosuppressive mechanisms in BMs is needed to develop more effective immunotherapy and strategies to reprogram the immune microenvironment of BMs to facilitate responses to immunotherapy.

As noted above, BMs are "cold tumors" and thereby less responsive to immunotherapy [1]. Therefore, techniques to activate the immune microenvironment in BMs have great clinical significance. For example, the abscopal effect is a presumed immune-mediated mechanism whereby local radiation to a single lesion resulting in release of tumor antigens and T-cell expansion can activate a dramatic generalized antitumor response distant from the site of radiation [115–117]. An experimental melanoma BM model demonstrated an abscopal effect with combined irradiation and PD-L1 blockade similar to the reported clinical potentiation of the abscopal effect with concurrent ICI therapy [118–121]. As several reports suggest, it may be possible to harness the abscopal effect to treat BMs through targeting a systemic lesion or conversely activate a systemic response through local irradiation of BMs [122, 123]. While the occurrence of an abscopal response is relatively rare, further investigations into its precise mechanisms are expected to provide insight into more effective strategies to activate the immune system to improve response for systemic and CNS-based cancers.

BM Cross-Talk with the Brain Metastasis Microenvironment

In addition to interactions with the vascular BBB/BTB niche and infiltrating immune cells described above, cross-talk with resident neural cells also plays an important role in BM biology (reviewed in Refs. [97, 99, 124]). The brain is generally a hostile microenvironment for extravasated cancer cells, the majority of which die; however, those that survive as dormant or actively propagating cells appear uniquely able to co-opt or adapt to the conditions in the brain microenvironment [81]. For instance, cancer cells that grow in the brain activate unique brain-enriched gene expression profiles, and undergo metabolic reprogramming so that they can effectively utilize non-glucose energy sources, like the brain (reviewed in Refs. [125, 126]). Expression of Serpins on BM cells counteracts with the cell-death and anti-migratory effects of brain-derived plasmin necessary for BM cell survival and engagement with brain microvascular cells for local invasion [127]. While neural cells can impede BM growth, specific interactions with neural cells have also been shown to promote BM survival and growth. For instance, astrocyte interactions promote BMs through gap junction-mediated transfer of cGAMP and astrocyte-derived exosomal miRNA-mediated suppression of PTEN function [72, 128]. Similarly, the activated state of microglia can either inhibit or promote BM growth [81]. BM cell secreted exosomal miRNAs can reprogram microglia to promote BM growth through immunosuppressive mechanisms [71]. Finally, based on the increasingly recognized impact of peripheral innervation in cancer metastasis and CNS neural activity to promote glioma proliferation, future studies should be directed to understanding how electrical activity may influence BM physiology [129–131].

Molecular Heterogeneity and Selection for Brain Metastasis

Given the complexity of mechanisms and environmental selection pressures summarized above, it is not surprising that primary cancers and their brain metastases exhibit extensive molecular heterogeneity. Since the seminal publication by Gerlinger et al. in metastatic renal cell carcinoma, intra-tumor molecular heterogeneity and branched evolution have been recognized to contribute to the genesis, progression, and treatment resistance of many cancers [5, 132–134]. Genomic instability and selective evolution are principle mechanisms driving heterogeneity at the genetic, epigenetic, and transcriptional levels [132, 133, 135]. Multiregional tumor biopsy sampling, research autopsies, spatial and temporal liquid biopsies, and single-cell sequencing are emerging approaches that will help decode the complex architecture of tumor, specifically as it relates to brain metastasis [132, 133, 135–137].

For BMs, studies of paired primary and metastatic lesions reveal several clinically relevant insights including (i) a high proportion of BMs possess mutations distinct from those of the primary site, (ii) BMs from individual patients share mutations distinct from those detected in the primary cancer, and (iii) BMs exhibit activation of oncogenic signaling pathways (e.g., PI3K/Akt/mTOR) distinct from those present in the primary cancer [5, 138–140]. These observations suggest that BMs may arise from unique cell subpopulations within the original cancer and/or that selection pressure for specific mutations and phenotypes drive successful BM formation and growth.

Genomic studies indicate that BMs retain ancestral mutations of their primary cancer but acquire additional unique mutations through branched evolution [136, 138, 139, 141]. In the largest study to date of paired primary and metastatic cancer samples, Brastianos et al. determined that BMs share mutations with the primary cancer but develop unique or "private mutations" in all cases, of which 53% represent potential actionable targets unique to their CNS disease [138]. As further shown by EGFR mutations shared by paired primary and BM specimens, these observations suggest that clonal selection during BM formation may be required for effective metastatic outgrowth and therapeutic resistance [133, 138, 142].

Activation of specific oncogenic signaling pathways occurs in BMs in concert with the evolution of genomic changes. In primary melanoma, lung and breast cancer patients, more than 50% of brain metastatic tissue contain clinically relevant oncogenic alterations in PTEN, PIK3CA, EGFR, and HER2 genes and cancer hot spot regions that activate PI3K–AKT–MTOR and EGFR/HER2 pathways involved in tumor cell growth and proliferation [5, 132]. Primary tumors treated with systemic therapy such as PI3K/AKT/mTOR, CDK, and HER2/EGFR inhibitors are more inclined to develop brain metastasis [138]. In squamous cell lung cancers (SQCLC), PI3K-aberrant tumors were associated with high metastatic tumor burden and increased incidence of brain metastasis [143]. However, colorectal cancer (CRC) shows less genetic heterogeneity (APC, KRAS, FBXW7, PIK3CA, BRAF, SMAD4, and ACVR2A mutations) with greater genetic concordance between matched primary and brain metastatic tumors [144].

Overall, brain metastases exhibit a branched evolution pattern reflecting primary tumor mutation profiles and acquisition of additional unique molecular profiles with respect to other non-CNS metastases. Additionally, molecular profiles of intracranial sites within individual patients suggest a high degree of homogeneity. This genomic concordance may provide guidance for systematic personalized therapy and facilitate our understanding of mechanisms involved in brain metastasis.

Spinal Metastasis

Metastatic spinal cord compression is considered an oncological emergency that may require immediate treatment either through surgical decompression, emergency radiotherapy, or a combination of the two. This occurs in 3–5% of cancer patients, with breast, lung, and prostate

being the most frequent source [145]. The majority of metastases affects the bone first and cause compression through direct mass effect or pathological fracture. Even more rare are intradural extramedullary and intramedullary metastases accounting for less than 6% and 1–2% of spinal metastasis, respectively [146–148]. The incidence of intramedullary spinal metastasis may be increasing perhaps with extended overall survival. Additionally, metastasis to the spine is generally a poor prognostic sign of overall patient survival with median survival of only 8 months in patients treated for intramedullary renal cell metastasis [149].

By virtue of their different recipient tissue microenvironments, it is not surprising that the cellular and molecular mechanisms that promote bone metastasis, and thereby osseous-based spinal cord compression, differ from those driving BMs (reviewed in Refs. [150–153]). Given the rarity of both extra- and intra-axial spine metastasis, studies of their specific mechanisms are scarce. Presumably, extramedullary spinal metastases result from local leptomeningeal growth of CSF-disseminated cells. Like BMs, molecular analysis of leptomeningeal cancer cells reveals mutations shared with and unique to the primary cancer site that can be monitored through analysis of ctDNA [154, 155]. By contrast, intramedullary spinal metastases are more likely to originate through mechanisms similar to those that regulate BMs. Intramedullary spinal cord metastasis (ISCM) is exceedingly rare with incidence of ~2% in systemic cancers [147, 156, 157]. It is most commonly seen with lung and breast cancers but has also been reported for colon cancer, Merkel cell carcinoma, renal cell carcinoma, gastric cancer, ovarian cancer, and thyroid cancer [148, 149, 158–162]. As with BMs, the increasing success of systemic therapies may be contributing to the increased incidence of ISCM [148]. Lung cancers frequently metastasize to the CNS, but intramedullary spine metastasis is detected in only 1.65% of 1215 autopsy cases and 1.8% of NSCLC patients; and these were highly associated with concomitant BMs suggesting common mechanisms for their colonization and growth [147, 157]. Anaplastic lymphoma kinase (ALK) gene mutations are associated with aggressive features in NSCLC including early CNS metastasis and higher rates of intramedullary spinal cord metastasis [146, 157, 163]. While rare, the consequences of spinal extra- and intramedullary metastases are devastating and warrant further study of their basic biology to develop more effective therapies. See the "Spinal Metastases" section of this book for in-depth coverage of this topic.

Conclusion

Brain metastasis is a devastating disease with increasing incidence. The increased rate is due to a lack of prognostic and diagnostic biomarkers at early disease stages. Systemic, longitudinal blood-based liquid biopsy (CTCs, cell-free DNA, exosomes, secretory proteins, etc.), alongside molecular imaging approaches, may provide novel biomarkers for designing early diagnostic tools (see Fig. 2.3). In brain metastatic patients, surgical resection is a key part of clinical management and provides an immediate opportunity for tumor molecular characterization for determining effective therapies. These studies can also assist in identifying therapeutic targets to eliminate residual disease or recurrence in brain metastatic patients with other primary cancers. Poor prognosis of brain metastatic patients is also related to drug resistance and tumor heterogeneity between primary and brain metastasis tumors. In the era of precision medicine and individualized therapy, deciphering the tumor heterogeneity based on spatiotemporal selection is clinically imperative. Multidisciplinary approaches are necessary to fill in the gaps in knowledge regarding the molecular landscape of brain metastasis. Preclinical models such as microfluidic device, organotypic 3D culture, and patient-derived xenografts may clarify both the interplay between metastatic cell and brain tumor microenvironment and the brain metastatic cascade (Fig. 2.3). These emerging tools overcome traditional cell-based technologies as they have

the potential to monitor real-time cancer progression and personalize therapy for patients. Further, the advancement in future multimodal studies will open new paradigms to understand the realm of brain metastasis and improve patient outcomes.

References

1. Achrol AS, Rennert RC, Anders C, Soffietti R, Ahluwalia MS, Nayak L, et al. Brain metastases. Nat Rev Dis Primers. 2019;5(1):5.
2. Martin AM, Cagney DN, Catalano PJ, Warren LE, Bellon JR, Punglia RS, et al. Brain metastases in newly diagnosed breast cancer: a population-based study. JAMA Oncol. 2017;3(8):1069–77.
3. Toyokawa G, Seto T, Takenoyama M, Ichinose Y. Insights into brain metastasis in patients with ALK+ lung cancer: is the brain truly a sanctuary? Cancer Metastasis Rev. 2015;34(4):797–805.
4. Hall WA, Djalilian HR, Nussbaum ES, Cho KH. Long-term survival with metastatic cancer to the brain. Med Oncol (Northwood, London, England). 2000;17(4):279–86.
5. Dagogo-Jack I, Carter SL, Brastianos PK. Brain metastasis: clinical implications of branched evolution. Trends Cancer. 2016;2(7):332–7.
6. Andrews DW, Scott CB, Sperduto PW, Flanders AE, Gaspar LE, Schell MC, et al. Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: phase III results of the RTOG 9508 randomised trial. Lancet (London, England). 2004;363(9422):1665–72.
7. Ramakrishna R, Rostomily R. Seed, soil, and beyond: the basic biology of brain metastasis. Surg Neurol Int. 2013;4(Suppl 4):S256–64.
8. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147(2):275–92.
9. Pantel K, Brakenhoff RH. Dissecting the metastatic cascade. Nat Rev Cancer. 2004;4(6):448–56.
10. Metastasis JE. Neoplastic Diseases: A Treatise on Tumors. 2nd ed. W.B. Saunders Company; 1922; 76–88.
11. Prasetyanti PR, Medema JP. Intra-tumor heterogeneity from a cancer stem cell perspective. Mol Cancer. 2017;16(1):41.
12. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. Cell. 2017;168(4):613–28.
13. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer. 2002;2(8):563–72.
14. Amelot A, Terrier LM, Mazeron JJ, Valery CA, Cornu P, Carpentier A, et al. Timeline metastatic progression: in the wake of the << seed and soil >> theory. Med Oncol (Northwood, London, England). 2017;34(11):185.
15. Fidler IJ, Yano S, Zhang RD, Fujimaki T, Bucana CD. The seed and soil hypothesis: vascularisation and brain metastases. Lancet Oncol. 2002;3(1):53–7.
16. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989;8(2):98–101.
17. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel). 1995;154(1): 8–20.
18. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2(6):442–54.
19. Jechlinger M, Grunert S, Beug H. Mechanisms in epithelial plasticity and metastasis: insights from 3D cultures and expression profiling. J Mammary Gland Biol Neoplasia. 2002;7(4):415–32.
20. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003;113(6):685–700.
21. Kokudo T, Suzuki Y, Yoshimatsu Y, Yamazaki T, Watabe T, Miyazono K. Snail is required for TGFbeta-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells. J Cell Sci. 2008;121(Pt 20):3317–24.
22. Kahn SA, Wang X, Nitta RT, Gholamin S, Theruvath J, Hutter G, et al. Notch1 regulates the initiation of metastasis and self-renewal of Group 3 medulloblastoma. Nat Commun. 2018;9(1):4121.
23. Rahmathulla G, Toms SA, Weil RJ. The molecular biology of brain metastasis. J Oncol. 2012;2012:723541.
24. Talbot LJ, Bhattacharya SD, Kuo PC. Epithelial-mesenchymal transition, the tumor microenvironment, and metastatic behavior of epithelial malignancies. Int J Biochem Mol Biol. 2012;3(2):117–36.
25. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol. 2019;20(2):69–84.
26. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004;117(7):927–39.
27. Jiang WG. E-cadherin and its associated protein catenins, cancer invasion and metastasis. Br J Surg. 1996;83(4):437–46.
28. Kafka A, Tomas D, Beros V, Pecina HI, Zeljko M, Pecina-Slaus N. Brain metastases from lung cancer show increased expression of DVL1, DVL3 and beta-catenin and down-regulation of E-cadherin. Int J Mol Sci. 2014;15(6):10635–51.
29. Barsky SH, Siegal GP, Jannotta F, Liotta LA. Loss of basement membrane components by invasive tumors but not by their benign counterparts. Lab Invest. 1983;49(2):140–7.

30. Langley RR, Fidler IJ. The biology of brain metastasis. Clin Chem. 2013;59(1):180–9.
31. Hagedorn HG, Bachmeier BE, Nerlich AG. Synthesis and degradation of basement membranes and extracellular matrix and their regulation by TGF-beta in invasive carcinomas (review). Int J Oncol. 2001;18(4):669–81.
32. Chaffer CL, Thompson EW, Williams ED. Mesenchymal to epithelial transition in development and disease. Cells Tissues Organs. 2007;185(1–3):7–19.
33. Hugo H, Ackland ML, Blick T, Lawrence MG, Clements JA, Williams ED, et al. Epithelial--mesenchymal and mesenchymal--epithelial transitions in carcinoma progression. J Cell Physiol. 2007;213(2):374–83.
34. Yao D, Dai C, Peng S. Mechanism of the mesenchymal-epithelial transition and its relationship with metastatic tumor formation. Mol Cancer Res. 2011;9(12):1608–20.
35. Franchino F, Ruda R, Soffietti R. Mechanisms and therapy for cancer metastasis to the brain. Front Oncol. 2018;8:161.
36. Nolte SM, Venugopal C, McFarlane N, Morozova O, Hallett RM, O'Farrell E, et al. A cancer stem cell model for studying brain metastases from primary lung cancer. J Natl Cancer Inst. 2013;105(8):551–62.
37. Tan BT, Park CY, Ailles LE, Weissman IL. The cancer stem cell hypothesis: a work in progress. Lab Invest. 2006;86(12):1203–7.
38. Strizzi L, Hardy KM, Seftor EA, Costa FF, Kirschmann DA, Seftor RE, et al. Development and cancer: at the crossroads of Nodal and Notch signaling. Cancer Res. 2009;69(18):7131–4.
39. Zhang L, Ridgway LD, Wetzel MD, Ngo J, Yin W, Kumar D, et al. The identification and characterization of breast cancer CTCs competent for brain metastasis. Sci Transl Med. 2013;5(180):180ra48.
40. Wurth R, Bajetto A, Harrison JK, Barbieri F, Florio T. CXCL12 modulation of CXCR4 and CXCR7 activity in human glioblastoma stem-like cells and regulation of the tumor microenvironment. Front Cell Neurosci. 2014;8:144.
41. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature. 2012;488(7412):522–6.
42. Beier D, Schulz JB, Beier CP. Chemoresistance of glioblastoma cancer stem cells--much more complex than expected. Mol Cancer. 2011;10:128.
43. Chu JE, Allan AL. The role of cancer stem cells in the organ tropism of breast cancer metastasis: a mechanistic balance between the "seed" and the "soil"? Int J Breast Cancer. 2012;2012:209748.
44. Lee G, Hall RR 3rd, Ahmed AU. Cancer stem cells: cellular plasticity, niche, and its clinical relevance. J Stem Cell Res Ther. 2016;6(10):pii: 363.
45. Dahan P, Martinez Gala J, Delmas C, Monferran S, Malric L, Zentkowski D, et al. Ionizing radiations sustain glioblastoma cell dedifferentiation to a stem-like phenotype through survivin: possible involvement in radioresistance. Cell Death Dis. 2014;5:e1543.
46. Auffinger B, Tobias AL, Han Y, Lee G, Guo D, Dey M, et al. Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy. Cell Death Differ. 2014;21(7):1119–31.
47. Dasgupta A, Lim AR, Ghajar CM. Circulating and disseminated tumor cells: harbingers or initiators of metastasis? Mol Oncol. 2017;11(1):40–61.
48. Joosse SA, Gorges TM, Pantel K. Biology, detection, and clinical implications of circulating tumor cells. EMBO Mol Med. 2015;7(1):1–11.
49. Alix-Panabieres C, Pantel K. Challenges in circulating tumour cell research. Nat Rev Cancer. 2014;14(9):623–31.
50. Riethdorf S, Pantel K. Disseminated tumor cells in bone marrow and circulating tumor cells in blood of breast cancer patients: current state of detection and characterization. Pathobiology. 2008;75(2):140–8.
51. Yu M, Stott S, Toner M, Maheswaran S, Haber DA. Circulating tumor cells: approaches to isolation and characterization. J Cell Biol. 2011;192(3):373–82.
52. Giuliano M, Shaikh A, Lo HC, Arpino G, De Placido S, Zhang XH, et al. Perspective on circulating tumor cell clusters: why it takes a village to metastasize. Cancer Res. 2018;78:845.
53. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014;158(5):1110–22.
54. Adkins CE, Mohammad AS, Terrell-Hall TB, Dolan EL, Shah N, Sechrest E, et al. Characterization of passive permeability at the blood-tumor barrier in five preclinical models of brain metastases of breast cancer. Clin Exp Metastasis. 2016;33(4):373–83.
55. Hanssen A, Riebensahm C, Mohme M, Joosse SA, Velthaus JL, Berger LA, et al. Frequency of circulating tumor cells (CTC) in patients with brain metastases: implications as a risk assessment marker in oligo-metastatic disease. Cancers (Basel). 2018;10(12):527.
56. Klinac D, Gray ES, Freeman JB, Reid A, Bowyer S, Millward M, et al. Monitoring changes in circulating tumour cells as a prognostic indicator of overall survival and treatment response in patients with metastatic melanoma. BMC Cancer. 2014;14:423.
57. Lin X, Fleisher M, Rosenblum M, Lin O, Boire A, Briggs S, et al. Cerebrospinal fluid circulating tumor cells: a novel tool to diagnose leptomeningeal metastases from epithelial tumors. Neuro Oncol. 2017;19(9):1248–54.
58. Uhr JW, Pantel K. Controversies in clinical cancer dormancy. Proc Natl Acad Sci U S A. 2011;108(30):12396–400.

59. Riethdorf S, Wikman H, Pantel K. Review: biological relevance of disseminated tumor cells in cancer patients. Int J Cancer. 2008;123(9):1991–2006.
60. Boral D, Vishnoi M, Liu HN, Yin W, Sprouse ML, Scamardo A, et al. Molecular characterization of breast cancer CTCs associated with brain metastasis. Nat Commun. 2017;8(1):196.
61. Wang H, Zhang C, Zhang J, Kong L, Zhu H, Yu J. The prognosis analysis of different metastasis pattern in patients with different breast cancer subtypes: a SEER based study. Oncotarget. 2017;8(16):26368–79.
62. Liu Y, Cao X. Organotropic metastasis: role of tumor exosomes. Cell Res. 2016;26(2):149–50.
63. Weidle HU, Birzele F, Kollmorgen G, RÜGer R. The multiple roles of exosomes in metastasis. Cancer Genomics Proteomics. 2017;14(1):1–16.
64. Shankar GM, Balaj L, Stott SL, Nahed B, Carter BS. Liquid biopsy for brain tumors. Expert Rev Mol Diagn. 2017;17(10):943–7.
65. Fong MY, Zhou W, Liu L, Alontaga AY, Chandra M, Ashby J, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. Nat Cell Biol. 2015;17(2):183–94.
66. Camacho L, Guerrero P, Marchetti D. MicroRNA and protein profiling of brain metastasis competent cell-derived exosomes. PLoS One. 2013;8(9):e73790.
67. Kuroda H, Tachikawa M, Yagi Y, Umetsu M, Nurdin A, Miyauchi E, et al. Cluster of differentiation 46 is the major receptor in human blood-brain barrier endothelial cells for uptake of exosomes derived from brain-metastatic melanoma cells (SK-Mel-28). Mol Pharm. 2019;16:292–304.
68. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527(7578):329–35.
69. Hoshino A, Costa-Silva B, Shen T-L, Rodrigues G, Hashimoto A, Mark MT, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527(7578):329–35.
70. Tominaga N, Kosaka N, Ono M, Katsuda T, Yoshioka Y, Tamura K, et al. Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood-brain barrier. Nat Commun. 2015;6:6716.
71. Xing F, Liu Y, Wu SY, Wu K, Sharma S, Mo YY, et al. Loss of XIST in breast cancer activates MSN-c-Met and reprograms microglia via exosomal miRNA to promote brain metastasis. Cancer Res. 2018;78(15):4316–30.
72. Zhang L, Zhang S, Yao J, Lowery FJ, Zhang Q, Huang WC, et al. Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. Nature. 2015;527(7576):100–4.
73. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014;6(224):224ra24.
74. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet. 2019;20(2):71–88.
75. Siravegna G, Geuna E, Mussolin B, Crisafulli G, Bartolini A, Galizia D, et al. Genotyping tumour DNA in cerebrospinal fluid and plasma of a HER2-positive breast cancer patient with brain metastases. ESMO Open. 2017;2(4):e000253.
76. Boire A, Brandsma D, Brastianos PK, Le Rhun E, Ahluwalia M, Junk L, et al. Liquid biopsy in central nervous system metastases: a RANO review and proposals for clinical applications. Neuro Oncol. 2019;21:571.
77. De Mattos-Arruda L, Mayor R, Ng CK, Weigelt B, Martinez-Ricarte F, Torrejon D, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. Nat Commun. 2015;6:8839.
78. Pentsova EI, Shah RH, Tang J, Boire A, You D, Briggs S, et al. Evaluating cancer of the central nervous system through next-generation sequencing of cerebrospinal fluid. J Clin Oncol. 2016;34(20):2404–15.
79. Huang WT, Lu NM, Hsu WY, Chang SE, Atkins A, Mei R, et al. CSF-ctDNA SMSEQ analysis to tailor the treatment of a patient with brain metastases: a case report. Case Rep Oncol. 2018;11(1):68–74.
80. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol. 2017;14(9):531–48.
81. Wilhelm I, Fazakas C, Molnar K, Vegh AG, Hasko J, Krizbai IA. Foe or friend? Janus-faces of the neurovascular unit in the formation of brain metastases. J Cereb Blood Flow Metab. 2018;38(4):563–87.
82. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. Nature. 2009;459(7249):1005–9.
83. Fan J, Cai B, Zeng M, Hao Y, Giancotti FG, Fu BM. Integrin beta4 signaling promotes mammary tumor cell adhesion to brain microvascular endothelium by inducing ErbB2-mediated secretion of VEGF. Ann Biomed Eng. 2011;39(8):2223–41.
84. Kusters B, Leenders WP, Wesseling P, Smits D, Verrijp K, Ruiter DJ, et al. Vascular endothelial growth factor-A(165) induces progression of melanoma brain metastases without induction of sprouting angiogenesis. Cancer Res. 2002;62(2):341–5.
85. Izraely S, Sagi-Assif O, Klein A, Meshel T, Tsarfaty G, Pasmanik-Chor M, et al. The metastatic microenvironment: brain-residing melanoma metastasis and dormant micrometastasis. Int J Cancer. 2012;131(5):1071–82.
86. Gorantla V, Kirkwood JM, Tawbi HA. Melanoma brain metastases: an unmet challenge in the era of active therapy. Curr Oncol Rep. 2013;15(5):483–91.
87. Eichler AF, Chung E, Kodack DP, Loeffler JS, Fukumura D, Jain RK. The biology of brain

metastases-translation to new therapies. Nat Rev Clin Oncol. 2011;8(6):344–56.
88. Avraham HK, Jiang S, Fu Y, Nakshatri H, Ovadia H, Avraham S. Angiopoietin-2 mediates blood-brain barrier impairment and colonization of triple-negative breast cancer cells in brain. J Pathol. 2014;232(3):369–81.
89. Ma SC, Li Q, Peng JY, Zhouwen JL, Diao JF, Niu JX, et al. Claudin-5 regulates blood-brain barrier permeability by modifying brain microvascular endothelial cell proliferation, migration, and adhesion to prevent lung cancer metastasis. CNS Neurosci Ther. 2017;23(12):947–60.
90. Connell JJ, Chatain G, Cornelissen B, Vallis KA, Hamilton A, Seymour L, et al. Selective permeabilization of the blood-brain barrier at sites of metastasis. J Natl Cancer Inst. 2013;105(21):1634–43.
91. Lyle LT, Lockman PR, Adkins CE, Mohammad AS, Sechrest E, Hua E, et al. Alterations in pericyte subpopulations are associated with elevated blood-tumor barrier permeability in experimental brain metastasis of breast cancer. Clin Cancer Res. 2016;22(21):5287–99.
92. Yonemori K, Tsuta K, Ono M, Shimizu C, Hirakawa A, Hasegawa T, et al. Disruption of the blood brain barrier by brain metastases of triple-negative and basal-type breast cancer but not HER2/neu-positive breast cancer. Cancer. 2010;116(2):302–8.
93. Kodack DP, Askoxylakis V, Ferraro GB, Fukumura D, Jain RK. Emerging strategies for treating brain metastases from breast cancer. Cancer Cell. 2015;27(2):163–75.
94. Fidler IJ. The role of the organ microenvironment in brain metastasis. Semin Cancer Biol. 2011;21(2):107–12.
95. Fidler IJ, Balasubramanian K, Lin Q, Kim SW, Kim SJ. The brain microenvironment and cancer metastasis. Mol Cells. 2010;30(2):93–8.
96. Shibahara I, Kanamori M, Watanabe T, Utsunomiya A, Suzuki H, Saito R, et al. Clinical features of precocious, synchronous, and metachronous brain metastases and the role of tumor resection. World Neurosurg. 2018;113:e1–9.
97. Doron H, Pukrop T, Erez N. A blazing landscape: neuroinflammation shapes brain metastasis. Cancer Res. 2019;79(3):423–36.
98. Kamath SD, Kumthekar PU. Immune checkpoint inhibitors for the treatment of central nervous system (CNS) metastatic disease. Front Oncol. 2018;8:414.
99. Quail DF, Joyce JA. The microenvironmental landscape of brain tumors. Cancer Cell. 2017;31(3):326–41.
100. Fischer GM, Jalali A, Kircher DA, Lee WC, McQuade JL, Haydu LE, et al. Molecular profiling reveals unique immune and metabolic features of melanoma brain metastases. Cancer Discov. 2019;9:628–45.
101. Raza M, Prasad P, Gupta P, Kumar N, Sharma T, Rana M, et al. Perspectives on the role of brain cellular players in cancer-associated brain metastasis: translational approach to understand molecular mechanism of tumor progression. Cancer Metastasis Rev. 2018;37(4):791–804.
102. Kim R, Keam B, Kim S, Kim M, Kim SH, Kim JW, et al. Differences in tumor microenvironments between primary lung tumors and brain metastases in lung cancer patients: therapeutic implications for immune checkpoint inhibitors. BMC Cancer. 2019;19(1):19.
103. Sobottka B, Pestalozzi B, Fink D, Moch H, Varga Z. Similar lymphocytic infiltration pattern in primary breast cancer and their corresponding distant metastases. Oncoimmunology. 2016;5(6):e1153208.
104. Ogiya R, Niikura N, Kumaki N, Yasojima H, Iwasa T, Kanbayashi C, et al. Comparison of immune microenvironments between primary tumors and brain metastases in patients with breast cancer. Oncotarget. 2017;8(61):103671–81.
105. Harter PN, Bernatz S, Scholz A, Zeiner PS, Zinke J, Kiyose M, et al. Distribution and prognostic relevance of tumor-infiltrating lymphocytes (TILs) and PD-1/PD-L1 immune checkpoints in human brain metastases. Oncotarget. 2015;6(38):40836–49.
106. Harary M, Reardon DA, Iorgulescu JB. Efficacy and safety of immune checkpoint blockade for brain metastases. CNS Oncol. 2019;8:CNS33.
107. Kono K, Mimura K, Kiessling R. Immunogenic tumor cell death induced by chemoradiotherapy: molecular mechanisms and a clinical translation. Cell Death Dis. 2013;4:e688.
108. Kirilovsky A, Marliot F, El Sissy C, Haicheur N, Galon J, Pages F. Rational bases for the use of the Immunoscore in routine clinical settings as a prognostic and predictive biomarker in cancer patients. Int Immunol. 2016;28(8):373–82.
109. Berghoff AS, Fuchs E, Ricken G, Mlecnik B, Bindea G, Spanberger T, et al. Density of tumor-infiltrating lymphocytes correlates with extent of brain edema and overall survival time in patients with brain metastases. Oncoimmunology. 2016;5(1):e1057388.
110. Teglasi V, Reiniger L, Fabian K, Pipek O, Csala I, Bago AG, et al. Evaluating the significance of density, localization, and PD-1/PD-L1 immunopositivity of mononuclear cells in the clinical course of lung adenocarcinoma patients with brain metastasis. Neuro Oncol. 2017;19(8):1058–67.
111. Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res. 2015;75(11):2139–45.
112. Xue D, Xia T, Wang J, Chong M, Wang S, Zhang C. Role of regulatory T cells and CD8(+) T lymphocytes in the dissemination of circulating tumor cells in primary invasive breast cancer. Oncol Lett. 2018;16(3):3045–53.
113. Plaumann J, Engelhardt M, Awwad MHS, Echchannaoui H, Amman E, Raab MS, et al. IL-10 inducible CD8(+) regulatory T-cells are enriched in patients with multiple myeloma and impact the gen-

eration of antigen-specific T-cells. Cancer Immunol Immunother. 2018;67(11):1695–707.
114. Wu SY, Watabe K. The roles of microglia/macrophages in tumor progression of brain cancer and metastatic disease. Front Biosci (Landmark Ed). 2017;22:1805–29.
115. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol. 1953;26(305):234–41.
116. Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys. 2004;58(3):862–70.
117. Okwan-Duodu D, Pollack BP, Lawson D, Khan MK. Role of radiation therapy as immune activator in the era of modern immunotherapy for metastatic malignant melanoma. Am J Clin Oncol. 2015;38(1):119–25.
118. Brix N, Tiefenthaller A, Anders H, Belka C, Lauber K. Abscopal, immunological effects of radiotherapy: narrowing the gap between clinical and preclinical experiences. Immunol Rev. 2017;280(1):249–79.
119. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366(10):925–31.
120. Hiniker SM, Reddy SA, Maecker HT, Subrahmanyam PB, Rosenberg-Hasson Y, Swetter SM, et al. A prospective clinical trial combining radiation therapy with systemic immunotherapy in metastatic melanoma. Int J Radiat Oncol Biol Phys. 2016;96(3):578–88.
121. Pfannenstiel LW, McNeilly C, Xiang C, Kang K, Diaz-Montero CM, Yu JS, et al. Combination PD-1 blockade and irradiation of brain metastasis induces an effective abscopal effect in melanoma. Oncoimmunology. 2019;8(1):e1507669.
122. Stamell EF, Wolchok JD, Gnjatic S, Lee NY, Brownell I. The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys. 2013;85(2):293–5.
123. Hamilton AJ, Seid J, Verdecchia K, Chuba P. Abscopal effect after radiosurgery for solitary brain metastasis from non-small cell lung cancer. Cureus. 2018;10(12):e3777.
124. Hoshide R, Jandial R. The role of the neural niche in brain metastasis. Clin Exp Metastasis. 2017;34(6–7):369–76.
125. Schild T, Low V, Blenis J, Gomes AP. Unique metabolic adaptations dictate distal organ-specific metastatic colonization. Cancer Cell. 2018;33(3):347–54.
126. Rondeau G, Abedinpour P, Desai P, Baron VT, Borgstrom P, Welsh J. Effects of different tissue microenvironments on gene expression in breast cancer cells. PLoS One. 2014;9(7):e101160.
127. Valiente M, Obenauf AC, Jin X, Chen Q, Zhang XH, Lee DJ, et al. Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell. 2014;156(5):1002–16.
128. Chen Q, Boire A, Jin X, Valiente M, Er EE, Lopez-Soto A, et al. Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. Nature. 2016;533(7604):493–8.
129. Venkatesh HS, Johung TB, Caretti V, Noll A, Tang Y, Nagaraja S, et al. Neuronal activity promotes glioma growth through neuroligin-3 secretion. Cell. 2015;161(4):803–16.
130. Futakuchi M, Singh RK. Animal model for mammary tumor growth in the bone microenvironment. Breast Cancer. 2013;20(3):195–203.
131. Allen JK, Armaiz-Pena GN, Nagaraja AS, Sadaoui NC, Ortiz T, Dood R, et al. Sustained adrenergic signaling promotes intratumoral innervation through BDNF induction. Cancer Res. 2018;78(12):3233–42.
132. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol. 2018;15(2):81–94.
133. Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, et al. Toward understanding and exploiting tumor heterogeneity. Nat Med. 2015;21(8):846–53.
134. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92.
135. Stanta G, Bonin S. Overview on clinical relevance of intra-tumor heterogeneity. Front Med. 2018;5:85.
136. Echeverria GV, Powell E, Seth S, Ge Z, Carugo A, Bristow C, et al. High-resolution clonal mapping of multi-organ metastasis in triple negative breast cancer. Nat Commun. 2018;9(1):5079.
137. Stanta G, Jahn SW, Bonin S, Hoefler G. Tumour heterogeneity: principles and practical consequences. Virchows Arch. 2016;469(4):371–84.
138. Brastianos PK, Carter SL, Santagata S, Cahill DP, Taylor-Weiner A, Jones RT, et al. Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets. Cancer Discov. 2015;5(11):1164–77.
139. Han CH, Brastianos PK. Genetic characterization of brain metastases in the era of targeted therapy. Front Oncol. 2017;7:230.
140. Neagu MR, Gill CM, Batchelor TT, Brastianos PK. Genomic profiling of brain metastases: current knowledge and new frontiers. Chin Clin Oncol. 2015;4(2):22.
141. Bertucci F, Finetti P, Guille A, Adelaide J, Garnier S, Carbuccia N, et al. Comparative genomic analysis of primary tumors and metastases in breast cancer. Oncotarget. 2016;7(19):27208–19.
142. Liao L, Ji X, Ge M, Zhan Q, Huang R, Liang X, et al. Characterization of genetic alterations in brain metastases from non-small cell lung cancer. FEBS Open Bio. 2018;8(9):1544–52.
143. Paik PK, Shen R, Won H, Rekhtman N, Wang L, Sima CS, et al. Next-generation sequencing of stage IV squamous cell lung cancers reveals an association of PI3K aberrations and evidence of clonal het-

erogeneity in patients with brain metastases. Cancer Discov. 2015;5(6):610–21.
144. Jesinghaus M, Wolf T, Pfarr N, Muckenhuber A, Ahadova A, Warth A, et al. Distinctive spatiotemporal stability of somatic mutations in metastasized microsatellite-stable colorectal cancer. Am J Surg Pathol. 2015;39(8):1140–7.
145. National Collaborating Centre for Cancer. National Institute for Health and Clinical Excellence: guidance. Metastatic spinal cord compression: diagnosis and management of patients at risk of or with metastatic spinal cord compression. Cardiff (UK): National Collaborating Centre for Cancer (UK) National Collaborating Centre for Cancer; 2008.
146. Karsy M, Guan J, Sivakumar W, Neil JA, Schmidt MH, Mahan MA. The genetic basis of intradural spinal tumors and its impact on clinical treatment. Neurosurg Focus. 2015;39(2):E3.
147. Costigan DA, Winkelman MD. Intramedullary spinal cord metastasis. A clinicopathological study of 13 cases. J Neurosurg. 1985;62(2):227–33.
148. Payer S, Mende KC, Westphal M, Eicker SO. Intramedullary spinal cord metastases: an increasingly common diagnosis. Neurosurg Focus. 2015;39(2):E15.
149. Weng Y, Zhan R, Shen J, Pan J, Jiang H, Huang K, et al. Intramedullary spinal cord metastasis from renal cell carcinoma: a systematic review of the literature. Biomed Res Int. 2018;2018:7485020.
150. Zheng H, Li W, Kang Y. Tumor-stroma interactions in bone metastasis: molecular mechanisms and therapeutic implications. Cold Spring Harb Symp Quant Biol. 2016;81:151–61.
151. Theriault RL, Theriault RL. Biology of bone metastases. Cancer Control. 2012;19(2):92–101.
152. Futakuchi M, Fukamachi K, Suzui M. Heterogeneity of tumor cells in the bone microenvironment: mechanisms and therapeutic targets for bone metastasis of prostate or breast cancer. Adv Drug Deliv Rev. 2016;99(Pt B):206–11.
153. Battafarano G, Rossi M, Marampon F, Del Fattore A. Cellular and molecular mediators of bone metastatic lesions. Int J Mol Sci. 2018;19(6):1709.
154. Magbanua MJ, Melisko M, Roy R, Sosa EV, Hauranieh L, Kablanian A, et al. Molecular profiling of tumor cells in cerebrospinal fluid and matched primary tumors from metastatic breast cancer patients with leptomeningeal carcinomatosis. Cancer Res. 2013;73(23):7134–43.
155. Cheng H, Perez-Soler R. Leptomeningeal metastases in non-small-cell lung cancer. Lancet Oncol. 2018;19(1):e43–55.
156. Potti A, Abdel-Raheem M, Levitt R, Schell DA, Mehdi SA. Intramedullary spinal cord metastases (ISCM) and non-small cell lung carcinoma (NSCLC): clinical patterns, diagnosis and therapeutic considerations. Lung Cancer (Amsterdam, Netherlands). 2001;31(2–3):319–23.
157. Okamoto H, Shinkai T, Matsuno Y, Saijo N. Intradural parenchymal involvement in the spinal subarachnoid space associated with primary lung cancer. Cancer. 1993;72(9):2583–8.
158. Haykal T, Towfiq B. Merkel cell carcinoma with intramedullary spinal cord metastasis: a very rare clinical finding. Clin Case Rep. 2018;6(6):1181–2.
159. Perez-Suarez J, Barrio-Fernandez P, Ibanez-Plagaro FJ, Ribas-Arino T, Calvo-Calleja P, Mostaza-Saavedra AL. Intramedullary spinal cord metastasis from gastric adenocarcinoma: case report and review of literature. Neurocirugia (Astur). 2016;27(1):28–32.
160. Kaballo MA, Brennan DD, El Bassiouni M, Skehan SJ, Gupta RK. Intramedullary spinal cord metastasis from colonic carcinoma presenting as Brown-Sequard syndrome: a case report. J Med Case Reports. 2011;5:342.
161. Isoya E, Saruhash Y, Katsuura A, Takahashi S, Matsusue Y, Hukuda S. Intramedullary spinal cord metastasis of ovarian tumor. Spinal Cord. 2004;42(8):485–7.
162. Zhou Z, Li Y, Yan X, Wang X, Chen S, Xiao J. Characteristics of a thyroid carcinoma cell line derived from spinal metastasis. Biosci Rep. 2016;36(6):e00426.
163. Gainor JF, Ou SH, Logan J, Borges LF, Shaw AT. The central nervous system as a sanctuary site in ALK-positive non-small-cell lung cancer. J Thorac Oncol. 2013;8(12):1570–3.