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The Role of Astrocytes in Tumor Growth and Progression

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Abstract

Current research is continually implicating the importance of astrocytes as active participants in neurological injury, disease, and tumor progression. This chapter will discuss some of these emerging concepts, especially as they relate to tumor biology. Astrocytes themselves can become tumorigenic, such as the case in gliomas, which often have aberrant signaling in key regulating genes of astrocyte development. Astrocytes secrete factors that maintain the tight junctions of the blood brain barrier (BBB), which in turn regulates the success or failure of metastatic cells extravasating into the brain. This astrocytic association with the brain vasculature also promotes brain tumor stem cell characteristics, which are known to be necessary for tumor initiation. Tumor cells within the brain make direct contacts with astrocytes through gap junctions, which subsequently lead to increased chemoresistance of the tumor cells. Astrocytes have also been shown to effect tumors cells via secretion of degradative enzymes, cytokines, chemokines, and growth factors, all of which have been shown to promote tumor cell proliferation, survival, and invasion. Thus, research in astrocyte biology and the role of astrocytes in the tumor microenvironment has and will likely continue to reveal novel targets for cancer intervention.

Keywords: astrocytes, metastasis, blood-brain barrier, reactive astrogliosis, cancer

1. Introduction

The tumor microenvironment plays a critical role in tumor progression. Tumors within the central nervous system (CNS) include primary brain tumors originating from a CNS resident cell, or secondary tumors that came from extraneural origins. The brain microenvironment consists of multiple cell types including the most abundant glial cell, astrocytes. Astrocytes have very diverse and microenvironment-dependent morphologies; for a long time, this

structural contribution was considered their main purpose. Present in gray matter, protoplasmic astrocytes are the most common types of astrocytes and are stellate in nature with branching processes or "endfeet" [1]. These endfeet make important contacts with neurons and other cells within the brain microenvironment. Importantly for this chapter, one of these interfaces, which we will discuss further, is the astrocyte endfeet connections made with endothelial cells and pericytes, commonly referred to as the blood brain barrier (BBB). This barrier allows for select metabolites to enter and toxic waste to exit the brain.

Homeostasis in the brain is of utmost importance to maintain neural function and prevent potentially detrimental immune responses from occurring. An invading tumor cell normally encounters enormous barriers before it can colonize the brain. On entering the brain, it will need to overcome brain defense mechanisms, which are partly mediated by astrocytes and brain macrophage cells called microglia. These and other mechanisms are in place to thwart tumor cell entrance, however, in some cases these mechanisms are either not adequate to prevent tumor cell invasion, or exploited to aid in tumor cell extravasation into the brain. In addition to regulating brain metastases, astrocytes, which develop from neural stem cells (NSCs), can become transformed and undergo developmental dysregulation due to aberrant gene activation, resulting in various types of brain tumors, including gliomas.

In this chapter, we will further discuss autocrine, paracrine, and juxtacrine mechanisms in which astrocytes influence surrounding cells in the brain microenvironment and tumor progression within the CNS. We will discuss the underlying mechanisms that regulate these processes, and provide examples of possible interventions that could eventually be translated into successful clinical treatment for patients.

2. Primary tumors of astrocytic origin

We begin this chapter by understanding how astrocytes themselves may become transformed and discuss the key features of these types of tumors. The cellular origin of many brain tumors can be traced back to multipotent NSCs, which are able to self-renew and differentiate into all subtypes of mature neurons and glial cells. However, many tumors with more distinct cellular origins exist along the glial cell differentiation axis, and are traced back to more restricted and differentiated astrocyte progeny [2]. During development of mature astrocytes, NSCs first partially differentiate into neuronal precursor cells where, in the presence of specific growth factors and their cognate receptors, they differentiate into various cellular lineages [3]. Early astrocyte precursors are characterized by their expression of fibroblast growth factor receptor (FGFR), nestin, and epidermal growth factor receptor (EGFR), while mature astrocytes express markers such as glutamate aspartate transporter (GLAST), FGFR3, S100β and glial fibrillary acidic protein (GFAP) [4–7]. These astrocyte precursor cells are perhaps most vulnerable to transformation, and depending on the stage of these cells, fatal adult primary brain tumors (gliomas) may arise. Because mature astrocytes maintain their ability to proliferate throughout adulthood (an uncommon characteristic of many CNS cells), it is hypothesized that this is a contributing reason for why astrocytic tumors are so common overall and most common in adults [8, 9].

The term "glial cells" describes a broader group of cells including astrocytes, ependymal cells and oligodendrocytes, not all gliomas are specifically astrocytic in nature. Gliomas which are thought to originate or histologically resemble astrocytes include astrocytomas, mixed gliomas or oligoastrocytomas, diffuse intrinsic pontine gliomas [10], and high grade astrocytomas called glioblastoma multiforme (GBM). There are also several types of mixed neuronal-glial tumors. These tumors are extremely heterogeneous, differing in histology, location in the brain, molecular biology, karyotype, age of onset, and survival prognosis of the patient. Gliomas share many characteristics with astrocytes, particularly activated astrocytes, which will be discussed in a later section of this chapter. Some of these include migration capabilities, growth factor expression pattern, stem cell-like characteristics, and the ability for anchorage-independent growth which is correlated with invasiveness of a tumor [11–13].

In general, cellular origins of the previously mentioned gliomas are astrocyte precursor cells. However, the diversity in the distinct molecular/genetic alterations of the tumors suggests that different stages or types of precursor cells have different sensitivities to specific genetic mutations. One of the most notable genetic signatures of GBM is EGFR amplification and overexpression which, as previously mentioned, is also involved in regulating astrocyte differentiation [14–17]. There have been several mechanisms associating EGFR overexpression with astrocyte tumor malignancy. Several known ligands of EGFR, including EGF and transforming growth factor-α (TGF-α), promote proliferation of astrocytes and astrocyte precursor cells, thus contributing to the malignancy of the tumors [14, 18, 19]. Additionally, cell cycle regulators such as Rb, p53 and CDKN2A are commonly mutated and inactivated in low grade gliomas and GBM, [15, 20–23]. Mutations in isocitrate dehydrogenase (IDH)-1 and -2 are also extremely common, but only in certain gliomas; they are present in 70% of grade II and III astrocytomas and oligodendrogliomas, as well as secondary GBMs, but are rare in primary GBMs [15, 24].

3. The role of astrocytes in tumor growth and progression

As will be discussed in more detail throughout this chapter, astrocytes are very heterogeneous in regards to function and influence on tumors within the CNS. This fact, combined with the heterogeneity that encompasses the transformation of cells results in unique tumor genotypes and phenotypes, plus many other contextual factors, equates to interactions that are both tumor promoting and tumor suppressive (**Figure 1**). Arguably, there is more evidence suggesting how astrocytes can be tumor promoting, which will be covered in this section. Some functions of astrocytes that are known within the literature to be tumor promoting to both primary and metastatic brain tumors are summarized and illustrated (**Figure 2**).

3.1. Metastatic tumors: Interactions with astrocytes at the blood-brain barrier

One of the critical steps in the life time of tumor progression is tumor metastasis, especially brain metastasis. This step results in catastrophic consequences from a patient perspective. The metastases from extraneural tumors in the brain are actually the most common sources of tumors in the CNS, as shown in **Table 1** [25, 26].
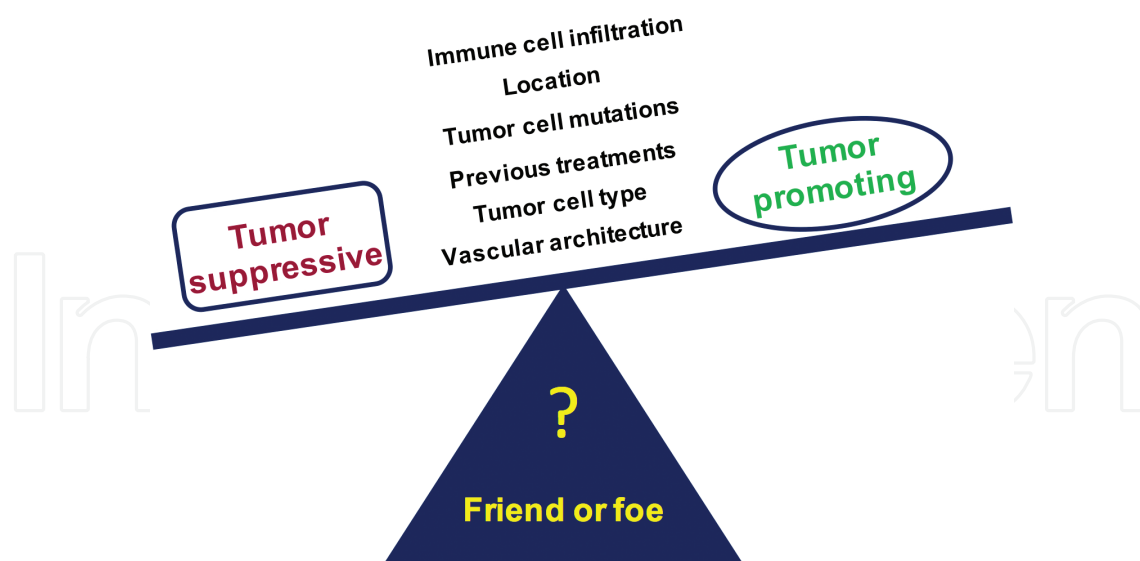
Figure 1. Research shows astrocytes have functions that result in tumor supporting and tumor suppressing mechanisms, and sometimes both. Like many physiological responses, this demonstrates the context dependent balancing act that occurs when homeostasis is breached, and the many factors that play a role in tipping the balance one way or another.

The process of metastasis, in brief, involves invasion of a tumor cell away from the tumor to a blood vessel, entry into and survival in the blood circulation, extravasation from the blood vessel into the secondary organ, and survival, engraftment, and proliferation into a secondary tumor. Extravasating into the brain provides an added challenge: that of getting past the BBB. The most functionally important component of the BBB are the tight junctions held between brain microvascular endothelial cells. Thus, substances that get into the brain parenchyma are tightly controlled. Both para-cellular and trans-cellular diffusion are low; most solutes that get in and out through the BBB, such as glucose and other nutrients, do so through transporters expressed on endothelial cells [27–29]. Despite this added barrier, many extraneural tumors have a strong tendency to metastasize to the brain. To note, there are regions within the brain that lack BBB, and could also be a potential avenue of metastasis [30]. The recent discovery of brain lymphatics is also suggestive of an alternative route given the already known function of lymphatics to carry tumor cells [31].

Breast cancer, melanoma, and lung cancer are three tumor types that show proclivity to go to the brain. The most common type of brain metastases originate from lung cancer, accounting for up to 56% of brain metastases, followed by breast cancer metastases at 13–30% [32, 33]. Interestingly, specific subtypes of these tumors have a much higher frequency of brain metastases, including non-small cell lung cancer (NSLC), triple negative breast cancer cells that are estrogenic receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor-2 (HER2) (ER$^-$, PR$^-$, HER2$^-$), and HER2-enriched (HER2$^+$) breast cancer cells [32–35]. One theory behind a specific tumors' proclivity to the brain is explained by Paget's seed and soil hypothesis, which suggests that for a seed (tumor cell) to take up in a soil (brain), it must adapt itself and make changes that will favor the soil [26, 36]. In support of this idea, genes associated with breast cancer metastasis to brain have been discovered and efforts continue to identify new targets for lung cancer cell metastasis to the brain and for other cancers as well [37–40]. However, the question raised by the
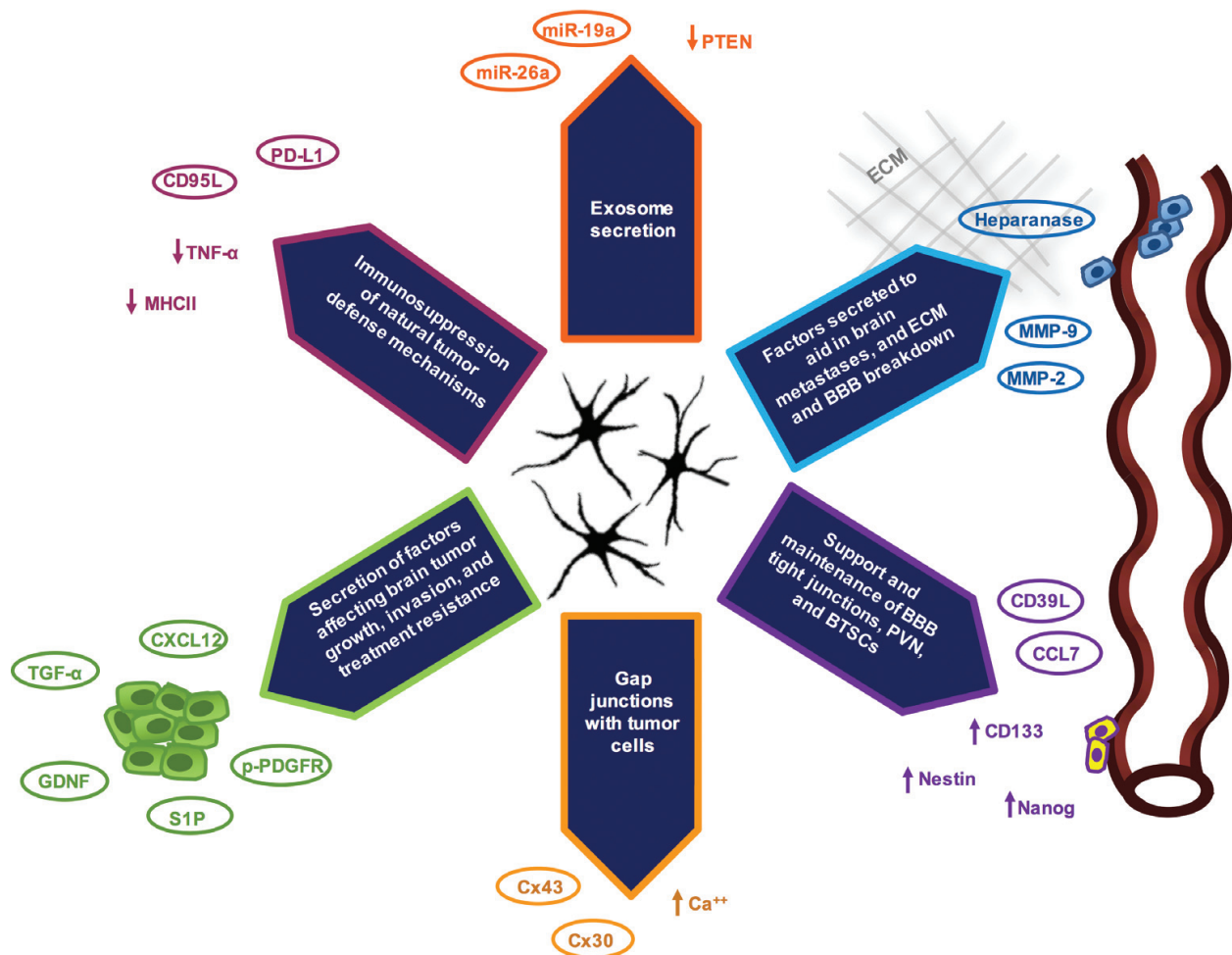
Figure 2. Many signaling mechanisms have been identified that implicate astrocytes in tumor progression. Some of these functions include secretion of factors that have been shown to increase invasiveness and malignancy of established brain tumors, in addition to factors that enable brain metastasizing cells to enter the brain. Astrocytes have also been shown to maintain the vascular niche in the brain which can promote stem-like characteristics in BTSCs. Astrocytes directly interact with tumor cells and communicate via gap junctions, leading to increased intracellular calcium and resistance to treatments. Indirect or paracrine communication with surrounding cells often occurs via secretion of exosomes known to carry miRNAs to target key tumor suppressor genes in tumor cells and surrounding microenvironment. Lastly, astrocytes have been shown to regulate CNS immune suppression, weakening the innate tumor killing response of the body.

seed-soil hypothesis is whether the soil influences the seed, and if so, how it is accomplished. One could argue that in the first place, the soil is influenced by the seed. Therefore, in this "circular logic," both seed and soil appear to contribute together, ultimately, for the growth of the tumor cell.

Astrocytes are vital to the development and maintenance of the BBB, therefore understanding their role in this process is necessary to understand how they also may influence brain metastatic tumor cells attempting to breach the BBB. The tight junctions between the endothelial cells of the BBB are comprised of many junctional proteins, notably claudin-5 and occludin [41, 42]. Vascular endothelial (VE)-cadherin is also of importance within adherens junctions which associate with tight junctions, as well as cytoplasmic scaffolding proteins such as zonula occludens (ZO)-1 and -2 [27, 43–45]. In normal conditions, the BBB homeostasis and junctional complexes are partially supported at the structural and physiological functional levels by astrocytes. Astrocytes contact

CNS tumor type	Incidence rate (per 100,000 persons) (all ages)	References
All brain metastases	8.3/11.1/14.3	[195–197]
Lung cancer brain metastases	~ 3.2–8(estimation based on 39–56% of all brain metastases)	[32]
Breast cancer brain metastases	~ 1.1–4.3 (estimation based on 13–30% of all brain metastases)	[32]
Melanoma brain metastases	~ 0.5–1.6 (estimation based on 6–11% of all brain metastases)	[32]
Primary malignant CNS tumors	7.2	[198]
GBM	3.2	[198]
Nerve sheath tumors	1.82	[198]
Other astrocytomas	1.2	[198]
CNS lymphoma	0.43	[198]
Embryonal tumors (medulloblastoma, ATRT, and PNET)	0.62 (*ages 0–19 only*)	[198]

Table 1. Primary and metastatic CNS tumors with their respective incidence rates per 100,000 persons. All tumors are accounting for all ages, except embryonal tumors which only includes persons' age 0–19 in the population study.

brain endothelial cells via their end feet processes. This contact was shown to maintain BBB permeability characteristics. However, it was later found that secreted factors alone in astrocyte-conditioned media also upheld the tight junction characteristics in endothelial cells, demonstrating the importance of both astrocyte contact and paracrine actions in BBB function [46–48]. For example, Sonic hedgehog (Shh), an important developmental signaling protein, is known to be secreted by astrocytes and bind its cognate receptor, Patched-1, expressed at the cell membrane of brain endothelial cells. This induces a signaling cascade mediated by β-catenin that bolsters tight junctions by increasing expression of occludin [49, 50]. Other proteins secreted by astrocytes that regulate and maintain tight junctions of brain ECs (most often by increased gene expression of junctional proteins) include angiotensin-1, FGF, TGF-β, glia derived neurotropic factor (GDNF), and retinoic acid (RA) [51–55].

Just as astrocytes are important for maintaining the BBB in homeostatic conditions, astrocytes also play key roles when the BBB is disrupted, which can occur during extravasation of tumor cells metastasizing to the brain. Several groups utilized mouse models of melanoma, lung, and breast cancer combined with histological and fluorescent imaging modalities to visualize very early interactions with tumor cells and the BBB. This work demonstrates that tumor cells first arrest in the brain capillaries, often at branch points [56, 57]. Lorger et al. (2010) show that very early on, astrocytes become activated and associate around vasculature in the brain where breast tumor cells are present, but in some cases have not yet extravasated or visibly altered the BBB [56]. This suggests that signals secreted by tumor cells are reaching astrocytes either directly or indirectly through the endothelial cells; a topic that requires further investigation. This association of tumor cells with reactive astrocytes persists throughout metastases formation, characterized by increased astrocyte expression of GFAP, nestin, and matrix metalloproteinase 9 (MMP-9), all of which aid in tumor extravasation mechanisms that will be discussed in later sections.

3.2. Astrocytes' direct cell-cell interactions with tumor cells

Astrocytes have multiple primary and branching endfeet which expand and contract, allowing them to dynamically contact both synapses and the microvasculature. Also, astrocytes regulate communication between neuronal networks and glial-vascular coupling by forming independent contact network [58–61]. Therefore, it has been widely accepted that astrocytes directly contact and communicate with neurons to regulate neuronal function at the synaptic and network levels, which provides a significant impact on physiological and pathological state of the CNS. Subsequently, direct interactions with astrocytes and tumor cells, often in the form of gap junctions, has also been discovered to be significant for tumor progression and resistance to therapy.

As discussed, gliomas are the most lethal primary intracranial tumors. The proliferative dysfunction and invasion of gliomas are associated with changes in gap junction communication [62, 63]. In metastatic brain tumors, reactive astrocytes protect melanoma cells from chemotherapy induced cell death by sequestering intracellular calcium through gap junctions [64]. In the brain, metastases from breast and lung cancer show upregulation of many survival genes which is dependent on the direct contact through gap junctions between the astrocytes and tumor cells, which was found to be causal for developing resistance [65]. These data suggest that reactive astrocytes participate in tumor progression and chemo-resistance by their direct physical contacts and gap junctional communication with tumor cells in the brain.

Gap junctions are efficient tools for intercellular communication. In astrocytes, they are composed of connexins 30 and 43 (Cx30, Cx43) [66]. Cx43 is widely expressed in adult astrocytes and exhibits increased expression in reactive astrocytes induced by various brain pathologies and intercellular calcium signaling [67–73]. Also, Cx43-mediated intercellular communication between astrocytes plays an important role in the invasion of glioma cells in the brain [63]. A recent study has also revealed that breast and lung cancer cells express proto-cadherin 7 (PCDH7) to promote tumor-astrocyte gap junction formation by recruiting Cx43, which allows the transfer of cGAMP from tumor cells to astrocytes to trigger the secretion of inflammatory cytokines, which further promote tumor growth and chemo-resistance [74].

3.3. Astrocytes' secretome and paracrine signaling mechanisms that influence tumor cells

3.3.1. Cytokines and growth factors

Astrocytes can synthesize a host of biologically interesting growth factors and cytokines. Previous studies have shown that sphingosine-1-phosphate (S1P), which shows the highest expression in the brain and is only expressed by astrocytes, induces cell motility in GBM cell lines that express S1P receptor-1 and S1P receptor-3 [75, 76]. Other neurotrophic factors secreted by astrocytes, such as TGF-α, C-X-C motif chemokine 12 (CXCL12), and GDNF, have also revealed the potential to increase the invasive capacity of GBM cells [77, 78]. In brain metastatic tumors, an early study found that metastatic MDA-MB-435 breast cancer cells, when cultured with astrocyte conditioned media, exhibit better growth in response to the conditioned medium. However, the growth-stimulatory effect was partially reversed by anti-IL-6, anti-TGF-β, and anti-insulin like growth factor-1 (IGF-I) antibodies [79]. Another study showed that reactive astrocytes expressed phosphorylated platelet-derived growth factor receptor β at

tyrosine 751 (p751-PDGFRβ). Pazopanib, an inhibitor of PDGFRs, inhibited the activation of p-PDGFR expressing astrocytes, and thus prevented brain metastasis formation in the HER2-transfected MDA-MB-231 breast cancer cells [80]. Taken together, this work demonstrates that paracrine signaling by astrocyte secreted cytokines and growth factors facilitates tumor metastasis formation in the brain.

3.3.2. Extracellular matrix (ECM) proteins and degradative enzymes

ECM proteins are important participants in the tumorigenic process, as they are involved in not only the physical adhesion and migration of tumors cells, but also the regulation of intracellular signaling. The brain parenchyma is high in proteoglycans, glycoproteins, and matricellular proteins, all of which astrocytes express and secrete [4]. Specifically, some astrocyte secreted matricellular proteins have been studied in regulation of various brain tumors, including secreted protein acidic and rich in cysteine (SPARC) and CYR61/CTGF/NOV (CCN). Both SPARC and CCN2 have been shown to be secreted by activated astrocytes proximal to brain tumors or injuries [81, 82]. While increases in CCN2 secretion have been correlated with negative glioblastoma outcomes, expression of SPARC and its effect on tumor cells is tumor dependent. In gliomas and astrocytomas, tumor secretion of SPARC promotes invasion, angiogenesis, and a negative prognosis [83, 84]; however medulloblastoma tumor cells have increased loss of SPARC, which when rescued induces cell cycle arrest, neuronal differentiation, and limits radioresistant DNA damage response [85–87].

Previously, we discussed MMP-related mechanisms in which astrocytes assist tumor cells in extravasating the BBB. The secretion of these matrix degrading enzymes also supports brain tumor progression by breaking down the barriers induced by the ECM. Heparanase degrades the glycosaminoglycan side chains of heparan sulfate proteoglycans, which are essential and ubiquitous macromolecules associated with the cell surface [88–90]. Reactive astrocytes have been frequently found in areas surrounding melanoma-related lesions and produce nerve growth factor (NGF), the prototypic neurotrophin [91]. Neurotrophins can stimulate heparanase production in astrocytes and thus contribute to the brain colonization of melanoma cells [88].

MMP-2 and -9 have been observed in secretory vesicles in astrocytes [92]. Stimulation of astrocytes with lipopolysaccharide, IL1-α, IL1-β, or TNF-α induces MMP-2 and -9 secretion [93]. MMP-9 also promotes the growth of primary brain tumors by releasing vascular endothelial growth factor (VEGF) sequestered in the surrounding matrix [94]. The expression of MMP-9 was up-regulated in reactive astrocytes, which was involved in the brain metastases of MDA-MB-435 cells [56]. Moreover, both MMP-2 and -9 secreted by astrocytes contribute to breast cancer MDA-MB-231 cell invasion and brain metastases [95]. In addition to secreting MMPs themselves, astrocytes can also induce tumor cells to secrete MMPs, as shown by Mendes et al. (2007), where they found breast cancer cells to secrete significantly more MMP-2 in the presence of astrocyte conditioned media, aiding metastasis to the brain [96].

3.3.3. Exosomes

The topic of exosomes, which are endosome-derived microvesicles between 50 and 100 nm in size that carry specific protein and RNA cargo, has become a subject of intense interest in

tumor biology. Exosomes are released from cells by fusion of multi-vesicular structures with the plasma membrane through the process of exocytosis [97]. Exosomes are a general mode of intercellular communication and can interact with neighboring cells, thus mediating signals between astrocytes and other cells in the brain microenvironment [98, 99].

Among RNA cargo, microRNA (miRNA) transcripts, specifically miR-26a, is highly expressed in astrocytes and is present in astrocyte-derived exosomes [100, 101]. MiR-26a targets mRNAs that impact neuronal function and morphology, and was first implicated in many neuronal disorders [102–104]. Moreover, miR-26a can be sorted to exosomes and transported by these vesicles in the plasma, serum, whole blood, urine, or secreted *in vitro* by human umbilical vein endothelial cells [105–109]. In addition, miR-26a autonomously regulates primary gliomas by increasing *de novo* tumor formation and radiosensitivity through targeting of suppressor phosphatase and tensin homolog (PTEN) and ataxia-telangiectasia mutated (ATM), respectively [110, 111]. Therefore, it is plausible that miR-26a in astrocyte-derived exosomes may function to regulate the surrounding tumor environment. A recent study found that primary breast tumor cells express tumor suppressor PTEN, however this expression of *PTEN* was lost reversibly after tumor cells metastasized into the brain [112]. Astrocyte-derived exosomal miR-19a reversibly mediated the downregulation of *PTEN* expression in cancer cells, thus providing one mechanism for loss of PTEN in tumor cells that enter the brain. Further, miR-19a also increased C-C motif chemokine ligand 2 (CCL2) secretion and recruitment of myeloid cells, thus facilitating changes in the brain microenvironment to promote metastasis [112].

4. The role of astrocytes in brain tumor stem cell biology

An important attribute of brain tumor biology regarding tumor initiation and propagation is the existence of brain tumor stem cells (BTSCs). These cells have been found to, in many ways, resemble adult NSCs that exist in distinct regions of the brain, including the subventricular zone (SVZ) and the subgranular zone (SGZ) [113, 114]. Many groups identified CD133, Nestin, and sex determining region Y-box 2 (SOX2) as markers to isolate NSCs which maintain the essential properties of stem cells (self-renewal and ability to differentiate into multiple progeny) [115–118]. Using the NSC neurosphere culturing method, CD133 and/or CD15 have also been found to be expressed on BTSCs from GBM, medulloblastoma, ependymoma, and astrocytoma tumors [118–120]. Interestingly, Singh et al. (2003) found CD133$^+$ cells to be tumor initiating, whereas CD133$^-$ cells could not initiate a tumor or self-renew in a mouse xenograft model [118, 121]. The levels of CD133$^+$ BTSCs has since been correlated to negative prognoses in gliomas, and have been found to be particularly enriched in recurrent tumors after radiation and chemotherapy [122–124]. These findings highlight the importance of stem cells in the overall initiation, malignancy, and recurrence of brain tumors.

4.1. Astrocytes' direct influence on cancer stem cells

It is clear that BTSCs play an important role in the progression of all brain tumors. Therefore, cells in the microenvironment that influence BTSCs are of interest from a clinical therapy perspective. Interestingly, astrocytes seem to affect normal NSCs and BTSCs quite differently.

While astrocyte secreted factors have been shown to promote neurogenesis of normal adult NSCs, astrocytes within the microenvironment of brain tumors have also been shown to promote stem-like characteristics in BTSCs and enrich the stem cell population, thus worsening the malignancy of such brain tumors [125–128]. GBM CD133$^+$ stem cells co-cultured both directly and indirectly with astrocytes show gene expression signatures known to be involved in GBM invasion and metastasis, such as a disintegrin and metalloproteinase domain-containing protein 10 (*ADAM10*), hyaluronan synthase 2 (*HAS2*), and vascular cell adhesion molecule-1 (*VCAM1*). Interestingly, although there were many overlapping genes in conditions where astrocytes and tumor cells were and were not in direct contact, even the distinct gene expression changes in each condition were still related to tumor cell invasion. This emphasizes the role of astrocytes in GBM invasion, which is one of the most challenging traits of GBM [127]. Indeed, CD133$^+$ GBM cells were found to be more invasive, whereas CD133$^-$ GBM cells did not have the same gene expression and invasion changes [127]. Later, it was shown that indirect co-culture with CD133$^+$ GBM cells and astrocytes resulted in cytokine release from astrocytes that reduced radiosensitivity of the GBM cells; again, this same phenotype and crosstalk with astrocytes was absent in CD133$^-$ GBM cells [128]. Some of the astrocyte secreted cytokines that induced radioresistance include CXCL1, IL-4, IL-6, and CCL7 [128]. These differences suggest that cancer stem (or stem-like) cells signal differently with astrocytes compared to tumor cells lacking stem characteristics. The reverse effect, which is tumor stem cells influencing astrocytes has also been observed in GBM. GBM stem cells provide signals that block the expression of p53 in surrounding astrocytes [129]. P53 is a tumor suppressor often found mutated in many tumors [130], and is classically known for its function in DNA damage response. However, recently p53 has been shown to have non-autonomous cellular functions, particularly in the tumor microenvironment, by influencing secretion of proteins, including ECM proteins [129, 131, 132]. Thus, the interaction between astrocytes and BTSCs are bi-directional and influence each other's development.

In addition to primary brain tumors, cancer stem cells of brain metastatic tumors are also influenced by astrocytes. It has been shown that cyclooxygenase 2 (COX2) is highly expressed in breast cancer brain metastatic cells, which autonomously induces expression of MMP-1 and prostaglandins [133]. While MMP-1 allows for BBB tight junction and basement membrane degradation to aid brain metastasis, prostaglandins are able to activate astrocytes and subsequently increase astrocyte expression of CCL7, which was shown to significantly increase self-renewal and survival of breast cancer stem cells through increased expression of Nanog, a key stem cell regulator [133, 134]. This study provides evidence that astrocytes enrich breast cancer stem cells in brain metastases and aid in their ability to extravasate the BBB.

4.2. Astrocytes as a part of the perivascular niche

Normal NSCs in the SVZ and SGZ are maintained by specialized vascular regions called the perivascular niche (PVN) [135]. The PVN consists of the endothelial cells lining the vasculature, as well as astrocytes, pericytes, macrophages, microglia, fibroblasts, and vascular smooth muscle cells. These cells function and signal together to maintain structure and provide signals to NSCs. Evidence exists which demonstrates the vital role endothelial cells play in maintaining

and regulating NSC/BTSCs' survival and differentiation status [136–139]. Astrocytes also play a vital role within the PVN. First and foremost, they play an indirect but obvious role in the structural and chemical maintenance of endothelial cell-BBB phenotypes, as discussed earlier. Studies have shown that BTSCs maintain close proximity to angiogenic regions of the tumor microenvironment, providing evidence that these regions phenocopy the PVN within the SVZ/SGZ to provide enrichment signals to BTSCs [140].

5. Astrocytes as an immune regulator in the tumor microenvironment

The immune system within the CNS is tightly controlled. In addition to the BBB, there are other barriers that maintain the CNS as an immune-privileged system, including the blood-meningeal barriers and the blood-cerebrospinal fluid (CSF) barriers [141, 142]. During homeostasis, these barriers do not allow entry of pathogens or blood-borne immune cells. Only upon CNS injury do some of these cellular barriers become fenestrated to allow for immune cell entrance. Although microglia are thought to be the main regulator of immune responses within the brain, astrocytes (and other cells) also play key roles in this function [143]. A vast amount of work investigating the astrocyte function in either normal or activated state is often related to the regulation of the immune environment in the CNS, as shown in functional studies and astrocyte secretome studies, summarized well by Sofroniew et al. [144–147]. As suggested by Yang et al. (2013), the presence of classical immunological surface molecules, such as major histocompatibility (MHC) antigen and intercellular adhesion molecule-1 (ICAM-1) on astrocytes underlines their importance in CNS immune function [11, 148, 149]. We will discuss next how astrocytes control immune responses to invading tumor cells, and the immune-related concepts associated with this process.

5.1. Immune responses to tumor cell presence

As mentioned in the discussion of astrocytes and tumor cell interactions at the BBB, there have been a few key studies observing the cellular events that take place when metastatic cells extravasate into the brain parenchyma [56, 57]. From these studies, it is known that astrocytes are the first to respond to extravasating metastatic tumor cells entering the brain, followed by microglia [56]. Regardless of whether a CNS tumor is primary or metastatic, microglia and astrocytes control the immune response; therefore, it is upon their activation that other immune cells, such as macrophages or lymphocytes, may infiltrate [144–146, 150]. Activated astrocytes secrete pro-inflammatory molecules such as CXCL12, CCI2, Il15, CCL8, and CXCL1, all of which are known to regulate recruitment, activation and proliferation of T-cells, B-cells, or natural killer (NK) cells [144–146].

As it is often seen with any local or systemic inflammation, CNS immune responses can often persist or be dysregulated by tumor cells to become pathogenic. Many of these signaling responses are mediated by astrocytes. For example, Valiente et al. [151] reported that astrocytes produce FasL and plasmin ligands as defense mechanisms to kill brain-invading tumor cells. In response, tumor cells secrete serpins, which thwart the lethal action of plasmin [151]. Thus, Fas-mediated tumor cell apoptosis is blocked, leading to tumor survival. Other cells, such as endothelial cells, in the brain

microenvironment are also co-opted, which is facilitated by up-regulation of L1 cell adhesion molecules (L1CAM). All these mechanisms work together to initiate brain metastasis [151].

Astrocytes are also known to function in immunosuppression. This is accomplished by downregulating the pro-inflammatory cytokine TNF-α in surrounding microglia, and suppressing the antigen presenting abilities of various immune cells by downregulating their expression of MHCII and CD80 [152, 153]. Additionally, activated astrocytes can co-localize and induce apoptosis in T-cells attempting to infiltrate the brain parenchyma by expression of the "death ligand," CD95L, which binds the receptor on T-cells [152, 154].

5.2. Reactive Astrogliosis

Arguably, the most important feature of astrocytes in relation to their immune function is their ability to activate, a process called reactive astrogliosis. What determines whether an astrocyte is "activated" or not has not been clearly defined, however Sofroniew summarized the existing research into four key features. First, reactive astrogliosis is a spectrum of molecular, cellular, and functional changes among astrocytes in response to CNS injury of many kinds [147]. Second, the changes can vary in severity and the response can be sequential and/or progressive. Third, the changes are regulated by intra- and inter-cellular signals and lastly, signaling events can be both gain and loss of function in nature, resulting in both beneficial and detrimental outcomes [147, 155]. In other words, reactive astrogliosis is spectral in nature; the triggers can vary and therefore the "activation" or response can vary and is context dependent, which is also true in regards to how reactive astrocytes affect tumor progression and/or tumor death.

The activation responses can be as small as a transient upregulation of GFAP, to permanent structural changes in the brain from a process called glial scar formation. Scar formation occurs when astrocytes proliferate and overlap to a point that causes dense, compact barriers around necrotic tissue [147, 156]. In between these two extremes, other phenotypic changes that occur include hypertrophy of the cell body and processes, a vast array of gene expression changes, and varying degrees of proliferation up to the point of scar formation. Some of the chemical activators of astrocytes known to be secreted by or induced by tumor cells include EGF (glioblastoma and medulloblastoma), TGF-α (medulloblastoma), receptor activator of nuclear factor kappa-B (NFκB) ligand (RANKL) (glioma), macrophage migration inhibitory factor (MIF), interleukin-8 (IL-8), and plasminogen activator inhibitor-1 (PAI-1) (lung cancer metastases) [11, 157–160].

In addition to chemical activation, astrocytes can also be activated by tumor cells mechanically. Although extremely abundant, astrocytes hold a highly regulated, non-overlapping distribution that plays an important role in morphology and contact-dependent inhibition of proliferation [11, 61]. This distribution and homeostasis is mediated by contact inhibition and adherens junctions. Therefore, mechanical disruption occurs when processes such as migration and/or proliferation of surrounding cells is initiated. Such mechanical signals could come potentially emerge from tumor cells, subsequently triggering astrocyte activation via disruption of these cell surface complexes such as cadherins and β-catenin [161, 162]. The genes activated by β-catenin signaling are regulatory and often lead to proliferation and migration [11, 163]. Interestingly, Yang et al. (2012) found this contact initiated activation of astrocytes to parallel what occurs in the transformation of astrocytomas, further coupling the process of astrocyte activation and tumor progression [162].

6. Therapeutic opportunities for cancer emerging from astrocyte-tumor cross talk

As stated previously, homeostasis in brain environment is key for the functionality of the brain, and therefore key checkpoints, such as the BBB, are responsible for maintaining homeostasis [30]. The BBB also prevents access of key drugs into the brain for targeting tumor cells. Any surgical intervention in the brain clearly has quality of life considerations, and does not offer complete disease-free state. Therefore, it is of importance to prevent tumor cells from entering the brain or block the target routes and underlying mechanisms used by tumors to circumvent checkpoints. It is worth noting that the regions of the brain which are free from the protections of the junctional characteristics of the BBB, such as the stroma of the choroid plexus and area postrema have increased vascular permeability which can be problematic, and therefore must be considered when trying to block tumor cell entrance into the brain [30].

As we know, astrocytes are capable of signaling to trigger tumor cell (breast, lung, skin, and brain) migration, invasion and metastasis *in vivo* [88, 95, 127, 160]. There are many targets in the brain microenvironment that provide effective intervention strategies for metastasis, and is reviewed elsewhere [164]. Here, we will discuss targets and mechanisms at the signaling interface of tumor cells and astrocytes that offer fresh perspective on intervention strategies.

6.1. Enzyme targets

As discussed earlier, we and others have identified astrocyte secreted MMP-2, MMP-9, and MMP-1 to promote tumor progression, and blocking them with broad spectrum MMP inhibitors does influence tumor metastasis in pre-clinical models [95, 96, 165–167]. Interestingly, MMP-1 was one of 21 MMPs that showed clinical significance in regards to breast cancer brain metastasis, and expression analysis of brain-seeking triple negative breast cancer clonal cells confirm MMP-1 and MMP-9 as potential targets [133, 168]. Therefore, these studies suggest either MMPs or the underlying pathways that regulate their expression as pharmaceutical targets. Given that targeting MMPs in the past using first generation MMP inhibitors resulted in disappointing results in the clinic, we also suggest that next generation, highly-specific MMP inhibitors, applied locally, could be effective new strategies to consider in preventing further growth and movement of tumor cells to a second location in the brain [169].

6.2. Gap junction protein targets

Astrocytes are co-opted to up-regulate survival genes in tumor cells and induce protection from chemotherapy [65]. Downregulation of the astrocyte-initiated survival gene expression in tumor cells will render tumor cells sensitive to chemotherapy [65]. This chemoprevention role, however, appears to be contact dependent, utilizing gap junctions to mediate the changes in tumor cells. Previously, gap junction proteins Cx43 and Cx26 were utilized by breast cancer and melanoma cells to initiate brain metastatic lesion formation in cohort with the vasculature [170]. Indeed, patient data analysis revealed increased cancer recurrence and metastasis with increased expression of Cx26 and Cx43 in primary melanoma and breast tumor cells. The recent work done by Chen et al. shows that brain metastatic breast and lung cancer cells initiate contact with astrocytes through gap junctions, which produces a signaling

response (discussed in detail earlier in the chapter) resulting in chemoresistance [74]. Bio-available modulators of gap junctions, meclofenamate and tonabersat, could influence this paracrine signaling loop, and thus could be proposed for treatment of established brain metastases [74].

6.3. PTEN, exosomes and miRNA targeting

Breast cancer metastases often show common alterations in the EGFR and HER2 driven pathways, both of which are regulated by *PTEN* gene [171]. PTEN is mutated in human brain, breast and prostate cancer, and loss of PTEN was found in a substantial portion of breast cancer brain metastases samples significantly associated with triple negative breast cancer [172, 173]. Interestingly, PTEN loss promotes a feedback loop between tumor cells and glial cells, which contributes to disease progression. We already know one mechanism in which *PTEN* expression is lost; through the targeting and degradation of transcript by miR-26a and miR-19a from astrocyte secreted exosomes [110–112].

Blocking the astrocytes from secreting the PTEN-targeting microRNA rescues the PTEN loss and importantly suppresses brain metastasis *in vivo* [112, 174]. Similarly, miR-200 containing extracellular vesicles, which regulates the mesenchymal to epithelial transition, can be trans-ferred from metastatic cells to non-metastatic cells leading to promotion of metastasis [175]. Therefore, collectively, approaches that promote PTEN expression or prevent loss of PTEN expression has the potential to influence metastatic outcome in the clinic for select cancers such as breast, brain and prostate cancer.

6.4. Adaptations (environment)

The finding that breast cancer cells take up a neuronal phenotype when they are in the brain suggests co-evolution adaptive mechanisms associated with metastatic cells and their micro-environment. The variable PTEN expression in metastatic tumor cells in response to differ-ent organ environments suggests a genetic component that drives co-evolution adaptive behavior between metastatic cells and their microenvironment [176]. Brain homing MDA-MB-231 cells secrete bone morphogenic protein-2 (BMP-2), which mediates the differentia-tion of NSCs into astrocytes; subsequently, downregulation of BMP-2 in the brain homing tumor cells diminished their engraftment and colonization abilities [176]. Further, when co-cultured with NSCs, primary (non-brain homing) MDA-MB-231 cells fail to proliferate over 15 days, but brain homing MDA-MB-231 cells escaped this growth inhibition, and prolifer-ation occurred in parallel with NSCs' differentiation into astrocytes [176]. This suggests that both the brain homing MDA-MB-231 cells' adaptive phenotype and the NSCs' differentia-tion into astrocytes are codependent, meaning the brain homing MDA-MB-231 cells require astrocytic signals to survive. This group extended these observations further and demon-strated that human breast cancer cells found in the brain and not in the primary tumor, upregulated γ-aminobutyric acid (GABA) pathway genes, and displayed GABAergic pheno-types that are similar to neuronal cells [177]. This phenotype offers a proliferative advantage to tumor cells because GABA is catabolized into succinate which generates NADH, a critical metabolite necessary for tumor cell sustenance. It is noteworthy that GABA is abundant in

the brain, and perhaps tumor cells have adapted to this environment that gives them a proliferative advantage. Of the different cells in the brain, neurons, because of their function, require the majority of ATP [178]. As such, astrocytes expend less energy, and secrete lactate that is generated by glycolysis [179]. This evolutionary adaptation feature of tumors and their reliance on astrocytic signals open up avenues for targeting; several inhibitors of metabolites such as GABA are available and could be repurposed for brain metastases treatment. Of course, more research and context-specific treatment of such modalities will be needed.

6.5. CNS tumor immunotherapies and astrocytes

The emergence and success of immunotherapy techniques in many blood, lymph, and some solid tumors is bringing groundbreaking and exciting work in the cancer research field [180]. Currently, researchers are now looking for ways to modulate this therapy so it can be applied to more tumors, including tumors in the CNS [141]. Importantly, the effectiveness of strategies such as vaccine and immune checkpoint therapies rely on a strong response and presence of tumor infiltrating immune cells for antigen presentation, which is low in most brain tumors due to the limited presence of resident immune cells within the brain [141, 181]. Some strategies to stimulate the immune response, such as adjuvants or tetanus and diphtheria boosters with vaccine administration have increased effectiveness [141, 182, 183]. As discussed earlier, astrocytes play an important role in immunosuppression in the brain tumor microenvironment, however this function has not yet been targeted. Therefore, one could postulate investigation of a combination therapy targeting an immunosuppressive factor(s) produced by astrocytes as an additional option worthy of research.

Much excitement in the immune therapy world surrounds the programmed death ligand-1 (PD-L1), an immune checkpoint signal that is immunosuppressive by binding its cognate receptor, programmed death-1 (PD-1) receptor, expressed on T-cells to induce apoptosis [184]. Targeting and blocking PD-L1 or PD-1 with antibody therapies has been an effective treatment for several cancers [185, 186]. It has been shown that GBM tumors highly express PD-L1, in addition to infiltrating microglia [187, 188]. Normal astrocytes have also been found to highly express PD-L1, however astrocyte expression of PD-L1 in a tumor setting has not yet been investigated [189]. Future work investigating astrocyte (normal and reactive) expression of PD-L1 is needed and will provide mechanistic hypothesis to current clinical trial that utilizes nivolumab, a PD-1 antibody, in combination with temozolomide for treatment GBM. PD-L1 has also been investigated in metastatic brain tumors, however expression and correlation to outcomes appear to be tumor dependent, leading to conflicting reports on whether PD-L1 expression correlates to a positive or negative prognosis, therefore more research is needed [190–192].

7. Conclusions

Until recently, the complexities of astrocyte signaling and influence on human pathologies were not fully appreciated in the literature, especially in regards to astrocytes' influence on

tumor biology. Cancer researchers began recognizing that tumor cells themselves may not be the sole perpetrators in tumor initiation and progression, and the resulting research has made it increasingly clear that tumor cells and the host environment they reside in are constantly communicating to facilitate growth, sustenance and metastasis [193, 194]. To facilitate tumor progression, the host environment is either co-opted by tumor cells or defense mechanisms of host cells are overcome by the tumor cells. Whether astrocytes are "friends" or "foes" of tumor cells is a matter of context, as evidence exists for both scenarios. **Figure 1** depicts the balancing act of these functions, in addition to the outside factors which dictate them, eventually determining the fate of the respective tumor cells and tumor as a whole. There are many known (and unknown) factors that must be considered in understanding CNS tumors and their relation to astrocytes. We summarize some of the tumor promoting mechanisms of astrocytes, which have been highlighted in this chapter (**Figure 2**), effecting both primary brain tumors and secondary brain metastases. The diversity of astrocyte mechanism modalities will hopefully bring about unique and novel intervention strategies, some of which were also discussed in this chapter. In conclusion, astrocytes are a critical cell type that participate in various physiological and pathological conditions, and their role in the history of tumor progression is beginning to be appreciated.

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