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ASYMMETRIC CELL DIVISION: IMPLICATIONS FOR GLIOMA DEVELOPMENT AND TREATMENT

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Abstract

Glioma is a heterogeneous disease process with differential histology and treatment response. It was previously thought that the histological features of glial tumors indicated their cell of origin. However, the discovery of continuous neuro-gliogenesis in the normal adult brain and the identification of brain tumor stem cells within glioma have led to the hypothesis that these brain tumors originate from multipotent neural stem or progenitor cells, which primarily divide asymmetrically during the postnatal period. Asymmetric cell division allows these cell types to concurrently self-renew whilst also producing cells for the differentiation pathway. It has recently been shown that increased symmetrical cell division, favoring the self-renewal pathway, leads to oligodendroglioma formation from oligodendrocyte progenitor cells. In contrast, there is some evidence that asymmetric cell division maintenance in tumor stem-like cells within astrocytoma may lead to acquisition of treatment resistance. Therefore cell division mode in normal brain stem and progenitor cells may play a role in setting tumorigenic potential and the type of tumor formed. Moreover, heterogeneous tumor cell populations and their respective cell division mode may confer differential sensitivity to therapy. This review aims to shed light on the controllers of cell division mode which may be therapeutically targeted to prevent glioma formation and improve treatment response.

Keywords

Brain tumor; Astrocytoma; Oligodendroglioma; Asymmetric cell division; Symmetrical cell division; Stem cell; Progenitor cell; Cancer stem cell; Chemotherapy; Cell of origin

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Introduction

The regulation of brain tumor development and the ability of brain tumors to overcome conventional treatment have been intensely studied over past decades. Improved understanding of neural stem and progenitor cell division modes and mechanisms has led to the hypothesis that disruption of the tightly controlled ratio between asymmetric and symmetric cell division of stem and progenitor cells may lead to an aberrant predominance of symmetrical cell division, producing daughter cells with increased replicative potential and thus susceptibility to tumorigenic transformation [1]. Despite substantial insights provided by these studies, some open questions still remain. This review intends to discuss the cell division mode involved in the cell of origin and maintenance of a stem or progenitor like population within astrocytoma and oligodendroglioma and how the pathogenesis of these brain tumor types differ from each other in relation to cell division mode. Specific asymmetric cell division controllers will also be discussed for their potential role in tumorigenesis and treatment resistance, and for their actionable potential, as with further research these could be translated to a clinical setting.

The adult brain is predominantly made up of neurons, astrocytes, oligodendrocytes, ependymal cells and meningeal cells and there are brain tumors that histologically resemble each of these cell types, sometimes in combination with each other. Cancers are clonal in origin and until recently it has been unclear how a single cell type in the brain, including terminally differentiated neurons, has the ability to give rise to heterogenous brain tumors. However, the capability of stem cells to generate many differential progeny provides a probable explanation for this process, especially for brain tumors that may contain a mixture of cell types. Therefore stem and progenitor cells are likely cells of origin for the development of tumors in the brain and the disruption of asymmetric cell division is one mechanism for neoplastic transformation.

The term neural stem cell may be used to refer to radial glial cells due to their capacity for continued self-renewal in conjunction with production of cells for the differentiation pathway. Within gliomas there exists a subpopulation of cancer cells which have similar properties to the normal neural stem cells. Asymmetric cell division in the brain is a process by which neural stem or progenitor cells are able to divide to both self-renew and produce daughter cells for the differentiation pathway. This involves the asymmetric distribution of cell fate determinants between the daughter cells, through the establishment of cellular polarity prior to cell division. Thus stem and progenitor cells are able to produce two different cell types during a single mitosis. In contrast, symmetrical cell division is characterized by the production of two identical daughter cells that contain equivalent concentration of fate determining proteins.

Based on studies in *Drosophila*, the regulation of asymmetric cell division of neural stem cells requires a concerted effort of polarity proteins, mitotic spindle regulators and cell fate determinants. Many genes involved in asymmetric cell division are conserved and they range from kinases, phosphatases, GTPases, etc. Thus, many asymmetric cell division regulators have actionable potential. Therefore, pharmacological agents which target asymmetric cell division have high translational potential and may possibly be used to

prevent brain tumorigenesis or overcome treatment resistance. However, further basic and preclinical testing is required in order to clarify the regulation of cell division mode and the role of compounds targeting asymmetric cell division controllers in brain tumor treatment. Another comprehensive review of asymmetric cell division and cancer has recently been published [2], although the current review will be focused specifically on glioma and will discuss the translational implications of studies on asymmetric cell division regulators.

Cell division in mammalian brain development

As in most adult tissues, the differentiation hierarchy in the brain includes stem cells, transit amplifying cells, lineage-committed progenitor cells and mature cells of various types. During brain development in mammals, there is evidence of neural stem cells utilizing both symmetrical and asymmetric modes (Figure 1).

Neuroepithelial cells and radial glia cells

The brain is developmentally derived from the neural plate, which becomes the neural tube from which neuroepithelial cells arise [3]. During a subsequent proliferative period, neuroepithelial cells undergo predominantly symmetrical proliferative divisions to increase the pool of neuroepithelial cells with self-renewal capacity. It has been shown in dissected *Drosophila* optic lobe neuroepithelial cells that this cell type completes several symmetrical cell divisions before transitioning to asymmetric cell division in order to produce the multiple cell types that make up the central nervous system [4, 5] (Table 1). This transition from the proliferative to the neurogenic phase occurs when Sox 1 transcription factor expression is reduced in favor of Pax 6, which drives the formation of radial glial cells over the less differentiated neuroepithelial cells [6].

Neuroepithelial cells may produce one neuron or basal progenitor cell, also known as an intermediate progenitor, during asymmetric cell division [7] (Table 1). These more differentiated daughter cells lose both the apical and basal process to migrate to the subventricular zone. Basal progenitor cells divide symmetrically to produce two neurons, or rarely two basal progenitors [7–9]. In addition to being produced by neuroepithelial cells, basal progenitor cells may also be produced by asymmetric division of radial glial cells or outer radial glial cells.

Radial glial cells are located within the ventricular zone and have both apical and basal processes used to contact the lumen of the ventricle as well as the pial surface of the neural tube contacting the meninges [8]. Between radial glial cells are tight and adherens junctions at the apical end feet, maintained through the actions of Numb and Numbl and are required for the maintenance of radial glial cell polarity [10, 11]. Radial glial cells are able to divide either symmetrically or asymmetrically (Figure 1) and have been shown to undergo proliferative symmetrical cell divisions where radial glial cells or basal progenitors are produced, symmetrical neurogenic cell divisions where two neurons are produced or asymmetric cell divisions where radial glial cells, outer radial glial cells, basal progenitors or neurons are produced in combination with each other [12, 13] (Figure 1 and Table 1). Once radial glial cells become post-mitotic, they transition from Pax6 expression normally

exhibited by radial glial cells to Tbr2 when they become progenitor cells and then finally Tbr1 once they reach the neuronal phenotype in the developing cortex [14, 15].

The outer radial glial cells and outer subventricular zone

More recently, a second radial glial cell type was discovered in the subventricular zone. These are derived from asymmetric division of radial glial cells, after which they migrate from the ventricular zone to the subventricular zone. These cells retain the basal fiber previously belonging to the mother radial glial cell and like their mother radial glial cells express Pax 6 [16–18]. Thus they have been termed outer radial glial cells and the area that they populate is called the outer subventricular zone. Outer radial glial cells may divide asymmetrically to both self-renew and produce either a basal progenitor or neuron [17, 19] (Figure 1 and Table 1). This is a process which relies on integrin signaling and involves the more basally located daughter cell once again inheriting the basal fiber to become another outer radial glial cell and the more apical daughter cell undergoing differentiation [17, 20]. The outer subventricular zone in humans is much larger than in rodents and due to its high proliferative activity of outer radial glial cells and their transit amplifying progeny, it is believed to be crucial for the massive increase in neuron number in the human neocortex [21].

It was previously thought that the neurogenic phase was the only time during which new neurons were produced [22]. However, it is now clear that neurogenesis is sustained at postnatal stages and possibly throughout adult life in both vertebrates and invertebrates alike, indeed in the human brain [23]. This occurs largely in the subventricular zone surrounding the lateral ventricles and in the subgranular zone of the dentate gyrus within the hippocampus in mammals, where there is a resident pool of stem cells derived from radial glial cells that persists throughout adulthood [24]. A recent study has also shown that cortical interneurons may have a non-epithelial stem cell origin within the human ganglionic eminences, which are transitory brain structures evident during the embryonic and fetal stages of development [25]. This non-epithelial stem cell had not previously been reported and is characterized by the lack of radial fibers [25].

Gliogenesis

Astrocytes—Gliogenesis becomes predominant over neurogenesis following cortical development. Mek1, Mek2, Notch, STAT3 and bone morphogenic proteins have been experimentally shown to be required for this transition in radial glial cells generally referred to as the neurogenic-gliogenic switch [26–32].

Astrocytes are glial cells in the central nervous system, which support the structure of the blood-brain barrier and help to maintain brain homeostasis. It has been demonstrated that astrocytes are derived either directly from a radial glia cells or via a glia restricted progenitor [33] (Table 1).

Another pathway of astrocyte production may be through an intermediary progenitor cell [33]. There are likely several different classes of heterogenous glial restricted progenitor cells, although the scarcity of markers to differentiate between them has led to some

confusion and controversy in the field. Furthermore, there is great diversity in morphology and transcriptional regulation of gene expression between different populations of astrocytes, like the grey matter and white matter astrocytes, suggesting a differential origin [34]. However, there is also evidence of astrocyte production through an astrocyte specific progenitor, which is derived from a glial restricted progenitor cell characterized by A2B5 expression [35] (Table 1). Astrocytes may also be generated locally by symmetrical division of existing astrocytes (Table 1) [36]. Therefore it is likely that astrocytes are generated by multiple different pathways both including and excluding glial restricted progenitor cells [37].

Oligodendrocytes—Oligodendrocytes are the myelinating glial cells of the brain and are derived from oligodendrocyte progenitor cells characterized by platelet-derived growth factor receptor-alpha, NG2 proteoglycan and Olig2 expression [38, 39]. This is a separate progenitor cell lineage than that evident for neurogenesis, although this pathway may overlap with the genesis of astrocytes [40, 41]. Wnt, Olig1 and Olig2 signaling are important for the stimulation of oligodendrocyte production [40, 42], whereas Pax 6 has been shown to selectively inhibit production of oligodendrocytes [42]. Clonal analyses of O2A oligodendrocyte progenitor cells *in vitro* suggested that they initially undergo a finite number of symmetrical cell divisions, followed by a final asymmetric cell division to both self-renew and produce oligodendroglia. The oligodendrocyte progenitor division mode at adult stages may be more evenly distributed between asymmetric and symmetric divisions [41, 43, 44] (Table 1).

Cell division mode regulation and cancer

It has previously been shown that cells need to acquire several mutations before becoming cancerous [45], such that there is commonly intermediate transition to dysplasia before cells gain the final mutations to be classified as neoplastic. Furthermore, cancer cells often exhibit a multitude of genetic mutations, only a small subset of which may be required for tumorigenic transformation to occur - these mutations are known as driver mutations [45, 46]. Due to this stepwise accumulation of a few genes to produce the hallmarks of cancer, the number of cells with replicative potential and self-renewal capacity is tightly controlled, particularly for organisms that live a long time, as the lineage of such cells may be maintained throughout the lifespan. Therefore the control of cell division mechanisms which dictate stem cell self-renewal are of vast importance and are closely regulated in the brain.

Asymmetric cell divisions differ slightly between species and between cell types, but their general principle and many genes encoding for asymmetric cell division regulators are conserved. The induction and maintenance of asymmetric cell division is conducted through cooperation of several different factors, including intrinsic fate determinants, mitotic spindle orientation and extracellular cues. The full extent of the mechanisms controlling cell division mode in mammalian neural stem and progenitor cells are yet to be elucidated. However, this process has been extensively studied in *Drosophila* neural stem cells and studies showed that the mammalian homologs of fate determinants and polarity controllers play a similar role in the mammalian central nervous system. Therefore previous studies focused on *Drosophila* cell division mode controllers could have implications for

tumorigenesis in the human brain, although further investigation is required in order to validate this hypothesis.

Control of asymmetric cell division in *Drosophila* neuroblasts has been thoroughly reviewed in several recent papers [2, 47, 48]. In brief, neuroblast asymmetric cell divisions are characterized by the production of a self-renewing neuroblast daughter cell and concurrently a smaller, more differentiated ganglion mother cell from which mature neurons are derived. Cell polarity is established through the formation of an apical complex, which leads to correct spindle orientation and the creation of a basal complex [49].

The apical complex is made up of atypical protein kinase C (aPKC), Partition defective 3 also known as Bazooka (Par 3), Partition defective 6 (Par 6), Inscuteable (Insc), Partner of inscuteable (Pins), Gαi, Dig, Canoe and mushroom body defect (Mud) [50–54]. This apical complex is preferentially distributed into the self-renewing neuroblast daughter cell, whereas the basal complex becomes localized within the ganglion mother cell [55]. The basal complex is made up of Numb, Partner of numb (Pon), Prospero (Pros), Miranda (Mira), brain tumor (Brat) and Staufen (Stau) [56, 57]. These fate determining factors are known as the cell intrinsic controllers of asymmetric cell division. Many studies have indicated that all of the intrinsic controllers of asymmetric cell division are interrelated, such that if one of these factors is disrupted, then this may result in aberrant size ratios or cell fates of the daughter cells produced [49, 50, 58].

Asymmetric cell division regulation in mammalian neural cells

Many mammalian homologs of *Drosophila* cell fate determinants have been identified. These substances have been shown to act in the same manner within mammalian radial glial cells and in some cases have been able to perform the same function within *Drosophila* neuroblasts when used to replace their corresponding invertebrate homologs. Illustrating this, both *Drosophila* and mammalian Numb are able to perform normal interactions with aPKC *in vitro* [59]. Moreover, when applied to Numb mutant *Drosophila* embryos, mammalian numb is asymmetrically distributed into the cell fated to the neuronal pathway [60]. Similarly, the *Drosophila* fate determinant Brat is thought to be homologous to mammalian TRIM32, which during asymmetric cell division becomes preferentially located within the more differentiated daughter cell [61].

Current studies are directed at determining the degree of functional conservation in the regulators of cell fate determination and asymmetric cell division in mammalian radial glial cells. As described previously, radial glial cells have both an apical and basal process, which aids in their ability to interact with the mammalian brain microenvironment and effectively produce the heterogeneous cell types that make up the brain. During asymmetric cell division, the basal process of radial glial cells is inherited by the more basal daughter cell. Within this process, Cyclin D2 is also asymmetrically inherited by this daughter cell and is involved in the promotion of a self-renewing cell fate [62].

Despite the complex nature of mammalian asymmetric cell division and the specialized shape of radial glial cells a few of the key regulators have been identified, although it is expected that in due time more will be elucidated (Figure 2). As mentioned previously,

mammalian numb has been found to perform similar functions to its *Drosophila* counterparts. Indeed, demonstration of asymmetric distribution of Numb between daughter cells in embryonic mice has shown that Numb was preferentially segregated into the b-tubulin III-positive daughter cell destined for the neuronal pathway (Figure 2)[63]. Interestingly, this phenomenon was more pronounced as time progressed from embryonic days 12–14 [63]. Furthermore, Numb knockout mice have been shown to undergo significantly fewer asymmetric cell divisions than their wild type counterparts [63].

Studies have also found evidence of asymmetric distribution of epidermal growth factor receptor (EGFR), which was mediated by Notch signaling [64, 65]. The EGFR inheritance influenced cell fate, as FACS sorting of the cellular population with high EGFR expression revealed that these cells formed twice as many neurospheres than their low EGFR expressing counterparts [66]. Another study showed that the daughter cell with high levels of EGFR also displays markers for radial glial cells and thus is the self-renewing daughter cell (Figure 2) [65]. Additionally, the mRNA binding protein Stau2 has been found to be asymmetrically segregated during cortical development in the mouse into the more differentiated daughter cell to promote a neuronal cell fate, such that its knockdown resulted in neuronal mass formation in the peri-ventricular region [67].

As has been well characterized in *Drosophila* neuroblasts, Par 3 is also important for the asymmetric cell division of mammalian radial glial cells [68]. Par 3 becomes asymmetrically localized and is preferentially distributed into the less differentiated daughter cell during asymmetric cell divisions in the developing mouse neocortex (Figure 2)[68]. Furthermore, either the inhibition of Par 3 or its ectopic expression resulted in a significant reduction in the proportion of asymmetric cell divisions in favor of symmetrical cell division when compared with control constructs [68].

Musashi-1 (Msi1) is integral to the asymmetric cell division of precursor cells during *Drosophila* sensory organ development [69]. In mammals it has been shown to enhance Notch inhibition of Numb to increase self-renewal of neural stem cells [70]. However, when the Msi1 gene was disrupted in neural stem cells, there was no significant difference in their ability to selfrenew [71]. Therefore, further investigation is required to determine if Msi1 performs a similar asymmetric cell division regulatory function in mammalian neural stem cells as in *Drosophila* sensory organ precursors.

In contrast to the discussed asymmetry controllers, abnormal spindle-like microcephaly-associated protein (ASPM) promotes symmetrical cell division in mammalian cells. In mouse embryonic neuroepithelial cells ASPM is downregulated when the transition from proliferative symmetric cell divisions to neurogenic asymmetric cell divisions occurs [72]. The mechanism of this switch may be through decreased ASPM microtubule interactions that lead to alterations in the cleavage plane of the cells, influencing the mode of cell division [72]. Therefore ASPM knockdown results in decreased neuroepithelial cell numbers through reduced proliferative symmetrical cell divisions [72].

In addition to discussing the potential roles of classical asymmetric cell division controllers in brain tumor development, maintenance and treatment, this review will also demonstrate

the dysregulation of factors with a less well-characterized role in asymmetric cell division in central nervous system malignancies. The importance of this is that many asymmetric cell division controllers are able to be targeted pharmacologically, which in the future may have therapeutic benefits for patients suffering from glial brain tumors.

Glioma

The term glioma encompasses all brain tumors characterized by histological evidence of neoplastic glial cells, which includes astrocytoma, oligodendroglioma and ependymoma. This review is focused predominantly on astrocytoma and oligodendroglioma (Table 2). Glioma makes up a large proportion of brain tumor sufferers and may affect patients throughout any stage of life (Table 2).

Recently, glioblastoma multiforme (GBM) have been classified according to four molecular subtypes based on gene expression studies. These are the proneural, neural, classical and mesenchymal molecular profiles. The classical subtype has been characterized by EGFR mutation, and has been shown to be the variant which responds most favorably to aggressive treatment [83]. The mesenchymal subtype has a high frequency of *NF1* mutations and the proneural displayed evidence of alterations in *PDGFRA*/*IDH1* along with poor response to aggressive therapy [83].

The only treatment combination that has improved overall survival of glioma patients is radiation in conjunction with the alkylating agent temozolomide [84]. Currently under investigation is Avastin, also known as bevacizumab, a monoclonal antibody against vascular endothelial growth factor and as such is an anti-angiogenic drug. However, this treatment has only been able to provide symptomatic improvement for patients and ultimately makes gliomas more aggressive upon resistance development and relapse [85, 86]. Therefore the development of alternative treatment strategies is of vast importance and this review will discuss the role that asymmetric cell division controllers may have in improvement of therapy for glioma. Furthermore, the current review will be focused predominantly on the role of cell division mode and its control in the development, maintenance, recurrence and treatment-resistance of gliomas.

Brain tumors are largely distinguished from each other based on their histological features and cell types present. In the past it was thought that these histologically evident cell types were the cells of origin for different brain tumors. For example, astrocytomas were thought to be derived from astrocytes, oligodendrogliomas from oligodendrocytes, ependymomas from ependymal cells and meningiomas from meningeal cells that had undergone tumorigenic transformation. However, given this model, it remained unclear how terminally differentiated cells could become transformed and how cells of a committed lineage could give rise to a heterogeneous tumor.

Cancer is characterized by cells that do not rely on external proliferative stimulation, are able to evade growth suppression and death signals from the tumor microenvironment including the immune system, the ability to grow new blood vessels, invasion of surrounding tissue and the ability to metastasize [87, 88]. These features are brought about by genetic alterations which generate diverse cellular features and lead to a pro-

inflammatory environment conducive to tumor growth [89, 90]. Replicative ability is required for the acquisition of mutations that lead to neoplasia in the brain and throughout the body. Therefore stem cells, with their self-renewal properties and high replicative potential are likely candidates for brain tumor cell of origin. As such, it has recently been proposed and subsequently demonstrated in genetically engineered mouse models that instead of being resultant from differentiated cells acquiring tumorigenic capacity, that brain tumors originate rather from neural stem and progenitor cells, with multipotent radial glial cells being a likely candidate [91]. Moreover, stem cells with the ability to self-renew require fewer mutations in order to become cancerous, when compared with their more differentiated counterparts [92]. However, it remains unclear how temporal and regional differences in neural stem and progenitor cells may influence mechanisms of malignant transformation and the development of different brain tumor types.

Discovery of the exact cell of origin for different types of brain tumors is of vast importance as it may have implications for treatment and preventative measures [93]. However, it is important to note that the cell of mutation and cell of origin for cancer may be distinct entities, as the initial cell of mutation may not acquire enough mutations to allow for tumorigenic transformation, but rather a more lineage restricted progenitor cell derived from the cell of mutation may be the actual cell of origin through inheritance of the preliminary mutation [94, 95].

It is possible that control of asymmetric cell division is more robust in cells higher in the stem cell hierarchy, such as neural stem cells. Thus progenitor cells may be more susceptible to malignant conversion than their counterparts at an earlier developmental stage [95].

In contrast, neural stem cells have increased replicative potential over their derivative progenitor cells. Progenitor cells have been shown to complete a finite number of replications before becoming post-mitotic [96, 97], whereas stem cells have unlimited capacity for self-renewal [98]. Specific mutations may confer a predilection for malignant transformation at particular stages of differentiation and could be responsible for the heterogeneity of brain tumor histology and differential genetic profiles evident in each subtype. For example, K-ras activation is sufficient for tumorigenesis during the early stages of radial glial cell development, but must be combined with p53 inactivation to have the same effect in more differentiated cells at a later time point in development [99]. The variance in susceptibility of cells along the hierarchy of differentiation to tumorigenic transformation highlights the fact that genetic changes are context dependent and may involve complex interactions with the brain microenvironment at different stages of development [100]. This must be taken into account when formulating measures to prevent glioma development, which may be able to be achieved through manipulation of cell division mode in the pre-neoplastic brain.

In conjunction with the differences in self-renewal capacity between stem and progenitor cells in the brain, there are also regional differences in differentiation potential [101]. This has implications for brain tumor development, as demonstrated using a genetically engineered mouse models of common glioma driver mutations, where neurospheres were derived from the wall of the third ventricle and from the lateral ventricles [102]. *p53*

inactivation caused proliferation in both locations, whereas *PTEN* loss only affected the subventricular zone derived neurospheres and both *Nf1* loss or *KIAA1549:BRAF* overexpression only affected the third ventricle derived neurospheres in this way [102]. This may explain why gliomas with specific genetic lesions are commonly found in specific brain areas. This information is important, as the brain regions venerable to malignant transformation may be those that should be therapeutically targeted to preserve the normal cell division mode.

Dysfunction of asymmetric cell division through disruption of its control mechanisms is sufficient for cancer development in the brain, as has been shown in invertebrates [103]. For example, D*rosophila* neuroblasts with mutations in *Pins*, *Mira*, *Numb* or *Pros* had significantly increased proliferative potential, as evidenced by a 100 fold increase in brain tissue size resulting in mortality within 2 weeks [103]. Upon transplantation into new hosts, this tissue was found to be tumorigenic with associated genomic instability [103]. Improved understanding of this phenomenon may lead to the development of preventative treatments or improved therapeutic options for brain tumor patients through the identification of novel targets that are involved in the control of asymmetric cell division in human brain tissue.

Cell division mode in glioma origin

Some researchers have proposed a common cell of origin for all glioma tumors since astrocytes, oligodendrocytes and ependymal cells are all derived from a common ancestor, the radial glial cell. However it has also been suggested that any cell along the developmental pathway that leads to astrocyte and oligodendrocyte production, including the most mature cells, may be transformed to become tumorigenic. In contrast to the radial glial theory of glioma origin, other researchers have proposed that oligodendrogliomas originate predominantly from oligodendrocyte progenitor cells, whilst astrocytomas originate from the more stem-like radial glial cells (Table 3). Furthermore, many of the genetic mutations experimentally used to recapitulate human gliomas as closely as possible have been implicated in control of cell division mode. Therefore it is possible that disruption of asymmetric cell division, in favor of symmetrical cell divisions, promotes increased proliferative potential and thus commonly results in tumor formation. However, this hypothesis has yet to be exhaustively tested.

In an attempt to determine the glioma cell of origin, a *p53*/*Nf2* mutation driven mouse model of glioma was utilized to show that even when the initial cell of mutation is a neural stem cell, the cell of tumor origin is in fact an oligodendrocyte progenitor cell [95]. The loss of *p53* in cancer favors symmetrical cell division. This has been shown in mammospheres, where restoration of *p53* was correlated with resumed asymmetric cell division [117]. Therefore it is also possible that the *p53* mutation in neural stem cells may contribute to an aberrant increase in symmetrical cell divisions in their derivative oligodendrocyte progenitor cells, but not in the neural stem cells themselves. Although the effect of *p53* mutation on asymmetric cell division of neural stem and progenitor cells has yet to be established, it would be of interest for future studies to determine if there is a lineage and maturity-specific effect of *p53* mutation on the mode of cell division in the brain.

The *p53* gene is mutated in approximately 48% of anaplastic astrocytoma and 31% of GBM [118]. Mutation in *p53* alone is not sufficient for astrocytoma formation [119]. However it does confer proliferative advantage to the slow dividing neural stem cells and fast proliferating progenitor cells of the subventricular zone in adult mice carrying *p53* deletion [119]. However, in mice which harbor *p53* and *Nf1* deletion, astrocytomas develop, the majority of which display characteristics of GBM [120]. *Nf1* is deleted in approximately 18% of GBM and 53% of the mesenchymal molecular subtype of GBM [83]. When inactivated, *Nf1* results in pro-mitotic Ras signaling. Furthermore, these tumors first appear in the regions of the brain in which stem cell populations reside, like the subventricular zone [120].

Based on mathematical modeling, it has been proposed that mutations which promote symmetric cell division, like overexpression of *PDGF* or *p53* deletion, result in a progenitor cell of origin for astrocytoma, whereas other mutations which do not increase symmetrical cell division result in a neural stem cell of origin [121]. Recent studies from within our lab have shown that neural stem and progenitor cells have distinct sensitivities to MAPK pathway activation with regards to their cell division mode, such that asymmetric cell division is more sensitive to disruption in progenitor cells when compared to their neural stem cell counterparts (Lerner R., unpublished data).

Alternatively, it is also possible that several central nervous system cells in the differentiation hierarchy have the potential to become neoplastic, but that different mutation combinations may have a predilection for cell division mode disruption in specific cell types (Table 3)[105, 122]. In addition, there is evidence to suggest that neural stem cells at the same stage of differentiation, but from different germinal zones, may display differential reactions to the same genetic stimulus [102].

Interestingly when a construct carrying two shRNAs targeting *p53* and *Nf1* was used to transduce neural stem cells, astrocytes and mature neurons the tumors derived from mature neurons and astrocytes displayed high levels of progenitor markers, like Nestin and Sox2, suggesting de-differentiation is involved in tumor formation [105]. Evidence for the possibility of an astrocyte origin for astrocytic tumors was also provided when murine primary astrocytes on a *p53*−/− background were transduced with c-Myc and Akt [104]. Upon *in vitro* cultivation, these cells showed stem cell marker expression, indicative of de-differentiation, and *in vivo* were tumorigenic when implanted into C57BL/6 mice [104].

It has been proposed that c-Myc is integral to this de-differentiation step of tumor formation in mature cell types. For instance, neural stem cells deficient for *Pten* and *p53* showed increased self-renewal, proliferation and failed to respond to differentiation signals *in vitro* [123, 124]. In contrast, this same differentiation stimulus caused the cells with a wild type genetic profile or only one of these mutations to flatten and lose Nestin staining [123, 124]. The pro-differentiation environment also resulted in increased c-Myc in the *Pten* and *p53* double null neural stem cells. Furthermore, the resistance to differentiation stimuli was abolished when the activity of c-Myc was inhibited [123, 124].

Some studies have shown that only self-renewing cell types and not terminally differentiated cells are able to be genetically transformed to result in tumor formation particularly with Pten deletion [106, 110] (Table 3). The tumor suppressor gene *Pten* has been proposed to play a role in asymmetric cell division maintenance, although further studies are required to validate this theory [125]. This proposition is based on mouse studies where *Pten* knockdown was localized in central nervous system tissues at mid-gestation, resulting in increased cell proliferation, cell size and decreased apoptosis, which led to increased brain size and histological abnormalities [126]. In addition, when *Pten* is knocked down in patient derived glioblastoma cells, the tumor inhibitor lethal giant larvae (Lgl) is phosphorylated and thus inactivated by aPKC that leads to the maintenance of the self-renewing phenotype in these cells [127]. However, differentiation of this self-renewing cancer cell population was initiated when Pten levels were restored to normal levels, when Lgl was constitutively activated and when aPKC was knocked down [127]. Therefore each of these factors are in all likelihood involved in the maintenance of asymmetric cell division in astrocytoma during tumor development and should be tested as possible targets to disrupt this cell division mode and possibly sensitize GBM cells to standard chemotherapeutic agents.

Based on the discussed studies, it is likely that there is no single cell of origin for astrocytoma, but rather that they may arise from any of the cells along the neural stem cell, gliogenic and neurogenic differentiation hierarchy. In this scenario, there are multiple pathways by which astrocytic tumors can be formed, which may account for their high incidence. However, these studies also suggest that neural stem cells, progenitor cells and more differentiated cell types have distinct propensity for tumorigenic transformation, with the least differentiated cells requiring fewer mutations in key pathways driving tumor growth and therefore most likely to be transformed. A possible reason for this is increased replicative potential of cells at less differentiated stages. Nevertheless, it is also conceivable that de-differentiation is required before mature astrocytes or neurons may undergo malignant transformation, thus providing an extra barrier to tumor formation in this cell type. This could explain why experimental studies have shown that some mutations are unable to elicit tumor formation from these cell types.

Moreover, the literature suggests that the predominant mode of cell division that leads to tumor formation may be cell of origin specific, with differentiation state dictating whether asymmetric cell division is disrupted. Therefore, further studies are required to elucidate the exact mechanisms involved in astrocytoma development and if changes in cell division mode are causally involved in this process.

In contrast to astrocytic tumors, the cell of origin for oligodendroglioma is much more consistent within the literature. By far, the most common cell of origin for oligodendroglial tumors has been found to be oligodendrocyte progenitor cells [112]. This predominantly different cell of origin to those evident for astrocytomas may be responsible for the improved therapeutic response evident in oligodendroglioma patients when compared to patients with astrocytomas of the same grade.

As previously discussed in this document, oligodendrocyte progenitor cells may divide either symmetrically or asymmetrically. Our group has shown that increased symmetrical

cell division in lieu of asymmetrical cell division in oligodendrocyte progenitor cells leads to oligodendroglioma formation [44]. This study was performed using a genetically modified mouse model of glioma expressing *verbB* with a S100β promoter, to overexpress *EGFR* in neural stem cell progeny that have lost *GFAP* expression, like oligodendrocyte progenitor cells [112, 113]. The described mouse model produces oligodendroglioma tumors from oligodendrocyte progenitor cells in close association with white matter tracts, as identified by NG2 and olig2 expression [112]. Furthermore, gene expression profiling of the resultant murine tumors showed an expression profile that closely resembled oligodendrocyte progenitor cells rather than that of neural stem cells [112].

To evaluate asymmetric cell division, NG2 expression was characterized in daughter cells that had recently been produced by mitosis of an oligodendrocyte progenitor cell. NG2 proteoglycan was asymmetrically localized in 41% of oligodendrocyte progenitor cell pairs derived from wild type mice, whereas this was decreased to 22% in S100β-*verbB p53*+/− mice, as evaluated by an *in vitro* pair cell assay [44]. In conjunction with this decrease in asymmetric cell division, there was also evidence of aberrant self-renewal, impaired oligodendrocyte differentiation and increased tumor initiating potential [44]. This was mirrored by the human condition, as NG2+ cells isolated from oligodendroglial tissue showed reduced asymmetric cell divisions when compared to control tissue from epileptic patients with no evidence of neoplasia [44]. This study has far reaching implications, as cell division controllers may be able to be targeted to avoid disruption of asymmetric cell division in oligodendrocyte progenitor cells and perhaps prevent oligodendroglioma tumor formation. Therefore further studies should aim to identify pharmacological targets with the ability to preserve asymmetric cell division and thereby restore differentiation and prevent aberrant self-renewal in non-neoplastic oligodendrocyte progenitor cells.

Further supporting the oligodendrocyte progenitor origin theory of oligodendroglioma, a Ctv-a mouse model where PDGF-B driven tumor induction is restricted to oligodendrocyte progenitor cells, showed tumor formation in 33% of animals [114]. Likewise, retroviral infection of cells with the marker profile of oligodendrocyte progenitor cells to express a EGFR-GFP fusion protein on postnatal day 3 in rats, demonstrated proliferation resulting in hyperplasia of this cell type [116].

However, not all experimental models show that oligodendrocyte progenitor cells are the origins for oligodendroglioma. Oligodendroglial tumors and mixed oligoastrocytomas have also been produced from genetic manipulation of *GFAP* expressing cells, thus excluding oligodendrocyte progenitor cells. For example, both oligodendroglial tumors and oligoastrocytomas developed from GFAP-positive cells, like neural stem cells or astrocytes, in *GFAP-V*^{12}Ha-ras; *GFAP-EGFRvIII* transgenic mice [111]. Additionally, transfer of *PDGF-B* to nestin expressing cells, like neural stem and progenitor cells, led to formation of oligodendroglioma in 60% of mice as evaluated at 12 weeks of age [115]. In conjunction, transfer of *PDGF-B* to GFAP expressing cells resulted in a mixture of oligodendrogliomas and oligoastrocytoma formation in 40% of mice at the same time point [115].

Therefore, it is likely that the transformation of different cell types into tumors with histological features of oligodendroglioma may be mutation specific. However, the vast

majority of experimentally induced oligodendroglioma originate from an oligodendrocyte progenitor origin. This may have implications for treatment, as it has been proposed that the mechanism which controls asymmetric cell division in these cells is more susceptible to disruption than that evident in more stem like cells, although as of yet this has not been experimentally proven.

Effect of cell of origin and cell division mode on treatment

Tumors with the same genetic mutations from different cells of origin have been shown to respond differently to preventative treatment. Combination treatment with a Ptgs2 inhibitor and an EGFR inhibitor prevented tumorigenic growth of oligodendrocyte progenitor cells, but not neural stem cells transformed with amplified *HRas* and *p53* mutation [108]. It is unclear if this phenomenon was due to the hierarchal differentiation stage of these two cell populations. It is possible that the machinery that determined the cell division mode of these cells were affected differently, both by the initial genetic mutation and by the subsequent treatment. Thus, asymmetric cell divisions may have been maintained in neural stem cells to provide resistance to treatment, but disrupted to become more symmetrical in oligodendrocyte progenitor cells, rendering them more susceptible to therapeutic intervention.

Additionally, there is some evidence to suggest that tumors originating in stem and progenitor cells are inherently more difficult to treat and show increased aggressive behavior. Clinical and magnetic resonance imaging data from 91 patients with GBM were analyzed to determine if proximity to the germinal niche affected outcome [128]. Those patients with tumors in contact with the subventricular zone showed poorer outcome and more rapid disease progression when compared with the patients who had cortical GBM [128]. It is possible that derivation from a stem or progenitor cell population enriches astrocytic tumors with cancer cells that resemble these normal brain cell types.

Tumor propagating cells

Heterogeneous cancer cell populations have been identified within brain tumors [129–131]. In this context, cancer cell heterogeneity refers to cancer cell populations with distinct genetic mutations, differentiation status and responses to external stimuli. These include tumor populations characterized by greater tumorigenic potential as evidenced by transplantation assays into immune compromised mice, when compared to the remaining tumor cell subpopulations [132, 133]. These populations have been called cancer stem cells or stem-like tumor propagating cells (TPC) for their intrinsic similarities to neural stem cells, which include expression of stem cell markers such as CD133, drug resistance, self-renewal and the generation of multi-lineage progeny. TPC have repeatedly been shown to be associated with poorer outcome due to their resistance to chemotherapeutic agents and radiotherapy [92, 134, 135]. More recently it has been demonstrated that tumor cells with expression of lineage restricted progenitor markers OLIG2 and NG2 can be highly malignant as well [136] pointing to heterogeneity in the tumor propagating population.

In vitro sphere forming assays have also been used to identify TPC. It has recently become clear that these spheres are heterogeneous and contain both stem and progenitor-like TPC

[137]. Therefore, populations referred to as cancer stem cells in some studies, may in fact contain multiple populations of cancer cells at various stages of differentiation.

Stem-like TPC are slow growing with self-renewal properties [132, 138, 139], but progenitor-like TPC are faster dividing cells which make up a large bulk of the tumor cells [133, 137]. The distinction between these tumor cell subpopulations is imperative, as they may respond differently to therapeutic agents and thus if not correctly identified, may lead to the generation of misleading results for drug screening assays. Further investigation is also required to determine how stem and progenitor-like TPC relate to each other, whether progenitor-like TPC are hierarchically derived from stem-like TPC or if these cells are able plastically transition in both directions via differentiation and de-differentiation.

It is also important to note the distinction between neural stem cells and stem-like TPC. TPC are not necessarily derived from neural stem cells, although there are similarities between them in terms of marker expression and behavior *in vitro*. For instance, both neural stem cells and stem-like TPC commonly exhibit Y-box binding protein 1 (YB-1) expression, Sox-2, nestin, and musashi-1 (Msi1) expression and with increased differentiation these markers are lost [140]. It is possible that TPC are direct descendants neural stem cells as suggested in genetically engineered mouse models of glioma. For example, using *Nestin-ΔTK-IRES-GFP* transgenic mice that were used to mark the quiescent subventricular zone neural cell population, it was shown that when these mice were crossed with *Nf1*, *p53* and *Pten* deleted mice, tumor growth ensued and that a subpopulation of the tumor cells were also marked by green fluorescent protein, likely representing the TPC population [141]. However, it is also possible that TPC originate from dedifferentiation of other more differentiated tumor cell subpopulations. Furthermore, there is increased heterogeneity of marker expression and molecular profiles for TPC over neural stem cells [142].

TPC have been identified in many human gliomas [143, 144]. Populations of TPC are able to not only recapitulate the pathophysiology of the original tumor from which they were isolated, but are also able to produce the corresponding diversity of cell populations within the tumor mass in an experimental setting. Thus, a single glioma TPC is able to produce multiple heterogeneous cell types [139, 145]. It has been proposed that a combination of asymmetric and symmetric cellular divisions allows TPC to both expand the tumor mass and produce differential progeny.

Evidence of this is presented in an *in vitro* study using single cell lineage tracing analysis of glioma TPC isolated from human surgical glioma specimens, in order to determine the mode of TSC division. This study revealed that glioma TPC predominantly perform symmetric cell divisions under expansion conditions, functioning to build up the bulk of the tumor, whilst completing principally asymmetric cell divisions under growth factor deficient conditions [146]. In this case CD133 was asymmetrically segregated between the two daughter cells, as determined by mitotic paired cell analysis [146].

This abnormally low proportion of asymmetric cell divisions, which has been identified in gliomas, may contribute to impaired patient survival through influence on treatment response. Following treatment with gamma knife surgery, radiation or chemotherapy,

gliomas are enriched for TPC positively labeled with CD133 [147–149]. Whether stem-like TPC are being selectively spared from treatment induced cell death, or alternatively if the treatment stimulates expansion of this cancer cell population is difficult to address in human patients.

Two studies suggest that the first scenario is more likely. First, studies in genetically engineered mouse models suggest that the nestin-positive TPC survive treatment with temozolamide and grow the recurring tumor [141]. Secondly, clonal analyses of 118 human GBM samples obtained from The Cancer Genome Atlas has shown that the recurrent tumor is frequently genetically distinct from the primary tumor with a small proportion of overlap [131]. Thus, in some patients the recurrent tumor contains differential proportions of specific cancer cell subpopulations when compared to the primary tumor and in other patients it may contain additional subpopulations which were not present at all in the primary tumor [131].

The homeobox gene *PROX1* is the mammalian homolog of *Drosophila Pros*, which, is part of the basal complex that maintains asymmetric cell division of neuroblasts. *PROX1* has been associated with poor outcome in human glioma and was also more prevalent in high grade astrocytic gliomas, as determined by immunohistochemistry in a series of 56 human gliomas of differing grade [150, 151]. This poorer outcome could be due to increased asymmetric cell divisions in the PROX1 labeled cell populations within these tumors [152]. Asymmetric cell division has been proposed to contribute to therapy resistance in the slow replicating stem-like TPC populations of other cancer types including gastric cancer and acute lymphoblastic leukemia [153, 154]. Therefore, a similar mechanism may confer poorer outcome to brain tumor patients with malignancies which contain a proportion of asymmetrically dividing stem-like TPC.

As previously discussed, Msi1 is a RNA-binding protein and functions to enhance Notch signaling and thus inhibit Numb, promoting neural stem cell self-renewal [70]. In a study of primary human central nervous system tumors, Msi1 expression was increased, particularly in malignant gliomas, when compared to non-neoplastic tissue and other brain tumor types [155]. This expression was related to both tumor malignancy and proliferation rate [155, 156]. In GBM cells with shRNA knockdown of Msi1, an increased number of cells were evident in G2 or M phase of the cell cycle, meaning that this knockdown increased the time these cells spent in mitosis [157]. Further, when these cells were orthotopically xenografted into NOD/SCID mice, the resultant tumors were 96.6% smaller than their non-Msi1 knockdown counterparts [157]. Therefore, further studies should be performed to determine if targeting Msi1 could be used to improve treatment of gliomas by disrupting asymmetric cell division of TPC and possibly improving tumor response to standard glioma treatment strategies.

Numb is a classical controller of asymmetric cell division and has been found to be asymmetrically segregated between daughter cells of dividing GBM stem-like TPC [158]. In this study, Numb was localized into the CD133-positive daughter cell and excluded from its CD133-negative counterpart [158]. In contrast, a study using Lgl1 knockout mice showed that brain progenitor cells are only able to symmetrically distribute Numb between daughter

cells, resulting in a failure of asymmetric cell division [159]. This led to a hyperproliferative phenotype where cells lack features of differentiation [159]. These data suggest that normal Numb function and distribution is required for asymmetric cell division of TPC, which may inhibit excessive proliferation of this population, but may also contribute to treatment resistance and tumor propagation in astrocytomas.

Supporting this, studies have shown that Numb is expressed in all grades of astrocytomas [160]. *Numb* deletions or very low levels of Numb have been found within the proneural GBM subtype, whereas much higher expression is evident in the classical and mesenchymal subtypes, which are associated with comparatively poorer outcomes for patients [158]. Thus, increased Numb in astrocytoma, specifically in classical and mesenchymal GBM may contribute to an increased proportion of asymmetrically dividing stem-like TPC within the tumor mass, leading to impaired treatment response. However this theory has not yet been validated experimentally.

Notch is important for the maintenance of stem cells and inhibition of the differentiation pathway. In astrocytoma and GBM tissue samples, Notch is elevated when compared with non-neoplastic tissue [161, 162]. Although Numb acts to inhibit Notch signaling and Numb has been associated with poor treatment response in GBM, Notch is also a hallmark of poorer prognosis for glioma patients [163]. Notch signaling has been shown to induce increased colony forming capacity, increased self-renewal and decreased differentiation in GBM neurosphere cultures [164]. Similarly, in neural stem cells, *NOTCH2* activation results in hyperplasia of the neurogenic niche and impaired neuronal differentiation. In this same study, Notch 2 signaling in GBM TPC resulted in enhanced proliferation and decreased apoptosis [165].

NOTCH activation can be promoted by the neural stem cell niche endothelial cells [166, 167]. This may in part explain why tumors located in close proximity to the ventricles produce poorer patient outcomes [128]. In conjunction, using a three dimensional culture system for surgical GBM specimens to simulate the normal tumor and tumor stroma cellular arrangement, when Notch signaling is pharmacologically inhibited with the g-secretase inhibitor DAPT (N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester), not only is there inhibition of tumor cell proliferation and self-renewal, but also a decrease in endothelial cells [168]. Therefore, Notch signaling likely plays an important role in tumor associated angiogenesis and its inhibition may be beneficial in treating astrocytoma. When Notch 1 is inhibited in stem-like TPC, their tumorigenicity is impaired when injected into Balb/c nude mice [169]. Furthermore, siRNA knockdown of *NOTCH1* in established tumors significantly slowed the cancerous growth when compared to control tumors [169]. Studies have also shown that with Notch pathway inhibition, with either a g-secretase inhibitor or arsenic trioxide, depletes the stem-like TPC population within GBM [170–173]. Furthermore, CD133-positive and CD133-negative cells, thought to represent TPC and non-TPC respectively, were isolated from human glioma surgical specimens, and treated with g-secretase inhibitors [174]. This resulted in increased sensitivity to radiation therapy in the TPC population but not the non-TPC population, which is already considered to be radiosensitive [174]. Thus, Notch pathway blockade by a g-secretase inhibitor has been suggested for therapeutic intervention following GBM diagnosis and in conjunction with

standard treatment options. These standard treatment options have long been thought to selectively spare or possibly promote the expansion of stem-like TPC, meaning that this intervention aimed at increasing TPC sensitivity to treatment could have a substantial clinical impact.

Mutation or amplification of *EGFR* is very common among adult astrocytoma, particularly GBM. The asymmetric cell division regulators Numb and Notch have both been shown to regulate *EGFR* expression. Knockdown of *Numb* in U87 glioblastoma cells increased *EGFR* expression [158] and *NOTCH1* knockdown in U251 malignant glioma cells by shRNA downregulated *EGFR* expression [161]. EGFR has consistently been shown to be instrumental in the formation and propagation of many astrocytoma tumors. In cells derived from patient GBM samples, it has been demonstrated that EGFR-positive subpopulations sorted by FACS were more tumorigenic than their EGFR-negative counterparts as evaluated by intracranial implantation into nude mice [129]. As such, EGFR inhibitors have gone into clinical trials for the treatment of high grade glioma and have shown significantly improved patient survival, although treatment resistance is a significant issue with this treatment [175, 176].

Studies have shown that EGFR is unevenly distributed between daughter cells during asymmetric cell division of neural stem and oligodendrocyte progenitor cells [44, 66]. The role of EGFR is to promote proliferation and self-renewal in the daughter cell into which it has been distributed during asymmetric cell division [44]. However, if asymmetric cell division is disrupted, then EGFR may become active in both daughter cells, thus increasing the replicative potential of the cellular population. It is currently unclear if EGFR is an asymmetric cell division controller itself, or if it is simply maneuvered asymmetrically by other factors. Similarly, at present the effect of EGFR inhibition on cell division mode in astrocytoma TPC and the possible implications for patient outcome has not yet been determined.

The Wnt pathway is frequently mutated in astrocytoma and is associated with hereditary syndromes that predispose patients to central nervous system tumor formation [177, 178]. Wnt, through the actions of beta catenin, is important for the maintenance of asymmetric cell divisions in the brain [179, 180]. GBM cells treated with Wnt ligands exhibit decreased proliferation and promotion of the differentiation pathway [181]. Another study has shown that the Wnt pathway antagonist, recombinant sFRP1, is effective at inhibiting patient derived glioma TPC self-renewal and proliferation [182].

Therefore further investigation is warranted to understand the role of the Wnt pathway in regulation of TPC division mode and the possible implications for treatment response. Towards this goal, an intracranial xenograft model of U373MG and GBM578 GBM was used to determine that the Wnt pathway is activated following radiation treatment with concurrent enrichment for a stem cell population within the remaining tumor [183]. This result suggests that Wnt and its role in maintenance of asymmetric cell division, may contribute to astrocytoma treatment resistance. Furthermore, siRNA inhibition of the Wnt pathway resulted in reduced survival of GBM cells following radiation treatment when compared to the wild type cell line [183].

Polo-like kinase 1 (Plk1) is an important component of the machinery that governs spindle orientation and mitotic progression of mammalian neural progenitor cells [184] and has been shown to be important for asymmetric cell division in non-mammalian experimental models [185, 186]. *Plk1* expression is increased in a manner correlated with degree of anaplasia in human glioma cell lines [187]. Similarly, astrocytoma TPC show an increase in the expression of Plk1 more than 100 fold above that evident for non-neoplastic astrocytes [188]. Furthermore, inhibition of Plk1 in these TPC decreased proliferation and caused G2/M arrest, ultimately leading to apoptosis [188]. Concurrently, there was a decrease in SOX2 marker expression with Plk1 inhibition, indicating that the TPC lost some of their stem cell properties [188]. Therefore it is possible that Plk1 inhibition could be used in conjunction with standard therapeutic agents, to deplete the normally treatment resistant stem-like cancer cell population within astrocytoma. Further studies should be performed to determine if Plk1 is involved in the maintenance of asymmetric cell division in mammalian TPC and if its inhibition leads to a predominance of symmetrical cell divisions over asymmetric cell divisions.

Oligodendroglioma are far more susceptible to standard therapeutic approaches than astrocytoma. Both oligodendroglioma cells and oligodendrocyte progenitor cells extracted from genetically modified mouse models are more susceptible to temozolomide when compared with astrocytoma cells or neural stem cells [112]. The fact that both the normal cells and their derived tumors exhibit this sensitivity suggests that the therapeutic resistance seen in astrocytoma and susceptibility evident in oligodendroglioma stems from their proposed differential cell of origin.

Another possible reason for this difference is that oligodendrogliomas are less enriched for a stem-like TPC population, but rather harbor more progenitor-like TPC when compared to astrocytoma [112, 189]. Furthermore, NG2-positive progenitor-like TPC derived from human oligodendroglioma have a far greater tumor forming capacity when injected into mice, compared to their NG2-negative counterparts [112]. As previously discussed, it has been proposed that asymmetric cell division promotes resistance to therapeutic agents targeted towards actively cycling cells due to their slow proliferation rate. However, symmetrically faster dividing cells are suggested to be more susceptible to these same agents. Thus, the increased rate of symmetrical cell divisions evident in oligodendroglioma NG2-positive progenitor-like TPC may explain why oligodendroglioma are more chemosensitive than their stem-like TPC enriched astrocytoma counterparts [44].

Tumor microenvironment and cell division mode

As previously discussed for glioma, CD133 has been shown to be asymmetrically distributed between daughter cells suggesting that glioma TPC undergo asymmetric cell divisions. In GBM cell lines established from surgical tissue samples, the CD133-positive cells showed increased rate of proliferation in neurosphere culture conditions and increased potential for neuronal differentiation [190]. Whether these asymmetric cell divisions are part of a tumor cell hierarchy similar to that generated by radial glial cells in the developing brain remains to be determined.

In brain tissue placed under hypoxic conditions stem and progenitor cells populations expand [191]. Astrocytomas, in particularly GBM, commonly contain areas within the center of the tumor that are hypoxic due to the inadequate expansion of blood vessels for the nutritional needs of the neoplastic mass [192]. It is thought that these hypoxic regions enrich the tumor for TPC, in a similar manner to the normal brain, which under hypoxic conditions is enriched for neural stem cells [191]. This is supported by the fact that TPC have been found to be most prevalent within the center or inner core of GBM tumor masses, where hypoxic environments are commonly found [193].

CD133 expression increases when GBM cells are cultured in 7% oxygen, compared with 20% oxygen [194]. Low oxygen also decreased the proliferation and enhanced the differentiation capacity of these cells [194]. Similarly, when GBM cell lines were cultured in 21% oxygen and hypoxic conditions of 3% oxygen, the CD133-positive content of the cell lines were altered from 69% to 92% respectively [195]. Another effect of hypoxic conditions is an increase in the expression of Notch pathway ligands in GBM [196]. Further, in patient derived GBM TPC, culture in hypoxia not only increased CD133 expression, but also increased the ability of the cells to form neurospheres [197]. These data suggest that culture in low oxygen levels selects for CD133-labeled TPC cells or preferentially promote their expansion, which may be a predominantly asymmetrically dividing population. Supporting this, asymmetric cell division has previously been shown to be increased in lung cancer cells upon exposure to a hypoxic environment [198]. Thus extrinsic environmental factors like oxygen content may be important for regulation of cell division mode in astrocytoma TPC, in turn controlling treatment resistance.

Conclusion

Gliomas with different histological features are likely derived from a differential cell of origin with specific modes of cell division involved in this process. For instance, the disruption of asymmetric cell division in favor of faster and more expansive symmetric cell divisions in oligodendrocyte progenitor cells leads to oligodendroglioma formation, a process that has been well characterized. In contrast, astrocytomas seem to maintain asymmetric cell division of radial glial cells or dedifferentiated cells further along the gliogenic or neurogenic pathway. Thus, astrocytoma development and the cell of origin of astrocytoma are less well characterized than for oligodendroglioma in the context of cell division mode. There may also be some overlap between oligodendroglioma and astrocytoma development cell of origin. These tumors are likely on a spectrum, where the most oligodendroglial-like tumors are derived from oligodendrocyte progenitor cells and become enriched for progenitor-like TPC, whilst the most astrocytic tumors may be derived from multiple different cell types and become more enriched for stem-like TPC.

The cell types present within a glioma neoplastic mass and their cell division mode should inform tumor treatment, as faster symmetrically dividing progenitor-like TPC are thought to be more susceptible to standard therapeutic agents, whereas slower asymmetrically dividing stem-like TPC are resistant to therapeutic agents targeted at cycling proliferative cancer cells. Future studies should be aimed at conclusively determining the stem and progenitor-

like TPC population ratios in oligodendroglioma and astrocytoma with the intention of confirming the cell division modes prevalent within these subgroups in each tumor type.

There are multiple factors which control cell division mode and future studies ought to focus on identifying actionable proteins which can be manipulated to prevent the disruption of asymmetric cell division, which has been shown to result in oligodendroglioma development. Conversely, targets aimed at disrupting the asymmetric cell division of TPC in astrocytoma thought to be responsible for treatment resistance would also be of vast importance. A major challenge may be to alleviate the proposed negative aspects of asymmetric cell division maintenance in astrocytoma without inadvertently causing the expansion of tumor growth by symmetrically dividing oligodendrocyte progenitor cells. Therefore it is likely that therapeutic agents targeting cell division mode need to be cell type specific to avoid unwanted tumorigenic side effects. Furthermore, research into cell division mode controllers and their role in glioma formation and treatment response could have implications for many other tumor types, as stem and progenitor cells have been proposed as the cell of origin for cancers that develop in many organs other than the brain.

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Abbreviations

aPKC	atypical protein kinase C
ASPM	abnormal spindle-like microcephaly-associated protein
Brat	brain tumor
EGFR	epidermal growth factor receptor
Insc	inscuteable
Lgl	lethal giant larvae
Mira	Miranda
Msi1	musashi-1
Mud	Mushroom body defect
Nf1	neurofibromatosis type 1
Par 3	Partition defective 3
Par 6	partition defective 6
Pins	partner of inscuteable
Plk1	polo like kinase 1
Pon	partner of numb
Pros	prospero

Stau	staufen
TPC	tumor propagating cells
YB-1	Y-box binding protein 1

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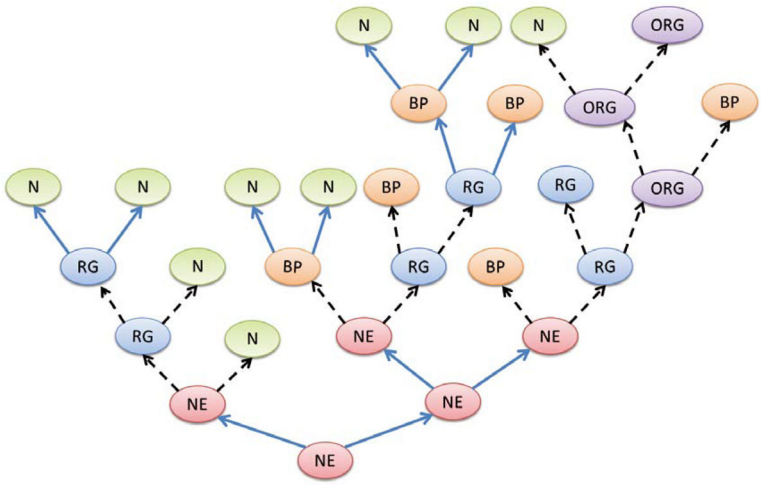

Figure 1.

Diagram depicting asymmetric and symmetrical cell divisions and their contributions to the differentiation hierarchy in the brain during neurogenesis. Blue filled arrow denotes symmetrical cell divisions and dashed black arrows indicate asymmetric cell divisions. NE- neuroepithelial cell, RG- radial glial cell, BP- basal progenitor cell, ORG- outer radial glial cell, N- neuron.

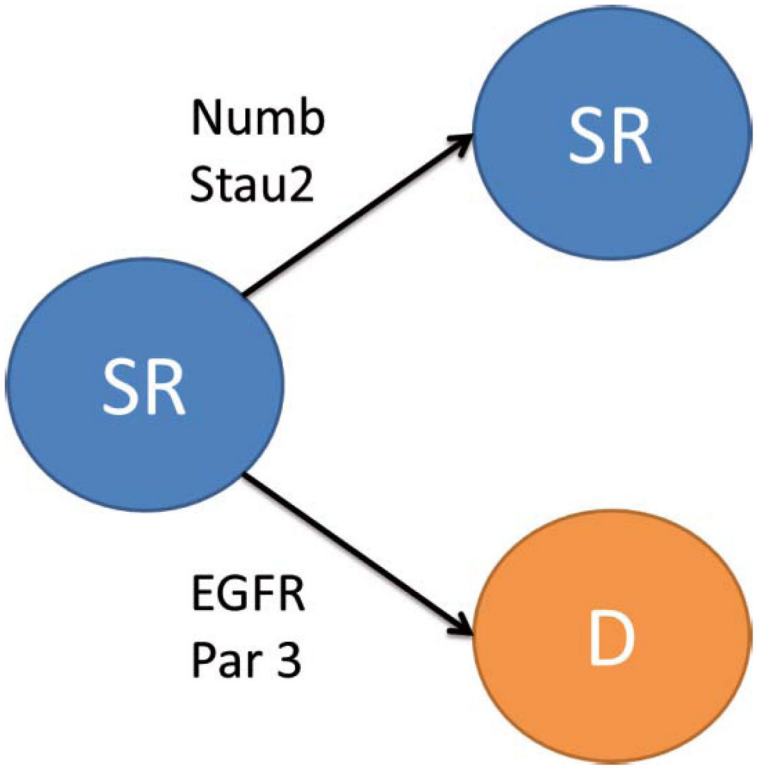

Figure 2.
Mammalian asymmetric cell division. SR- self renewing cell, D- cell for the differentiation pathway.

Table 1

Table of cell genesis in the mammalian brain.

Cell type	Location	Predominate mode of cell division	Possible daughter cells
Neuroepithelial cell	Neural tube	Symmetrical during expansion and asymmetric during the neurogenic period	Neuroepithelial cell, radial glial cell, basal progenitor and neuron
Radial glial cell	Ventricular zone	Symmetrical during expansion and asymmetric during the neurogenic period	Radial glial cell, outer radial glial cell, basal progenitor, neuron, astrocyte, ependymal cell
Outer radial glial cell	Subventricular zone	Asymmetrical	Outer radial glial cell, basal progenitor and neuron
Basal progenitor cell	Subventricular zone	Symmetrical	Basal progenitor and neuron
Glial restricted progenitor cell	Throughout the brain	Unknown	Glial restricted progenitor, astrocyte and oligodendrocyte progenitor
Oligodendrocyte progenitor cell	Throughout the brain, predominantly white matter	Symmetrical and asymmetrical	Oligodendrocyte progenitor cell, oligodendrocyte
Astrocyte	Throughout the brain	Symmetrical	Astrocyte

Table 2

Comparison of astrocytoma and oligodendroglioma.

	Astrocytoma	Oligodendroglioma
Incidence	most common primary brain tumor [73], most common childhood brain tumor (40% of all pediatric brain tumors)[74–77]	rarer than astrocytoma
Grade	Grades I and II-low grade, grade III and IV - high grade, Grade IV astrocytoma - (glioblastoma multiforme, GBM) most aggressive brain tumor currently incurable	Grades I and II -low grade, grade III - high grade
Progression	low grade commonly progress to higher grades	generally do not progress to higher grades
Treatment response	chemoresistant	chemoresponsive, nosignificant difference in survival for complete versus incomplete resections [78]
survival	overall survival low grade astrocytoma - 4.5 years [79], median overall survival grade III anaplastic astrocytoma- 15 months [80], median survival GBM - 8 months [81, 82]	overall survival anaplastic oligodendroglioma grade III - 42 months [80]

Table 3

Cell type specific mutations which result in glioma formation.

Mutation	Cell type which forms tumors	Cell type unable to form tumors	Tumor histology	Reference
p53 deletion, active c-Myc, Akt	primary astrocytes		astrocytoma	[104]
p53 deletion, *Nf1*	neural stem cells, astrocytes, neurons		astrocytoma	[105]
Nf1, *Pten*, inactivation of *p53*	stem and progenitor cells	mature astrocytes	astrocytoma	[106]
p53 and *Pten* mutation	oligodendrocyte progenitor cells		proneural GBM	[107]
p53 mutation, *Hras* amplified	oligodendrocyte progenitor cells, neural stem cells		GBM	[108]
Ink4a/*Arf* deletion, EGFR activated	neural stem cells, astrocytes		high grade glioma	[109]
Pten deletion		GFAP-positive cells	no tumor	[110]
Pten deletion, *p53* deletion	GFAP-positive cells		high grade astrocytoma	[110]
EGFR		GFAP-positive cells	increased astrocyte number, no tumor	[111]
EGFRvIII		GFAP-positive cells	increased astrocyte number, no tumor	[111]
verb B	oligodendrocyte progenitor cells		oligodendroglioma	[44, 112, 113]
PDGF-B	oligodendrocyte progenitor cells		oligodendroglioma	[114]
PDGF-B	nestin expressing cells		oligodendroglioma	[115]
PDGF-B	GFAP-positive cells		oligodendroglioma, oligoastrocytoma	[115]
EGFR	oligodendrocyte progenitor cells		hyperplasia	[116]
V12Ha-ras, *EGFRvIII*	GFAP-positive cells		oligodendroglioma, oligoastrocytoma	[111]