The Role of Stem Cells in Pediatric Central Nervous System Malignancies

Branavan Manoranjan, Neha Garg, David Bakhshinyan, and Sheila K. Singh

Abstract Representing the leading cause of childhood cancer mortality, pediatric brain tumors are comprised of diverse histological features, genetic perturbations, cellular populations, treatment protocols, and clinical outcomes. In this chapter we discuss recent and emerging data that implicate cancer stem cells (also known as brain tumor-initiating cells) in initiating and maintaining the growth of a number of pediatric brain tumors including: medulloblastoma, supratentorial primitive

B. Manoranjan
McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada L8S 4K1

Michael G. DeGroote School of Medicine, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

N. Garg
McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada L8S 4K1

D. Bakhshinyan
McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada L8S 4K1

Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

S.K. Singh, M.D., Ph.D., F.R.C.S(C). (✉)
McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada L8S 4K1

Michael G. DeGroote School of Medicine, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

Department of Surgery, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5
e-mail: ssingh@mcmaster.ca

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neuroectodermal tumor, atypical teratoid/rhabdoid tumor, ependymoma, low-grade glioma, glioblastoma, diffuse intrinsic pontine glioma, germ cell tumor, and craniopharyngioma. The development of a stem cell framework for the study and treatment of these tumors will enable future clinical approaches to harness the heterogeneous cellular and genomic landscape of these solid tumors as an avenue for developing targeted patient-oriented therapies, thereby improving the overall survivorship for the most lethal childhood cancer.

Keywords Pediatric brain tumor • Cancer stem cell • Brain tumor-initiating cell • Medulloblastoma • Ependymoma • Supratentorial primitive neuroectodermal tumor • Low-grade glioma • Pediatric glioblastoma • Diffuse intrinsic pontine glioma • Atypical teratoid/rhabdoid tumor • Germ cell tumor • Craniopharyngioma

Introduction

Central nervous system (CNS) tumors represent the leading cause of childhood cancer mortality with an incidence of 30 cases per million [1]. Current diagnostic and therapeutic parameters are dependent on clinical history, radiological imaging, and histological confirmation. Although the present era of molecular classification has aided in the recognition of several distinct subgroups in a variety of pediatric brain tumors, the WHO classification of childhood brain tumors remains dependent on tumor location, histopathological features, and immunohistochemical marker expression [1]. These features primarily determine tumor grade, which in turn reflects patient outcome. Prognostically, there have been minimal improvements in the outcome of pediatric brain tumors, but the overall survivorship remains dismal. Consequently, novel biological frameworks must be applied to elucidate mechanisms that may yield high clinical and therapeutic utility.

The cancer stem cell (CSC) hypothesis suggests that a relatively small fraction of tumor cells termed, CSCs, have the ability to proliferate and maintain tumor growth [2]. This is in sharp contrast to all other cells of the bulk tumor, which are characterized by limited proliferative capacity and a more specified lineage potential. More specifically, a CSC maintains two key properties: self-renewal and multilineage differentiation. Self-renewal is defined as the ability of a parental cell to generate an identical daughter cell and a second cell of the same or different phenotype, whereas through the process of differentiation a CSC is able to give rise to the heterogeneous cell lineages that comprise the original tumor [2]. In the recent past, such CSC populations (also termed, tumor-initiating cells, and in the case of brain cancer, brain tumor-initiating cells (BTICs)) have been identified in a number of hematopoietic and solid tumor malignancies based on cell surface markers and stem cell assays, of which the generation of tumors in human–mouse xenograft models has become the gold standard. Moreover, unlike current genomic platforms, the CSC framework takes into account intratumoral heterogeneity by having a developmentally primitive cell at the apex of the hierarchy with a spectrum of more differentiated cells as one goes down this hierarchy [3].

The concept of a CSC suggests that tumors are organized into distinct clonal populations of cells with only the CSC demonstrating the properties of self-renewal and differentiation in vitro and in vivo [2]. Using in vitro assays originally developed to purify neural stem cells (NSCs) [4–6], Singh et al. [7] reported the identification and purification of a cell from primary human medulloblastoma (MB) and glioblastoma (GBM) that had a marked capacity for proliferation, self-renewal, and differentiation. The BTIC represented a minority of tumor cells and was marked by expression of the cell surface marker CD133. Additional in vivo characterization of the BTIC using a human–mouse xenograft assay formally established the identification of CSCs in brain tumors [8]. This work was corroborated in pediatric BTICs, which expressed high levels of NSC genes *CD133*, *Sox2*, *Musashi1*, and *Bmi1*, providing credence to NSC-driven brain tumorigenesis [9]. Furthermore, by taking advantage of unique stem cell properties such as self-renewal, the BTIC model has been clinically validated as a correlative indicator of patient outcome in pediatric brain tumors, suggesting more aggressive tumors to have a higher stem cell self-renewal index. [10] Overall, the BTIC framework of oncogenesis not only takes advantage of developmental genes and pathways implicated in pediatric brain tumorigenesis but also provides an avenue for studying cancer at a cellular level as distinct differences in subsets of tumor cells may not otherwise be appreciated using the current strategy of bulk tumor genomic profiling.

Medulloblastoma

Medulloblastoma (MB) represents the most frequent malignant pediatric brain tumor, comprising 18 % of pediatric intracranial tumors and 350 new diagnoses each year in the United States. The incidence peaks at two timepoints during childhood: 3–4 years and 8–9 years of age [11]. Histologically, MB is classified into several subtypes: classical, desmoplastic/nodular, MB with extensive nodularity, anaplastic, and large cell. Although these subtypes speak to the cellular and morphological heterogeneity of the bulk tumor, their clinical and prognostic utility has remained futile. More recent molecular classifications of MB have re-conceptualized the heterogeneity that exists within these pathological subtypes by identifying multiple distinct molecular subgroups that differ in their demographics, transcriptomes, somatic genetic events, and prognostic outcomes [12–18]. These studies have also given context to the role of key developmental signaling pathways in MB pathogenesis, providing greater support for subgroup-specific BTICs [18–21].

The current consensus for the molecular classification of MB consists of four subgroups, each distinct in terms of prognosis and predicted therapeutic response [18]. Groups 1 and 2 are characterized by upregulation of genes in the Wnt (7–8 % of patients) or Sonic hedgehog (Shh) (28–32 % of patients) pathways, respectively. These two subgroups are associated with improved clinical outcomes, when compared to Groups 3 (26–27 % of patients) and 4 (34–38 % of patients), which are characterized by a greater propensity for metastatic disease and poor clinical outcomes [12–14, 17, 18, 22–24]. Although recent transgenic murine models have

identified amplification of *c-myc* signaling to characterize Group 3 MBs [19, 21], both Group 3 and 4 MBs are collectively considered aggressive and refractory to current treatment modalities [25].

Since its initial identification in 1910 by James Homer Wright, MB has been thought to arise from restricted neuronal precursors termed, "neuroblasts" [26]. The concept of a common cell of origin in MB was further supported in 1925 by Percival Bailey and Harvey Cushing in their observation of both glial and neuronal cells, which they proposed to have originated from a primitive embryonic neuroepithelial cell termed, "medulloblast" [27]. Anatomically, the developing cerebellum provides a reservoir of cells susceptible to malignant transformation. Interestingly, pathways implicated in characterizing MB Groups 1 and 2 have also been described in the proliferation, migration, and maturation of cerebellar stem/progenitor cells. In normal cerebellar development, Shh signaling drives the proliferation and migration of a subset of cerebellar precursor cells termed, granule neuron precursors (GNPs). GNPs contain the Shh receptors Ptch and Smo, and thereby respond to a concentration gradient established by the release of Shh ligand from Purkinje cells [28–31]. Genomic alterations in components of the Shh signaling pathway have been identified in up to 25 % of sporadic human MBs and consist of inactivating mutations of *Ptch1* and *Suppressor of fused* (*Sufu*), and/or activating mutations of *Smo* [32–36]. The canonical Wnt signaling pathway is responsible for defining the midbrain-hindbrain boundary from which the entire cerebellum develops [37]. More recently, Wnt signaling has been shown to differentially regulate cerebellar NSCs and GNPs [38]. Although Wnt activation in vitro *and* in vivo was shown to promote proliferation of NSCs but not GNPs, the proliferative NSCs did not undergo prolonged expansion or neoplastic growth, suggesting Wnt to function as a regulator of cerebellar stem cell growth and differentiation. Nevertheless, these developmental pathways must be tightly regulated since the cerebellum reaches complete maturation only several months after birth [29], making it a vulnerable target for oncogenic mutations as the developmental phase is prolonged and active beyond in utero.

While the molecular profiling of MB has been credited with providing a developmental approach to studying its pathogenesis, the direct isolation and characterization of subgroup-specific MB BTICs has largely been attributed to several transgenic mouse models. The overlapping expression of genes unique to the human Wnt subgroup in the fetal mouse dorsal brainstem has enabled the discovery of a distinct germinal zone within the hindbrain believed to contain the cell of origin for Wnt-driven MB [20]. Consequently, BTICs may not only contribute to the cellular heterogeneity within MB but may also be responsible for the spatial heterogeneity associated with specific subgroups. Transgenic mice haploinsufficient for *Ptch1* (*Ptch1*+/−), have greatly contributed to elucidating the role of Shh signaling in MB pathogenesis [33]. Through an increase in the proliferative potential of NSCs, the incidence of MB is 15–20 % in these mice [33, 39, 40]. Initial work with this model identified cells resembling GNPs, which retained their proliferative potential suggesting GNPs to promote Shh-dependent MB [33]. More conclusive evidence for the acquisition of a GNP phenotype as being necessary and sufficient for the initiation of Shh-driven MB has been shown with dysregulated Shh-signaling in unipotent

Nestin+GNPs [41], unipotent Math1+ GNPs, or multilineage embryonic NSCs [42, 43]. Although recent evidence has also alluded to a non-cerebellar cell of origin in Group 2 MB [44], similar to that of Wnt-driven MB [20], further characterization of these cell populations is required to truly implicate their involvement in initiating and contributing to MB pathogenesis. While a GNP may very well serve as the cell of origin for Group 2 MB, the identification of tumor propagating cells within this subgroup is required for targeted therapeutic interventions. Interestingly, CD15 has been shown to serve as a putative marker of MB stem cells in the *Ptch1+/−* model [39]. CD15+ cells comprised a small fraction of normal GNPs (as indicated by the co-expression of Math1), and exhibited a higher proliferative capacity, and elevated levels of Shh target genes when compared to CD15- cells. However, unlike CD133+ cells [7, 8], CD15+ cells did not display multilineage differentiation or neurosphere formation when cultured at clonal densities. Consequently, it was believed that these cells marked progenitor populations as opposed to a more primitive stem-like cell. However, upon culturing these cells in serum-free conditions, they were shown to propagate as multipotent MB stem cells, suggesting CD15 to be an additional marker of MB BTICs [40]. Although the evidence for a GNP as the cellular origin for Shh subtype MB is paramount, it remains unclear as to how these cells propagate and transform over the course of tumorigenesis as defined by their regulatory mechanisms and marker expression. The characterization of Group 3 MB BTICs has only recently benefited from discoveries in transgenic mouse models [19, 21]. Using postnatal murine cerebellar stem cells based on the expression of Prominin1 and lacking expression of lineage-specific markers for GNPs, Pei et al. [19] introduced a mutant, stabilized *myc* construct with a dominant negative p53, which induced in vivo MBs distinct from Shh and Wnt murine MBs. In contrast, Kawauchi et al. [21] generated Group 3 MBs in vivo by introducing *myc* ex vivo into *Trp53* null GNPs sorted for the neuronal lineage marker Atoh1 (Math1). Both groups demonstrated the in vivo MBs generated from their transgenic cells to recapitulate many histopathological and genomic features of the human Group 3 MB. Most interestingly, protein and genomic expression profiles of tumors generated by both groups overlapped most with those of NSCs, induced-pluripotent stem cells, and embryonic stem cells. Although Kawauchi et al. [21] had initially injected cells sorted for GNPs negative for stem cell markers such as Prominin1, the resulting tumors had lost Atoh1 expression and instead displayed increased expression of Prominin1 and other stem cell markers. Similarly, Pei et al. [19] observed an increase in markers of undifferentiated cells in the resulting tumors, suggesting Group 3 MB to either arise from cerebellar stem cells or through a process of dedifferentiation in which distinct tumor cells take on a stem-like phenotype. Clinically, the identification of a cerebellar stem cell as the target for initiation and propagation of Group 3 MB is in keeping with the treatment-refractory, metastatic characteristics observed in these patients.

Although Group 4 MB has yet to be described using a transgenic mouse model, stem cell properties and pathways may still be used to further elucidate novel regulatory mechanisms unique to this subgroup. While these tumors share the clinical features of metastatic disease and poor treatment response with Group 3 MBs, their signaling and genomic frameworks are quite distinct [18]. Unlike Group 3 MBs

characterized by *myc* signaling, Group 4 MBs are identified by isochromosome 17q and loss of the X chromosome [12–14, 17, 18]. Additional genomics features include the involvement of genes implicated in neuronal development and differentiation. However, the clinical utility and relevance of these genes have yet to be assessed. Given the identification of fate-determination genes in Group 4 MB, the chromatin-modifying Polycomb-group (PcG) gene *Bmi1* has been considered as a novel regulator of Group 4 MB BTICs [45, 46]. Bmi1 functions as a critical regulator of NSC self-renewal through repression of the $p16^{Ink4a}$ and $p19^{Arf}$ senescence pathways [47, 48]. The Bmi1 signaling pathway is also consistently dysregulated or overexpressed in several emerging CSC populations, most recently being cited as a marker of recurrence, poor treatment response, metastatic potential, and death in many cancer models [49, 50]. With respect to MB, *Bmi1* is preferentially expressed in Group 4 tumors [45, 46] and has been shown to recapitulate NSC self-renewal pathways in MB BTICs [45]. The metastatic properties of Group 4 MBs may also be attributed to the interaction of Bmi1 with Twist1 [51]. Twist1, a transcription factor upstream of *Bmi1*, promotes epithelial–mesenchymal transition (EMT) in normal development and metastatic/invasive properties in cancer [51]. Given the preferential expression of both genes in Group 4 MB [45, 46], their interaction may facilitate the invasive and migratory features of Group 4 MB BTICs.

It is apparent that although specific therapeutic targets have yet to be associated with stem cell pathways in MB, several mediators of NSC self-renewal, proliferation, and differentiation continue to demonstrate preferential segregation towards this childhood brain tumor. The continued demonstration of BTICs in transgenic murine models and their evaluation in primary human patient samples will prove to be invaluable in the development of targeted therapies at subgroup-specific BTICs.

Supratentorial Primitive Neuroectodermal Tumor

Supratentorial primitive neuroectodermal tumor (sPNET) accounts for 3–5 % of all pediatric brain tumors and is considered a member of the embryonal family of malignant childhood brain tumors [1, 52]. Although sPNETs resemble the small blue cell histological phenotype attributed to MB, the molecular framework of these tumors is quite distinct. The recent molecular classification of sPNET has categorized this tumor into three distinct subgroups: primitive-neural, oligoneural, and mesenchymal [53]. Clinically, the primitive-neural subgroup represents a younger age of onset ($\leq$4 years), increased metastatic potential within this age group, and the worst overall survivorship among all three subgroups [53]. Of particular interest, developmental genes such as the *Hox* family and pathways including Wnt and Shh are also enriched in the primitive-neural subgroup, establishing the adequate cellular machinery for regulating putative sPNET BTICs. Further evidence for the presence BTICs in maintaining sPNETs is based on the histological heterogeneity observed in these tumors consisting of variable neuronal, ependymal, and glial differentiation [1].

Interestingly, given the strong precedent for the role of BTICs in driving tumorigenesis in sPNETs, a paucity of data remains in the characterization of these cells. Currently, the literature on sPNET BTICs is restricted to one report, in which BTICs were cultured from a human mesenchymal sPNET [54]. The sPNET BTICs from this patient sample were shown to maintain multilineage differentiation into glial, neuronal, and oligodendrocytic lineages along with a sustained self-renewal potential over several in vitro and in vivo passages. CD15+/CD133+ comprised 25–40 % of the bulk tumor population over several passages and demonstrated the greatest in vitro self-renewal capacity. This observation was supported with a reduced overall survival in murine intracranial xenografts of CD15+/CD133+ cells. Therefore, CD15+/CD133+ cells demonstrate a novel cellular target for mesenchymal sPNET BTICs. Future work into the regulation of these cells by pathways enriched in the mesenchymal subgroup such as TGF-β signaling may provide novel small molecules for a tumor that has largely remained refractory to current therapeutic efforts. It also remains to be addressed if the CD15+/CD133+ cells may represent sPNET BTICs irrespective of the molecular subgroup, which may then yield significant clinical utility and improved survivorship for one of the most malignant childhood cancers.

Atypical Teratoid/Rhabdoid Tumor

Atypical Teratoid/Rhabdoid Tumor (AT/RT) is a highly aggressive and malignant intracranial embryonal tumor occurring in children less than 2 years of age [52, 55]. Histologically, these tumors contain a mixture of rhabdoid, primitive neuroepithelial, epithelial, and mesenchymal structures [56]. AT/RT accounts for 2–3 % of all pediatric brain tumors and has a predilection for arising in the posterior fossa [57]. Unlike the heterogeneous histological composition of these tumors, approximately 80 % of AT/RTs contain mutations or deletions in chromosome 22, which account for the inactivation of the *INI1* (*hSNF5*/*SMARCB1*) gene [58–60]. INI1 functions as a protein component of the ATP-dependent SWI/SNF chromatin-remodeling complex, which regulates genes responsible for proliferation and differentiation [61]. The clinical prognosis is extremely poor with a median survival of 11–17 months, leaving several avenues for future research and targeted therapies at treatment-refractory cell populations [62].

Given the aggressive nature and diverse cell types present in AT/RT, it may be postulated that these tumors contain a BTIC population responsible for maintaining tumor growth, promoting treatment-resistance, and accounting for the distinct cellular architecture [63–65]. Gene expression profiling in CD133+ AT/RT BTICs has shown an increased expression of developmental genes such as *Oct4, Nanog, Sox2, Nestin, Musashi1, and Bmi1*. Interestingly, the identification of drug-resistant/ABC transporter genes including *MDR-1, MRP1,* and *ABCG2* are in keeping with functional data demonstrating CD133+ AT/RT BTICs to be radioresistant [66–68]. Further evidence in support of AT/RT BTICs relates to the overexpression of the

chromatin-remodeling Polycomb group complex member, EZH2, which functions to maintain self-renewal, cell growth, proliferation, and radiation-resistance in AT/RT. [69] Although several gene expression studies have been conducted to evaluate the differences between distinct cell populations that may serve as putative AT/RT BTICs, additional mechanistic and in vivo studies may be of greater clinical utility for developing targeted therapies at AT/RT BTICs.

Ependymoma

Ependymoma (EP) is the third most common pediatric brain tumor, representing approximately 9 % of primary brain tumors in children, with an estimated incidence of 200 per year. The median age of diagnosis of pediatric patients is 5 years [11]. Anatomically, EPs occur throughout the CNS, including the supratentorium, posterior fossa, and spinal cord. In the pediatric population, the posterior fossa is the most frequent site of tumorigenesis with 70 % of cases occurring in the fourth ventricle, whereas supratentorial and spinal tumors present more often in adults [70, 71]. According to the WHO, EP may be classified into grades I–III. Although there are distinct anatomical locations for the pathogenesis of EP, histologically, these tumors remain indistinguishable. In keeping with poor histological parameters and a high mortality rate of 45 %, novel genomic markers have been investigated to further delineate genomic subtypes and therapeutic targets [70, 71].

In an attempt to merge genomics with clinical utility, recent molecular analyses of two large independent EP cohorts have revealed the presence of two demographically, transcriptionally, genetically, and clinically distinct groups of pediatric posterior fossa EPs [72]. When compared to Group B patients, Group A patients are younger, associated with laterally located tumors with a balanced genome, and are much more likely to exhibit recurrence, metastasis, and death. Consequently, Group B patients may be treated less aggressively, while novel adjuvant therapies remain critical in the treatment of Group A posterior fossa EPs. Although the most striking candidate markers for distinguishing the two molecular subgroups are *LAMA2* and *NELL2* in Group A and B, respectively, they're functional significance in regulating EP BTICs remains to be investigated.

The first report of EP BTICs was described in an analysis of 100 human EPs, in which the expression of developmental genes was correlated with distinct anatomical origins of tumor formation [73]. Supratentorial, spinal cord, and posterior fossa EPs were found to overexpress members of the *EphB-Ephrin/Notch* pathways, *Hox* gene family, and *AQP1*, respectively. While the pathways and genes associated with supratentorial and spinal cord EPs had been implicated in the regulation of normal NSCs [74] and the anteroposterior patterning of the spinal cord [75], respectively, posterior fossa EPs were continuously found to arise in the SVZ by projecting near the fourth ventricle. Consequently, all three anatomical subgroups demonstrated cell-intrinsic properties in keeping with their anatomically oriented precursor cells.

Through mapping the expression of these genes in the developing mouse, it was found that a distinct type of neural precursor cells termed, radial glial cells, displayed a gene expression profile similar to each of the anatomically distinct human EPs [73]. While this data supported a developmental origin for EP, the generation of a human–mouse EP BTIC xenograft model provided the necessary functional evidence for EP BTICs [73]. Using the NSC markers CD133, Nestin, and RC2 in conjunction with the radial glial cell marker, BLBP, 10,000 CD133+Nestin+ RC2+BLBP+ cells were intracranially injected into the brains of immunocompromised mice. Tumors resembling human EP were identified 4–5 months following transplantation. In contrast, intracranial injections with 2×10 [6] CD133- cells or 2×10 [6] unsorted EP cells did not lead to engraftment or tumor formation even 1 year following transplantation [73]. Consequently, radial glial cells have been considered to function as putative EP BTICs. More recently, tumors resembling human supratentorial EPs have only been capable of developing from the overexpression of EphB2 in mouse embryonic cerebral NSCs [76]. While the genomic anomalies between anatomic subtypes of EP may be distinct, the cellular target continues to function as a BTIC with properties associated with normal radial glial cells. Therefore, continued work in understanding the pathways that promote the differentiation of radial glial cells may be harnessed for therapies targeting the EP BTIC.

Low-Grade Glioma

Pediatric low-grade glioma (LGG) represents the most common pediatric brain tumor, of which the pilocytic astrocytoma (PA) histological subtype accounts for the majority of cases (~20 % of all pediatric brain tumors) [52]. Although these tumors are considered slow-growing and benign, surgically inaccessible midline LGGs remain a therapeutic challenge and account for considerable morbidity and mortality. PAs have classically been described in conjunction with the NF1 (neurofibromatosis) inherited tumor predisposition syndrome [1]. PAs resulting from the mutational inactivation of the *NF1* tumor suppressor gene are primarily located along the optic pathway, while sporadic PAs that do not harbor the *NF1* inactivation predominantly arise in the cerebellum [1]. Recent whole-genome sequencing of PAs has uncovered several recurrent activating mutations in *FGFR1* and *PTPN11* [77, 78]. Although the literature in support of a LGG BTIC is minimal at best, the development of tumors resembling pediatric low-grade optic gliomas from the inactivation of *NF1* in murine third ventricle NSCs has provided a putative cell of origin within the third ventricle for NF1-PAs [79]. Consequently, the application of cell surface markers specific to normal human third ventricle NSCs may further enrich and assist in characterizing putative LGG BTICs. With the discovery of novel LGG driver gene mutations and the presence of LGG BTICs, the future of targeted therapies for inoperable pediatric LGGs remains dependent on the integration of whole-genome sequencing data with cell-intrinsic functional pathways unique to BTICs.

Glioblastoma

Pediatric glioblastoma (GBM) accounts for approximately 3 % of all childhood brain tumors [11, 52]. While pediatric GBMs are histologically identical to adult GBMs, several genomic alterations distinguish these tumors [80, 81]. With the advent of whole-exome sequencing, genomic anomalies unique to pediatric GBM have been identified for the first time [82, 83]. Recurring gain-of-function heterozygous mutations in the *H3F3A* gene, which encodes histone H3.3 have been shown to regulate telomere maintenance and/or heterochromatin stability. Although the comprehensive examination of these mutations in regulating BTICs remains to be investigated, the G34R mutation in *H3F3A* has been shown to promote H3K36me3 enrichment and subsequent activation of transcription factors responsible for NSC proliferation, maintenance, and maturation [84]. In keeping with a primitive transcriptional state, pediatric GBM BTICs have shown an enhanced self-renewal capacity that exceeds septenary spheres with variable expression of putative BTIC markers: CD15, Sox2, Bmi1, Nestin, and Olig2 [85]. The clinical significance of these developmental markers has been established through a *Hox* gene signature that is predictive of temozolomide-resistant pediatric GBM BTICs [86]. Although targeted therapies using cell-intrinsic, treatment-refractory pathways have yet to be pursued with pediatric GBM BTICs, preclinical in vitro and in vivo BTIC models have displayed a reduction in self-renewal capacity and survival advantage in mice, respectively, following treatment with oncolytic viruses [87]. Consequently, pediatric GBMs may provide a novel platform for targeted therapies through the elucidation of epigenetic regulatory mechanisms unique to BTICs that may be amenable to surface marker-based immunotherapies. The integration of diverse research platforms such as cancer genomics, stem cell biology, and immunotherapy may thereby provide a novel paradigm for collaborative research efforts, targeted therapies, and an improvement in the overall survivorship of children diagnosed with pediatric GBM.

Diffuse Intrinsic Pontine Glioma

Diffuse intrinsic pontine glioma (DIPG) is an anatomical variant of high-grade pediatric glioma, which has remained a therapeutic challenge for several decades due to its location in the neurologically delicate brainstem. Brainstem gliomas account for approximately 10–15 % of all pediatric brain tumors, with a median age at presentation of 6–7 years [52, 88]. Clinically, the most common presentation is that of a mass arising in the pons, which is amenable only to radiotherapy. Unfortunately, radiotherapy has shown minimal improvements in mean progression-free survival with an increase to 5.8 months from 5 months for those who do not receive radiotherapy [89]. Overall, 90 % of children succumb to their illness within 2 years of diagnosis, making DIPG one of the leading causes of death in children with brain tumors [88].

Similar to pediatric GBM, whole-genome sequencing has only recently identified novel mutations in the *H3F3A* and *HIST1H3B* genes, which encode histone H3.3 and H3.1, respectively [82, 90]. Histones are basic nuclear proteins responsible for the nucleosome structure of chromosomes. As the nucleosome is formed from DNA being wrapped in repeating units around an octamer consisting of two molecules of each core histone (H2A, H2B, H3, and H4), mutations in histone coding genes provide an epigenetic mechanism of tumor formation. The functional and cellular significance of these mutations in distinct cell populations such as BTICs remains to be established. In keeping with the current era of integrated molecular profiling, DIPGs have recently been classified into two distinct molecular subgroups [91, 92]. Subgroup 1 is associated with activation of the Shh pathway [92] or the presence of mesenchymal/pro-angiogenic markers and the enrichment of developmental genes such as *Sox2, Musashi1,* and *Nestin* [91]. In contrast, subgroup 2 tumors are reflected by *myc* (*N-myc*) activation [92] or the presence of oligodendroglial features with PDGFRA activation [91]. A recent DIPG BTIC human–mouse xenograft model has provided further evidence in establishing a developmental phenotype for subgroup 1 DIPGs [93]. Aside from being regulated by Shh signaling, a regulator of ventral pons precursors, subgroup 1 DIPG BTICs also displayed variable expression of typical BTIC markers (*CD133, Sox2*) along with markers of normal progenitors of the ventral pons (*Nestin*, *Olig2*). In vivo characterization of the xenograft tumors revealed infiltrative tumors throughout the murine brain involving the cortex, cerebellum, and pons. A second DIPG mouse model in support of a primitive cell of origin for the initiation and maintenance of DIPGs was established using the (RCAS)/tv-a system to overexpress PDGFB in primitive nestin-expressing cells [94]. The malignant transformation of these cells lining the fourth ventricle and aqueduct also lead to the formation of tumors resembling DIPGs. However, unlike human DIPGs, which are thought to arise from the ventral brainstem [93], these transgenic tumors mostly developed from precursor cells near the neonatal dorsal brainstem. The targeted therapy of DIPG BTICs is largely dependent on elucidating the mechanisms that regulate these cells. A recent tissue microarray of human DIPG samples has identified the overexpression of Sox2, Olig2, and Bmi1 in the majority of DIPG samples [95], and thereby provides novel avenues for investigating BTIC mechanisms in a childhood cancer that is only beginning to enter the age of molecular diagnostics, classification, and BTIC-based therapeutics.

Germ Cell Tumor

Intracranial germ cell tumors (iGCTs) represent a rare fraction of pediatric brain tumors, which arise from primordial cells of the developing embryo [96]. Germ cells typically form the reproductive system, but arise in GCTs when they aberrantly migrate and proliferate in sites other than the gonads (i.e., chest, abdomen, and less frequently, the brain) [96]. iGCTs are most commonly found in young people aged 10–20 and account for approximately 2–5 % of all pediatric brain tumors [52, 97].

Gender differences have been reported for both the incidence and localization of iGCTs with a higher incidence in males (3:1) and the localization of tumors in the pineal and suprasellar regions in males and females, respectively [98].

As with many other malignancies, iGCTs possess histopathological subtypes: germinoma, immature/mature teratoma, and non-germinomatous (yolk sac tumor, embryonal carcinoma, and choriocarcinoma) [97, 99, 100]. The primary regimen of treatment includes chemotherapy and/or radiotherapy [101]. Surgery, although not typically recommended due to the inaccessibility of tumors, is preferred in cases of well-encapsulated mature teratomas [96, 102]. Overall prognosis remains relatively poor for non-germinomatous tumors (60 %) when compared to pure germinomas (90 %) [103].

The proposed cell of origin for iGCTs remains controversial as these cells do not maintain a neural lineage. Nevertheless, iGCTs and gonadal GCTs share several molecular features such as chromosomal alterations, mutations in developmental genes, and epigenetic modifications [104]. The expression of several stem cell genes such as *c-kit*, *Oct3/4*, and *Nanog* implicates an embryonic stem cell-like phenotype in these tumors, the hallmark of primordial germ cells [105]. The case for the presence of BTICs in iGCTs has been recently established with the ectopic expression of *Oct4* in NSCs leading to the formation of teratomas in murine xenografts [98, 106]. The identification of elevated Nestin expression, a putative marker for NSCs [107] and BTICs [9], in iGCTs with dissemination and metastatic potential [108] has provided additional evidence for the role of BTICs in driving intracranial germ cell tumorigenesis. Nevertheless, a clear distinction remains to be established between migratory germinal cells or neural lineage-derived BTICs as the cells of origin in iGCTs.

Craniopharyngioma

Craniopharyngioma (CP) is a rare type of low-grade malignancy originating in the sellar and parasellar regions of the brain. 30–50 % of CPs occur in the pediatric population with common symptoms including headaches, visual impairments, growth retardation, and additional symptoms relating to hypothalamic dysfunction [52, 109–111]. Current treatment for CP involves complete tumor resection in cases where the optic nerves or normal functioning of the hypothalamus may not be compromised. However, in cases where complete resection is not possible, surgical resection is complemented by local irradiation [109]. Although current treatment protocols ensure high survival rates (87–95 %), it is common for patients to experience a significant reduction in quality-of-life resulting from surgical complications relating to the optic nerves, pituitary gland, or hypothalamus [112].

CP is a non-glial tumor that originates from the malformation of embryonal tissue [113]. Histopathological features of CP in the pediatric population are in keeping with an adamantinomatous feature with possible cysts and in 70 % of the cases accompanied by stabilizing mutations in *CTNNB1,* which codes for the key downstream effector protein of the canonical Wnt pathway, β-catenin. In contrast, adult cases of CP are in keeping with a squamous-papillary histology [114].

Two possible cellular origins for CP include: the ectopic remnants of Rathke's Pouch (RP) or embryonal epithelial cells of the anterior pituitary gland and infundibulum [115, 116]. A recent transgenic mouse model of adamantinomatous CP with constitutive Wnt pathway activation in progenitor cells of RP has aided in determining a putative TIC [117]. Although all pituitary cells contained the β-catenin mutation, only a small population of cells showed the accumulation of β-catenin in the nucleus and cytosol during the pre-tumoral stages [118]. Further analysis of the β-catenin-enriched fraction of cells revealed the absence of the proliferation marker Ki67 along with the presence of long telomeres—two properties commonly associated with a quiescent state, a feature of stem cells. A subsequent gene expression analysis of the β-catenin-enriched cells showed increased activity of Shh pathway target genes, which are active during cell specification and proliferation of early RP progenitors [119]. Additional immunocytochemical analyses of human CP samples identified an increase in the expression of genes previously implicated in stem cells and BTICs: Sox2, Oct4, KLF4, and Sox9 [118]. Despite the activation of developmental signaling pathways and genes responsible for fate determination, it is still unclear whether RP progenitors may truly function as CP BTICs. The paucity of in vitro and xenograft data from putative human CP BTICs further confounds the identification and characterization of these cells for therapeutic targeting.

Conclusion

The study of pediatric brain tumorigenesis has drastically evolved over the past 100 years, with several key discoveries having been made in only the past decade. With the advent of deep genome sequencing of malignant tissue, the identification of additional molecular classification systems rooted in clinical outcome and risk stratification has begun to emerge. However, the heterogeneous nature of childhood brain tumors remains a burden to be reckoned with as recent reports have shed light on the extent of intratumoral heterogeneity within solid cancers. Given the urgent desire for targeted therapies and the observation of a heterogeneous genomic landscape, other frameworks and model systems should be investigated for exploring the dynamic nature of brain tumors. One such model system is that of the cancer stem cell (CSC) or brain tumor-initiating cell (BTIC). Since rare stem cell populations typically comprise a minority of cells within a heterogeneous tumor and these cells may be underrepresented on bulk tumor analyses, it is possible that very low transcript levels identify critical BTIC regulatory genes when profiling bulk tumors. Consequently, current molecular profiling techniques may not truly account for those genes preferentially expressed within the BTIC population. Moreover, the CSC model provides a framework to study the interplay between BTIC and their tumor niche, offering researchers with multiple perspectives regarding tumor biology and differential gene expression patterns in specific subsets of tumor cells.

Although the CSC model provides several advantages in studying tumor heterogeneity, one must not neglect the limitations accompanied with this framework. These challenges primarily surround our ability to characterize these rare clonal

populations of cells, which is particularly true for rare tumors such as MB, pediatric GBM, and DIPG. The current use of cell surface markers to prospectively identify BTICs has proved to be quite controversial. For example, based on differences in cell culture methods CD15 has been shown to identify cells either lacking [39] or displaying [40] multilineage differentiation and neurosphere formation, respectively. CD133 has also recently been identified as a contentious marker for BTICs [120–122]. The original work that prospectively established CD133 as a BTIC marker was restricted to minimally cultured, primary human cells [7, 8]; however, this may not be extended to the long-term culture methodologies applied in several recent papers, in which human BTICs have been passaged as tumor spheres in culture for greater than 3 weeks [123] or more than 20 passages [124]. It is highly possible that these long-term cultured cells have acquired transformation events in vitro that are independent of CD133 expression status or that CD133 protein expression levels no longer correlate with CD133 transcript levels or intracellular receptor activity. Consequently, the utility and readout of cell surface markers in distinguishing BTICs may be highly dependent on cell culture methods. Furthermore, primary human pediatric BTIC cultures are technically challenging, provide limited cell numbers for data acquisition, and require specific infrastructure; therefore, this platform is unlikely to be widely adapted for routine laboratory use at this point, unlike current genomic platforms. However, continued study of larger numbers of human BTIC specimens will eventually elucidate key stem cell signaling pathways and molecular mechanisms of self-renewal that could provide specific targets for tumors that remain refractory to current therapies.

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