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BRAIN TUMOR STEM CELLS: ROLE IN NEURO-ONCOGENESIS AND THERAPEUTIC IMPLICATIONS

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ABSTRACT

Malignant brain tumors are notorious for high morbidity and mortality. Our deficient understanding of brain tumor pathogenesis is reflected in our inability to cure this disease. Treatment remains palliative at best. The cancer stem cell hypothesis of brain tumors promises to consolidate many observations which have previously eluded neuroscientists and may reveal why aberrations in developmental programs are among the commonest findings in brain tumors. It is ironic that brain ontogeny and cancer - two processes with very different outcomes - exploit similar mechanisms to multiply, migrate, and survive. Implications of this hypothesis extend beyond mere academic interest. It may explain our current failures in the clinic and sets the stage for novel therapeutic paradigms aimed at altering the developmental adaptations of brain malignancies.

Diseases of the nervous system have been in focus as targets of stem cell therapies. Recently however, these cellular reservoirs have also become suspect in the pathogenesis of brain tumors.

Interest in stem cell therapies for neurological diseases has led to concurrent progress in developmental neurosciences. These studies have given way to surprising findings that contradict classical notions. It now seems that glial cells - originally named for their glue-like function in the CNS - are in fact the neural stem cells in the developing brain. They are the embryological predecessor of astrocytes known as radial glia, giving rise to neurons, astrocytes, and oligodendrocytes, as well as ependymal cells.[1,2] Furthermore, it is now known that radial glia persist into adult life in mammalian brain as more restricted astrocyte-like neural precursor cells (NSCs) within two mutually exclusive regions - the dentate gyrus of the hippocampus and a region immediately surrounding the lateral ventricles known as the subventricular zone (SVZ)[1,3,6] (Figure 1). In these regions, NSCs are responsible for neurogenesis - the process of formation of new neurons - throughout life3. Although NSCs are found at comparable locations in adult human brains[6,8], it is not known with certainty if they are neurogenic in situ.[9] It has also been demonstrated that approximately 3% of adult human white matter is composed of white matter precursor cells that, given the right environmental signals, can regain their ability to form the various cell types of the CNS.[10,12] These white matter precursor cells are thought to be derived from the embryonic SVZ and represent a quiescent pool of multipotent cells left over from developmental processes.[10,13]

TUMORS OF THE CNS

Primary brain parenchymal tumors (PBTs) are a heterogeneous group of neoplasms that originate within the CNS. PBTs encompass mainly glial cell tumors called gliomas. Gliomas can be low- to high- grade astrocytomas, pure oligodendrogliomas, ependymomas, mixed oligo-astrocytomas, and other rare variants. Other than gliomas, PBTs include a long list of less common brain neoplasms. Among these, primitive neuroectodermal tumors such as medulloblastoma (MB) are a special subset. Of the high-grade gliomas, glioblastoma multiforme (GBM) stands out as the most malignant of cancers and can be broadly divided into primary and secondary GBM. Primary GBM arise de novo in the absence of a pre-existing low-grade lesion, while secondary GBM develop progressively from low-grade gliomas. Many PBTs presenting with one histological picture may frequently recur as or progress to a high-grade tumor of a different histology, and it is not uncommon to observe neoplastic cells of distinct histologic subtypes within a given PBT.

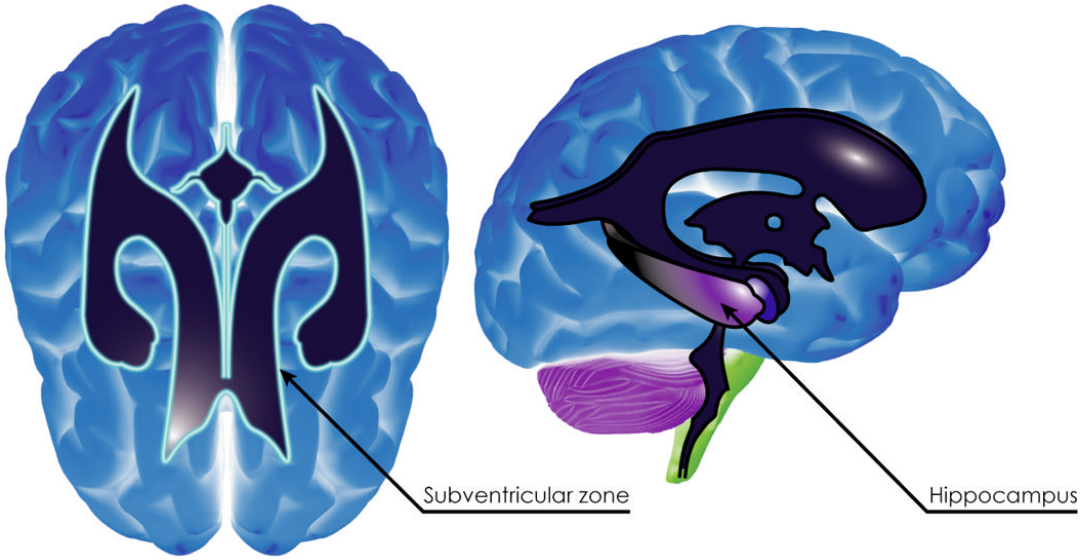

Figure 1 - Germinal niches in the adult human brain. The subventricular zone, shown as a blue halo surrounding the lateral ventricles from above, and the hippocampus, seen on either side of the lateral ventricles from the side, are two sites where NSCs reside in the adult brain. Additionally, NSC-like white matter progenitor cells are widely dispersed in the white matter and display properties of NSCs in vitro and in vivo.

Current therapy for most PBTs is surgical resection followed by radiotherapy and/or adjuvant chemotherapy; nevertheless, the mortality and morbidity of PBTs remains strikingly high, as does their recurrence rate.[14] Median survival of GBM, the most common PBT in adults, remains at 9-12 months even after intervention.[14] This grim prognosis justifies the search for novel therapies, which have hitherto been limited by our incomplete understanding of brain tumor biology. Defining the cellular and molecular origins of PBTs would hence have implications for a more cogent classification, as well as early diagnosis, and ultimately a curative treatment for PBTs.

NEURAL STEM CELLS

NSCs are operationally defined as primitive cells derived from the developing neuroepithelium that possess the ability to (1) proliferate; (2) self-renew by giving rise to daughter cells with identical properties from generation to generation; and (3) give rise to all the cell types (also known as lineages) of the CNS, a property known as multipotency.[3] The series of relatively differentiated intermediate cells generated by NSCs prior to their terminal differentiation into neurons, astrocytes or oligodendrocytes are collectively referred to as lineage restricted progenitors (LRPs) which retain the ability to proliferate and give rise to more differentiated progeny, but are unable to self-renew or display multipotency (Figure 2); these are short-lived and collectively serve to amplify the progenitor pool.

An exact definition of NSCs has been unforthcoming, largely because of absence of well-defined molecular markers for the developmental milestones of neural lineages. With this consideration, NSCs have been described on the basis of expression of nestin (an intermediate filament), CD133 (surface glycoprotein),[15,16] Sox1/2 (DNA binding proteins), Bmi1 (epigenetic transcriptional repressor),[17,18] Mushashi1 (RNA binding protein) and SSEA.[19,20] Additionally, NSCs have also been shown to express GFAP (glial fibrillary acidic protein; an intermediate filament) - a marker previously considered to be specific for astrocytes and radial glia.[4,5]

PRIMARY BRAIN TUMORS AND THE CANCER STEM CELL HYPOTHESIS

The concept that cancers may arise from cancerous stem cells is not entirely novel, being initially suggested as early as 150 years ago.[21] A series of publications in the 1960s and 1970s revisited this idea for human acute myeloid leukemias (AML) using in vitro colony-forming assays and suggested that rare cancerous cells in AML had properties expected of stem cells, i.e. they could proliferate, self-renew, and give rise to new tumors.[22] John Dick and colleagues extended this idea in vivo using serial xeno-transplantation experiments into bone-marrow ablated non-obese diabetic / severe combined immunodeficiency (NOD-SCID) mice. Even though the vast majority of the leukemic cells were unable to recapitulate the original malignancy, rare cells within these leukemias were able to repopulate the bone marrow and give rise to leukemic progeny typically seen in blood.

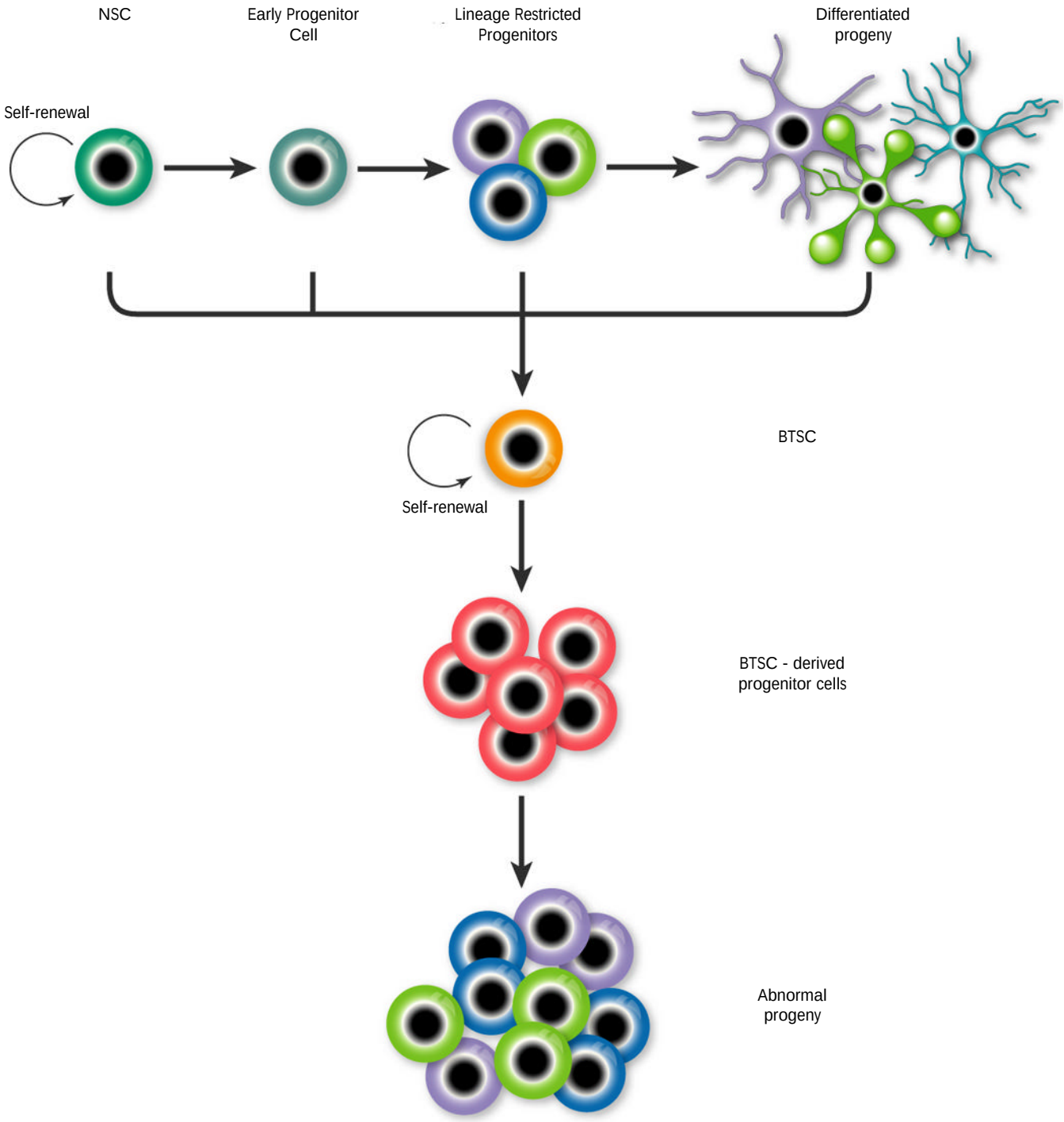

Figure 2 - Life history of a normal and cancerous stem cell. NSCs are able to self-renew, proliferate, and give rise to multiple lineages, including neurons (blue), astrocytes (lavender), and oligodendrocytes (green). Prior to terminal differentiation, NSCs give rise to increasingly restricted progenitor cells which serve to amplify the reserve pool; the LRPs of various lineages are shown here. The cancer stem cell hypothesis posits that cancers arise from cancerous stem cells - either from transformation or dedifferentiation of normal cells - and give rise to the various lineages typically representing the bulk of cancer cells.

The cancer-initiating cell forms the foundation of the cancer stem cell hypothesis, which posits that cancers arise from rare cancerous stem cells which give rise to the main bulk of tumor cells representing various lineages typically seen in malignancies. This hypothesis has now been expanded to include other hematopoeitic malignancies (chronic myeloid leukemia, acute lymphoblastic leukemia and multiple myeloma), breast, colon, and stomach cancer, and possibly prostate cancer.[23,25]

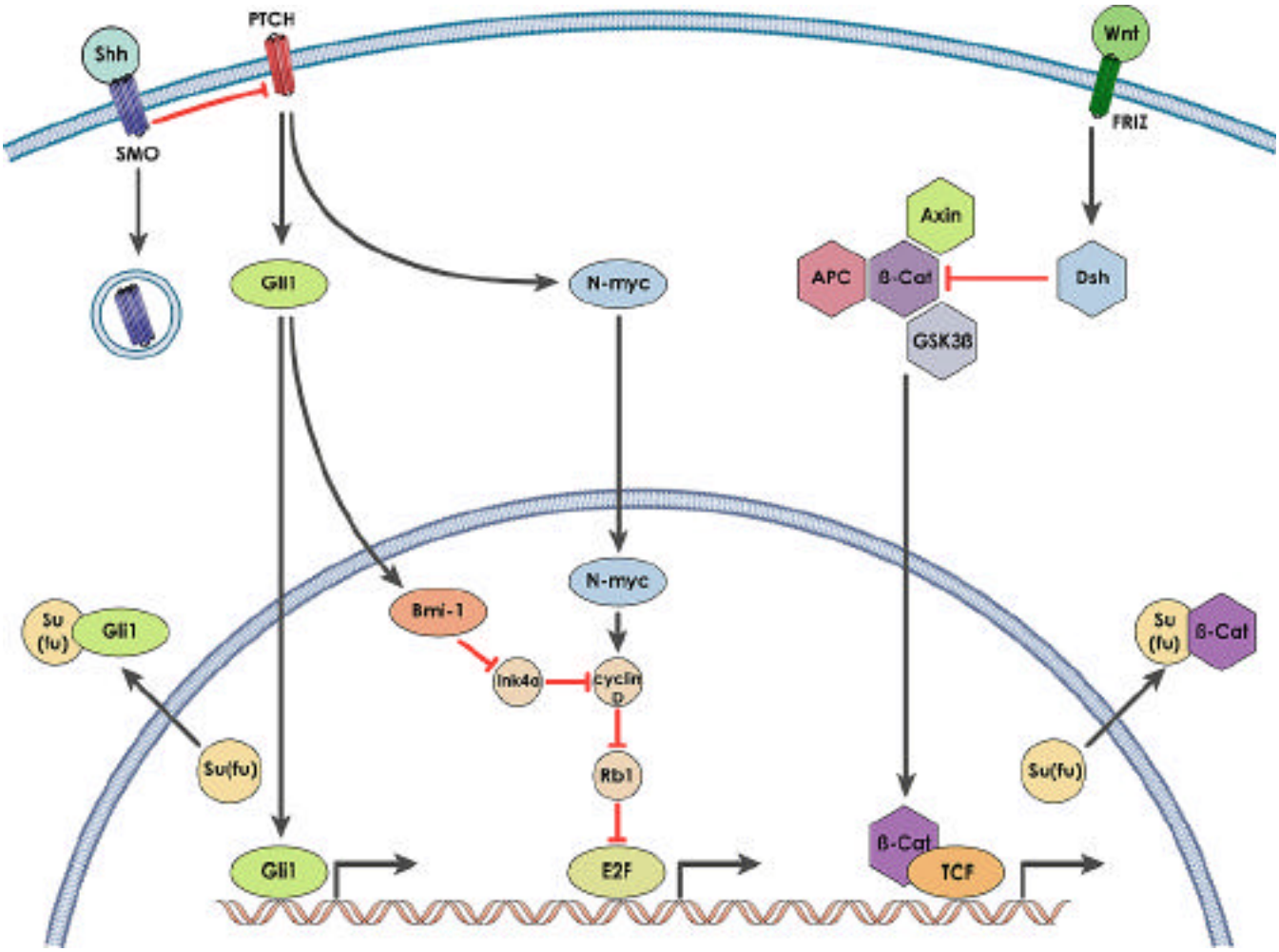

Figure 3 - Evolutionarily conserved signaling cascades involved in stem cell self-renewal. Three pathways that control self-renewal/proliferation of NSCs/LRPs include Shh, Bmi1, Wnt and are shown in this figure. Shh activates N-myc and Gli transcription factors as well as Bmi1 to induce changes in gene expression of several genes including Ptch, Gli1, Wnts, Pdgf, Igf2, N-myc, cyclins, and Foxm1. It is possible that Bmi1 and N-myc are activated by other signaling pathways as well. By activating cyclin D, Shh signaling also influences cell-cycle regulatory pathways. Wnt canonical signaling culminates in translocation of ≤-catenin into the nucleus to alter expression of developmental genes by interacting with its target transcription factor, TCF. Su (fu) is a terminator of Shh and Wnt signaling and acts by diverting Gli and ≤-catenin respectively away from the nucleus and targeting them for destruction; mutations in Su (fu) have been described in MBs.

Peter Dirk and colleagues probed the cellular origins of PBTs using tumor cells isolated from clinical samples of PBTs (GBMs and MBs) on the basis of NSC surface-marker expression (CD133+).[26] They elegantly demonstrated that as few as 100 cells from the CD133+ cell fraction could found tumors in NOD-SCID mice, while several thousand cells of the CD133- compartment were unable to do so, establishing the CD133+ fraction as the source of brain tumor initiating cells.[26] Furthermore, the CD133+ cells were able to recapitulate the original tumor morphology, lineage expression, and mitosis both in vitro and in vivo.[26,27] In addition to tumor generation, CD133+ cells also displayed the classic properties of stem cells in vitro: proliferation, self-renewal, and multipotency.[26,27] Serial re-transplantation of these likely brain tumor stem cells (BTSCs) verified the ability of this cell population to self-renew and remain multipotent in vivo.[26] However, they differed from normal NSCs by virtue of an abnormal karyotype, neoplastic proliferation in vivo, and/or epidermal growth factor receptor (EGFR) amplification,[26,27] depending on the original tumor sample.

The presence of brain tumor initiating cells has been reproduced in other studies as well.[17,28,29] It has also been shown that at least some of these BTSCs display other properties of NSCs such as Bmi1, Mushashi1, Sox2 mRNA expression,[17] high levels of telomerase reverse transcriptase associated with lack of replicative senescence,[28] as well as nestin and vimentin staining,[30,34] lending further support to notion that the transformed cell is now indeed a stem cell, albeit cancerous. That BTSCs derived from a range of different tumor phenotypes and patient samples are able to express several common markers of an undifferentiated lineage and display properties of NSCs suggests that the initial transforming event lies in the stem or progenitor cell compartment, rather than being a mere consequence of dedifferentiating mutations in terminally differentiated cells.[35]

Many strategies utilized by PBTs to infiltrate, grow and survive are an aberrant extension of characteristics already possessed by NSCs. The presence of a BTSC adds a new dimension to the heterogeneity of brain tumors, and may explain why several lineage markers may be identified in tumor specimens. The recent discovery that the developmental plasticity of NSCs may also include vascular endothelial cells leads to the exciting premise that the abnormal endothelial cells seen in tumors may also be a derivative of the BTSC itself.[36]

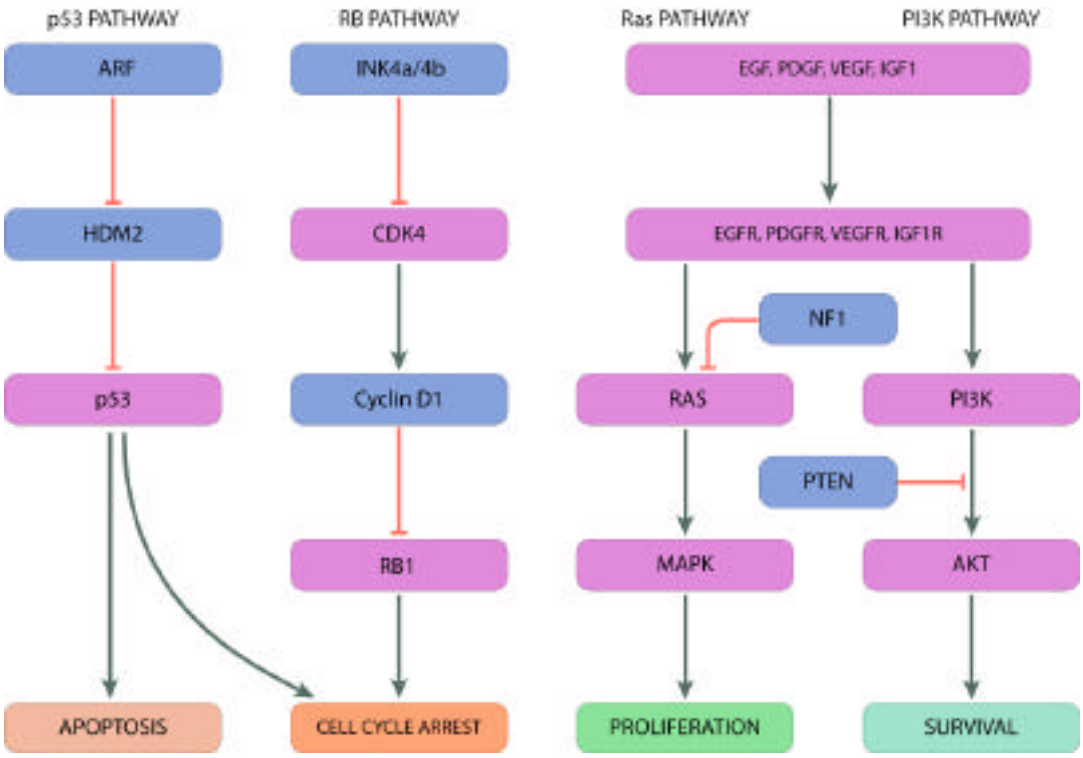

Figure 4 - Signaling pathways affected in primary brain tumors. Flow chart representing the two signaling cascades that are most commonly involved in tumorigenesis: the RTK-associated growth factor signaling cascade, represented by Ras and PI3K pathways and the cell-cycle regulatory cascade, represented by p53 and RB pathways. Human PBTs typically display alterations in any component of the listed pathways the net result of which is activation of RTK-signaling (e.g. EGFR overexpression) and/or inhibition of cell-cycle control (e.g. deletion of Ink4a-Arf). Alterations occurring in a cell-specific context in various components either within or between these pathways also influence the behavior and grade of tumor. Grey arrows signify activation while red hatchets signify inhibition.

BRAIN ONCOGENESIS - DEVELOPMENT GONE AWRY?

Organogenesis and oncogenesis are very similar processes. Both involve massive mitosis and self-renewal of the basal cell population, as well as extensive migration, differentiation, neoangiogenesis, and cell death.

The Hedgehog pathway is an evolutionarily conserved signaling cascade involved in tissue induction, patterning, growth and differentiation of the developing embryo.[37-41] Hedgehogs are secreted glycoproteins that act as morphogens, i.e. they exert variable effects in a concentration gradient by regulating expression of developmental Hox genes. Hedgehogs antagonize their transmembrane receptor Patched (Ptch) to release inhibition on Smoothened (Smo), culminating in activation of Gli zinc-finger transcription factors (Figure 3). Other downstream mediators of Hedgehog signaling include N-myc, Cyclin D and Bmi1.

Among the Hedgehogs, Sonic hedgehog (Shh) is prominently expressed in the CNS during development where it is known to participate in establishment of the dorso-ventral axis in the developing neural tube, specification of certain dopaminergic neurons, oligodendrogliogenesis, and maintenance of germinal niches in the embryonic forebrain.[42,47] Shh signaling has also been implicated in the maintenance of adult hippocampal and SVZ NSCs[48,51] both in vitro and in vivo, possibly by influencing proliferation and facilitating survival of the progeny, suggesting a conserved and generalized role of Shh signaling in maintaining stem cell niches in the brain even after completion of developmental programs.
Alterations in the Shh pathway is the single consistent abnormality ascribed to sporadic MBs.[52] Conversely, patients of Gorlin syndrome - having inherited mutations in the Ptch gene - have an increased incidence of sporadic MBs. Shh pathway involvement is as significant in MB oncogenesis as in normal proliferation of granule cell precursors from which they originate. Experimental evidence suggests that the Shh/Gli pathway may also be involved in the initiation and/or maintenance of gliomas. An analysis of primary CNS tumor samples and cell lines encompassing astrocytomas, GBM, and oligodendrogliomas revealed a consistent upregulation of Gli1.[53-55] The implications of these findings are two-fold: (1) expression of Gli1 may suggest an origin of these tumors within SVZ NSCs or LRPs, since most Gli1 expressing cells lie in this compartment in postnatal life;[48] and (2) the Shh pathway may be involved in self-renewal of the BTSC population within gliomas. Inhibition of Shh signaling has been demonstrated to induce tumor growth arrest in glioma cell lines in vitro using cyclopamine.[56,57] The potential involvement of the Shh-Gli1 axis in gliomagenesis needs to be explored further.

Phosphatase and tensin homologue on chromosome 10 (PTEN) is a tumor suppressor gene that is commonly deleted in secondary GBM. The functions of PTEN have been elucidated using transgenic mice and suggest that PTEN regulates several aspects of neural development including NSC self-renewal, cell migration, and apoptosis.[58-60]
PTEN deletion targeted to nestin+ NSCs leads to a massive increase in brain size, which can be partially attributed to an increase in proliferation and a decrease in apoptosis.[58]

Notch receptor signaling is another phylogenetically conserved pathway that can alter transcription of developmental genes by interacting with its ligands Delta/Jagged. Notch and its ligands have been shown to be upregulated several-fold in primary human oligodendrogliomas and grade II/III astrocytomas, but to a lower extent in GBM. Also, upregulation of Mushashi1, a negative regulator of Notch antagonists, has been demonstrated in MBs.

Bmi1 is a member of the polycomb repressor group of proteins that are involved in epigenetic modifications of DNA.[61] Bmi1 is crucial for self-renewal of normal hematopoeitic and neural stem cells and for proliferation of progenitor cells in their respective compartments, both in vitro and in vivo. This is associated with an upregulation of Ink4a-Arf proteins, members of the p53 and RB pathways which are known to inhibit NSC self-renewal and promote a terminally differentiated state[18] (next section), suggesting that Bmi1 permits NSC self-renewal at least in part by repressing Ink4a-Arf. Bmi1 upregulation as a mechanism by which Shh might promote self-renewal/proliferation in stem/progenitor cell populations,[62] and possibly in brain tumors.

Other potential developmental mechanisms that are active during development and may be worth investigating in BTSCs include the nuclear orphan receptor TLX, Sox group of DNA binding proteins, and the N-Cor co-repressor.

Based on available knowledge, it seems that development and cancer are two faces of the same coin, differing only in the strength, timing, or order of their responses to normal regulatory cues. Notwithstanding the similarities between NSCs and BTSCs, to date it remains unclear whether the BTSC is derived from brain-resident NSCs or LRPs, or from dedifferentiation of terminally differentiated cells. This is a fundamental question in understanding tumor initiation, progression, and behavior, and more importantly, in developing and designing targeted therapies.

TABLE.1
Cooperativity between signaling pathways and the possible cell of origin in mouse models of PBTs. Abbreviations: AA - anaplastic astrocytoma, DSR - double strand repair, GBM - glioblastoma multiforme, LGA - low-grade astrocytoma, MB - medulloblastoma, NHEJ - non-homologous end joining, ODG - oligodendroglioma, OAC - oligoastrocytoma. pGBM signifies primary GBM while sGBM signifies secondary GBM.

Tumor model	Gene Product targeted	Signal-transduction cascade affected	Lineage targeted/ Probable origin	Penetrance	Related references
MB	-Ptch	Shh	None / GCPs	30%	69
MB	+Smo*	Shh, Notch	None / GCPs	48%	70
MB	-p53, -Rb1	RB, p53	GFAP$^+$ / Astrocytes or NSCs	100%	71
MB	-p53, -Ptch	Shh, p53	None / GCPs	95%	72
MB	+Shh, +c-myc	Shh, Myc	Nestin$^+$ / NSCs or LRPs	23%	73
MB	+Shh, +IGF2 +Shh, +Akt	Shh, PI3K	Nestin$^+$ / NSCs or LRPs	39% 48%	74
MB	-p53, -DNALig4	p53, NHEJ (DSR)	None / GCPs	100%	75
MB	-p53, -PARP1	p53, DSR	None / GCPs	49%	76
ODG, OAC	+Pdgfb +Pdgfb, -Ink4a/Arf	Ras, PI3K	1. GFAP$^+$ / Astrocytes or NSCs 2. Nestin$^+$ / NSCs or LRPs	40-60%	77
ODG	+Pdgfb,-Ink4a/Arf +Pdgfb, -p53	Ras, PI3K Ras, p53	None / Unknown None / Unknown	60-83% 56-89%	78 78
ODG	+v-erb +v-erb, Ink4a/Arf	Ras, PI3K Ras, PI3K, RB, p53	S-100 / Glia or glial LRPs	60% 80%	79 79
ODG	+EGFR*, +Ras	Ras, PI3K	GFAP$^+$ / Astrocytes	50%	80
pGBM	+Ras, +Akt +Ras, +Akt, +c-myc	Ras, PI3K Ras, PI3K	Nestin$^+$ / NSCs or LRPs GFAP$^+$ / Astrocytes	26% -	81 81
pGBM	-Ink4a/Arf, Ras/Akt	Ras, p^{53}, RB	1. GFAP$^+$ / Astrocytes or NSCs 2. Nestin$^+$ / NSCs or LRPs	42-49%	66
pGBM	-Ink4a/Arf, +EGFR*	Ras, PI3K, p53, RB	1. GFAP$^+$ / Astrocytes 2. Nestin$^+$ / NSCs or LRPs	100%	82
AA, pGBM	+Ras*	Ras→PI3K, p^{53}, RB	GFAP$^+$ / Astrocytes	95-100%	83
LGA→sGBM	-p^{53}, -Nf1	Ras, p^{53}→Rb, PI3K	GFAP+ / Astrocytes or NSCs	100% LGA → 70% GBM	84
AA	+T$_{121}$, Pten$^{+/-}$	Rb, PI3K	GFAP$^+$ / Astrocytes	100%	85
LGA, AA	-p^{53}/Nf1 *cis*	Ras, p^{53}	GFAP$^+$ / Astrocytes or NSCs	55-77%	86,87

BTSCS AND THE MOLECULAR MECHANISMS OF TUMORIGENESIS

Oncogenesis may result from dysregulation of signal-transduction pathways due to accumulation of mutations, gene amplification, transcriptional activation, loss of regulatory cues, or any combination of these. The most frequent genetic abnormalities found in human PBTs either activate signal-transduction pathways downstream of growth factor receptor tyrosine kinase (RTK; RAS/MAPK and PI3K/AKT) or disrupt cell-cycle pathways that maintain cells in G1 phase and mediate apoptosis (p^{53}/ARF and RB/INK4a) as shown in Figure 4.

Evidence from mouse models with somatic mutations introduced in specific neural cell populations or germline mutations indicates that, in parallel with hematological malignancies, neoplastic transformation of NSCs/LRPs or de-differentiation of terminal astrocytes may both be viable routes to tumor formation.[63-65] The most common mutation in primary GBM occurs at the INK4a-ARF locus which simultaneously dismantles both p^{53} and RB pathways (Figure 4), increasing the likelihood of de-differentiation and cell-cycle entry.[66] However, alterations in Ink4a-Arf alone are insufficient to give rise to tumors, and require coupling with alterations activating RTK-associated mediators, such as Ras-Akt or Egfr. Conversely, activated RTK/Ras signaling also needs to recruit disabling alterations in cell-cycle control pathways to eventuate in malignant transformation suggesting that cooperativity between these two pathways is an essential feature of glioma initiation and generation of the founder BTSC. Inappropriate activation of RTK/Ras-PI3K pathway is a crucial event in brain gliomagenesis (and probably BTSC formation) and cooperates with inactivation of p^{53} and RB pathways in tumor initiation and progression.

THERAPEUTIC IMPLICATIONS AND CONCLUSIONS

The search for therapies for PBTs has so far remained elusive, partly due to our incomplete understanding of the tumor pathophysiology. The recent discovery of BTSCs in brain tumors provides some clues into their origins and may suggest why PBTs are so resilient to known chemotherapies. One reason may be that stem cells are known to express several classes of ATPase-binding cassette transporters (ABC) that actively efflux most lipophilic agents, including therapeutic drugs that would ordinarily kill most cells.[67] Chemotherapeutic agents target actively dividing cells; since NSCs (and presumably BTSCs) spend more time in G0, they are inherently resistant to such treatments. Stem cells also have a greater capacity for DNA repair compared to other cells allowing them to endure DNA-damaging agents.[68] Consequently, even though a therapeutic agent may be able to reduce tumor bulk and lead to clinical remission, it may be unable to cure the cancer owing to persistence of drug-resistant BTSCs which are capable of giving rise to recurrence over time.

What leads to the generation and maintenance of BTSCs? Evidence from tumor models favors a dominant role of dysregulation in signal-transduction pathways, usually a combination of RTK-associated pathways and cell-cycle regulators, as well as many pathways that are involved or implicated in NSC maintenance. These findings go well with retrospective genetic and expression analyses of human PBTs. Accumulation of mutations in these pathways either in the parent BTSC or its progeny may alter tumor characteristics such as invasiveness, growth, phenotype, and consequently, tumor grade. At least in some instances, antagonizing these pathways can lead to differentiation, apoptosis, or even complete regression of the tumor. The fact that BTSCs can be induced to differentiate into cells expressing markers of a mature lineage suggests that differentiation therapy of tumors may not be a far-fetched idea. It seems plausible that the treatment of PBTs may be as simple as inhibition or stimulation of a signaling cascade in BTSC.

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