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Neural Stem Cells and their Role in the Pathology and Classification of Central Nervous System Tumors

Nöral Kök Hücreler ve Santral Sinir Sistemi Tümörlerinin Patolojisinde ve Sınıflandırmasında Rolleri

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ABSTRACT

Today, one of the most popular and controversial topics in medicine is undoubtedly the rapidly developing field of stem cell research. Some of the controversy in this field arises from lack of uniform terminology and different interpretation of concepts such as brain tumor stem cells. In addition, lack of reliable and universal markers that can identify stem cells and define precursor cells in a particular differentiation pathway further confounds the interpretation of results in many studies.

Stem cells are undoubtedly critical in normal cellular development as well as tumor biology and better characterization of these cells is likely to have profound influence on the classification schemes of tumors. In this manuscript, we present the generally accepted definitions of key concepts in stem cell biology and review some of the related molecular pathways. In addition, we put forth our position on how progress in this field should be affecting the future classification schemes of central nervous system neoplasia. We strongly believe that the ever increasing knowledge in the field of neural and brain tumor stem cells should be influential in the subsequent attempts to classify brain tumors.

Key Words: Stem cells, Neural stem cells, Brain neoplasms, Classification

ÖZ

Günümüz tıp dünyasındaki en popüler ve tartışmalı konulardan biri kuşkusuz büyük bir hızla gelişen kök hücre alanındaki araştırmalardır. Bu alandaki uzlaşmazlıkların bir kısmı üniform bir terminoloji olmamasından ve beyin tümörleri kök hücreleri gibi kavramların farklı yorumlanmasından kaynaklanmaktadır. Ayrıca, kök hücreleri ve öncül hücreleri tanımlayan güvenilir ve evrensel belirteçlerin olmaması birçok çalışmadaki sonuçların yorumlanmasını daha da zorlaştırmaktadır.

Kök hücreler, gerek normal hücresel gelişimde gerekse tümör biyolojisinde kuşkusuz büyük önem taşırlar ve bu hücrelerin daha iyi karakterize edilmesi, tümörlerin sınıflandırma şemaları üzerinde büyük etki oluşturabilir. Bu yazıda, kök hücre biyolojisindeki temel kavramların genel olarak kabul edilen tanımlarını ve gözden geçirdiğimiz bazı ilişkili moleküler yolakları sunuyoruz. Yanısıra, bu alandaki gelişmelerin santral sinir sistemi neoplazilerinin gelecekteki sınıflandırma şemalarına nasıl etki etmesi gerektiği ile ilgili düşüncelerimizi belirtiyoruz. Nöral tümör ve beyin tümörü kök hücreleri alanındaki giderek artan bilgilerin, beyin tümörlerinin ilerideki sınıflandırma çabaları üzerinde etkili olması gerektiğine kuvvetle inanıyoruz.

Anahtar Sözcükler: Kök hücreler, Nöral kök hücreler, Beyin neoplazmları, Sınıflandırma

INTRODUCTION

Two problems plague the issue of stem cells and specifically the neural stem cells: ambiguity of definitions and the highly controversial debate fueled by personal or social beliefs. There is no doubt that the latter problem is partially exacerbated by the former. The goal of this review is to provide a forum for the use common terminology and to highlight how the advances in the understanding of neural stem cells can influence brain tumor classification. We present a brief review of the literature to clarify the critical concepts such as stem cell, neural stem cell, precursor cell, cell of origin, brain tumor stem cell and tumor-initiating cell. We also discuss our perspective on the impact of advances in stem cell biology on pathological classification of central nervous system tumors. We hope that our attempt will help to eliminate some controversies that arise from ambiguity in the definitions and encourage the framers of the WHO classification scheme to consider the findings in this ever advancing field of research.

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Stem Cells

The concept of a "stem cell" in biology has been in existence since the 19th century in the works of scientists like Alexander A Maksimov or Ernst Heinrich Haeckel (1). These scientists have postulated the existence of stem cells that give rise to mature cells. Today, the term stem cell identifies the cells that are capable of replication and possess a number of unique characteristics. First, all stem cells are required to be unspecialized cells capable of renewing through asymmetric cell division even after long periods of inactivity (**self-renewal**) (2). Second, under certain conditions, stem cells can be induced to differentiate into numerous types of specialized cells (**pluripotency**) (2). In addition, a stem cell is subject to physiological regulatory mechanisms of the organism (**regulability**) (3). In some organs, stem cells regularly divide to repair and replace damaged cells, while in others stem cells only divide under special conditions. There is much debate on the types and nature of stem cells, yet most agree that these cells exist mostly in the embryo and to a much lesser extent in the adult (4). The three cardinal features (self-renewal, pluripotency, regulability) are critical in the identification of "normal" stem cells in organisms.

Numerous proteins that also exist in mature cells have been postulated as stem cell markers. While the expression of these markers has been considered sufficient for stemness, cells harboring such markers often do not have all three cardinal features of normal stem cells (5). On the other hand, bona fide stem cells do not seem to harbor some of these markers (6). As of today, a perfect stem cell marker is elusive and the most reasonable studies utilize a panel of markers to confirm "stemness" of a given cell in addition to demonstration of pluripotency and asymmetric division capacity.

Neural Stem Cells

Neural stem cells (NSCs) are the self-renewing, pluripotent cells that can give rise to all types of differentiated neuroepithelial cells. The first putative NSCs were isolated in 1992 from the subventricular zone of mice brain (7). Following these studies, NSCs have been isolated from various areas of the fetal and adult brain of many species (8, 9). The discovery of NSCs during adult life significantly altered our understanding of brain physiology and suggested that neurogenesis is possible in the adult and may confer some plasticity to the CNS (4).

As a source of NSCs, the subventricular zone of the lateral ventricle (SVZ), its cellular composition and architecture have been well studied (10). These studies suggest that NSCs in SVZ (so called B cells) give rise to more restricted, "transiently amplifying progenitor cells" (so called C cells), which eventually differentiate into neuroblasts (so called A cells) and oligodendrocytes (4). NSCs have also been identified in other parts of the adult brain. As a caudal extension of the SVZ, the subcallosal zone between the hippocampus and corpus callosum was also found to contain cells that behaved in a stem cell fashion in vitro (11). Furthermore, subgranular zone of the dentate gyrus (SGZ) (12), the boundary between internal granular layer and white matter of the cerebellum (13), and spinal cord (14) were shown to harbor cells with stem cell properties. In all these sites, either the cells identified as NSCs lack pluripotency or the demonstration of their stem cell properties predicates special conditions.

The historical model for NSC maturation proposed the emergence of distinct neuronal and glial precursors early in the development. This theory was based on the idea that neurogenesis and gliogenesis occurred independent of each other (15). Others suggested that NSCs in ventricular zone of the embryo give rise to neuronal precursors as well as radial glia (16). However, recent evidence suggests that NSCs can also be induced to differentiate into mesenchymal cell types (2). Studies in avian and mammalian species also demonstrated presence of radial glia in adults (17, 18).

Some authors argue that most mature cells in the CNS are not directly derived from NSCs, but arise through formation of the transitional cell types known as intermediate precursor cells (19). **Precursor cell** defines an immature embryonal cell with limited differentiation potential that will give rise to mature cells along a committed pathway. Most importantly, the precursor cells may not undergo asymmetric mitotic division and are more limited in their potency as opposed to the stem cells.

NSCs can be identified by the neurosphere assay in which the cells of interest form three-dimensional spheroids in serum-free media on a non-adhesive substrate in the presence of βFGF and EGF (7). Neurospheres include precursor cells and mature cells in addition to a small number of putative stem cells. Thus, neurosphere formation is not specific for NSCs and precursor cells can also form neurospheres in culture. However, precursor cells often lose this ability in repeated passages unlike stem cells that can continuously form neurospheres. NSCs can also be isolated by the neural colony-forming cell assay. This assay allows discrimination between NSCs and precursor cells, but also has significant limitations (20).

There is no doubt that understanding NSC biology and stages of neurogenesis can provide greater insight into CNS tumor biology. Through this insight, we could better identify the **cell of origin** for each mature cell type and characterize specific markers along the differentiation pathways. The latter will greatly enhance our understanding of CNS tumors and allow us to better classify them based on an improved cell of origin paradigm.

Markers of Neural Stem Cells

One of the first reported NSC markers is nestin, which is an intermediate filament and had been associated with both stem cells and precursor cells (21). Nestin expression has been correlated with the stemness of a cell and its expression decreases with concomitant increase in neuronal or glial differentiation marker expression (22). Another interesting marker is CD133 that was initially demonstrated in mouse brains (23). CD133 positive cells isolated from the human brain are capable of neurosphere formation *in vitro* and proliferation, migration and differentiation *in vivo* (24). However, this marker has been found in differentiated as well as precursor cells in humans, and its utility has been questioned (5). A similar protein, CD34 that can reliably identify hematopoietic stem cells is not as helpful in the identification of NSCs, and is less specific and less sensitive than CD133 (25). Musashi-1, an RNA-binding protein, was also reported as being highly expressed in NCSs, glial precursors as well as astrocytes and it is down-regulated in mature neural cells (26).

Many other markers have been suggested but their utility as stem cell marker has been limited (27). None of the above mentioned markers are specific to stem cells and sorting the cells by these markers may increase the number of stem cells in cultures but do not necessarily isolate them in purity.

The Concept of Brain Tumor Stem Cell

Existence of cells with stem cell properties in tumors was initially suggested in studies with human acute myeloid leukemia (28). Later, cells that share NSC properties were identified in brain tumors leading to the concept of **brain tumor stem cells** (BTSCs) (29). Despite many publications on BTSCs, an accurate and consistent description of these cells has not emerged. Therefore, BTSC is still a concept without a common definition. One perspective considers BTSC synonymous with (normal or abnormal) **brain tumor initiating cells** that are assumed to give rise to brain tumors. Others define BTSC as neoplastic cells that have all the properties of NSC including self-renewal and expression of stem cell markers. In most instances, pluripotency of these cells are not well documented.

There is much debate about the cell of origin for BTSCs and whether they are NSCs, precursor cells that have lost cell-cycle regulation or mature cells that have gained stem cell features. Most tumor cells have the ability to proliferate, but it is not clear if this is self-renewal capability akin to stem cells or to precursor cells. Furthermore, there is insufficient evidence that BTSCs actually undergo asymmetric cell division and generate both differentiated and undifferentiated cells.

While putative BTSCs share many features with normal NSCs, they also show significant differences. BTSCs often form neurospheres more efficiently at a faster rate and can be sustained in culture much longer than NSCs (30). In addition, injection of BTSCs into immunodeficient mice is tumorigenic whereas injection of normal NSCs constantly fail to produce tumors. Tumors caused by BTSCs are locally invasive and can migrate along white matter tracts to distant sites in the host brain (30). Most importantly, cells differentiating from BTSC exhibit aberrant morphology and dual-fate markers in contrast to cells differentiating from normal NSCs (31). The expression of NSC markers such as nestin, CD133 or Musashi-1 have been different in BTSC (29, 31). While the expression of stem cell markers in tumors does not imply stem cell character, the absence of these markers does not exclude tumor-initiating properties of the cells in question (32). All of these challenges raise doubt about the validity of studies that identify BTSCs based on markers that have not been conclusively proven to distinguish stem cells from other cell types.

CNS Tumor Classification and Stem Cells

Primary CNS tumors constitute a diverse group of neoplasms that range from indolent to highly malignant, and our understanding of their diversity is best reflected by the recent WHO classification (33). This classification scheme, similar to earlier classifications, is based on the presumed tumor **cell of origin**. The cell of origin paradigm relies on the morphological resemblance of tumor cells to their normal counterparts in the adult CNS. In tumors such as medulloblastoma and ependymoblastoma, the cell of origin is considered to be precursor cells. However, not all tumors, entities or variants in WHO classification can be traced to a normal cellular element. This fact is reflected in tumors such as angiocentric glioma, chordoid glioma or dysembryoplastic neuroepithelial tumor.

Current WHO classification provides biologically and clinically relevant scheme for the majority of the CNS tumors and gives some insight for the others. Most of the studies that provide critical information regarding

the molecular and genetic features of the CNS tumors use the WHO classification (34). Further refinement of the classification through better Identification of cell of origin and tumorigenic pathways can lead to significant improvements in our understanding of CNS neoplasms. It will be equally beneficial to correctly define brain tumor initiating cells and to identify whether such cells originate from NSCs. Thus, the characterization of precursor cells and NSCs in the CNS and their differentiation pathways becomes crucial in to answer the above questions. A better insight will further allow better and biologically more relevant tumor classification schemes and hopefully better management of patients. One of the helpful ways in this effort is the study of molecular pathways that are common to both NSC biology and CNS tumors.

Common Molecular Pathways in NSC Biology and CNS Tumors

A number of pathways involved in cell proliferation, differentiation and migration were shown to be critical in the regulation and function of NSCs. Some of these pathways and their dysregulation were also implicated in CNS neoplasia. While it is hardly possible to construct a comprehensive list, some of the critical pathways are briefly mentioned in this section. As more common pathways are discovered for both normal and tumor stem cells, we expect to determine a stronger association between NSC dysregulation and CNS tumorigenesis.

Epidermal Growth Factor Receptor, Phosphoinosite 3-Kinase and the Phosphatase and Tensin Homologue

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with an external ligand binding domain and a cytoplasmic region that is homologous to other tyrosine kinases (35). EGFR family of proteins is widely expressed in many tissues and mediates various cellular processes including cell division, adhesion, differentiation and apoptosis. Ligand binding induces receptor dimerization and tyrosine kinase activation, which further initiates various downstream signaling cascades

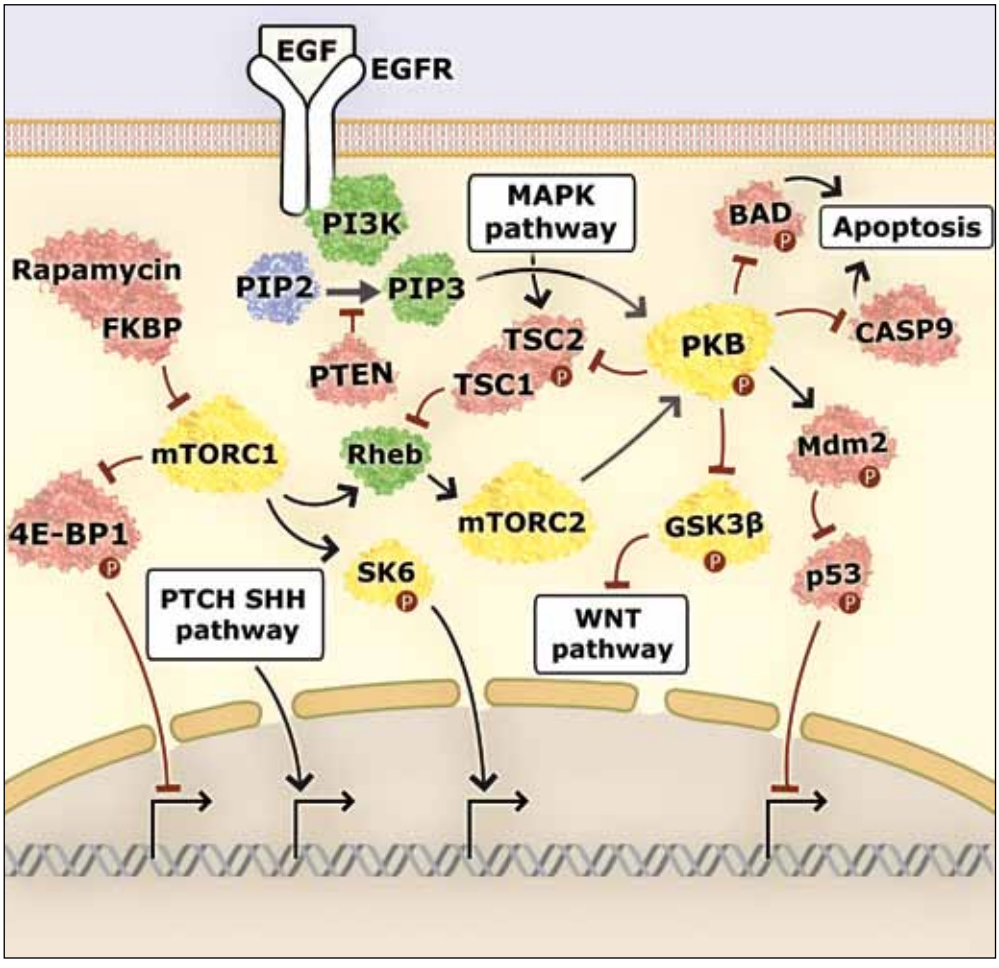

Figure 1: Simplified diagram demonstrating critical elements of the EGFR/PI3K/mTOR pathway. In this diagram, the molecules labeled with the green color are predominantly pro-proliferative or activating molecules, while red-colored molecules identify anti-proliferative molecules as well as tumor suppressors and checkpoints. The yellow-colored molecules can be either pro or anti-proliferative depending on the specific cell or circumstance. Red lines indicate inhibitory reactions while dark arrows are activating reactions. **EGF(R):** epithelial growth factor (receptor); **FKBP:** FK506 binding protein; **4E-BP1:** translation initiating factor 4E binding protein-1; **mTOR:** mammalian target of rapamycin; **PI3K:** phosphoinositide 3-kinase; **PIP:** phosphatidylinositol 4,5-bisphosphate; **PIP3:** phosphatidylinositol (3,4,5)-trisphosphate; **TSC1 and 2:** tuberous sclerosis complex proteins 1 and 2; **PKB:** protein kinase B; **GSK3b:** glycogen synthase kinase 3 beta; **BAD:** Bcl-2-associated death promoter; **CASP9:** caspase 9; **Mdm2:** murine double minute oncogene 2 protein.

including the phosphoinositide 3-kinase (PI3K) (Figure 1), mitogen activated protein kinase (MAPK) (Figure 2) and signal transducer and activator of transcription 3 (STAT3) pathways.

Activated EGFR signaling enhances proliferation and survival, and inhibits differentiation of NSCs (36). Dysregulation of EGFR signaling is one of the most common genetic alterations in malignant gliomas (37). EGFR gene overexpression, with or without amplification, occurs in 40-70% of primary glioblastomas and various clinical trials have utilized therapies targeting EGFR (38). In addition, activating mutations such as EGFRvIII are found in approximately half of the tumors overexpressing EGFR and have been associated with poor prognosis (39).

One of the principal enzymes activated by the EGFR-ligand interaction is PI3K. Activation of PI3K triggers phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3) which in turn phosphorylates protein kinase B (PKB) also known as Akt (40). Phosphorylated PKB subsequently affects various substrates that regulate cell survival, growth, proliferation and metabolism including Bcl-2/Bcl-XL-associated death promoter (BAD), tuberous sclerosis complex 2 (TSC2), proline-rich AKT substrate 40 (PRAS40), cyclin-dependent kinase 27 and 21, and glycogen synthase kinase-3β (GSK3β) (41). The diversity of PKB targets leads to significant convergence between the PI3K pathway and others such as Wnt/β-catenin and sonic hegdehog and Myc in many tumors (3, 42). One of the best-conserved functions of PKB is its role to promote cell growth mainly through activation of mTOR (mammalian target of rapamycin) complex 1(mTORC1). PKB phosphorylates the TSC2 within TSC1-TSC2 (Tuberin-Hamartin) complex and blocks their ability to inhibit the formation of mTOR-raptor complex (mTORC1) (43). mTORC1 regulates protein synthesis by phosphorylating translation initiation factors and increasing the level of proteins that are needed for cell cycle progression, proliferation, angiogenesis, and survival pathways.

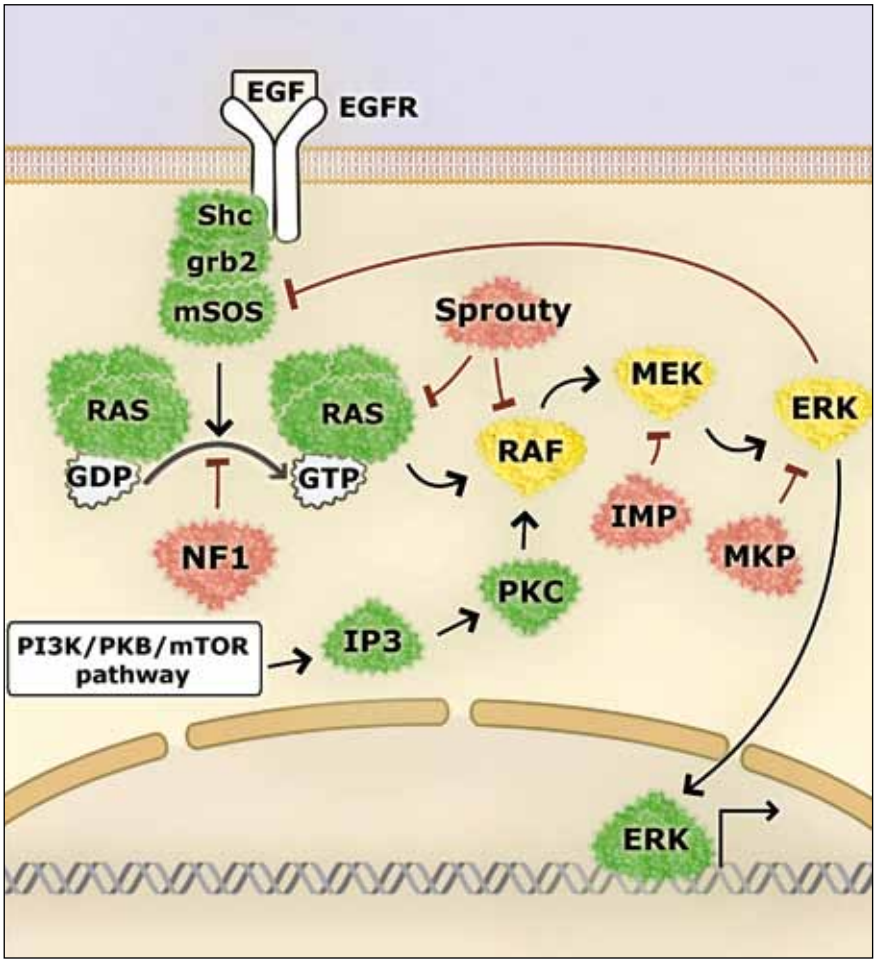

Figure 2: Simplified diagram showing the critical elements of the MAPK pathway. The colors for each molecule as well as the arrows are used in an identical fashion as Figure 1. This pathway highlights the central role of RAS and RAF proteins in the MAPK pathway, which has been identified as a critical pathway for oncogene-induced senescence.
RAS: the superfamily of gene products originally identified from a "rat with sarcoma"; **Shc:** SH2 containing sequence; **grb2:** growth factor receptor bound protein 2; **mSOS:** mouse guanine nucleotide exchange factor; **NF1:** neurofibromatosis 1 protein; **RAF:** serine-threonine kinase family of proteins; **MEK:** mitogen activated protein kinase kinase; **IMP:** impedes mitogenic signal propagation E3 ligase; **MKP:** MAPK phosphatase; **ERK:** extracellular signal regulated kinase.

The phosphatase and tensin homologue (PTEN) negatively regulates PI3K/PKB/mTOR signaling pathway by dephosphorylating PIP3 to PIP2 (40). It is the second most commonly mutated tumor suppressor gene only after TP53, and inactivated in various malignancies including brain tumors (44). PTEN antagonizes tumor proliferation induced by the PI3K/PKB pathway, and PTEN loss is associated with PI3K/PKB pathway activation. Loss of PTEN is quite common in high grade gliomas and the analysis of PTEN either by immunohistochemistry or mutational analysis has found significant use in clinical practice (45).

Mitogen Activated Protein Kinase Pathway

The MAPK pathway is a genetically conserved signaling cascade that involves a series of protein kinases most of which are activated by phosphorylation (Figure 2). Binding of receptor tyrosine kinases by growth factors leads to downstream phosphorylation of Ras, Raf, Mek and Erk which regulate transcriptional factors related to the cell cycle, migration, angiogenesis, and self-renewal of NSCs (46). Ras activation can be reversed by GTPase-activating proteins such as Neurofibromin 1 (NF1) (47). The MAPK pathway is dysregulated in a wide range of malignant tumors including high grade gliomas (48). The importance of the MAPK pathway in the pathogenesis of pilocytic astrocytomas was initially suggested by the association of Neurofibromatosis-1 (NF-1) and optic gliomas. Although mutations of NF1 gene cannot be shown in sporadic pilocytic astrocytomas, further studies revealed duplication of KRAS and BRAF genes leading to fusion proteins (49).

Tropomyosin-Related Kinase and Pan-Neurotrophin Receptor

The tropomyosin-related kinase (Trk) receptors (TrkA, TrkB and TrkC) are receptor tyrosine kinases that can be activated by one or more of neurotrophins including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins 3 and 4 (NT3 and NT4) (50). Activated receptor provides recruitment of adaptor proteins that will initiate signaling cascades including Ras/Raf/Erk and PI3K/PKB. Neurotrophin-mediated activation of Trk receptors regulates cell proliferation, survival and remodeling. Expression of TrkA has been linked to positive prognosis in medulloblastoma (51). Expression of TrkA and TrkC was correlated with apoptosis in medulloblastoma (52). In addition, Trk receptor expression has been suggested in reactive astrocytes as well as astrocytic tumors, in contrast to oligodendroglial tumors (53).

All the neurotrophins also bind to the pan-neurotrophin receptor p75NTR which is an unusual member of the Fas tumor necrosis factor (TNF) receptor family (54). P75NTR regulates affinity and specificity of Trk receptors to neurotrophins so that Trk receptors are more sensitive to low levels of their preferred ligands in the presence of p75NTR (54). In addition to potential attenuation of Trk signaling by limiting the non-preferred ligand activation, studies have shown that p75NTR has autonomous pro-apoptotic effects. Recent studies showed that p75NTR is a specific marker of cerebellar external granular layer cells and is expressed in a subset of medulloblastomas (55).

WNT Signaling Pathway

The WNT signaling pathway is composed of a complex set of proteins that lead to activation of nuclear transcription factors and regulation of cell fate. The so-called "canonical WNT signaling pathway" describes the series of events that occur when WNT proteins bind to their transmembrane receptors (Frizzled) and activate the cytoplasmic signaling proteins (Dishevelled, DSH) (Figure 3A). Activated Dsh disrupts the complex of Axin, adenoma poliposis coli protein (APC), GSK3β and β-catenin. This complex targets β-catenin degradation and its disruption causes accumulation of β-catenin that leads to transcription of genes related to cell cycle entry/proliferation and survival such as Cyclin D1 and cMYC (56).

WNT signaling induces proliferation of progenitor cells as well as neural differentiation (57). Recent studies demonstrate β-catenin accumulation in gliomas and medulloblastomas via mutations of β-catenin, APC or Axin1 (58,59). In addition, medulloblastomas showing nuclear β-catenin positivity were reported to have better prognosis compared to β-catenin negative medulloblastomas (60).

Sonic Hedgehog Signal Transduction Pathway

Hedgehog signal transduction pathway is a ubiquitous signaling cascade that is common to many species (Figure 3B). Three secreted hedgehog ligands have been identified in mammals and sonic hedgehog homolog (Shh) is the best studied ligand. Shh binds its receptor patched (Ptch) leading to disinhibition of the transmembrane proto-oncogene, Smoothened (Smo). Smo initiates a signaling cascade that activates transcription factors such as Gli proteins (61). Activated Gli proteins promote the transcription of numerous genes including cell cycle regulators (*Cyclin D1, Cyclin E, cMYC* and *nMYC*), growth factors and their receptors (62).

The Shh pathway is essential for the normal development of human cortex (63), granular cell precursors in the cerebellum (64) and formation and regulation of adult neural stem cells in the SVZ (65). SHH gene mutations are

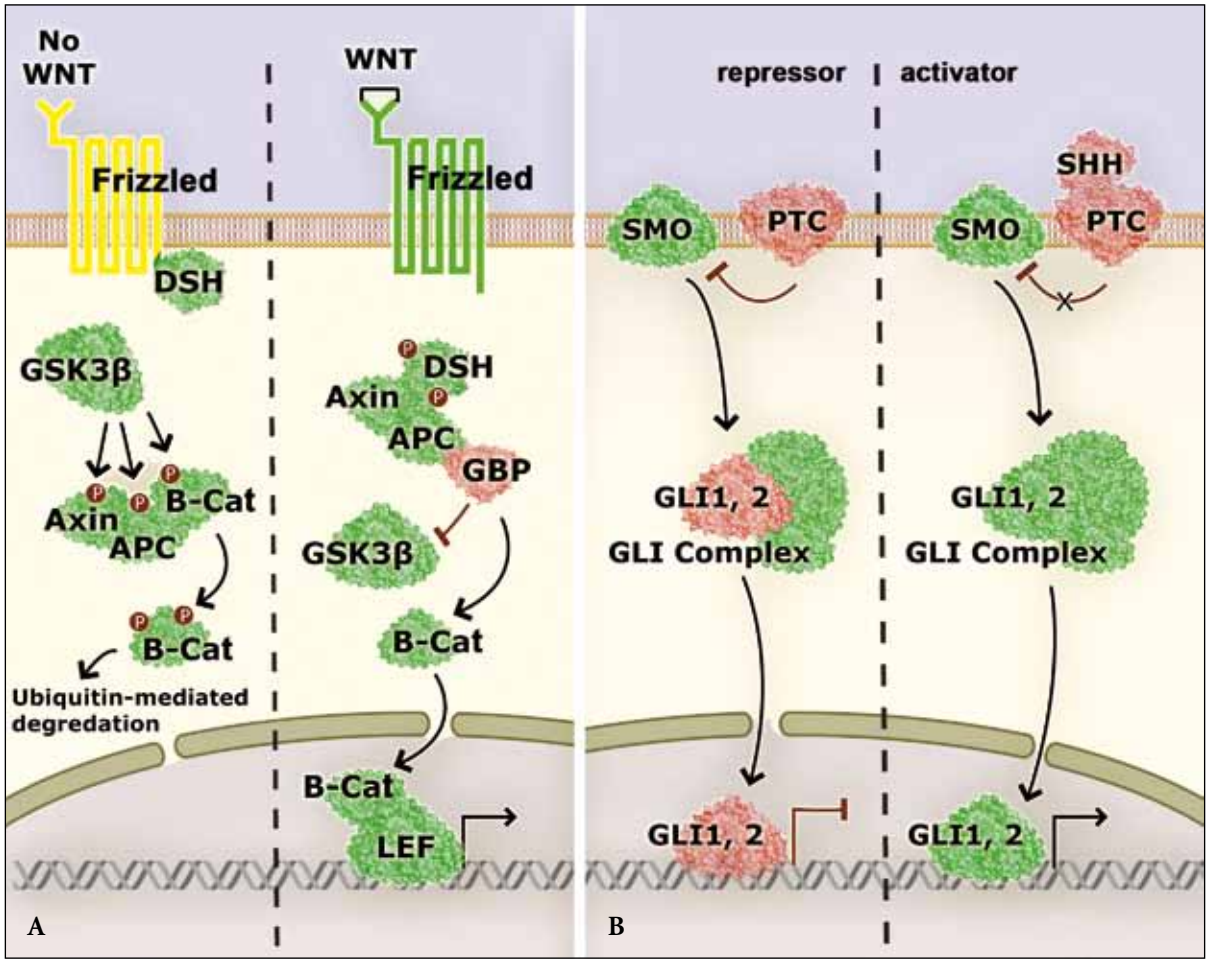

Figure 3: Simplified diagram for **a)** WNT pathway and **b)** SHH pathway. Again the colors of the protein reflect their activator/pro-proliferative (green) or inhibitory (red) nature. **A)** Beta catenin is a critical factor that affects the transcription of many genes and this effect can vary in different cells and under different circumstances. **WNT:** combination of wingless and INT genes and defines a group of proteins that directly interact with the membrane receptor Frizzled. **DSH:** dishevelled protein; **GSK3b:** Glycogen synthase kinase 3 beta; **B-Cat:** Beta catenin; **APC:** adenomatous polyposis coli protein; **GBP:** GSK3 binding protein. **B)** The SHH- sonic hedgehog pathway uses both repressor or activator forms of GLI proteins to affect the transcription and in some cases these seemingly opposite effects work in tandem to elicit the desire biological result. The GLI gene was originally isolated from a glioblastoma cell line. **SMO:** smoothin; **PTC:** patched.

associated with a number of developmental anomalies and Shh pathway dysregulation has been found in some gliomas and medulloblastomas (34).

BMI Polycomb Ring Finger Oncogene

B-cell specific Moloney murine leukemia virus integration site 1 (BMI-1) is a member of Polycomb group (PcG) gene family of chromatin modifiers and transcriptional repressors. These molecules are necessary for efficient self-renewal of adult hematopoietic stem cells and NSCs. BMI-1 promotes cell proliferation and stem cell renewal by inhibiting the transcription of the cyclin-dependent kinase inhibitors, $p16^{INK4A}$ and $p19^{ARF}$ that are responsible for RB1 and TP53 gene activation, respectively (66).

BMI-1 has a very broad tissue distribution and its deficiency results in severe neurological, skeletal and hematopoietic defects (67). BMI-1 is also thought to function as an inhibitor of senescence by inhibiting p53 protein. BMI-1 over-expression has been related to glial neoplasms (68). Furthermore, BMI-1 overexpression was reported in medulloblastomas as an alternative mechanism for SHH pathway activation (69).

Notch Signaling Pathway

The Notch signaling pathway (NSP) is highly conserved throughout the species and is critical for cell differentiation during embryogenesis and adult life. The Notch gene encodes a heterodimeric transmembrane receptor in the

plasma membrane and NSP is initiated upon cell to cell interaction. Activation of the receptor leads to cleavage of the intracellular domain by presenilin-γ-secretase complex and the cytoplasmic region is transferred to the nucleus. Notch then binds to the transcriptional regulator complex resulting in displacement of co-repressors and recruitment of co-activators that regulate cell-cycle entry and progression.

Notch is involved in lateral inhibition, which ensures that distinct cell types are produced in correct numbers from a pluripotent cell population (70). NSP is required for the maintenance of the NSC pool and regulation of cell differentiation and attenuation of notch signaling induces differentiation of neuronal and glial cells (71). NSP dysregulation is found in numerous developmental defects as well as in gliomas, embryonal tumors and meningiomas (72, 73).

Bone Morphogenic Proteins

Bone morphogenic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) superfamily that interact with specific cell surface receptors through a class of proteins (SMADs). Approximately 20 BMP family members have been identified to date and each one has a distinct role in the development and maintenance of different tissues (74). BMPs interact with signaling pathways such as Notch, Wnt and MAPK (75, 76). BMPs promote self-renewal and maintenance of NSCs, and are critical for the regulation of cell fate (76). BMP signaling was shown to be dysregulated in tumors such as glioblastoma and medulloblastoma (77, 78). In these tumors, BMPs were found to induce apoptosis, reduce proliferation and trigger differentiation.

Myc Transcription Factor

Although Myc does not represent a pathway, members of the Myc family of basic helix-loop-helix transcription factors are downstream targets of the several of the above mentioned pathways. Particularly cMyc and nMyc have critical roles in the control of cell-cycle progression and cell immortality. They regulate the NSC proliferation, cell fate determination of proliferating progenitor cells, and inhibit neural differentiation (3).

Myc protein expression has been reported in up to 64% of medulloblastomas and cMYC or nMYC amplification has been identified in 10-15% of medulloblastomas (79). MYC gene expression or amplification is also associated with a poor prognosis (80). MYC gene upregulation can act in combination with other pathways in the pathogenesis of medulloblastoma (81).

SUMMARY

Despite the increasing number of studies involving NSCs, there is still a need to provide uniform definitions of concepts and reliable markers that can identify stem cells. On the other hand, the immediate benefit of identifying BTSCs is not quite clear and there is still much to be learned about them. The similarities and differences between normal NSCs and BTSCs may help us understand the mechanisms of CNS tumorigenesis. In this effort, the first critical step appears to be the recognition of regulatory steps in formation, maintenance and proliferation of NSCs and mature cells of the CNS. Common molecular pathways to both normal and neoplastic cells may provide us an insight about what goes wrong, and what can be done to prevent or reverse it. Through this insight, we could develop a more advanced model of "cell of origin" for each CNS tumor type and hopefully provide better prognostic markers and targets for future therapies.

The current WHO classification of the tumors of the CNS has been very successful in providing a reproducible and clinically relevant scheme to accurately classify the majority of the tumors. There is no doubt that the 2007 classification scheme is a "living document" that is prone to modifications. Emerging knowledge about the NSC biology and BTSCs will present the opportunity to develop complementary data to improve this classification system in the near future. We believe that the next modification of the WHO scheme can be achieved through identification of pathways from NSCs to the mature cells of the CNS, and the mechanisms involved in their dysregulation.

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