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Biomarkers of Neural Stem Cells: Roles in Self-Renewal, Differentiation, and Neurogenesis

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Abstract

Background: Neural stem cells (NSCs) have a unique ability to self-renew and differentiate into various neural lineages, including neurons, astrocytes, and oligodendrocytes. This self-renewal process is governed by a complex interaction of intrinsic factors and external signaling pathways, which are essential for maintaining neural populations during development and supporting regenerative processes in the adult brain. Over the years, numerous markers have been identified to characterize NSCs and help elucidate the mechanisms behind their self-renewal.

Methods: This review summarizes key findings from the literature, focusing on these markers and their roles. Given the potential of NSCs in treating neurological disorders, understanding the molecular mechanisms that regulate their fate is critical. This review incorporates research published between 1998 and 2024, sourced from databases such as Web of Science, PubMed, and ScienceDirect. It highlights various markers, including Bcl11b, Musashi-1 (Msi1), Oct-4, Emx2, Nanog, Cux1, Cux2, Sox2, Prx1, Nr2f1, Prion protein (PrPC), PH3, PSA-NCAM, bHLH, Nestin, Vimentin, NeuroD, EGFR, BMI1, and CD133, all of which are crucial for identifying NSCs.

Results: Given their significant potential in addressing debilitating nervous system diseases and advancing genetic research, understanding the defining markers of NSCs at different stages of differentiation is of utmost importance.

Conclusion: This knowledge is essential for developing stem cell-based therapies for neurodegenerative diseases and injuries. Future research may uncover additional markers and pathways, further enhancing our understanding of NSC biology.

Keywords: Neural stem cells, Neurogenesis, Self-renewal, Differentiation

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Introduction

The concept of "stemness" describes the unique ability of stem cells to remain undifferentiated and sustain their self-renewal capabilities indefinitely, driven by specific molecular mechanisms (1). NSCs are self-renewing cells found in the central nervous system (CNS) of mammals, and can also be derived from germline stem cells. They can proliferate, migrate, and differentiate into various glial cells. NSCs are essential for CNS development, learning, memory, and trauma response. They generate progenitor cells capable of self-renewal and differentiating into neurons and glial cells through multiple stages. In germinal regions, NSCs undergo self-renewal, precursor cell amplification, neuroblast formation, cytoskeletal reorganization, and differentiation into neurons, astrocytes, and oligodendrocytes (2, 3). These cells can be cultured, genetically altered, and reprogrammed into specialized cells for growth, adulthood, or therapeutic applications. Furthermore, they demonstrate mobility and seem to migrate toward injured regions of the brain. The phenomenon of neurogenesis in advanced mammalian brains was first identified by Altman and Das in 1965 (4). Neurogenesis is the process of forming the nervous system through the development of neurons from stem cells and progenitor cells. In the adult mammalian brain, this process is largely restricted to specific areas, such as the lateral ventricles and the hippocampus, where NSCs are located and neurogenesis occurs (5). NSCs have significant healing potential and offer promising avenues for treating injuries to the central nervous system. To maximize their therapeutic applications, it is crucial to understand the mechanisms that regulate their proliferation, differentiation, and migration. Research has underscored the pivotal role of transcription factors (TFs) and conserved genes in shaping the essential properties of NSCs (6, 7). Transcriptional regulation is fundamental to controlling gene expression in tissues and responding to diverse stimuli. Transcription factors, proteins that bind to specific DNA sequences, serve as key regulators of gene expression and play a vital role in maintaining stem cell pluripotency. Over the past decade,

studies have revealed that transcription factors encoded by the OCT4, SOX2, and Nanog genes are crucial for preserving the unique properties of NSCs (8). In addition, various markers, including Musashi-1, embryonic stage-specific antigen (SSEA-1/Lewis X/CD15), nestin, and CD133, have been used to identify NSCs (9, 10). Signaling pathways, including Wnt, Ras-MAPK, and JAK/STAT, are central to regulating neural stem cell functions such as self-renewal, proliferation, differentiation, survival, and apoptosis. Despite their importance, the precise mechanisms governing these processes remain largely unknown. These pathways are activated by interactions between cell surface receptors and environmental cues, such as growth factors, the extracellular matrix, and adhesion molecules (11). A deep understanding of the molecular mechanisms that govern neural stem cell fate is critical for advancing cell-based therapies and treatments for neurological disorders. These therapies have demonstrated considerable promise in animal models of central nervous system injuries. The ability to maintain stem cells effectively and control their differentiation with precision provides significant therapeutic benefits (12, 13). Abbaszadeh et al. have demonstrated that transplantation of neural stem cells derived from bone marrow into a rat model of contusive spinal cord injury significantly enhances motor function recovery compared to control groups, particularly during the period spanning the second to fourth weeks post-injury. These results emphasize the therapeutic potential of adult tissue-derived neural stem cells in facilitating neural repair and functional restoration following central nervous system damage. Such findings further underscore the critical importance of accurately identifying and characterizing neural stem cell markers to improve the efficacy and precision of stem cell–based regenerative therapies (14).

Given the crucial role of neural stem cells (NSCs) in regeneration, repair, and the formation of new neurons within the central nervous system, accurate and stage-specific identification of these cells using distinct molecular markers is essential for both basic and clinical research. A wide range of biomarkers has been identified to date, each associated with specific stages of the NSC lifecycle, including self-renewal, differentiation, migration, and survival. In this study, we focus on a comprehensive set of these biomarkers, encompassing transcription factors, surface receptors, signaling pathway components, and cytoskeletal proteins. The following sections systematically explore the biological roles of key markers such as Bcl11b, Sox2, Nestin, NeuroD, CD133, EGFR, and others that are involved in regulating NSC self-renewal and differentiation, providing a detailed understanding of their applications in various phases of neurogenesis.

Methods

Search strategy: This narrative review aimed to collect and synthesize current knowledge about neural stem cell (NSC) markers with an emphasis on those involved in self-renewal, proliferation, and differentiation.

***Study selection and data extraction*:** To identify relevant articles, a systematic search was conducted in scientific databases including PubMed, Scopus, Web of Science, and Google Scholar up to March 2025. The following keywords and their combinations were used: neural stem cells, NSC markers, self-renewal, differentiation, neurogenesis, transcription factors, cell surface markers, and signaling pathways.

Inclusion Criteria:

- Articles published in English.
- Peer-reviewed original research and review papers.
- Studies focusing on NSC biology and related biomarkers in mammalian systems.
- Articles discussing molecular markers associated with NSC self-renewal, proliferation, or differentiation.

Exclusion Criteria:

- Non-English articles.
- Conference abstracts or case reports without full text.
- Studies unrelated to neural stem cells or without clear marker relevance.

Additional references were identified by manually screening the reference lists of selected articles. The selected literature was then organized thematically based on marker type and function (e.g., transcription factors, cytoskeletal proteins, and membrane receptors), allowing comprehensive and focused analysis in the Results and Discussion sections.

Results

Bcl11b

B-cell leukemia 11b (Bcl11b) is a zinc finger protein transcription factor that regulates gene expression by directly binding to promoter regions and indirectly interacting with other transcription factors. Bcl11b is a key factor in fetal development, playing a crucial role in neuronal subtype differentiation and central nervous system (CNS) development. It marks subclass V projection neurons and interneurons (3). Bcl11b significantly influences NSCs by regulating their proliferation and differentiation, which is crucial for promoting neural regeneration. Dysregulation of Bcl11b has been linked to various developmental disorders, including intellectual disabilities, autism spectrum disorder, and developmental delays. Mutations in the BCL11B gene have been associated with brain development defects, with insights provided by animal models on the underlying mechanisms of these disorders (15). As a key transcription factor, Bcl11b governs the differentiation of NSCs into specific neuronal subtypes, such as pyramidal neurons, while suppressing glial cell formation. This regulation is particularly

prominent in regions such as the cortex and hippocampus. Additionally, Bcl11b supports neuronal identity by influencing chromatin remodeling and maintaining the differentiation trajectory of neurons (16). Bcl11b interacts with critical developmental pathways, including Notch, Wnt, and Sonic Hedgehog, to regulate neural development comprehensively. Dysregulation of this transcription factor has been implicated in neurodevelopmental disorders like autism and schizophrenia, emphasizing its pivotal role in maintaining proper CNS function (17).

Musashi-1

Another biomarker, Musashi-1 (Msi1), has remained unchanged throughout evolution. It is a conserved family of RNA-binding proteins, primarily expressed in the nervous system. In the CNS, Musashi is specifically expressed in proliferating neuroblasts, where it plays a unique role. Musashi-1, the mammalian equivalent, is an RNA-binding protein that is highly expressed in both fetal and adult NSCs (3). Musashi-1 enhances Notch signaling and plays a critical role in the self-renewal of NSCs. Additionally, it is involved in the regulation of epithelial stem cells, such as those found in the mammary and intestinal glands, highlighting its importance across different tissue types (18). This protein plays a crucial role in maintaining the undifferentiated state of NSCs by initiating Notch signaling. Notch signaling is key in regulating NSC self-renewal and preventing premature differentiation, ensuring that NSCs retain their ability to proliferate and differentiate into various cell types when needed (19, 20). The Notch signaling pathway plays a central role in maintaining NSC quiescence and self-renewal. Hes1, a downstream target of Notch, represses pro-neuronal genes to prevent differentiation. Disruption of Notch signaling can lead to NSC depletion and aberrant development. Mouse-Musashi-1 is an RNA-binding protein essential for neural development, particularly in the CNS. It is highly expressed in NSCs, where it regulates precursor cell differentiation into neurons and glial cells. Unlike the Hu protein, which is found in postmitotic neurons, Mouse-Musashi-1 binds preferentially to poly (G) RNA. These proteins likely have distinct roles in neurogenesis, potentially through sequential regulation or mRNA sorting during asymmetric divisions of neural precursor cells (21). Musashi-1 is an RNA-binding protein that regulates neural stem cell fate and differentiation. It maintains NSC self-renewal by preventing premature differentiation and promoting undifferentiated states. Musashi-1 also controls the differentiation of NSCs into neurons and regulates the balance between neurogenesis and gliogenesis. It interacts with the Notch signaling pathway to ensure proper NSC renewal and differentiation. Additionally, Musashi-1 regulates key genes like Numb and p21, influencing NSC proliferation (22). Dysregulation of Musashi-1 is linked to neurodevelopmental disorders and cancers, including gliomas. Musashi-1 is an RNA-binding protein crucial for the maintenance of NSCs. It regulates the stability and translation of mRNAs involved in stemness, including those in the Notch signaling pathway, which helps prevent differentiation. Elevated expression of Musashi-1 is linked to enhanced self-renewal and proliferation of NSCs.

Oct-4

Oct-4 is a protein expressed in vitro in both mouse and human stem cells. It consists of three domains: the N-terminal, C-terminal, and POU domains. In humans, Oct-4 is encoded by the POU5F1 gene (23). Oct-4 (also known as Oct-3) is a member of the POU (Pit-Oct-Unc) family of transcription factors (24). Oct-4 is a homeodomain transcription factor that activates the transcription of target genes by binding to an octameric sequence motif (AGTCAAAT) (25, 26). Oct-4 is not essential for the initiation of pluripotency, but it plays a critical role in maintaining it. Recent findings have suggested that Oct-4 is predominantly expressed in embryonic stem cells (8). During fetal development, Oct-4 is initially expressed in all blastomeres and throughout the blastocyst until the inner cell mass (ICM) forms, where it helps maintain the pluripotency of embryonic stem cells. Inhibition of Oct-4 expression leads to the differentiation of ES cells into trophectoderm. During puberty, Oct-4 expression becomes restricted to developing germ cells (8, 26-28). During differentiation, changes in chromatin structure, such as methylation and histone acetylation, along with alterations in gene expression, occur through epigenetic mechanisms. These changes lead to a decrease in Oct-4 expression, ultimately resulting in a reduction of stem cell pluripotency (29, 30). Recent studies have shown that the expression of four transcription factors—Oct-4, Sox2, Klf4, and c-Myc—can induce pluripotency in mouse fibroblast cells (31). Studies have also demonstrated that the expression of the Oct-4 gene promotes the pluripotency of NSCs (31-33). Transferring OSKMs (Oct-4, Sox2, Klf4, and c-Myc) into cells induces stem cell-like properties. Oct-4 is crucial for maintaining the pluripotency of embryonic stem cells, and blocking its expression leads to differentiation into trophectoderm. Suppression of Oct-4 expression by GCNF triggers the differentiation of NSCs into mature nerve cells (33). Recent studies have found that mature astrocytes from the spinal cord can revert to a pluripotent state when provided with the appropriate signals. Previous efforts to reprogram astrocytes into induced NSCs (iNSCs) were often unstable, ineffective, and frequently resulted in the formation of intermediate precursors (33). A single factor, Oct4, is capable of converting astrocytes into induced NSCs (iNSCs), which exhibit neurosphere morphology, NSC gene expression, self-renewal, and multipotency. The reprogramming of astrocytes into iNSCs driven by Oct-

4 was further enhanced by continuous Shh stimulation, leading to accelerated reprogramming and improved conversion efficiency. Additionally, the neurons derived from iNSCs displayed typical neuronal functionality. The crosstalk between Sox2/Shh downstream signals and PI3K/Cdk2/Smurf2 signaling pathways likely contributes to this cellular reprogramming process (34). Oct4 and Nanog are transcription factors associated with pluripotency and self-renewal. While they are most commonly linked to embryonic stem cells, they are also expressed in NSCs during early development. These factors help maintain the undifferentiated state of NSCs and suppress differentiation.

Emx2

Emx2 (empty spiracles homeobox 2) plays a significant role in regulating NSCs in the hippocampus, particularly in the dorsal ventricular zone (dVZ). Studies on Emx2 mutant mice have revealed alterations in neurogenesis, with an increase in proliferating progenitor cells. In these mutant models, an increase in the density of PH3-positive (a marker for cell division) and Tbr2-positive (intermediate progenitor marker) cells was observed, suggesting that Emx2 affects the proliferation and differentiation of NSCs in the hippocampus. These findings indicate that Emx2 is crucial for regulating cell cycle dynamics and influencing neurogenic processes, potentially by controlling progenitor cell proliferation and migration (35). Emx2 is a transcription factor that plays a crucial role in the development of the rat cortex. It is involved in the proliferation of neuroblasts from the neural epithelium and ventricular zone (VZ), as well as the development of post-mitotic Cajal-Retzius cells. Emx2 is highly expressed during cortical initiation and is primarily used as a marker for the dorsal region during cortical angiogenesis. Its role is essential in both early cortical patterning and in guiding the development of specific neuronal populations in the brain (36). Emx2, a transcription factor, plays a critical role in regulating the proliferation, migration, and differentiation of neuroblasts during development. Studies on Emx2-null embryos demonstrate that the absence of this gene disrupts these processes, highlighting its essential function in cortical development. The lack of Emx2 leads to abnormalities in neuroblast behavior, underscoring its role in maintaining proper neuronal development and regionalization. These findings emphasize the importance of Emx2 in both the formation and the guidance of neural progenitor (37).

Nanog

Transcription factor Nanog plays an important role in the self-renewal of embryonic and NSCs (38). The identification of Nanog has significantly advanced our understanding of stem cell culture. Nanog, which encodes a homeobox transcription factor, is expressed in key developmental stages. It is found in the inner cells of the blastocyst, which are crucial for early development, and continues to be expressed in the embryonic body as well as in germ cells. Nanog plays a pivotal role in maintaining pluripotency and promoting the self-renewal of stem cells. This discovery has helped refine protocols for culturing stem cells, especially by highlighting the factors needed to preserve their undifferentiated state during in vitro expansion (39). Nanog plays a crucial role in regulating stem cell differentiation. Overexpression of Nanog has been shown to delay differentiation, maintaining stem cell properties, while its deletion accelerates differentiation. Recent studies have suggested that in NSCs of the cerebellum, Nanog expression is essential for continued self-renewal even after birth. This highlights Nanog's importance in sustaining the undifferentiated state of stem cells, especially in the postnatal brain. Additionally, research underscores the importance of a balanced network of factors, including Nanog, in regulating the self-renewal of embryonic stem cells. Disruptions to this delicate balance can lead to premature differentiation (40). Nanog and Oct4, two key transcription factors, are involved in regulating various cellular processes during the differentiation of embryonic stem (ES) cells. One of their important functions is facilitating chromatin repair and maintaining transcriptional integrity as ES cells transition to differentiated states. Specifically, Nanog plays a vital role in regulating Kat6b, a histone acetyltransferase that is involved in the acetylation of histones, influencing chromatin structure and gene expression during differentiation. As differentiation progresses, Kat6b activity is inhibited, and Nanog is crucial in controlling this inhibition, ensuring that chromatin remains in a transcriptionally active state at appropriate stages of differentiation (41).

Cux1 and Cux2

Cux1 and Cux2 are critical transcription factors involved in neural stem cell differentiation. Cux1 is involved in the differentiation of neural progenitors, influencing cell fate decisions, while Cux2 is highly expressed in specific subsets of neural progenitors in the developing forebrain. These transcription factors are particularly important for cortical development and contribute to the generation of specific neuronal subtypes, such as late-born neurons in upper cortical layers (42). Additionally, studies suggest that Cux2+progenitors are pre-committed to specific neuronal fates before the onset of neurogenesis, a concept that has been supported by recent fate-mapping studies (42). Cux1 and Cux2 are transcription factors that serve as key markers for upper-layer neurons, particularly in the developing cerebral cortex. They play a crucial role in regulating late neuronal differentiation and are involved in processes such as dendritic branching and the formation of specific neuronal subtypes. Cux1 is typically expressed in

post-mitotic neurons, and its expression increases as these cells move toward the cortical plate, where they contribute to the formation of cortical layers II and III significant regulator of cortical development, and it has been shown to mark a distinct subset of upper-layer cortical neurons. It plays a role in ensuring proper neuronal migration and positioning, as well as in the differentiation of these neurons into specific layers. Both Cux1 and Cux2 are involved in the fine-tuning of dendritic arborization and synaptic development, which are essential for proper neuronal connectivity (37, 43).

Sox2

Sox2 is a key transcription factor that maintains the pluripotency and self-renewal of NSCs by regulating cell cycle genes and suppressing differentiation pathways. Expressed in both embryonic and adult NSCs, Sox2 ensures their undifferentiated state, with its absence leading to reduced proliferation and premature differentiation. It is crucial for neural development, maintaining embryonic stem cell pluripotency, and supporting brain development. Recent research has highlighted Sox2-related chromosomal interactions in brain-derived NSCs, emphasizing its essential role in neural biology (44). Sox2 is a transcription factor widely utilized in various differentiated tissues to reprogram adult stem cells into induced pluripotent stem cells (iPSCs) (45). Sox2 is expressed early during development and influences a wide range of embryonic tissues. It is part of the SRY-related gene family and encodes a transcription factor belonging to the SOX9 gene family (46). NSCs in the subventricular zone (SVZ) exhibit overexpression of Sox2, GFAP, and Nestin, markers commonly associated with their undifferentiated and multipotent state (Sox2 + /GFAP + / Nestin +) (47). These NSCs differentiate into neuroblasts and subsequently into adult neurons, including olfactory neurons, as well as astrocytes (48). Sox2 is a transcription factor expressed early in neurodevelopment, serving as a marker for neurodevelopment. It plays a crucial role in maintaining neural stem cell properties, including proliferation, survival, self-renewal, and neurogenesis (49). The Survivin gene is regulated by Sox2 in NSCs, protecting them from cell death and controlling cell division. The influence of Sox2 on Survivin expression highlights the connection between reduced Sox2 levels in NSCs, decreased cell growth, and the onset of apoptosis (49). Researchers have demonstrated that Sox2 plays a crucial role in the proliferation of glioblastoma tumor cells (50). Sox2 is essential for stem cell generation through the iPS method and plays a key role in tissue development, especially in nervous tissue. As we age, the expression of Sox2 decreases in various tissues (51). It has also been reported that Sox2 is a significant new clinical target of microRNA-21 in glioblastoma, with important prognostic implications (52). Sox2 expression is influenced by promoter CpG methylation levels and the regulation of various transcription factors. As a stem cell marker, Sox2 is expressed in many stem cells and regulates the expression of downstream target genes, impacting stem cell function. Sox2 plays a crucial role in both stem cells and tumor cells (53).

Prx1

Prx1 (paired-related homeobox 1) is a transcription factor expressed in various stem and progenitor cells, including those involved in tissue regeneration. Research indicates that Prx1 + cells, particularly from the calvarial suture, actively contribute to bone regeneration, especially in response to injury. These cells are essential for the repair of skeletal defects, including the formation of osteoblasts and osteocytes during wound healing. Prx1 + cells also exhibit stem cell-like properties, with potential applications in regenerative medicine for treating cranial and other skeletal injuries (54). The paired homeobox protein Prx1 (MHox1/Prrx1) is crucial for the maintenance of neural progenitor cells. It acts as a coactivator with the transcription factor Sox2, and its suppression in NSCs reduces their self-renewal capacity. Prx1 is expressed in Sox2 + /GFAP + /Nestin + astrocytes located in the forebrain's germinal centers in adult mice (55).

Nr2f1

The NR2F1 (Nuclear receptor subfamily 2 group F member 1), also known as COUP-TF1, plays a crucial role in the development and regulation of NSCs. In particular, it influences the neurogenic niche in regions like the hippocampus, where it regulates neural stem cell function and neuronal lineage specification. Research has highlighted that NR2F1 is essential for the proper differentiation of neurons and their integration into neural circuits. Additionally, NR2F1's activity in neural progenitors can affect mitochondrial function, a crucial aspect for the survival and proper functioning of newly born neurons. In animal models, particularly in the hippocampal dentate gyrus, NR2F1 has been shown to regulate both the survival and integration of adult-born neurons. Disruption of NR2F1 expression leads to impaired neurogenesis, which can affect cognitive functions and adaptive behaviors. Moreover, mutations in NR2F1 are linked to developmental disorders, such as Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS), which impacts neurodevelopment and can result in intellectual disabilities and other neurological symptoms (56, 57). Nr2f1, also referred to as Coup-tf1, plays a crucial role in the development of the neocortex. This biomarker is integral to the regionalization of the neocortex, influencing how different regions of the brain are specified during development. Additionally, Nr2f1 is essential for the differentiation of corpus callosum projection neurons (CPNs) and the migration of late-born

neurons. Its regulatory effects ensure proper neuronal development, migration, and integration into the cortical circuits (37, 58).

Prion Protein (PrPC)

The prion protein (PrP(C)), in collaboration with the secreted stress-inducible protein 1 (STI1), plays a role in promoting neuronal survival and neurogenesis. The interaction between these two proteins influences various cellular functions that are crucial for neural stem cell (NSC) physiology. However, the specific mechanisms through which the PrP(C)-STI1 complex affects NSC behavior are not fully understood. Research indicates that this complex may contribute to the regulation of neurogenesis, though further studies are needed to explore its precise role in NSC survival, proliferation, and differentiation. Both proteins have been implicated in maintaining brain homeostasis and neuroplasticity, yet their joint impact on stem cell physiology remains an open area of investigation (59, 60). Studies have indicated that the PrP(C)-STI1 complex plays a critical role in the self-regulation of neural progenitor cells by influencing cell proliferation and modulating their stem cell potential. This interaction appears to regulate the balance between cell renewal and differentiation, which is essential for proper nervous system development. The PrP(C)-STI1 complex likely contributes to the maintenance of neural stem cell properties, promoting both neurogenesis and cell survival during development. However, the precise mechanisms by which this complex regulates neural progenitor behavior remain an area of ongoing research (59).

PH3

Phosphohistone H3 (PH3) is a marker for identifying cells in the M phase of the cell cycle, specifically during mitosis. It is valuable in studying NSCs and neurogenesis, particularly in regions like the subventricular zone (SVZ) and subgranular zone (SGZ) of the hippocampus. PH3 staining helps quantify proliferating cells, providing insights into neurogenesis, brain development, injuries, and therapies aimed at promoting neural regeneration (61, 62). Expression of PH3 occurs during late G2 and throughout M phase during cell division. The characterization of PH3 indicates that it is commonly used to screen cell subsets in proliferative and mitotic states (37).

PSA-NCAM (Polysialylated Neural Cell Adhesion Molecule)

PSA-NCAM is a protein that regulates NSCs in processes like proliferation, differentiation, migration, and plasticity (63). It reduces cell adhesion, allowing NSCs to remain flexible and migrate, which is essential for brain development and regeneration. PSA-NCAM influences NSC differentiation into neurons and glial cells, particularly in regions like the hippocampus. It also supports neuroplasticity and memory in the adult brain. Additionally, PSA-NCAM interacts with pathways like Notch and Wnt to balance NSC behavior. PSA-NCAM, a polysialylated form of the neural cell adhesion molecule, serves as a key biomarker primarily expressed in neural and glial progenitor cells during brain development (64). In the adult brain, PSA-NCAM-positive cells are often concentrated in newly formed or developing granule cells (65). Additionally, this biomarker is recognized as an indicator of migratory neuroblasts that differentiate into neurons within the olfactory bulb in the SVZ in vivo (66). Also, most PSA-NCAM-positive cells express markers such as NeuroD, Doublecortin, and NeuN. However, they do not express GFAP, as they are GFAP-negative (37, 65). Polysialylated Neural Cell Adhesion Molecule (PSA-NCAM) plays a key role in the plasticity and migration of NSCs. PSA-NCAM reduces cell adhesion, allowing NSCs to remain flexible and enhancing their migratory capacity. It is particularly associated with actively proliferating and migrating NSCs within neurogenic niches.

bHLH

The bHLH (basic Helix-Loop-Helix) transcription factors are crucial in regulating NSCs and their differentiation into neurons and glial cells (67). These proteins control multiple aspects of neurogenesis, including NSC self-renewal and neuronal subtype specification. bHLH factors like Neurogenin and NeuroD promote neuronal differentiation (68), while factors like Ascl1 maintain NSC self-renewal. They also influence gliogenesis and interact with pathways such as Notch and Wnt to regulate NSC fate. Dysregulation of bHLH proteins can lead to neurological disorders and cancers, including gliomas (69). Certain bHLH factors, particularly Ascl1, help maintain NSC self-renewal by preventing premature differentiation (70). These factors interact with other signaling pathways such as Notch signaling, which also plays a critical role in keeping NSCs undifferentiated (71). This allows for the continuous production of new neural cells.

Nestin

Another biomarker of NSCs is nestin, an intermediate filament (nanofilament) protein, whose role in neurogenesis, including adult neurogenesis, remains unclear (72). It supports cytoskeletal organization during cell division and is downregulated upon differentiation. Nestin is widely used as a marker to identify undifferentiated NSCs in both the developing and adult brain. Nestin is a protein that serves as a marker for stem cells, particularly neuroepithelial stem cells. It is found in CNS progenitor cells, the developing nervous system, neuroepithelial cells, and cultured cells in vitro, but is not present in differentiated and mature neurons (73, 74). The expression of the Nestin gene and

protein is essential for differentiation. It is also present in non-NSCs, such as pancreatic and hematopoietic progenitors. Predicted Nestin protein sequences suggest that it is a unique type VI intermediate filament, playing a critical role in neuronal differentiation (75). These changes in gene expression are vital for neuronal differentiation. Interestingly, nestin's role in the astrocyte niche can influence the proneurogenic effect caused by nestin deficiency. Nestin negatively regulates neuronal differentiation and survival through Notch signaling from astrocytes to NSCs, although its expression in NSCs is not required for normal neurogenesis (72). Nestin plays a vital role in maintaining cell structure during differentiation. Nestin is commonly found in radial glial cells, which give rise to neurons, astrocytes, and oligodendrocytes. Its expression decreases as cells differentiate into specialized types. In addition to its role in embryonic development, Nestin is also involved in adult neurogenesis, particularly in the hippocampus (76). It is found in non-NSCs as well, such as pancreatic and hematopoietic progenitors. Nestin is also involved in signaling pathways like Notch, which regulate neurogenesis. Notch signaling helps maintain NSCs in an undifferentiated state and regulates the balance between self-renewal and differentiation by promoting Notch activity (77). Nestin modulates Wnt/β-catenin signaling, which regulates NSC self-renewal and differentiation (78). Nestin influences the MAPK/ERK pathway, which plays a role in promoting NSC differentiation into neurons and glial cells (79). **PI3K/Akt pathway,** involved in NSC survival and proliferation, is regulated by Nestin to maintain a balance between survival and differentiation (80). Nestin also interacts with TGF-β signaling, promoting the differentiation of NSCs into glial cells (81).

Vimentin

Vimentin is an important marker for NSCs, particularly during early neurogenesis (82). It is an intermediate filament protein expressed in mesoderm-derived cells, including radial glial cells, which are considered NSCs. Vimentin plays a crucial role in maintaining cell shape and stability and is often co-expressed with other markers like Nestin and Sox2 (83). As NSCs differentiate into neurons or glial cells, vimentin expression decreases, but it continues to assist in cell migration and cytoskeletal remodeling (84). Vimentin is widely used in both in vitro and in vivo studies to track NSCs and plays a key role in neural differentiation, tissue regeneration, and neurogenesis research (85). Two key intermediate filament proteins in glial cells are vimentin and GFAP. Vimentin is primarily expressed in radial glial cells and immature astrocytes during early brain development, but disappears towards the end of pregnancy. GFAP, on the other hand, replaces vimentin in astroglial cells. However, studies have indicated that vimentin is still expressed in radial and horizontal cells of the subgranular zone (SGZ) (86). A study found that the development of the human central nervous system is critical during embryogenesis. In early pregnancy, three key markers for radial glial cells are vimentin, nestin, and WT1, while Sox2 is expressed in cells from the ventricular zone (87). Vimentin plays a significant role in cellular processes such as differentiation, migration, and tissue integrity. It interacts with several key signaling pathways, influencing cell functions in neurogenesis and inflammation. Vimentin interacts with MAPK/ERK, regulating cell proliferation, differentiation, and migration in response to growth factors (88). Vimentin influences the PI3K/Akt pathway, affecting cell survival, stress responses, and tissue repair (89). TGF-β regulates vimentin expression, which is crucial for processes like epithelial-mesenchymal transition, wound healing, and glial cell differentiation in the nervous system (90). Vimentin regulates NF-κB, which is involved in inflammation and immune responses, especially during tissue repair and injury (91). Vimentin interacts with Notch signaling, influencing stem cell maintenance and neural differentiation (92). Vimentin modulates the Wnt/β-catenin pathway, crucial for cell proliferation, differentiation, and maintaining neural progenitors (93).

NeuroD

NeuroD is a crucial protein that regulates neuronal differentiation by influencing identity genes and determining the fate of neural precursor cells. It plays a vital role in balancing neurogenesis and glial differentiation, particularly during early brain development in the hippocampus and cortex, which are important for learning and memory. NeuroD1, an isoform of NeuroD, has the potential to reprogram non-neuronal cells, like astrocytes and microglia, into neurons, offering new possibilities for treating neurodegenerative diseases. NeuroD is a helix-loop-helix transcription factor, serving as a marker for differentiated cells in neurogenesis. NeuroD1 can reprogram microglia into neurons both in vitro and in vivo by activating genes like Scrt1 and Meis2, which leads to the loss of microglial gene expression and erasure of epigenetic marks in microglial promoter and enhancer regions. This suggests that NeuroD1 drives global epigenetic changes, facilitating neuronal identity acquisition while erasing microglial characteristics (94). NeuroD is commonly used as a marker for early neuronal lineages and to identify mitotic neurons (86). NeuroD also contributes to epigenetic regulation by controlling the expression of genes involved in chromatin remodeling. It has been shown to drive epigenetic reprogramming during neuronal differentiation, as evidenced by experiments in which NeuroD1 induces the expression of genes like Scrt1 and Meis2, which are essential for determining neuronal fate (95). Due to its unique expression pattern, NeuroD

is widely used as a marker for differentiating neurons and early neuronal lineages. It is commonly employed in research to track neuronal development in both in vitro (cell culture) and in vivo (living organism) models. NeuroD1 is a member of the proneural gene family, which plays a critical role in embryonic neurogenesis, acting as a key factor in neuronal differentiation (96). The NeuroD signaling pathway is essential for neuronal differentiation, maintenance, and neurogenesis. NeuroD, a bHLH transcription factor, interacts with pathways such as Notch, Wnt/β-catenin, and MAPK/ERK to regulate the differentiation of neural progenitors and facilitate the formation of mature neurons (71). It also integrates PI3K/Akt and TGF-β signaling to ensure cell survival and subtype specification (97). Additionally, NeuroD is involved in activity-dependent neurogenesis via calcium/CREB signaling and epigenetic regulation, activating neuronal genes (98). Overall, NeuroD coordinates multiple pathways to maintain neural progenitors and drive the development of functional neurons.

Epidermal Growth Factor Receptor (EGFR)

EGFR signaling plays a vital role in the proliferation and survival of NSCs. Mechanistically, EGFR activation initiates downstream pathways, including MAPK and PI3K/Akt, which promote self-renewal. Elevated EGFR expression is observed in NSCs during active neurogenesis. Selim et al. investigated the critical role of EGFR signaling in maintaining NSCs, supporting their self-renewal and differentiation, as well as its involvement in the regulation of cancer stem cells, particularly in gliomas (99). Yao et al. discuss the importance of EGFR signaling in both normal neural development and neural repair following injury. They emphasize its significant role in neurogenesis and the development of reactive glia (100).

BMI1

BMI1 is a crucial gene for maintaining NSCs and their self-renewal. As a member of the Polycomb group of proteins, it plays a key role in chromatin remodeling and gene regulation. In both the central and peripheral nervous systems, BMI1 supports efficient NSC self-renewal. Its absence impairs self-renewal and reduces NSC populations, though it does not affect progenitor cell differentiation. BMI1 is also linked to tumorigenesis, particularly in gliomas, where it is vital for the maintenance of cancer stem cells, including those in glioblastoma. This underscores its importance in both normal and cancerous stem cell functions (101).

CD133

CD133 (Prominin-1) is an essential marker for identifying NSCs, especially in normal development and diseases like gliomas. It is expressed variably in NSC populations, contributing to the maintenance of stem cell properties. Research shows that CD133+NSCs have self-renewal, differentiation potential, and clonogenicity. This marker is commonly used alongside others, such as CD15, to identify and isolate specific NSC subpopulations (102, 103). Interestingly, while CD133 is typically linked to stem cells, its expression can vary among different neural cell types and even within the same stem cell population. Studies have found that this variability does not always correlate with cell potency, as populations with varying CD133 levels can still exhibit stem cell-like behaviors, such as differentiating into neurons, oligodendrocytes, and astrocytes under the right conditions (103). This highlights the complex role of CD133 in NSC biology and its potential application in regenerative medicine and glioma research (102).

Conclusion

Despite advancements in stem cell research and cell therapy, there is still no conclusive evidence supporting the functionality of essential cells in neurogenesis. The absence of well-established markers remains a significant concern for researchers. By applying a systematic knowledge framework, researchers can utilize their findings to discover new technologies for therapeutic approaches involving NSCs, which can be differentiated from other stem cells through specific biomarkers. The study identified several markers, including Bcl11b, Musashi-1 (Msi1), Oct-4, Emx2, Nanog, Cux1, Cux2, Sox2, Prx1, Nr2f1, Prion protein (PrPC), PH3, PSA-NCAM, bHLH, Nestin, Vimentin, NeuroD, EGFR, BMI1, and CD133, which show the highest expression levels. This study identifies NSCs and neurons at different developmental stages and time points. Each specific cell type expresses unique surface proteins that distinguish it from others in a stepwise manner. These proteins can be used as markers to identify distinct cell types and track their development over time. Numerous biomarkers have been identified for this purpose. During embryogenesis and neurogenesis, various cell lines generate different cell types at specific time points. Each cell type is characterized by unique surface proteins that identify different stages of the NSC pool. Therefore, cell markers and various biomarkers can be employed to identify specific cells.

It is important to acknowledge that several commonly used neural stem cell (NSC) markers are not exclusively expressed in NSCs (Table 1). For example, Nestin is also found in pancreatic progenitors and hematopoietic stem cells, indicating its expression is not strictly limited to neural lineage cells. Similarly, Vimentin is widely expressed in mesenchymal cells and various progenitor populations beyond the nervous system. Many stem cell markers, such as Sox2 and CD133, have overlapping expression patterns in different stem and progenitor cells, including non-neural tissues and cancer stem

Table 1. Summary of Neural Stem Cell Markers and Their Specificity

Marker	Cell Type/Stage	Specificity to NSCs?	Function/Role	Additional Notes
Nestin	Neural stem/progenitor cells	Not fully specific	Cytoskeletal organization: a marker of undifferentiated NSCs	Also expressed in non-NSCs like pancreatic progenitors
Vimentin	Early NSCs, radial glial cells	Low specificity	Maintains cell shape, involved in migration	Expressed in mesoderm-derived cells; also in immature astrocytes
Sox2	NSCs, progenitors	Moderately specific	Maintains stemness and self-renewal	Also expressed in other stem/progenitor cells
CD133	NSCs, cancer stem cells	Variable specificity	Stem cell marker with self-renewal properties	Expression varies within NSC populations
Prx1	NSCs in calvarial sutures, forebrain astrocytes	Limited specificity	Tissue regeneration; coactivator with Sox2	Also in non-neural progenitors
NeuroD	Early neuronal lineages	Specific to neuronal progenitors	Drives neuronal differentiation and epigenetic reprogramming	Marker for differentiating neurons
EGFR	Active NSCs	Not specific	Promotes proliferation and survival	Involved in normal neurogenesis and cancer
BMI1	NSCs	Relatively specific	Maintains self-renewal via chromatin remodeling	Also implicated in tumorigenesis
PSA-NCAM	Migratory neuroblasts, progenitors	Moderately specific	Regulates migration, plasticity, and differentiation	GFAP-negative cells
PH3	Mitotic cells (M phase)	Not NSC-specific	Marker of proliferation	Identifies proliferating NSCs and others
Prion Protein (PrP(C))	NSCs, neurons	Low specificity	Promotes survival and neurogenesis	Works with STI1; mechanism not fully clear
bHLH (Neurogenin, Ascl1, NeuroD)	NSCs and progenitors	Moderately specific	Regulates differentiation and self-renewal	Interacts with Notch, Wnt pathways

cells. This overlap underscores the importance of using combinations of markers, rather than relying on a single marker, to improve the specificity of NSC identification. To enhance the accuracy of NSC characterization, it is recommended to use panels of multiple markers that include both general stem cell markers and those more specific to neural lineage. For instance, co-expression of Sox2, Nestin, and GFAP is more indicative of NSCs in specific brain regions compared to any single marker alone. Since marker expression alone may not definitively identify NSCs, functional assays such as neurosphere formation, differentiation potential, and lineage tracing should be combined with marker analysis to confirm stemness and neural identity. Expression patterns of NSC markers can vary depending on developmental stage, tissue microenvironment, and physiological or pathological conditions. For example, CD133 expression may be higher in proliferative NSC populations during active neurogenesis, but variable in quiescent stem cells. Future studies should focus on identifying novel markers or molecular signatures that more specifically distinguish NSCs from other stem or progenitor cell types. Advances in single-cell transcriptomics and proteomics hold promise for refining marker specificity.

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