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REVIEW ARTICLE

Signaling in NSC

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ABSTRACT

The subventricular and the subgranular zones are the uniquely designed niches loaded with adult neural stem cells (NSCs) that are considered as key areas for adult neurogenesis in the central nervous system. These neurogenic cells are regulated by many significant factors and signaling pathways, which control their cell fate choices, survival and proliferation rates. NSC properties such as self-renewal and multipotency are modulated by both cell-intrinsic and cell-extrinsic factors. The architecture of stem cell niche *in vivo* enables adult NSCs to generate continuously functional neurons in specific regions of brain throughout life. The cell-intrinsic regulators coordinate with extrinsic signals to control the balance between NSC self-renewal and differentiation. The signaling processes take place in the microenvironment of the NSC known as "Niche". Recently the cell intrinsic transcription regulators & extrinsic cellular signaling have gained a lot of ground in the last few years and how these molecular and cellular mechanisms have influenced the behavior of niche and NSCs are discussed by the authors. This review deals with neurgonesis, cellular signaling pathways for the self-renewal & differentiation of NSCs in the central nervous system, role of transcription factors, cell intrinsic & extrinsic regulators at length.

Key words: Neural stem cells, niche, neurogenesis, intrinsic transcription regulators, extrinsic signaling.

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INTRODUCTION

Earlier it was believed neuronal proliferation of brain was meant and limited to glial cells which function as supportive cells of neurons. Later potential neurogenesis was demonstrated in lower mammals and in humans [1]. The potential development of CNS begins as a sheet of cells made up of primary progenitors known as neuroepithelial cells. Then gradually and progressively the neuroepithelial cells were replaced by different NSCs [2]. The discovery of adult neural stem cells (NSCs) marked the dawn of a new era in the neuro-physiology of stem cells. This has opened new doors for future understanding of NSC biology and the importance of cellular signaling among and within NSCs. One of the most exciting aspects of stem cell biology is to unravel the molecular mechanisms underlying the stem cell re-newal. NSCs are the undifferentiated precursors capable of proliferation and self-renewal; and have the ability to form both neural and glial lineages [3]. Some Researchers believe that NSCs are more preferable than human embryonic stem cells (hESC) for clinical applications because NSCs have less potential to form tumors and considered safer bet for transplantation [4].

NSCs- identity, origin and development

NSCs were first isolated and characterized *in vitro* by Reynolds and Weiss in the year 1992. There are 2 types of NSCs; they are CNS stem cells and Neural crest stem cells. CNS stem cells give rise to neurons, astrocytes and oligodendrocytes. While the Neural crest stem cells give rise to neurons and glia of the peripheral nervous system [5]. NSCs are multipotent cells with the potential to differentiate into neurons, oligodendrocytes and astrocytes which can be efficiently propagated *in vitro* [6]. These cells can be obtained from the spinal cord and their characteristics are different from NSCs obtained from the forebrain [7]. NSCs undergo differentiation in 2 ways: Neurogenesis and Gliogenesis. Normally in the developing mammalian nervous system, neurogenesis precedes gliogenesis. In this order of sequence, it is understood that the glial differentiation pathways are actively suppressed during the neurogenic period [8]. After the identification of growth factors like Fibrous growth factor (FGF) and Epidermal growth factor (EGF), which have critical trophic and mitogenic actions, have led to sea changes in the culture and maintenance of variety of neural cells *in vitro*. Since then, the rapid growth of science in this field has marked for the first time that the NSCs are present specifically in the subventricular zone (SVZ) of the lateral ventricle wall and the subgranular zone (SGZ) of the hippocampal dentate gyrus. Though there is some proof to show that origin of new neurons is from multipotent adult NSCs, yet there is considerable amount of debate regarding their origin. This led to the belief that unique niche architectures present in these regions permit functional neurogenesis from NSCs *in vivo* [9, 10, 11, 12]. Growing evidence shows adult neurogenesis also occurs in the peripheral nervous system (PNS), like generation of olfactory neurons in the olfactory epithelium and neural crest lineages in the carotid body [13, 14]. Once isolated and grown in culture, adult NSCs undergo self-renewal and generate multiple neural lineages, including neurons, astrocytes and oligodendrocytes. Despite general acceptance of their existence, the exact identity and location of adult NSCs *in vivo* have long been controversial [15]. In the midst of this controversy, recent results have shown some

promise, that the cell bodies of adult NSCs are located in the SVZ and they contact the ventricles through ependymal cell-like apical surfaces [16].

NSCs- Growth, Maintenance & Proliferation

Adult neurogenesis recapitulates the complete process of neuronal development in the mature CNS environment. The local environment or "niche" maintains the adult NSCs and regulates their development *in vivo*. Multiple stimuli emerging from physiological, pathological and pharmacological sources regulate cell proliferation during adult neurogenesis in the SGZ and SVZ, such as physical exercise, seizures, stroke and antidepressant treatments. [17]. Growth factor signaling is the major regulating mechanism of cell proliferation, apart from FGF, EGF, neuregulins (NRGs), vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF). Several neurotransmitters such as GABA, also regulate cell proliferation during adult neurogenesis [18, 19]. Generation of new neurons is mainly restricted to the adult SVZ and SGZ, whereas astrocytes and oligodendrocytes are continuously born throughout the mature CNS. The fate of adult NSC is regulated by the neurogenic "niche" signals. Astrocyte-derived Wnts regulate neuronal specification of adult NSCs *in vitro* and Wnt-signaling promotes new neuron production in the adult dentate gyrus *in vivo* [19]. On the other hand, bone morphogenic proteins (BMPs) promote glia differentiation of NSCs derived from the adult SVZ and hippocampus *in vitro* [20]. In the adult SVZ, conditional deletion of Smad4, a downstream effector of BMP, in adult NSCs or infusion of noggin impairs neurogenesis and leads to an increases in the oligodendrocyte production [21]. It was shown that activity of the cAMP-PKA pathway is required for mediating the mitogenic signal of EGF [22].

NSC-Niche

The "niche" is defined as the microenvironment that intimately supports and tightly regulates stem cell behaviors, including their maintenance, self-renewal, fate specification and development [23, 24]. This unique local niche is highly influential and seems to restrict active neurogenesis from adult NSCs to two discrete regions: SVZ and SGZ [9]. Though there are some specific dissimilarities, interestingly there are some common features like cellular niche components and extra-cellular niche signals that regulate behavior of adult NSCs and their development. The major cellular neurogenic niche components are astroglia, ependymal cells, vascular cells, NSC progeny and mature neurons. There is enough evidence both in vitro and in vivo showing astrocytes have got a critical role in the process of neurogenesis involving self-renewal, fate specification of adult NSCs, migration, differentiation and final synaptic integration of new neurons [25, 26]. There are widespread reports which claim that neurogenesis may also occur in other areas of brain, including amygdala neocortex , substantia nigra , and striatum [27, 28]. The question is do these NSCs derived from other regions have the same capacity of neurogenesis under physiological conditions in vivo. Mature ependymal cells regulate the quiescence and self-renewal of adult NSCs in the SVZ by direct cell to cell contact and other diffusible signals [29].

Specific Brain Niches of NSC

SVZ- contains astrocytes *in vivo* which function as NSC. SVZ astrocyte NSCs are popularly called Type-B cells. Currently Type-B cells are divided into two subtypes: B1 and B2. B1controls cell proliferation with the help of cilium protruding into the ventricular cavity [30]. Recently, it has been described that Type-B cells *in vivo* generate oligodendrocytes that migrate into the corpus callosum and fimbria fornix [31]. Blood vessels play a significant role in the SVZ and there is strong evidence that the activation of neurogenic niches is regulated by vascular network [32]. Type-B1 cells give rise to amplifying progenitors or Type-C cells, where as Type-C cells produce migrating neuroblasts or Type-A cells [33].

SGZ- of the dentate gyrus in the hippocampus is a proliferative region which contains neuronal progenitors that give rise to granular neurons. *In vivo*, SGZ Type-B cells have a limited differentiating capacity. This property made researchers to believe that SGZ precursors may act as neuronal progenitors instead of NSCs. SGZ Type-B cells divide and give rise to type-D cells which differentiate locally to form mature granular neurons. Such newly generated neurons have got an important role in memory processing, learning and depression [34]. Surrounding neuronal activity has got an influence on SGZ neurogenesis. This indicates that mature neurons close to the neurogenic site can function as niche cells, providing a significant spatiotemporal regulation of adult neurogenesis in response to surrounding neuronal activity [35, 36]. Extensive studies done on SGZ have shown regulatory role of vascular cells in the prolifereation of adult NSCs, this has been substantiated by three-dimensional imaging revealing that SVZ vasculature has so much dense network of interconnecting vessels [32, 37]. Hence this demonstrates the potential contacts between adult SVZ NSCs and vessels are unusually permeable and there is devoid of other cellular interferences such as astrocytes and pericytes, suggesting that blood-derived cues are gaining access to regulate adult NSCs.

Microglia in neurogenic niche- Microglia occupies the entire CNS throughout the adult life of an individual and it's the chief immune cell of CNS [38]. The entire CNS is ably protected by microglia from the infectious agents and repairs the damaged neurons [38, 39]. In both, the adult SVZ and SGZ, microglia is abundant and is in close contact with NSC (Type-B cells), which is a fair indication to suggest that local interactions are possible between immune cells and multipotential progenitors. Microglia essentially forms the first line of defense against CNS injury and infections with effective endogenous and exogenous chemotactic factors influencing the total mechanism [38]. During inflammation or brain injury, microglia get stimulated and activated by secreting neurotrophic or neuron survival factors [39]. Recent evidence strongly favors that microglia only instructs NSC by secreting factors essential for neurogenesis, but not concerned with NSC maintenance, self-renewal, or propagation [40]. The factors which have a significant influence are interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) that serve as autocrine activators , interferon gamma (IFN-γ) producing a cytotoxic response, insulin-like growth factor-1 (IGF-1) that promotes cell proliferation that increases phagocytotic properties of microglia; leukemia inhibitory factor (LIF) concerned with growth promotion and cell differentiation [41, 42, 43, 44]. It has been suggested that microglia modulation of NSC is mediated by activation of mitogen activated protein kinase and phosphatidylinositol-3-

kinase/Akt signaling pathways [45]. Apart from the whole bunch of factors listed above, other varieties of inflammatory cells produce chemokines and cytokines, which can also influence proliferation, differentiation, survival, and migration of NSC [46].

Signaling pathways in self-renewal and differentiation of CNS

Many molecular niche signals have been observed in the recent times which can influence adult NSCs. A battery of developmental cues, signaling pathways and physiological humoral factors have been identified and demonstrated extensive progenitor proliferation and maintenance such as Wnt, Sonic Hedgehog (Shh), Bone Morphogenic Protein (BMP) antagonists , membrane-associated Notch signaling , leukemia inhibitory factor (LIF), transforming growth factor-alpha (TGF-a) and cytokines. Growth factors, including FGFs and neurotrophins such as brain-derived neurotrophic factor (BDNF), also significantly contribute to proliferation, survival and dendritic development of newborn neurons in the adult brain [47, 48]. The extracellular matrix (ECM) provides a platform for presentation of molecular cues and cellular interaction within neurogenic niches [49]. The above different factors and signaling pathways show specific effects on both SVZ and SGZ NSCs in the adult brain, thereby regulating the NSCs in the developing nervous system.

Wnt pathway - Wnt signaling plays an important role in the control of cell growth and differentiation during CNS development. Recent studies indicated that this signaling induces neuronal and astroglial differentiation but suppresses oligodendroglial differentiation [50]. Wnt signaling promotes proliferation of early neuronal progenitor cells and has influencing role in neurogenic phase [51]. Wnt signaling is a key pathway for embryonic development and has a crucial role in controlling the self-renewal in many of the adult tissues [52].

Notch pathway – The Notch proteins are 300-kDa single-pass transmembrane receptor, which function in CNS development by guiding cell fate determination. The unique feature is it promotes the glial cell fate while inhibiting neuronal fate. It also promotes the differentiation of astrocytes, while the differentiation of both neurons and oligodendrocytes is selectively inhibited. This shows notch signaling affects the development of nervous system at different levels. Hence in a nutshell Notch signaling plays active roles in maintaining neuronal progenitor renewal, inhibiting neuronal commitment, promoting astrocyte-oriented glial fates, preventing oligodendrocyte formation as well as brain morphogenesis and neuronal migration [53]. Notch signal pathway is an important determinant of NSC cell fate during development and has multiple critical roles in the regulation of NSC differentiation throughout the neurogenic to gliogenic *switch* [54]. Reports suggest that Notch signal pathway promotes glial differentiation while it prohibits premature neuronal differentiation. Notch signal pathway could promote gliogenesis and inhibit neurogenesis of NSC directly or shares cross-talk with STAT-signal pathway activated by diffusible signaling factors such as LIF (leukemia inhibitory factor), CNTF (ciliary neurotrophic factor), and BMPs [55].

BMP - BMPs are subclass of the transforming growth factor-beta (TGFβ) super family, which are expressed in many tissues under physiologic conditions. Various extracellular antagonists like

noggin, chordin, follistatin etc are responsible for regulating the BMPs through reversible interactions [56]. BMPs are involved in many developmental processes, including cell proliferation, differentiation, apoptosis and intercellular interactions during morphogenesis in a gene dosage dependent manner. Apart from promoting bone formation, BMPs specially BMP2 & BMP4 exhibit diverse activities in brain development [57]. They act at different stages of neural development and in different regions of the CNS to regulate proliferation and determine the cell fate and the manner of differentiation. BMP signaling exhibits cross-talk with other key pathways such as Wnt and Sonic Hedgehog (Shh) to coordinate cell proliferation and patterning thereby forming different types & numbers of differentiated neurons. BMPs inhibit pre-existing astrocyte precursors and oligodendrocyte specification while inducing astrocyte specification [58].

STAT3 pathway - Signal transducer and activators of transcription 3 (STAT3) act as signal messenger and transcription factor. STAT3-mediated signaling is one of the main mechanisms for promoting astrocyte differentiation by inhibiting neuronal differentiation in the embryonic cortex. Evidence shows STAT3 can promote NSC proliferation and in the presence of activated BMP and Notch signaling, it induces astrogenesis [59, 60]. Since it has crucial role to play in embryonic development, any deviation from this normal molecular effects can lead to developmental defects [61].

Shh pathway - Sonic hedgehog (Shh) is a member of the hedgehog family. Gli is the key transcription factor for Shh signaling. Shh promotes proliferation of both neural and non-neural tissues by regulating the expression of genes - N-Myc, cyclin D1, E2f1 and E2f2 [62]. Alongside the main roles in proliferation and differentiation, Shh signaling has a role in controlling cell death, because some of its downstream genes such as N-Myc and cyclin D1 are involved in the regulation of cell survival [63]. Shh signaling has been implicated in the specification and subsequent development of oligodendrocytes.

PDGF/EGF pathway - PDGF/EGF belongs to receptor tyrosine kinase superfamily, which exerts their stimulatory effects by binding to the tyrosine kinase receptors. Binding of ligands to the receptors causes autophosphorylation, leading to activation and signaling. Activation of protein tyrosine kinases (Tyk) in this cascade belong to the Janus kinase (JAK) that leads to activation of JAK-STAT signaling and initiation of astrogenesis. In addition to this mechanism, PDFG/EGF co-ordinates with other signaling pathways such as Notch and beta 1-integrin in NSCs [64]. The above mechanism involves JAK-1 and Tyk-2, these 2 important tyrosine kinases in neural precursors, resulting in nuclear translocation of 2 cytoplasmic transcription factors, STAT-1 and STAT-2. PDGF and EGF promote neuronal differentiation in committed NSCs, while promoting proliferation during the early phase of NSC development [65].

Cell - intrinsic Transcription regulators

Muti-faceted battery of transcription factors are involved in regulation of stem cell renewal and differentiation. They are Orphan nuclear receptor (TLX), Bmi1, The Sox family, Multiple basic helix-loop-helix (bHLH) genes, the tumor suppressor gene (Pten), the membrane-

associated protein Numb and its cytoplasmic homolog Numblike and nuclear receptors such as estrogen receptors (ER), thyroid hormone receptors (TR), and peroxisome proliferator-activated receptor γ (PPARγ). Each of them is touched upon briefly in this review to provide an insight for understanding the role of transcription factors in NSC biology.

TLX is an essential transcriptional regulator of NSC maintenance and self-renewal in the adult brain. *In vitro* all different varieties of neural cell types are formed by proliferation, self-renewal and differentiation when the TLX-expressing cells are isolated from adult TLX-heterozygote brains [66]. At the adult stage, TLX is expressed in NSCs in the SVZ and hippocamal dentate gyrus, the two adult neurogenesis areas.TLX is a key regulator that acts by controlling the expression of a network of target genes to establish the undifferentiated and self-renewable state of NSCs. Studies have shown that Tlx is essential for the transition of glial cells to astrocyte-like NSCs. *In vivo* Tlx's crucial role in the generation and maintenance of NSCs in the adult SVZ has been demonstrated [67]. Recent experiments conducted on mouse brain by different recombination techniques have shown a significant reduction of stem cell proliferation and a marked reduction in learning and behavior changes when Tlx is knocked off. [68]. Recent studies revealed that the interplay between miRNAs and nuclear receptor TLX plays an important role in NSC fate specification and determination [69, 70]. Interestingly, miR-9 and TLX form a feedback regulatory loop, which in turn regulates NSC proliferation and differentiation [69]. The TLX-positive NSCs in the dentate gyrus play an important role in spatial learning and memory [68] . The role of TLX in the developing brain has unraveled the molecular and cellular mechanisms underlying embryonic NSC proliferation and differentiation [71].

Bmi1 is a transcriptional repressor required for post-natal maintenance of neural stem cells in the peripheral and central nervous system [72]. Hence deficiency in Bmi1 leads to progressive postnatal growth retardation and neurological defects. The best way Bmi1can act is by repressing the cyclin-dependent kinase inhibitors, p16Ink4a and p19Arf, therefore it can promote the maintenance of adult stem cells [73].

The Sox family containing SoxB1 factors (Sox1, Sox2, and Sox3) are expressed in proliferating neural stem/progenitor cells throughout the development and in adulthood [74]. The Sox2 transcription factor is expressed at high levels in neural stem and progenitor cells in both embryonic and adult brain. The primary function of Sox is seen in the early development of brain; in addition to this, it is also essential for the proper maintenance of NSCs in adult neurogenic areas [75]. Regulatory mutations of Sox2 cause neurodegeneration and impaired adult neurogenesis [76]. Overexpression of Sox2 in neural progenitors resulted in upregulation of Notch1 and its downstream effector, Hes5 [77]. While Sox2 expression is regulated by sonic hedgehog (Shh) signaling pathway through its downstream effector, Gli2.

bHLH - Mammalian neural development is controlled by multiple basic helix-loop-helix (bHLH) transcription factors. bHLH genes play a critical role in regulation of NSC maintenance and differentiation [78]. Hes genes are repressor-type bHLH genes. Hes1 and Hes5 are highly expressed by NSCs, if there is a mis-expression of these genes, they would cause inhibition of neuronal differentiation and maintains NSCs in the embryonic brain. Hes1 and Hes5 are

essential effectors of Notch signaling. Another member of Hes family, Hes3 is also expressed by neural precursor cells in the ventricular zone of the developing nervous system. Triple knockout of Hes1, Hes3, and Hes5 led to accelerated neuronal differentiation and many defects in brain formation, suggesting that Hes genes are essential for the maintenance of NSCs in the developing brain [79].

The Pten tumor suppressor gene is a phosphatase which frequently undergoes mutation in human cancers and plays an important role in brain development. Knockout experiments have revealed that Pten is indispensable for control of NSC proliferation and self-renewal in the developing brain [80]. The Pten deficient NSCs show increased proliferation due to shortened cell cycle. Pten regulates cell cycle progression by suppressing cell cycle related genes like cyclin B1, cyclin B2, cyclin D1, and cyclin E1 [81]. By these actions it's understood that Pten is critical in keeping NSCs under check in the developing brain. Pten regulates NSCs in the adult brain apart from serving an important function in neural development. By a procedure known as conditional deletion of Pten in adult NSCs in the SVZ led to enhanced NSC self-renewal. This indicates Pten is also an important player in adult NSC self-renewal and neurogenesis.

Numb and Numblike play critical role in maintaining neural progenitor cells during brain development by allowing their progenies to choose progenitor over neuronal fates [82]. Numb suppresses Notch signaling by physical interaction with the intracellular domain of Notch 1. When Numb and Numblike are knocked out in mice, it led to the emergence of neurons at the expense of progenitor cells which resulted in complete depletion of dividing cells shortly after the onset of neurogenesis [83]. The main function of Numb and Nuunblike by this experiment is to maintain progenitor cells in mouse neurogenesis. Recently Numb was attributed with a critical role in the maintenance of cadherin-mediated cell adhesion and polarity of radial glila cells, subsequently responsible for the integrity of the ventricular zone and cerebral cortex [84]. Hence Numb has diverse roles in the neural development, such as regulation of SVZ homeostasis, neural progenitor cell adhesion and polarity.

Recently, different types of nuclear receptors such as estrogen receptors (ER), thyroid hormone receptors (TR), and peroxisome proliferator-activated receptor γ (PPARγ), have shown to regulate NSC proliferation and differentiation [85]. The role of ERβ has been proved beyond doubt that it is essential in neuronal maintenance of CNS by conducting experiments on mice. In the same way, experimental evidence from mice shows that TRα role is also equally important in the neurogenesis of mammalian adult brains. NSCs derived from PPARγ-deficient mouse brains have significantly reduced cell growth and its controlling role in NSC proliferation has been demonstrated [86, 87, 88].

Micro RNAs (miRNAs) regulators – Novel miracle molecule

miRNAs are short 20-22 nucleotide RNA molecules which function as negative regulators of gene expression in a variety of eukaryotes in a regulated manner. miRNAs are involved in numerous cellular processes including development, proliferation, and differentiation [89]. Recently identified miracle molecule of this modern era - miRNA is a large family of small non-

coding RNAs, which are key post-transcriptional players in stem cell self-renewal and differentiation. Epigenetic mechanisms, including DNA methylation and histone modification, are known to play significant roles in the modulation of NSC proliferation and differentiation [90]. One of the miRNAs, miR-137 has been implicated in neuronal maturation by translational repression of Mind bomb-1, a ubiquitin ligase known for its neurogenesis and neurodevelopment [91]. DNA methylation represses gene transcription by direct and indirect mechanisms. The direct is through blocking the access of transcription factors to their binding sites and indirectly recruiting proteins that bind methyl-CpGs (MBDs or Kaiso family proteins) which contains multiple sub-classes like MBD1, MBD2, MBD3, MBD4 and MECP2 [92]. It was found that MeCP2 regulates specific miRNAs in mouse NSCs [93]._Recent experiments discovered that MBD1 regulates the expression of a miR-184 (subset of miRNA) in adult NSCs but not by MeCP2 [94, 95]. miR-184 promotes NSC proliferation and inhibits differentiation by targeting Numblike [96]. Hence MBD1, miR-184, and Numblike may together form a homeostatic regulatory network between the proliferation and differentiation of NSCs. The most abundant of all the miRNA is miR-124 and its association with the neuronal differentiation has been talked about and its one of the most sought after miRNA. Recently miR-124's role has become predominant in the promotion of neuronal differentiation and cell cycle exit of NSC in the SVZ, one of the neurogenic niches of the adult mammalian brain. The deletion of REST (repressor element-1 silencing transcription factor) in SVZ progenitors *in vitro* led to precocious neuronal differentiation, this is similar to the over-expression of miR-124 [97].

The role of miRNAs have been spreading into many cellular domains and their predominant influence in various stages of neural differentiation, synapse development and synaptic plasticity have gone beyond reach [98]. Studies revealed that miR-132 regulates dendritic growth, activity-induced spine growth, spine morphology, hippocampal miniature excitatory postsynaptic current (mEPSC) frequency and circadian rhythms [99, 100].

Cell - extrinsic signaling

The microenvironment or niche is essential for stem cell renewal and differentiation ( ).To maintain the properties of stem cells intact absolute direct physical contact is mandatory between NSCs and their niches. This forms the backbone for cellular signaling between the NSCs. Once these contacts in the form of signaling disappear NSCs enter into disarray and microenvironment is in chaos. Signaling molecules in the niche are composed of various soluble factors, membrane bound molecules, and extracellular matrix (ECM), including many crucial pathways described earlier such as Wnt, Notch and Sonic hedgehog (Shh) [101]. Receptor tyrosine kinase (RTK) signaling has also been implicated in regulation of NSC proliferation and self-renewal.

For signaling to take place, the pre-requisite is to establish stem cell retention. To perform this duty, integrins are required, which mediate adhesion of cells to a basal lamina composed of ECM. Both integrins and adherens junctions play critical roles in maintaining the location, adhesiveness and proliferative status of epithelial cells within tissues [102]. The ability of the niche to retain its stem cells and then it has that special property of recruiting stem cells

is by a process called “homing” [103]. Once niches are established, they serve an important role in acting as signaling centers to attract stem cells. This is essential as stem cells need to stick and be there in niche to show the property of self-renewal and differentiation.

A study has shown that the cerebrospinal fluid sends intrinsic and extrinsic signaling to the progenitors which respond positively to the signals emanating from the CSF of brain [104]. There is a constant interplay between miRNAs and both intrinsic and extrinsic stem cell signaling mechanisms which are coupled with transcription factors [95]. Recent evidence shows that ECM surrounding the NSCs, vasculature, glial cells, and other neurons are components of the niche where NSCs are located. This surrounding microenvironment is the source of multiple extrinsic signals that instruct NSCs to either self-renew or differentiate [105]. In both adult neurogenic zones (SVZ & SGZ), the NSCs are in close contact with endothelial cells, astrocytes, ependymal cells, neurons and derived progenitor cells [106]. So it’s a clear indication that all these play a major role in preserving the homeostasis of NSC niche.

Recent evidences report that energy metabolism is an important regulator of NSC function. Energy metabolism influences many molecules and signaling pathways such as insulin/insulin-like growth factor I (IGF-1)-FoxO and insulin/IGF-1-mTOR signaling, AMP-activated protein kinase (AMPK), SIRT1, and hypoxia-inducible factors are immensely gaining ground and strongly implicated in NSC biology. Adding on to this, it was also proved that these specific signaling modules are coupled with other pathways involved in NSC maintenance and differentiation [107].

With the influence of local environment cues, decisions are made regarding fate. NSCs exist in niche in which extrinsic signals modulate the intrinsic signals that drive self-renewal and determination of cell fate. The extrinsic signals found in the niche can be a set of soluble signals emanating from either a distant or a local source. Some examples of soluble signals are stem cell mitogens such as fibroblast growth factor2 (FGF2), glycosylated cysteine protease inhibitor cystatin C, Epidermal growth factor (EGF), neureglin-1, BMP, and transformation growth factor beta (TGF-β) and Wnt families of signaling proteins. In addition to the above list of soluble factors, contact-mediated factors such as the Notch signaling pathway can regulate the cell fate determination. Finally, proteins such beta1 integrin found in ECM are another significant contact-mediated signaling in NSC niches [102]. So, in the presence of these multiple cues, the NSC integrates all the signals and has the right to choose either self-renewal or the pathway of differentiation (Gage FH, 2000). These extrinsic signals interact with receptors on the surface of the NSC. Normally neighboring cells in developing tissues communicate through Notch signaling pathway to direct cell fate decisions. In this mechanism one cell in a neighboring relationship in the niche is the signaler and the other cell is a receiver. This is how cells communicate in the niche with various signaling molecules and factors to change the behavior of the NSC.

A Study indicates that the Wnt/β-catenin signaling plays an important role in the proliferation and self-renewal of neural precursors, through its downstream target genes like cyclin D1. While Wnt proteins usually promote neuronal differentiation in NSC culture and in adult hippocampus [108], clearly demonstrating that signaling by the canonical Wnt pathway

has multiple functions in stem cells. Wnt pathways are critical regulators of both NSC self-renewal and neurogenesis.

Notch activity is needed for NSC maintenance; increased neuronal differentiation was detected in Notch1 mutant brains. Notch pathway components are also expressed in postnatal and adult mouse brains, both in germinal zones and in neurons. Apart from its critical role of maintaining NSC state in germinal regions, Notch can also promote terminal glial differentiation.

Sonic hedgehog is a potent mitogen for neural progenitor cells of adult brains. There is an increase in proliferation and neurogenesis of hippocampal SGZ cells due to over-expression of Shh near the dentate gyrus. There is also an increase in SVZ cell proliferation and maintenance proliferation of adult hippocampal neuronal progenitors *in vitro* [109, 110].

EGF, transforming growth factor α (TGFα), and fibroblast growth factor (FGF) are all extracellular ligands of RTKs and play critical roles in the proliferation of NSCs [111]. *In vivo* intraventricular infusion of EGF and TGFα increased NSC proliferation in the adult brain. *In vitro* experiments demonstrated that SVZ-derived progenitor cells can be expanded by EGF and FGF administration [112]. EGF and TGFα bind with Epidermal growth factor receptor, which is also a member of the RTK family. EGFR is expressed in neurogenic region adult (SVZ). Cyclin D2 is an effector of the FGF signaling, by promoting NSC proliferation influencing early phases of cell cycle.

CONCLUSION

Niche forms the basis for the origin of multi-centric signaling. NSCs in the brain are influenced by a convergence of extracellular signals may interact with intrinsic regulators by various signaling cascades, All these various mechanisms are coordinated to regulate the development, maintenance, self-renewal, and differentiation of stem cells. Understanding more about these signaling pathways will help reveal how the niches influence NSC self-renewal and tissue development. Unraveling how individual signaling cascades integrate into the global regulatory networks will be essential to better understand neural stem cell biology.

Undoubtedly, the knowledge generated from the understanding of various signaling mechanisms will expand possibilities for future regenerative therapies. As more and more inducible systems are developed and more signaling pathways interrogated, the cell-intrinsic requirements for adult neurogenesis will be established.

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