Treating Brain Disorders by Targeting Adult Neural Stem Cells

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**Adult neurogenesis**, a developmental process of generating functionally integrated neurons from neural stem cells, occurs throughout life in the hippocampus of the mammalian brain and highlights the plastic nature of the mature central nervous system. Substantial evidence suggests that new neurons participate in cognitive and affective brain functions and aberrant adult neurogenesis contributes to various brain disorders. Focusing on adult hippocampal neurogenesis, we review recent findings that advance our understanding of the key properties and potential functions of adult neural stem cells. We further discuss the key evidence demonstrating the causal role of aberrant hippocampal neurogenesis and various brain disorders. Finally, we propose strategies aimed at simultaneously correcting stem cells and their niche for treating brain disorders.

**Endogenous Neural Stem Cells in the Adult Brain**

The adult mammalian brain is a dynamic structure, capable of remodeling in response to various physiological, pathological, and pharmacological stimuli. One dramatic example of brain plasticity (see Glossary) is the birth and subsequent integration of newborn neurons into the hippocampus of the adult mammalian brain, including humans [1–5] (Box 1). Adult hippocampal neurogenesis has garnered significant interest because substantial evidence suggests that adult-born new neurons contribute to learning and memory, stress response, and mood regulation [6–8]. A general model has proposed that adult hippocampal neurogenesis is not merely a cell-replacement mechanism, but instead maintains a plastic hippocampal neural circuit via continuous addition of adult-born new neurons with unique properties and structural plasticity of mature neurons induced by new neuron integration. In addition, many studies have implicated dysfunction of adult hippocampal neurogenesis in an increasing number of brain disorders, such as epilepsy, major depression, and neurodegenerative diseases [7,9–11]. Continuous hippocampal neurogenesis in the mature brain reflects large-scale plasticity unique to this region, and could potentially serve as a target for modulation of a subset of cognitive and affective behaviors caused by various brain disorders. Here we review recent findings that advance our understanding of the key properties and potential functions of neural stem cells (NSCs) in the adult hippocampus. We further discuss the key evidence of how aberrant adult hippocampal neurogenesis contributes to several forms of brain disorders, including epilepsy, mental disorders, and neurodevelopmental disorders. Finally, we propose strategies aimed at simultaneously correcting both NSCs and their niche for potential treatment of these brain disorders.

**Key Properties of Adult NSCs**

NSCs with radial morphology in the dentate gyrus (DG) are thought to be the most primitive NSCs in the adult brain and are essential substrates for continuous neurogenesis throughout life. Recently, it has become clear that NSCs face multiple decision points during the initial
stages of adult neurogenesis, including decisions for quiescence versus activation, fate specification, and long-term maintenance (Figure 1). Importantly, these decision points for NSCs are subject to activity-dependent regulation.

Quiescence versus Activation of NSCs
In the subgranular zone (SGZ) of the adult DG, radial NSCs (rNSCs) are largely quiescent, but they can become activated in response to various external stimuli, such as exercise, antidepressants, and epilepsy [13]. Quiescence is thought to allow adult stem cells to withstand metabolic stress and to preserve genomic integrity over a lifetime. The quiescent state has long been viewed as a dormant and passive state of NSCs. However, accumulating evidence points to the opposite view. Recent single-cell transcriptome analysis of quiescent adult SGZ NSCs showed active expression of various receptors for niche signals and downstream signaling components [14], thus suggesting that quiescence is an actively maintained state. NSCs reside in a specialized local environment within the DG that consists of multiple distinct cell types, and signaling from these local cells can potentially control the key behavior of NSCs through the surface receptors expressed in NSCs. For instance, recent studies showed that local interneurons and mossy cells serve as critical niche components to regulate the activation versus quiescence state of the NSCs through the gamma-aminobutyric acid (GABA) and glutamate receptors expressed in rNSCs, respectively [15,16]. Besides local interneurons and mossy cells, ample evidence has demonstrated that NSCs are dynamically regulated by a barrage of niche signals [17], which may exert synergistic and antagonistic effects on rNSC regulation. This has raised a key question on how NSCs interpret diverse niche signals from the local environment to make the ultimate decision to stay in quiescence or become activated.

Fate Decisions of NSCs
Two hallmarks of NSCs are their abilities to self-renew and generate differentiated neuronal or glial progeny [1]. The initial studies using in vitro expansion and differentiation of adult neural precursor cells have suggested that adult NSCs are capable of self-renewal and are tri-potent, with the capacity to generate neurons, astrocytes, and oligodendrocytes [18]. However, recent single-cell lineage tracing and fate-mapping of adult hippocampal NSCs in vivo have demonstrated the generation of neurons and astrocytes, but not oligodendrocytes [19] (Figure 1), thus highlighting different niche environments between in vitro and in vivo, which dictates fate decisions of NSCs. Furthermore, these studies revealed a significant heterogeneity of individual adult NSCs in their fate choices, ranging from symmetric divisions for self-renewal to asymmetric divisions for generating neurons and/or astrocytes (Figure 1). Consistent with this notion, accumulating evidence has suggested that SGZ consists of NSCs with different morphologies [20] and behaviors in response to external stimuli [21]. With recent advances in microscopy technology, it has become possible to directly image the behavior of individual adult NSCs in vivo for an extended period of time [22,23]. Two-photon imaging analysis of individual NSCs in the adult mouse DG has revealed a new model, which shows that radial NSCs typically undergo two to three cell divisions with initial symmetric or asymmetric divisions,

Box 1. Adult Neurogenesis in Human
Recently, two prominent studies have elicited scientific debate about adult hippocampal neurogenesis in humans. A report by Sorrells et al. concluded that neurogenesis in the human dentate gyrus drops to an undetectable level during childhood [12]. In another study, Baldini et al. came to the opposite conclusion and reported lifelong neurogenesis in humans [4]. To facilitate the discussion on this topic, a recent article led by Kempermann et al. reviewed the key evidence from many laboratories on this topic, proposed future directions of how to further investigate human neurogenesis, and discussed how the current state of knowledge about adult hippocampal neurogenesis applies to the human situation [5]. They concluded that ‘currently there is no reason to abandon the idea that adult-generated neurons make important functional contributions to neural plasticity and cognition across the human lifespan’.

Glossary
Asymmetric division: the process of neural stem cells giving rise to a daughter cell identical to the mother cell and a second cell of a different cell type.
Autocrine signaling: the production and secretion of an extracellular mediator by a cell, followed by the binding of that mediator to receptors on the same cell to initiate signal transduction.
Brain plasticity: the ability of the brain to change based on different activities.
Chemogenetics: a technique that uses small novel molecules to activate genetically engineered receptors.
Clonal analysis: a study observing clones derived from single cells.
Dlx: Dlx family genes encode homeodomain transcription factors related to the Drosophila distal-less (Dll) gene. Dlx5/6 genes are specifically expressed by all forebrain GABAergic interneurons during embryonic development.
D-serine: synthesized in neurons, it serves as a neuromodulator by coactivating NMDA receptors, enabling them to open if they also bind glutamate.
Episomal dilution: DNA molecules that replicate independently of chromosomal DNA will dilute after cell division.
Feedforward inhibition: collateral branches of the excitatory afferent fibers that excite inhibitory interneurons that in turn inhibit neurons in the forward direction.
Gamma-aminobutyric acid (GABA): the chief inhibitory neurotransmitter in the mammalian central nervous system; acts on GABAA or GABAB receptors.
Glutamate: the major excitatory neurotransmitter in the mammalian central nervous system; acts on NMDA, kainate, AMPA, or metabotropic glutamate receptors.
Hypothalamic-pituitary-adrenal (HPA) axis: a major neuroendocrine system that controls reactions to stress and regulates body functions.
Interneurons: form GABAergic synapses to inhibit their target neurons.
Lineage tracing: identification of all progeny of a single cell.
followed by a final self-depleting symmetric division generating two nonradial daughter cells [23,24]. These findings are consistent with previous studies in fixed adult mouse brain sections revealing several rounds of neuronal differentiation followed by astroglial differentiation [25]. However, the other study showed that NSCs undergo long-term renewal [19]. This may be explained by the distinct NSC subtypes labeled by different NSC promoter driven Cre mouse lines: the in vivo imaging analysis traced Ascl1 (Achaete-scute complex-like 1)-expressing NSCs, whereas other NSC subtypes that do not express Ascl1 may be capable of long-term self-renewal.
Long-Term Maintenance of NSCs
Precise control of somatic stem cell properties is essential for the long-term maintenance of tissue homeostasis and has been shown to be closely linked to tissue demands at any given time. The balance of NSC maintenance and neurogenesis is essential to ensure continuous generation of new hippocampal neurons throughout life without depleting the NSC pool. For example, the long-term consequence of excessive activation is subsequent depletion of the NSC compartment and impaired maintenance of NSCs, which ultimately leads to the loss of regenerative capacity of the NSC population and subsequent neuronal production in the adult hippocampus [26–28]. Therefore, the total NSC pool reflects a summation of NSC decisions over time: maintenance through quiescence or asymmetric self-renewal, reduction through terminal differentiation, and expansion through symmetric self-renewal. Under normal conditions, NSCs are mostly quiescent, but once activated, they undergo several rounds of cell division to produce neural progenitors followed by astrocytic differentiation, thus resulting in a progressive depletion of the NSC pool over time [25]. Utilizing in vivo clonal analysis for lineage tracing, NSCs were found to self-renew by generating copies of themselves. However, this mechanism for the repopulation of NSCs does not seem to counteract the depletion of the NSCs, occurring naturally over time. This could explain the age-dependent decline of the NSC pool in both rodents [25] and humans [4].

Functional Roles of Adult NSCs
Functions of Adult-Born Neurons in the Local Hippocampal Circuitry
When adult NSCs were initially discovered, it was proposed that their function was to provide a regenerative source for new neurons upon neurodegeneration and injury. Now it is widely accepted that the primary function of these endogenous adult NSCs is to confer an additional layer of plasticity to the mature brain via continuous addition of adult-born new neurons with unique properties. This raises a fundamental question: how is hippocampal activity modulated by continuous addition of newborn neurons?

It has been proposed that adult-born neurons could impact brain functions by serving as an active modulator of local circuitry to shape mature neuron firing, synchronizion, and network oscillations [29]. It has become apparent that unique physiological properties of immature neurons allow them to participate differentially in the hippocampal network [30]. Immature neurons exhibit elevated excitability and plasticity compared with mature neurons during a critical time window between 4 and 6 weeks after birth [31–34], suggesting that immature neurons have a privileged role in regulating hippocampal functions. Therefore, preferential recruitment of excitable immature neurons with enhanced plasticity would allow this population to be a major player in information processing within the trisynaptic hippocampal circuit. Adult-born neurons make synaptic contacts through the mossy fiber axons to the interneurons and pyramidal cells of the hippocampal CA3 region (Figure 2). The connectivity of the dentate granule cells and CA3 pyramidal cells is important for certain cognitive and memory functions [35]. The sparse connectivity between granule cells and CA3 cells was thought to be critical for pattern separation, a process by which overlapping or similar inputs are transformed into less similar outputs [36]. Immature neurons form connections first with the CA3 pyramidal cells and later with the local interneurons [37], suggesting that immature neurons preferentially excite pyramidal neurons, thus bypassing feedforward inhibition mediated by interneurons. In contrast, mature neurons activate considerable feedforward inhibitory inputs onto the CA3 pyramidal cells, thus suppressing their activation. Interestingly, during the critical window of 4 weeks after birth, new neurons transiently form strong anatomical, effective, and functional connections with local inhibitory circuits in CA3 [38]. Supporting the unique properties of adult-born neurons, a recent study using two-photon calcium imaging to monitor the activity of young NSC-derived newborn neurons.
adult-born neurons in awake behaving mice demonstrated that adult-born neurons fire at a higher rate in vivo but exhibit less spatial tuning than their mature counterparts when animals are presented with different contexts [39]. These findings highlight the unique role of adult-born neurons in context encoding and discrimination, consistent with their proposed role for pattern separation.

Functions of Adult-Born Neurons in the Global Network

Adult-born neurons not only could exert direct impact on the local hippocampal circuit through axonal projections from newborn neurons to their downstream CA regions, they could also exert indirect impact on the global neural network through axonal projections of newborn neurons.
neurons to the local interneurons that project to other brain regions [40–43] (Figure 2). For instance, local somatostatin-expressing neurons can send long-distance projections to the medial septum (MS) and entorhinal cortex (EC), thus modulating the inhibitory tone and rhythmic activity in those target brain regions [44,45]. These findings may account for the highly synchronized theta activity in the hippocampus, MS, and EC. Furthermore, the hippocampus has been shown to have significant functional connectivity with other brain regions through direct or indirect neuroanatomical connections, including (but not limited to) the prefrontal cortex, striatum, septum, amygdala, and insular cortex [41,46–50]. Such broad connectivity from the hippocampus to other brain regions suggests that disruption of adult hippocampal neurogenesis may degrade optimal neuronal network dynamics, which in turn may shift large-scale brain activity changes to promote maladaptive states and contribute to the deficits associated with various brain disorders. Supporting this notion, recent studies demonstrated that disconnection of the hippocampus from other brain regions impairs animals’ performance on various learning-related tasks [51–53]. These findings raise the possibility that dysfunction of adult neurogenesis itself may play a causal role in various brain disorders and that adult neurogenesis could serve as a novel therapeutic target to prevent, ameliorate, or restore some of the cognitive and affective deficits associated with various brain diseases.

Functions of Adult-Born Astroglia
Though adult NSCs primarily generate new neurons, the role of adult NSCs in brain function is now expanding beyond being a mere source of neuronal progeny. Besides generating neuronal progeny, adult NSCs also give rise to astroglia through asymmetric cell division (Figure 1). In the adult SGZ, astroglia exhibit horizontal and bushy morphology and express astrocyte markers, including glial fibrillary acidic protein (GFAP), $S100$ calcium-binding protein B ($S100B$), and aldehyde dehydrogenase 1 family member L1 (Aldh111) [54]. Recent morphological studies using electron microscopy demonstrate that the radial processes of NSCs share vasculature with astrocytic processes and adhere to the adjacent processes of astrocytes [55], suggesting the potential functional interaction between NSCs and astrocytes. Under normal conditions, astroglia are not considered as neuronal precursors because they lack NSC marker expression, such as nestin [19,25,56]. However, a small portion of astroglia are labeled with cell cycle markers and share many molecular similarities with NSCs such as Asc1 [54,57]. Interestingly, new astroglia are generated from NSCs in the adult SGZ before they migrate to the hilus or the molecular layer of the DG [19]. Under pathological conditions such as epilepsy, which induces hippocampal hyperexcitability, NSCs become overactivated and generate reactive astrocytes with accompanying NSC pool depletion [58]. Though hippocampal SGZ NSCs generate astrocytes under both physiological and pathological conditions, the functions of newborn astrocytes remain largely unknown under these conditions. Astrocytes are connected by gap junctions and form networks that can modulate the surrounding neural circuitry activity and plasticity through gliotransmitters, including glutamate, ATP, and D-serine [59]. Additionally, transcriptome analyses of reactive astrocytes shows that neuroinflammatory reactive astrocytes upregulate many genes shown to be destructive to synapses, such as complement cascade genes [60]. Therefore, the reactive astrocytes generated from NSCs under pathological conditions may negatively contribute to hippocampus-dependent learning and memory. Further studies are needed to determine the contribution of adult gliogenesis to brain functions in order to fully understand the functions of adult NSCs.

Direct Contribution of NSCs to the Neurogenic Niche
It remains unknown whether adult SGZ NSCs (not their newborn progeny) make functional contributions to the hippocampal neurogenic niche. So far, no studies have exclusively manipulated SGZ NSCs for behavioral analysis. Therefore, the role of NSCs in regulating hippocampal
network activity, hippocampal functions, and hippocampal neurogenesis remain largely unknown. It remains to be determined whether NSCs are capable of releasing potent chemicals such as gliotransmitters and other secretory molecules into the neurogenic niche, which could in turn influence both mature and newborn progeny. Supporting this view, NSCs and their immediate progeny are found to secrete diazepam-binding inhibitor in the adult rodent brain; this antagonizes GABA signaling and promotes proliferation of neuroblasts through paracrine signaling [61]. In addition, NSCs might directly influence each other through autocrine signaling; NSCs in the adult SGZ express both the vascular endothelial growth factor (VEGF) receptor 3 and its ligand VEGF-C, and VEGF receptor stimulation promotes NSC activation [62].

Adult NSCs in Mediating Brain Disorders
Immediately after the initial discovery of neurogenesis in the postnatal rat hippocampus [63], Altman suggested that new neurons are critical for learning and memory. Since then, mounting evidence from sophisticated genetic approaches and computational modeling has implicated that adult hippocampal neurogenesis plays a central role in fine-tuned, spatially discrete memory processes and affective behaviors [34,36,64–66]. Furthermore, a substantial body of literature addresses changes of adult hippocampal neurogenesis mostly in rodents, in the context of various pathophysiological conditions, including aging, epilepsy, stroke, degenerative neurological disorders, and neuropsychiatric disorders [10,11,67,68]. It remains unknown whether these changes represent adaptive responses to various pathological conditions, or are part of the pathophysiology that contributes to these conditions. Examples in animal models now suggest that dysfunction of adult hippocampal neurogenesis may play a causal role in brain disorders with memory and mood deficits. Here we review recent studies that provide compelling evidence for the contribution of aberrant neurogenesis in various brain disorders, including epilepsy, mental disorders, and neurodevelopmental disorders.

Epilepsy
Recent studies have supported an emerging view that adult-born DG granule cells are directly involved in the pathogenesis of temporal lobe epilepsy, one of the most common human seizure-related disorders [69]. In animal models of epilepsy, pilocarpine-induced status epilepticus leads to a prolonged increase in dentate neural progenitor proliferation [70]. Additionally, newborn neurons born during and after epilepsy displayed aberrant synaptic integration, hilar basal dendrites with spines, ectopic hilar localization of the soma, and mossy fiber sprouting [71,72]. These aberrant morphological features of DG granule neurons in mice are similar to those observed in postmortem dentate gyri of patients with temporal lobe epilepsy [69]. Eliminating cohorts of newborn neurons decreases status epilepticus-induced mossy fiber sprouting and ectopic granule cells [72]. Furthermore, genetic ablation of hippocampal newborn neurons immediately after status epilepticus induction effectively reduced the development of spontaneous recurrent seizures [73]. These studies suggest that aberrantly integrated new neurons contribute to the morphological abnormality of DG granule neurons and epilepsy. Separately, deletion of phosphatase and tensin homolog deleted on chromosome ten (PTEN) in a small percentage of adult-born dentate granule cells is sufficient to cause spontaneous seizures within 4 weeks [74], suggesting that pathological changes in a small population of hippocampal adult-born neurons are sufficient to induce epilepsy. Collectively, these studies provide strong evidence that dysfunction of adult hippocampal neurogenesis plays a causal role in epileptogenesis.

Mental Disorders
An increasing number of studies show that the behavioral symptoms typical for neuropsychiatric disorders can be produced by manipulating DG neurogenesis [9,75]. One example is that
retrovirus-mediated knockdown of Disrupted in Schizophrenia 1 (DISC1), a risk gene for major mental disorders, including schizophrenia, major depression, and bipolar disorders [76], leads to aberrant morphogenesis and integration of newborn dentate granule neurons in the adult mouse hippocampus, due in part to hyperactivation of the mTOR pathway in newborn neurons [77–79]. Strikingly, dysregulated adult hippocampal neurogenesis following DISC1 knockdown in a cohort of retrovirally labeled newborn neurons is sufficient to cause cognitive and affective deficits, including pronounced learning and memory deficits (in the object-place recognition task and Morris water maze), anxiety and depression-like phenotypes (in the forced-swim test and elevated plus maze). Inactivation of these aberrant new neurons reverses specific behavioral phenotypes, indicating a causal role for adult neurogenesis dysfunction in behavioral impairments.

It is now well-established that stress negatively regulates progenitor proliferation and new-neuron survival [80], whereas antidepressant treatments promote proliferation of neural progenitors and maturation of newborn neurons during adult hippocampal neurogenesis [10,81]. Ablation of adult neurogenesis does not appear to alter affective behaviors at the basal level but abolishes some antidepressant-induced phenotypes in both rodents [82] and non-human primates [83]. Emerging evidence suggests a critical role for adult hippocampal neurogenesis in the stress response by suppressing the hypothalamic–pituitary–adrenal (HPA) axis. In mice with adult neurogenesis ablated, mild stress leads to increased stress hormone level and greater stress responses [84,84]. Furthermore, blockade of adult neurogenesis abolishes the antidepressant effect of hippocampal regulation of the HPA axis after chronic stress [85]. Interestingly, a recent study found that adult hippocampal neurogenesis confers resilience to chronic stress by inhibiting the activity of mature granule cells in the ventral DG, a subregion implicated in mood regulation. This study provided a novel circuit mechanism by which adult-born neurons regulate DG information processing to protect from stress-induced anxiety-like behavior [86].

**Intellectual Disability**

Fragile X syndrome, a neurodevelopmental disorder that leads to intellectual disability, is caused by the functional loss of fragile X mental retardation protein (FMRP). Fmrp null mice exhibit deficits in some forms of hippocampus-dependent learning, accompanied by reduced adult hippocampal neurogenesis due to impaired neuronal differentiation and survival [87]. Genetic deletion of Fmrp selectively in NSCs and their progeny results in defects in both adult neurogenesis and hippocampus-dependent learning. Furthermore, restoration of Fmrp expression in NSCs and their progeny is sufficient to rescue the learning deficits in Fmrp null mice [88]. These striking results suggest a critical role for adult neurogenesis dysfunction in learning impairments associated with fragile X syndrome. Whether this is generalizable to other neurodevelopmental disorders (such as autism spectrum disorder) remains to be determined.

Though these studies have provided compelling evidence for the critical role of aberrant hippocampal neurogenesis in brain disorders, the mechanisms by which aberrant adult-born neurons contribute to impaired brain functions are largely unknown. Future studies will be needed to dissect the circuit and signaling mechanisms underlying this interaction.

**Targeting NSCs and Their Niche in Treating Brain Disorders**

The promise of endogenous NSCs for the development of novel therapeutic strategies lies in the regenerative properties of NSCs that allows them to repair the damaged and diseased brain. Yet such strategies must consider and address biological constraints imposed by both NSCs and their local environment. To harness the brain’s endogenous capacity to generate
hippocampal NSCs as a potential therapeutic strategy, it is necessary to understand the properties of NSCs and their local microenvironment (also termed as ‘niche’), supporting NSCs to proliferate and differentiate. The intrinsic and extrinsic mechanisms underlying adult NSC regulation have been extensively reviewed elsewhere [2,13,17,89,90]. Activity-dependent adult NSC regulation is proposed, such that extrinsic niche signals activate NSC surface receptors, which in turn trigger intracellular signaling cascades to control the key behaviors of adult NSCs. Here we propose that strategies aimed at simultaneously correcting both NSCs (Figure 3) and their niche (Figure 4) would be likely to provide more effective treatments, compared with targeting only one of these aspects. Supporting this view, a recent study showed that increasing hippocampal neurogenesis along with brain-derived neurotrophic factor (BDNF) overexpression mimics the effects of exercising on cognitive improvement in a mouse model of Alzheimer’s disease (AD) [91], suggesting that promoting neurogenesis in an improved niche environment can ameliorate AD-associated cognitive deficits. This timely report highlights the importance of the combination of healthy neurogenic niche and increased neurogenesis for improving hippocampus-dependent cognitive functions.

Figure 3. Targeting Adult Hippocampal Neural Stem Cells (NSCs) in Treating Brain Disorders. Under pathological conditions, aberrant NSCs exhibit disrupted lineage, resulting in decreased number of newborn neurons, increased number of reactive astrocytes, and impaired brain functions. To correct the deficits, we illustrate a strategy to target NSCs for treating brain disorders: (1) the gene profiles can be obtained and then compared via single-cell sequencing analyses under both normal and pathological conditions. (2) Candidate genes will be selected to target NSCs using the rAAV4 vector with selective tropism to NSCs. (3) By manipulating a set of candidate genes to increase and/or decrease their expression in NSCs, the functions of NSCs are expected to be restored. (4) Restored NSCs are expected to exhibit increased number of newborn neurons and decreased number of reactive astrocytes, which in turn lead to restored brain functions.
Figure 4. Targeting the Adult Hippocampal Neural Stem Cell (NSC) Niche in Treating Brain Disorders.
Here we illustrate a strategy for treating brain disorders by targeting the local niche cells in order to create an improved
(See figure legend on the bottom of the next page.)
Targeting Adult Hippocampal NSCs in Treating Brain Disorders

Adult NSCs are difficult to study because they are rare and heterogeneous. To date, various viral and transgenic strategies have been developed to target and genetically manipulate adult hippocampal NSCs [92]. These approaches have undoubtedly revolutionized our understanding of developmental processes during adult hippocampal neurogenesis. However, a number of limitations in these approaches prevent efficient targeting of NSCs. For instance, classical onco-retroviral-mediated labeling approaches require active cell proliferation, thus preferentially labeling proliferating neural precursors and progenitors, not quiescent rNSCs [2,93,94]. Additionally, lentivirus and adenovirus with an NSC-specific promoter (Sox2 and GFAP) have been used to target NSCs [94,95], but the targeting specificity is a concern, as almost all of the NSC promoters are not active in the astroglia lineage, thus also labeling a substantial number of astrocytes as the starting population. Transgenic approaches using indelible labeling of NSCs and their progeny are the most commonly used strategies to target NSCs for lineage tracing, which requires the generation of double transgenic animals with the combination of an NSC-specific Cre recombinase and a floxed reporter gene. Furthermore, to manipulate gene(s) regulating NSCs in a cell-autonomous fashion, it requires generation of mice with multiple transgenes harboring NSC-specific Cre recombinase, floxed reporter gene, and floxed gene(s) of interest. Besides the prolonged animal generation time for multiple transgenes, the transgenic strategy is difficult for immediate translation in genetically intractable animal species, including humans. Therefore, developing a readily manufactured viral vector that allows flexible packaging of transgenes and supports high-level transgene expression selectively in NSCs is a pressing need for efficient targeting and manipulations of NSCs in both genetically tractable and intractable animal species. Recently, Crowther et al. have developed a recombinant adenovirus-associated virus serotype 4 (rAAV4)-based vector with highly selective tropism in adult SGZ NSCs [96]. They demonstrated that rAAV4-mediated expression is specific and robust in NSCs, thus holding promise for gene therapy by selectively targeting NSCs for treating human patients with hippocampal dysfunction. Currently, a major gap in targeting adult hippocampal NSCs to treat neurological and mental disorders is the lack of molecular profiles of adult hippocampal NSCs associated with these diseased conditions. However, with recent success in single-cell RNA-sequencing of adult SGZ NSCs [14] and adult SVZ NSCs upon brain injuries [97], this gap will be filled in the near future. In particular, these studies will reveal molecularly distinct groups of NSCs that respond differentially to various physiological or pathological stimuli in the adult neurogenic niche. Therefore, the discovery of unique molecular targets from unbiased gene profiling at the single-cell level associated with pathological conditions will lead to novel therapeutics by selectively targeting endogenous NSCs (Figure 3).

The non-Cre dependent AAV4 system holds great promise in its application as a research tool for studying adult NSCs and as a potential gene therapy platform for treating some functional domains in brain disorders arising from aberrant hippocampal neurogenesis, such as diseases

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neurogenic niche, which could lead to healthy neurogenesis and cognitive improvement. (A) Under normal conditions, local niche cells maintain the proper neurogenic niche activity, thus providing a healthy niche environment for neurogenesis and hippocampal functions. (B, C) Under pathological conditions, neuronal connections could either be weakened due to potential neuronal death or degeneration of the nerve terminals (B1), or enhanced due to increased neural transmission or overgrowth of nerve terminals to their postsynaptic targets (C1), thus leading to impaired neurogenesis and hippocampal functions. To repair these deficits, we propose to target specific niche cells using designer receptors exclusively activated by designer drugs (DREADDs) in order to modulate their activity. Specifically, excitatory DREADDs (hM3Dq) or inhibitory DREADDs (hM4Di) can be delivered through viral gene therapy to the dentate gyrus (DG), so that the DREADDs-expressing neurons can be stimulated or inhibited through the administration of an inert ligand clozapine-N-oxide (CNO). Stimulating the hM3Dq-expressing neurons under pathological decreased neuronal connections (B2), or inhibiting the hM4Di-expressing neurons under pathological increased neuronal connections (C2), will lead to restored hippocampal neurogenesis and improved hippocampal functions.
with memory and mood deficits, including epilepsy, major depression, and AD. However, the concern for this system is the episomal dilution associated with neural stem/progenitor cell division [98] which could lead to underestimation of the production of neuronal progeny derived from NSCs in the context of stem cell biology and insufficient labeling of NSCs to achieve functional recovery in the context of gene therapy application. Future studies by incorporating the CRISPR/Cas9 gene editing system into the non-Cre dependent AAV4 delivery system will circumvent this issue and achieve permanent genome editing.

While the prospect of using adult NSCs therapeutically as a regenerative source for neural repair is very exciting, a major issue that must be addressed is the functional consequences of new neuron generation, especially under pathological conditions. Vigorous examination of neuronal integration and the impact of new neurons on the surrounding neuronal circuitry will be imperative for this line of research to be clinically relevant.

Targeting the Adult Hippocampal NSC Niche in Treating Brain Disorders

Activity-Dependent Regulation of NSCs by Local Niche Cells

A growing body of data in many tissue systems indicates that stem cell function is critically influenced by the microenvironment in which stem cells reside. Therefore, the stem cell niche represents a critical entry point for therapeutic modulation of stem cell behavior. In addition to classic niche factors described for other somatic stem cell compartments, such as morphogens and growth factors [17], dynamic regulation by ongoing network activity is a hallmark of adult neurogenesis [1,2,90,99]. NSCs and their progeny reside in a specialized local environment that consists of a diverse group of local cells with distinct molecular, morphological, and functional properties [100], and signaling from these local cells can potentially control the NSC niche activity and key behaviors of NSCs. Despite lacking synapses, NSCs ‘listen to’ the neural network and take proper actions in response to ongoing network activity [90]. For instance, recent studies identified local parvalbumin-expressing (PV) interneurons and mossy cells as critical niche cells in regulating DG network activity and the key behaviors of NSCs in vivo [15,16,101]. While DG PV interneurons and mossy cells release GABA and glutamate as their main neurotransmitters, respectively, many local interneurons co-release neuropeptides. Neuropeptides are neuromodulators and therefore exert broad actions on multiple types of local niche cells. This raises an important question on how endogenous neuropeptides control NSC niche activity and key behaviors of NSCs at the neural circuit level.

Targeting Local Niche Cells for Treating Brain Disorders

Local interneurons and mossy cells in the hippocampal circuit have been shown to correlate with many physiological and pathological conditions, such as aging, AD, chronic stress, schizophrenia, and epilepsy [102,103]. This suggests that these local niche cells may be vulnerable to certain pathological conditions, which in turn may contribute to aberrant neurogenesis and impaired cognitive functions. For instance, local GABA interneuron dysfunction and impaired hippocampal neurogenesis have been observed in AD [104,105] and several forms of neuropsychiatric disorders [9,102]. In addition, mossy cell dysfunction and impaired hippocampal neurogenesis have been reported in mouse models of temporal lobe epilepsy [58,70,106]. These findings suggest that targeting local niche cells may provide a strategy to restore hippocampal neurogenesis by creating a permissive niche environment for NSCs to generate healthy neurons. To elaborate this view, here we illustrate a strategy for treating brain disorders by targeting the local niche cells in order to create an improved neurogenic niche, which could lead to healthy neurogenesis and cognitive improvement (Figure 4). Under pathological conditions, neuronal connections could either be weakened due to potential neuronal death or degeneration of the nerve terminals, or enhanced due to increased neural...
transmission or overgrowth of nerve terminals to their postsynaptic targets. Therefore, manipulating the activity of specific niche cell types under diseased conditions can provide an efficient strategy to restore neurogenic niche activity and promote healthy neurogenesis. Recent success in rAAV-mediated targeting of local interneurons through the Dlx enhancer in non-genetically tractable species [107] and the advent of the chemogenetic approach using designer receptors exclusively activated by designer drugs (DREADDs) to manipulate the activity of specific neuronal cell types and circuits provide such a possibility [108], though the safety of expressing viruses in human neurons remains to be tested for an extended period of time (>10 years). Nevertheless, DREADDs can be delivered through viral gene therapy to the hippocampus, and the DREADDs-expressing neurons can be stimulated or inhibited through the administration of an inert ligand clozapine-N-oxide (CNO). Despite the undisputed success of CNO as an activator of muscarinic DREADDs, it has been known for some time that CNO is subject to a low rate of metabolic conversion to clozapine, which exhibits high DREADD affinity and potency in vivo [108]. To address this concern, a new DREADD agonist C21 has been developed [110]. C21 has been characterized to be a potent and selective agonist at both excitatory and inhibitory DREADDs and has excellent bioavailability, pharmacokinetic properties, and brain penetrability, therefore, has great translational potential for human application.

Concluding Remarks

Adult NSCs and neurogenesis confer a unique mode of plasticity in the mature mammalian brain. One overarching goal of adult neurogenesis research is to manipulate NSCs to improve brain health. Although we have gained tremendous understanding of adult NSCs and their niche in the past decade, many questions remain (see Outstanding Questions). To take advantage of the regenerative capacity of adult NSCs, we need to continue our efforts in investigating and manipulating NSC regulatory mechanisms to enhance functional regeneration and repair of the adult brain, particularly, in the context of pathological conditions. Future research will benefit from identification of NSC subpopulation-specific markers via single-cell sequencing analysis to dissect properties and unique responses from subpopulations of NSCs that are responsive to certain brain pathology or injury. Moreover, the endogenous recovery capacity in the adult brain is rather limited under pathological conditions or following injury, likely due to the lack of a permissive niche environment for neuroregeneration. Future research will need to focus on interactions between signaling pathways mediated by distinct neural circuits to identify circuit and signaling hubs and the hierarchy that coordinates the barrage of incoming signals. In addition, although human and rodents share the same neurogenic region (i.e., the hippocampus), there are many differences between human and rodent neurogenesis. Future development of noninvasive tools that can selectively target and track human NSCs would bring us one step closer to the application of adult NSCs to treat human diseases.

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