Neural stem and progenitor cells in health and disease

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Neural stem/progenitor cells (NSPCs) have the potential to differentiate into neurons, astrocytes, and/or oligodendrocytes. Because these cells can be expanded in culture, they represent a vast source of neural cells. With the recent discovery that patient fibroblasts can be reprogrammed directly into induced NSPCs, the regulation of NSPC fate and function, in the context of cell-based disease models and patient-specific cell-replacement therapies, warrants review.

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INTRODUCTION

Neural stem/progenitor cells (NSPCs) exist at various locations and times throughout embryonic and adult development. For the sake of this review, we will define NSPCs to be any self-renewing neural cells capable of differentiation to neurons, astrocytes, and/or oligodendrocytes. During embryogenesis, NSPCs are responsible for the development of the growing brain; in adults, NSPCs play a role in learning and memory but do not typically contribute to regenerative repair. Although various subtypes of NSPCs can be described by their expression of unique markers, the extracellular signals and intracellular factors responsible for the regulation of NSPC fate and differentiation frequently overlap. Aberrations in NSPC regulation can lead to diseases ranging from psychiatric disorders to neurodegenerative disease to cancer. With the discovery that induced NSPCs (iNSPCs) can be generated from somatic cells of healthy and diseased individuals, the regulation of NSPC fate and function is increasingly important; iNSPCs have the potential to serve as a novel platform for cell-based replacement therapies and drug-based high-throughput screening for new therapeutics.

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SPATIAL AND TEMPORAL CUES AFFECT NPSC IDENTITY

NSPCs are responsible for both embryonic growth and adult neurogenesis. During embryonic development, NSPCs can be found in the neural crest (NC) and cortex. Although the adult brain was once thought to be post-mitotic, neurogenesis occurs in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus in the adult brain.1,2 Although we will focus on markers that distinguish NSPC populations (Table 1), a number of genes broadly identify NSPCs, particularly SRY (sex-determining region)-box 2 (Sox2)3,4 and Nestin,5,6 and Pax6, which is expressed in anterior NSPC populations,7,8 including some, but not most, NC NSPCs.9,10

Embryonic Neural Crest Cells

The NC is a multipotent migratory cell population that transiently exists during embryonic development. Unlike the other NSPCs discussed in this review, NC cells are unique in that they contribute to the peripheral nervous system (PNS). NC cells originate between the dorsal ectoderm and neural tube but migrate and differentiate to sensory neurons, Schwann cells, melanocytes, and cells that make up the craniofacial structures such as bone and cartilage.11 WNT, bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs) are responsible for NC induction, regulating expression of key NC genes including...
**TABLE 1** Partial List of NSPC Markers Helpful in Distinguishing NSPC Subpopulations

| NSPC Subpopulation | Marker | References |
|--------------------|--------|------------|
| Embryonic NC       | MSX1/2, PAX3/7, ZIC1, SNAI1, FOXD3, SOX9/10, ID3, p75, AP2 | Hong et al.13; Light et al.14; Bellmeyer et al.15; Mori et al.16; Stemple et al.17; de Croze et al.18 |
| Embryonic cortical | DLX1/2, NXX2.1, TBR2 | Basak et al.25; Mizutani et al.24; Englund et al.26 |
| Adult SVZ          | Type B: GFAP; Type A: DCX, PSA-NCAM, DLX2; Type C: MASH1 | Doetsch et al.32; (reviewed by Ming et al.1 and Zhao et al.2) |
| Adult SGZ          | RGC (type 1): GFAP, BLBP | Garcia et al.39; Suh et al.40; (reviewed by Ming et al.1 and Zhao et al.2) |

MASH homeobox 1 and 2 (MSX1/2), paired box 3 and 7 (PAX3/7), and zinc finger protein 1 (ZIC1).12 Together, PAX3 and ZIC1 act in a WNT dependent manner to increase snail (SNAI1), forkhead box D3 (FOXD3), SOX9, and SOX10 proteins.13 Inhibitor of DNA binding (ID) proteins are dominant negative antagonists of the basic helix-loop-helix transcription factors; of these, ID3, a downstream target of c-MYC, is required for the formation and maintenance of NC cells.14,15 A few other well-established NC markers include p75 nerve growth factor receptor, which can be used to purify NC cells,16,17 and AP2, a key regulator of NC specification and maintenance.18 Embryonic stem cell (ESC)-derived NC cells have been reported by several independent groups.19–21 A single-step, highly efficient method generates NCs from ESCs by combining small molecule SMAD inhibition and WNT activation.22

**Embryonic Cortical NSPCs**

Cortical development begins in the anterior neural tube and is specified by homeobox proteins such as DLX1, DLX2, and NKKX2.1.23 Two types of NSPCs contribute to embryonic cortical development in vivo: radial glia cells (RGCs) and basal (intermediate) progenitors (BPs).24 RGCs produce neurons and glia, and divide at the ventricular surface. BPs are derived from RGCs, produce only neurons, and divide away from the ventricular surface. In addition to SOX2 and NEStIN, RGCs express astroglial markers including glial fibrillary acidic protein (GFAP), glutamate aspartate transporter (GLAST), and brain lipid-binding protein (BLBP); BPs lack expression of transcription factors that maintain NSPC self-renewal, such as SOX2 and PAX6. RGCs are maintained by NOTCH signaling,24,25 and their transition from RGC to BP is associated with upregulation of Tbr2, a T-domain transcription factor, and downregulation of Pax6.26

ESCs are robustly differentiated into neuroectodermal precursors.26–28 An early report suggested that ESC-derived neural rosettes could be converted to a RGC-like population by treatment with mitogens such as FGF2 and endothelial growth factor (EGF).29 New methods for the differentiation of ESCs have become increasingly sophisticated, claiming to recapitulate cortical neogenesis in vitro and generating, via a NSPC intermediate, cortical neurons that can be transplanted in vivo to form fully mature cortical neurons.29–31 Following neural induction in the presence of two inhibitors of SMAD signaling, the addition of vitamin A efficiently induces a cortical progenitor population that can be expanded in the presence of FGF2 and differentiated into functional cortical neurons following an extended period of corticogenesis.30

**Adult SVZ Progenitors**

SVZ neurogenesis leads to the generation of new neurons, astrocytes, and oligodendrocytes in the olfactory bulb.32 The principal precursors in the SVZ are type B cells, a primarily quiescent RGC-like population. Type B cells produce type C cells, a type of transit-amplifying cell that divides rapidly to produce neuroblasts, also known as type A cells (B→C→A). Type A cells migrate along the rostral migratory stream to the olfactory bulb where they integrate with existing circuitry.33,34 Type B cells are characterized by expression of GFAP, VIMENTIN, and NEStIN; proliferating type C cells express Achaete-scute complex-like 1 (MASH1) and NEStIN; migrating and differentiating type A neuroblasts express doublecortin (DCX), PSA-NCAM and homeobox protein DLX2 (reviewed in Refs 1 and 2). Similar to embryonic cortical RGCs, adult SVZ type B cells are maintained by NOTCH signaling.35 Just as in embryonic cortical development, the fate of adult SVZ progenitors in vivo is determined by positional information; populations of adult SVZ progenitors appear to be restricted and diverse in vivo36, but much more plastic when cultured in vitro.37

To our knowledge, SVZ progenitors have not yet been generated from ESCs. Primary SVZs,
when cultured, form neurospheres \textit{in vitro} and are propagated with FGF2 and EGF\textsuperscript{28,38} (Table 1).

**Hippocampal SGZ NSPCs**

As in the adult SVZ, the hippocampal SGZ is maintained by a population of quiescent RGC-like cells (reviewed in Refs 1 and 2). Often referred to as type 1 cells, these progenitors have long radial processes, express GFAP, BLBP, NESTIN, and SOX2 and are generally considered to be the primary progenitors of SGZ neurogenesis.\textsuperscript{39,40} Once activated, these cells upregulate TBR2 and DNA replication licensing factor MCM2, and become a replicative cell population, sometimes referred to as type 2 progenitors. These intermediate NSPCs express DCX and BLBP-NCAM, but not GFAP, have only short processes, and in turn give rise to neuroblasts. Type 2 cells may arise from type 1 cells through a SOX2-dependent reciprocal relationship between the two cell types.\textsuperscript{40} The multipotency of SGZ NSPCs remains unclear as under certain conditions, hippocampal SGZ NSPCs appear to display significant plasticity in their lineage choice, both \textit{in vivo} and \textit{in vitro}.\textsuperscript{41,42}

Primary SGZ progenitors form neurospheres and monolayers \textit{in vitro}, and like SVZ cells, are propagated with FGF2 and EGF.\textsuperscript{28}

**GENETIC REGULATION OF NSPCs**

Although embryonic and adult NSPCs have different characteristics, likely due to differences in the expression of key proteins described above, it should be noted that NSPCs retain significant plasticity and can robustly alter lineage choice as a consequence of altered environmental signals.\textsuperscript{41,42} While the mechanism of plasticity remains unknown, it is well established that external signaling cues regulate many aspects of the replication, differentiation, migration, maturation, and death of NSPCs. Despite their differences, many regulator pathways are shared between populations of NSPCs; for example, FGF can be used as a multifunctional growth factor to expand many types of NSPCs \textit{in vitro} and \textit{in vivo}.\textsuperscript{43–45} The differences between populations of NSPCs may not be inherently intrinsic but instead reflective of their different niche environments.

A number of cell types, including endothelial cells, ependymal cells, astrocytes, microglia, and NSPCs themselves, contribute to the neurogenic niche and help to regulate all aspects of neurogenesis. Endothelial cells secrete vascular endothelial growth factor (VEGF), which promotes the replication of both embryonic and adult NSPCs.\textsuperscript{46,47} Conversely, spatial restriction of other growth factors can confer lineage restriction to specific populations of NSPCs. For example, unique to the SVZ, the ependymal cell layer of the lateral ventricles secretes NOGGIN, a protein that antagonizes BMP-mediated astrocyte differentiation.\textsuperscript{48–51} Astrocytes, both a product of NSPC differentiation and a component of the neurogenic niche, have been shown to regulate proliferation, fate specification, migration, maturation, and synapse formation during neurogenesis, at least in part through modulating the effects of factors secreted from blood vessels and ependymal cells.\textsuperscript{52,53} A multitude of signaling pathways regulate these diverse functions. Astrocyte-derived WNT signaling influences NSPC replication and differentiation.\textsuperscript{53,54} ROBO receptors regulate the rapid migration of SLIT1-expressing neuroblasts.\textsuperscript{55} Astrocyte-derived cholesterol supports synaptogenesis,\textsuperscript{56} while astrocyte-secreted extracellular matrix proteins, such as thrombospondins and Sparc, modulate synapse formation.\textsuperscript{57,58} Astrocyte-released glutamate stimulates NMDR and regulates the activity-dependent survival of newborn neurons during adult neurogenesis.\textsuperscript{59} Under basal conditions, microglia are responsible for phagocytosis of dead neurons,\textsuperscript{60} while under inflammatory conditions, reactivated microglia secrete both pro- and anti-inflammatory molecules.\textsuperscript{61} Even cell–cell interactions between NSPCs, through EGFR and Notch signaling, help to maintain the balance between NSCs and NPCs.\textsuperscript{24,25,62}

Although external factors regulate NSPC function, an intracellular network ultimately directs NSPC self-renewal and differentiation. Several common transcription factors are required to maintain many NSPC populations in an undifferentiated state; notable among these are SOX2, PAX6, and TLX. These transcription factors are key targets of cell cycle regulators, microRNAs, and epigenetic factors that are major intracellular regulators of neurogenesis; many of these regulators act either cooperatively or in opposition.

Loss of cell cycle inhibitors, including p16, p21, and p53, results in the activation and subsequent depletion of NSPCs.\textsuperscript{63–66} For example, p21 directly binds and represses the enhancer of SOX2.\textsuperscript{63} SOX1/2/3 have overlapping and redundant activity;\textsuperscript{67} the overexpression of any promotes NSPC proliferation, whereas their loss induces cell cycle exit and onset of differentiation.\textsuperscript{3,68} TLX also maintains NSPCs in the undifferentiated state\textsuperscript{69} by recruiting histone deacetylase (HDAC) to repress the transcription of several cell cycle genes.\textsuperscript{70} MicroRNAs are short (\textasciitilde22 nucleotides) non-coding RNAs involved in gene silencing through translational repression and/or
TABLE 2 | Partial List of Extrinsic and Intracellular Factors Regulating NSPCs

| Factors | Proliferation | Differentiation | Survival | References |
|---------|---------------|-----------------|----------|------------|
| **Extrinsic factors** | | | | |
| **Growth factors and neurotrophins** | | | | |
| BDNF | + (SVZ) | + (SGZ) | | Henry et al.83; Scharfman et al.84 |
| EGF | + | | | Doetsch et al.37; Aguirre et al.62 |
| FGF | + | | | Westermann et al.43; Jin et al.44; Zhao et al.45 |
| PDGF | + | + (astrocyte) | | Jackson et al.85 |
| VEGF | + | + (neuron) | | Leventhal et al.46; Cao et al.47 |
| **Morphogens** | | | | |
| BMP | + (astrocyte) | | | Nakashima et al.48; Yanagisawa et al.49 |
| Noggin | + (neuron) | | | Lim et al.50 |
| Notch | + | − (neuron) | − (glia) | Mizutani et al.24; Basak et al.25; Aguirre et al.62 |
| SHH | + | | | Ahn and Joyner86; Banerjee et al.87; Machold et al.88; Palma et al.51 |
| WNT3 | + | | | Lie et al.54; Song et al.53 |
| **Intracellular pathways** | | | | |
| **Transcription factors** | | | | |
| SOX2 | + | | | Graham et al.3; Thomas et al.4 |
| TLX | + | | | Shi et al.60; Sun et al.70 |
| PAX6 | + | (neuron) | | Ericson et al.8; Zhang et al.7 |
| Olig2 | + | (oligodendrocytes) | | Zhou et al.89 |
| **Epigenetic regulators** | | | | |
| MBD1 | + (neuron) | | | Zhao et al.81; Li et al.82 |
| Histone deacetylase | + (with TLX) | | | Hsieh et al.90; Sun et al.70 |
| **Cell cycle regulation** | | | | |
| p16INK4a | − | | | Molofsky et al.64 |
| p21 | − | | | Kippin et al.66; Marques-Torrejon et al.63 |
| p53 | − | | | Gil-Perotin et al.65 |
| **microRNAs** | | | | |
| miR9 | − | + (neuron) | | Krichevsky et al.71; Yoo et al.76 |
| miR124 | − | | | Yoo et al.76 |
| miR137 | − | + | | Sun et al.77; Smrt et al.78; Szulwach et al.79 |
| miR184 | − | − (neuron) | | Liu et al.80 |

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mRNA destabilization. miR-9 was one of the first microRNAs shown to regulate neurogenesis; it functions to decrease NSPC proliferation and increase neuronal differentiation. Known targets of miR-9 include TLX, FOXG1, and HES1, as well as key components of the FGF signaling pathway. Mitotic exit of NSPCs is accompanied by a subunit switch in the chromatin-remodeling SWI/SNF complex mediated by miR-9 and miR-124. Similarly, miR-137 has decreases proliferation and promotes differentiation of NSPCs, via targets found primarily in the epigenetic machinery. Conversely, the epigenetic machinery
also regulates microRNA expression. Methyl-CpG binding protein 1 (MBD1) directly represses miR-184; miR-184 promotes NSPC proliferation and inhibits neuronal differentiation. DNA methylation at CpG dinucleotides is bound by a family of methyl-CpG binding proteins (MBDs), including MBD1, MBD2, MBD3, MBD4, and MeCP2, leading to the recruitment of HDAC repressor complexes and inactive chromatin structures. MBD1—/− NSPCs exhibit reduced neuronal differentiation and increased genomic instability, which may be mediated, at least in part, due to the ability of MBD1 to repress FGF2 expression.

Thus, we come full circle. Cell cycle regulators repress transcription factors. Transcription factors and microRNAs regulate the epigenetic machinery. Epigenetic modifiers regulate key growth factors essential in the in vivo and in vitro NSPC niche (Table 2).

SYSTEMS BIOLOGY APPROACHES TO UNDERSTANDING NSPC REGULATION

Systems biology applies both experimental and computational approaches to explain how the numerous components of a cellular network interact to regulate molecular and cellular fate. Two approaches are generally employed. First, computational models simulate the intracellular interactions of key regulators, such as ligand–receptor dynamics, signal transduction pathways or transcription factor networks. Second, statistical analyses reduce large gene expression or protein datasets into principal components critical for regulating cell fate choice. While these analyses have helped to explain ESC fate regulation, their application to NSPCs to date has been limited.

Deterministic computational models, which always yield the same result given the same set of initial conditions, have been used to mathematically model signaling pathways downstream of key growth factors and cytokines in NSPCs. Such work has already led to insights into both the threshold levels of FGF2 required for NSPC maintenance as well as the neurotrophin-3 (NT-3) stimulation and downstream MAPK pathway activity required for neuronal differentiation. Because computational models require precise knowledge of the rate and binding constants of molecular interactions within the network, the lack of experimentally measured constants in NSPCs has somewhat restricted the application of these methods at this point in time.

One common outcome of deterministic models is network bistability, a situation where the continuous change in one input results in a transition between two steady-state solutions, converting a graded input signal into an ‘all or nothing’ biological response. For example, fluctuations in NOTCH signaling cause oscillations in Hes1 expression. In NSPCs, this results in a bistable switch regulating fate decisions between NSPC proliferation and differentiation; inhibition of NOTCH signaling leads to downregulation of Hes1, ultimately upregulating proneural genes such as Neurogenin2 (Ngn2) and the Notch ligand δ-like1 (Dll1), and increasing neuronal differentiation. A subsequent study modeled the signaling cross talk between the NOTCH, SHH, WNT, and EGF signaling pathways in the regulation of Hes1.

The incorporation of stochastic statistical models, which include the effects of noise in intracellular signaling pathways, will greatly improve computational simulations. Similar to ESCs, NSPCs are heterogeneous, with cells moving between two or more metastable states, each defined by specific patterns of transcription factor expression, chromatin modifications and biases in their differentiation potential.

For example, ESCs with high Nanog expression are less likely to differentiate than low Nanog expressing cells, an observation that might be explained by state transitions resulting from stochastic gene expression. Given that variable levels of SOX2, GFAP and HES1 can define distinct NSPC subpopulations in the SVG and SVZ, the application of stochastic models to NSPC behavior will be an important aspect of future computational models of NSPC regulation.

The increasing availability of gene expression and proteomic data sets from proliferating and differentiating NSPCs in vitro and in vivo should facilitate statistical analyses to elucidate regulatory networks. Gene expression changes during in vitro neural differentiation of ESCs were used to identify a principal component of approximately 4000 genes that described degree of neural commitment. Subsequently, Bayesian network analysis of ESC neural differentiation found that GFAP upregulates genes in a neural gene set created through principal component analysis. Additionally, the gene expression profiles of NSPCs derived from human ESCs, human fetal NSPCs, oligodendrocyte precursor cells and astrocyte precursor cells were compared in order to identify common and unique characteristics of each examined NSPC population.

Although ESC NSPC samples were generated through different methods in multiple labs, Shin et al. identified a distinct ESC NSPC gene expression profile and concluded that ESC NSPCs had limited overall
similarity to fetal NSPCs. They further speculated that the high expression of WNT molecules in ESC NSPCs may partially explain their broader differentiation potential relative to fetal NSPCs.105

Large data sets can also be used to ask specific questions concerning individual signaling pathways or biological processes. For example, Wang et al. superimposed those genes differentially expressed before and after NSPC differentiation106 with the protein–protein interaction network, in order to identify a signaling network regulated by rho-GDI-γ (guanine nucleotide dissociation inhibitor) during NSPC differentiation.107 Finally, Fietz et al. identified genes differentially expressed between cortical zones by using mRNA sequencing of fetal human and embryonic mouse tissue from various cortical zones. Because the expression pattern also correlated to the relative abundance of RGCs, these genes, the majority of which involved cell adhesion and the cell-extracellular matrix, were predicted to promote the proliferation and self-renewal of NSPCs in the developing neocortex.108

Advances in high throughput experimental techniques are rapidly creating large ‘omic’ datasets. It is our hope that these will be fruitfully mined by systems biology approaches in order to improve our understanding of the complicated intracellular mechanisms regulating NSPC fate.

DISCOVERY OF INDUCED NSPCs

The induction of iNSPCs from somatic cells provides a near limitless source of neural cells for cell-replacement therapies in vivo and cell-based in vitro models of neurological disease. iNSPC technology provides a fast and robust protocol to obtain proliferative neural precursors and generates more homogeneous populations than current induced Neuron (iNeuron) methods,109–112 while bypassing time-consuming induced pluripotent stem cell (iPSC) generation.113,114 This year, five groups reported the generation of iNSPCs from fibroblasts115–118 and a sixth reported iNSPC generation from urine.119 Approaches for the iNSPC reprogramming generally follow one of two strategies: (1) incomplete iPSC reprogramming combined with neural growth conditions and (2) overexpression of neural transcription factors.

Incomplete sets of the original iPSC reprogramming cocktail (OCT4, SOX2, KLF4, and c-MYC) can reprogram iNSPCs from fibroblasts. Both constitutive expression of SOX2, KLF4, and c-MYC, when paired with transient OCT4 activity,115 as well as overexpression of OCT4, SOX2, and KLF4 in the presence of another pluripotency gene, ZIC3,116 are sufficient to generate iNSPCs. For reasons not yet understood, the former method generated CNS iNSPCs, while the latter generated PNS iNSPCs. iNSPCs were also generated from exfoliated renal epithelial cells present in urine by transfection with episomal vectors carrying the reprogramming factors OCT4, SOX2, SV40LT, and KLF4, as well as the microRNA cluster MIR302–367 and a cocktail of small molecules.119 This last report is generated integration-free iNSPCs, demonstrating that iNSPC multipotency can be maintained without persistent transgene expression.

Combinations of neural transcription factors can also be used to reprogram iNSPCs from fibroblasts. Overexpression of SOX2, BRN2, NR2E1, BMI1, HES1, HESS, and c-MYC produced iNSPCs,120 as did a smaller combination of just three factors, SOX2, BRN2, and FOXG1.117 Most recently, a third group used just SOX2 overexpression to generate tripotent iNSPCs,118 though this method is markedly less efficient, requiring several rounds of selection by neurosphere suspensions. Generating iNSPCs with just SOX2 lends itself to the possibility of patterning iNSPCs to specific identities by inducing with SOX2 in conjunction with other subtype-specific growth conditions or transcription factors.

To date, the combinations of factors responsible for iNSPC generation represent some of the most critical genes in the maintenance of NSPC populations in vivo. This is unlikely to be a coincidence. New methods to permit patterning of specific regional identities of iNSPCs are critical. We can imagine that at least two possible approaches are feasible: cellular patterning and environmental signaling. In the first, iNSPC cellular identity is further specified by overexpression of cell-type specific NSPC transcription factors or microRNAs unique to the desired identity. For example, genes such as FOXG1 might help to specify embryonic cortical NSPCs. Alternately, expansion of iNSPCs in growth conditions supportive of a particular fate may provide reinforcing patterning cues. For example, ESC-derived NSPCs are typically cultured in vitro with FGF2 to maintain forebrain identity and with SHH/FGF8/CHIR99021 (a WNT agonist) to maintain midbrain identity.121 By better considering what distinguishes extracellular niche signals and endogenous transcriptional, epigenetic and microRNA modulators of cellular identity, protocols for iNSPC generation will be refined. While patient derived iNeurons enable functional characterization of mature neurons, they bypass neuronal development; iNSPCs permit studies of
aberrant neuronal specification, maturation and function that may contribute to disease.

**ROLE OF NSPCs IN DISEASE**

While many neurological disorders are traditionally thought to be diseases of mature neurons, new lines of evidence now suggest that aberrant NSPC function may contribute to psychiatric diseases, such as schizophrenia and autism spectrum disorders (ASDs), neurodegenerative diseases, such as Parkinson’s disease (PD) and Alzheimer’s disease (AD) and certain brain tumors (Table 3). Mutations in Disrupted-in-Schizophrenia (DISC1) result in an extremely rare monogenic form of schizophrenia. It has long been known that dominant negative DISC1 expression during mouse embryonic cortical development leads to cellular and behavioral phenotypes consistent with schizophrenia. Now, it has also been shown that silencing of DISC1 specifically in adult hippocampal NSPCs leads to accelerated dendritic growth, soma hypertrophy, and aberrant neural organization, mediated in part by depolarizing GABA signaling, in addition to aberrant behaviour. This was an important proof of concept that loss of DISC1, specifically in adult neurogenesis, can lead to behavioral phenotypes even more severe than those observed by complete loss of neurogenesis.

A deletion at 22q11 (DiGeorge syndrome) significantly increases genetic risk for schizophrenia and ASD. In a mouse model of this disease, significantly reduced expression of six cell cycle-related genes located in the 22q11 region was observed during embryonic development, in conjunction with reduced NSPC proliferation, aberrant migration, and altered connectivity. Notably, of the two populations of embryonic cortical NSPCs discussed earlier in the review (multipotent RGCs and...
transit-amplifying BPs), only BPs were observed to be affected in this 22q11 mouse model.

At onset, ASD is often characterized by excessive brain volume. MRI studies have found increased cortical white matter in 2- to 4-year-old autistic children, and it has been hypothesized that the surplus of neurons in the prefrontal cortex in ASD may be due to excessive proliferation of NSPCs. Among other roles, myocyte enhancer factor 2 (MEF2) regulates the NSPC differentiation and is a key regulator of signaling pathways that play a role in the pathogenesis of ASD. When MEF2 was removed from NSPCs using a Nestin-Cre-floxed-MEF2C, the conditional knockout mice had normal NSPC proliferation and survival, neurons were abnormally organized and showed immature electrophysiological properties. Additionally, these MEF2C null mice showed abnormal anxiety and decreased cognitive function, recapitulating those observed in the MECP2 mouse model of Rett syndrome. This demonstrates that loss of an autism risk gene in NSPCs may contribute to cellular and behavioral phenotypes consistent with this disorder.

Evidence for abnormal adult neurogenesis has been accumulating in both PD and AD. While α-synuclein (α-SYN) protein accumulation is associated with the death of dopaminergic neurons in PD, in the SGZ NSPCs, it leads to down-regulation of NOTCH1 signaling, ultimately leading to increased NSPC proliferation and impaired neuronal differentiation and maturation. This phenotype can be rescued by knockdown of the cell cycle gene p53, suggesting that p53 moderates the effects of α-SYN on repression of NOTCH1 and disruption of neurogenesis. Similarly, aberrant expression of a number of candidate AD genes in NSPCs has been reported to cause aberrant NSPC replication and differentiation in the adult hippocampus. Apolipoprotein E (APOE) deficiency leads to increased proliferation of SGZ NSPCs, and ultimately to the depletion of the NSPC pool. Knockdown of presenilin (PS1) promotes increased differentiation in the adult SGZ, reducing neurogenesis. Finally, over-expression of amyloid precursor protein (APP) causes reduced survival and proliferation of SGZ NSPCs. These findings imply that dysregulation of neurogenesis may play a role in neurodegenerative disease pathology.

Medulloblastoma (MB) is the most frequent form of malignant brain tumor in children, and in a subset of cases, the cell type of origin is thought to be NSPCs. When microRNA expression profiles of MB patient samples were analyzed, the target genes of the down-regulated microRNAs in MB tissue included those involved in NSPC migration, cell adhesion, and development, particularly reelin (RELN) and myelin transcription factor 1 (MYT1). The target genes of the up-regulated microRNAs, conversely, included many associated with metastatic disease. In addition to regulating healthy NSPCs, described earlier in this review, abnormal microRNA-regulated networks may be associated with transformation of normal NSPCs into brain tumor stem cells. This is consistent with the stem cell hypothesis of cancer, which supposes that cancer can result from the dysregulation of growth and survival pathways in normal stem and progenitor cells.

It seems likely that aberrant NSPC function may cause or exacerbate a number of neurological disorders. We hope that the study of NSPCs may lead to insights into the origins of these disorders and ultimately to therapies by which to correct these malfunctioning cells and ameliorate the disease phenotypes.

FUTURE CLINICAL USES OF NSPCs

While laboratory use of iNSPCs could directly lead to insights into the genetic and cellular predisposition of a number of human diseases, iNSPCs can also be used as a source of cells for either cell-based human therapies or drug-based high throughput screens for novel therapeutics.

Neurodegenerative diseases result from the loss of neurons, either locally (such as the dopaminergic neurons of the substantia niagra in PD) or globally (throughout the cortex in AD). Although fetal ventral mesencephalic transplants into PD patients have demonstrated that transplanted cells survive and integrate into existing circuits, these methods have yet to deliver clinical benefit. With their ability to expand in vitro and to differentiate into various neural lineages, iNSPCs serve as a promising source for genetically matched neural cells for cell-replacement therapies for many neurodegenerative diseases. In 2011, midbrain dopaminergic neurons were derived from human iPSCs and successfully engrafted into three animal models of PD. Long-term transplantation in the mice and rat models demonstrated robust survival of the dopaminergic neurons and improvements in movement-based phenotypes, though subsequent grafts into monkey models demonstrated the scalability of the process but failed to show phenotypic improvements (Figure 1). A number of technical concerns need to be addressed, particularly the relatively inefficient integration after transplantation. Nonetheless, such progress suggests that iNSPCs are a viable cell source for regenerative medicine.
Successful cell transplantation of human embryonic stem cell (ESC)-derived dopaminergic neurons into a monkey model of Parkinson’s disease (PD) (MPTP-lesioned rhesus monkeys). (a) Representative graft 1 month after transplantation, showing expression of the dopaminergic neuron marker tyrosine hydroxylase (TH), with surrounding TH$^+$ fibers (arrows). (b) Co-expression of human specific cytoplasm marker SC-121 (red) and TH (green) in graft. (Reprinted with permission from Ref 121 Copyright 2011 Nature).

In addition to cell-replacement therapies, NSPCs hold great potential as a platform for high-throughput screening to identify novel drug-based therapies. As a validation for future high-throughput screens, Marchetto et al. demonstrated the ability of two compounds, IGF1 and genamicin, to ameliorate the neuronal phenotype of Rett syndrome human iPSC-derived neurons in culture. Furthermore, we demonstrated the ability of a clinically utilized antipsychotic, loxapine, to improve the neural connectivity of schizophrenia iPSC-derived neurons in culture and Israel and colleagues demonstrated that treatment of AD iPSC neurons with $\beta$-secretase inhibitors led to significant reductions in phospho-Tau levels. A major technical issue is the scalability of both neural differentiation and phenotypic screening to permit testing of thousands of compounds. While we are unaware of any high throughput screening using iNSPCs for neurological diseases to date, a human ESC-based phenotypic assay successfully screened for small molecules that inhibit neuronal degeneration induced by activated microglial cells. Their phenotypic screen of >10,000 compounds identified approximately 0.3% hits across a number of biological pathways, providing an excellent proof-of-principle that such screens are both feasible and can yield meaningful hits (Figure 2).

We predict that iNSPC-based high-throughput screens will soon be routinely performed to identify novel therapeutics across a range of neurological diseases. Furthermore, by screening complex genetic diseases using iNSPCs derived from an increasing number of individuals characterized by heterogeneous clinical outcomes and drug-responsiveness, iNSPC-based high-throughput screens will identify drugs suitable for individuals with known treatment resistance and should ultimately realize the potential system for personalized medicine.

SUMMARY

Until recently, it was thought that adult humans could not generate new neurons. Now, it is widely accepted that neurogenesis continues throughout adult life, although the role for this process in health and disease is still being unraveled. Evidence continues to accumulate that aberrant replication, differentiation, or migration of NSPCs can lead to a variety of neurological conditions, and we predict that NSPCs may one day be a therapeutic target in the treatment of psychiatric and neurodegenerative disorders.

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