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Neural stem and progenitor cells in health and disease

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Abstract

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.

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. Though the 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.

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 the cortex. Although the adult brain was 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]. Though 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], as well as *Pax6*,

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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 NPSCs 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 MSH 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 NKX2.1 [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 that maintain NSPC self-renewal. 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 EGF [29]. New methods for the differentiation of ESCs have become increasingly sophisticated, claiming to recapitulate cortical neogenesis *in vitro* and form, via a NSPC intermediate, cortical neurons that can be transplanted *in vivo* to generate 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.

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 by [1, 2]). Similar to RGCs, adult SVZ type B cells are maintained by NOTCH signaling [35]. Just an 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 vivo [36], 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 *in vitro* and are propagated with FGF2 and EGF [28, 38].

Hippocampal SGZ NSPCs

As in the adult SVZ, the hippocampal SGZ is maintained by a population of quiescent RGC-like cells (reviewed by [1, 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 [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 PSA-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 [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 *in vivo* and *in vitro* [41, 42].

Primary SGZ progenitors form neurospheres and monolayers *in vitro*, and like SVZ cells, are propagated with FGF2 and EGF [28].

Genetic regulation of NSPCs

Though 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 [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 *in vitro* and *in vivo* [43–45]. The differences b

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 [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 [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 [52, 53]. A multitude of signaling pathways regulate these diverse functions. Astrocyte derived WNT signaling influences NSPC replication and differentiation [53, 54]. ROBO receptors regulate the rapid migration of SLIT1-expressing neuroblasts [55]. Astrocyte-derived cholesterol supports synaptogenesis [56], while astrocyte-secreted extracellular matrix proteins, such as Thrombospondins and Sparc, modulate synapse formation [57, 58]. Astrocyte released glutamate stimulates NMDRs and regulates the activity-dependent survival of newborn neurons during adult neurogenesis [59]. Under basal conditions, microglia are responsible for phagocytosis of dead neurons [60], while under inflammatory conditions, reactivated microglia secrete both pro- and anti-inflammatory molecules [61]. Even cell-cell interactions between NSPCs, through EGFR and Notch signaling, help to maintain the balance between NSCs and NPCs [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 [63–66]. For example, p21 directly binds and represses the enhancer of SOX2 [63]. SOX1/2/3 have overlapping and redundant activity [67]; the overexpression of any promotes NSPC proliferation, whereas their loss induces cell-cycle exit and onset of differentiation [3, 68]. TLX also maintains NSPCs in the undifferentiated state [69] by recruiting histone deacetylase (HDAC) to repress the transcription of several cell cycle genes [70]. MicroRNAs are short (~22 nucleotides) non-coding RNAs involved in gene silencing through translational repression and/or mRNA destabilization. miR-9 was one of the first microRNAs shown to regulate neurogenesis [71]; it functions to decrease NSPC proliferation and increase neuronal differentiation. Known targets of miR-9 include TLX [72], FOXG1 [73], and HES1 [74], as well as key components of the FGF signaling pathway [75]. Mitotic exit of NSPCs is accompanied by a subunit switch in the chromatin-remodeling SWI/SNF complex mediated by miR-9 and miR-124 [76]. Similarly, miR-137 decreases proliferation and promotes differentiation of NSPCs, via targets found primarily in the

epigenetic machinery [77–79]. 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 [80]. DNA methylation at CpG dinucleotides are bound by a family of methyl-CpG binding proteins (MBDs), including MBD1, MBD2, MBD3, MBD4, and MeCP2, leading to the recruitment of histone deacetylase (HDAC) repressor complexes and inactive chromatin structures. MBD1−/− NSPCs exhibit reduced neuronal differentiation and increased genomic instability [81], which may be mediated, at least in part, due to the ability of MBD1 to repress FGF2 expression [82].

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 helped to explain ESC fate regulation [91, 92], 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[93], 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 [94] as well as the neurotrophin-3 (NT-3) stimulation and downstream MAPK pathway activity required for neuronal differentiation [95]. 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 [96–98]. 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 Delta-like1 (*Dll1*), and increasing neuronal differentiation [99]. A subsequent study modeled the signaling cross-talk between the NOTCH, SHH, WNT and EGF signaling pathways in the regulation of *Hes1* [100].

The incorporation of stochastic statistical models, which include the effects of noise in intracellular signaling pathways [93], 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 [101]. For example, ESCs with high *Nanog* expression are less likely to differentiate than low *Nanog* expressing cells [92], an observation that might be explained by state transitions resulting from stochastic gene expression [102]. 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 identifiy a principal component of approximately 4,000 genes that described degree of neural commitment [103]. Subsequently, Bayesian network analysis of ESC neural differentiation found that GFAP upregulates genes in a neural gene set created through principal component analysis [104]. 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 [105]. 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 differentiation [106] 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], all while bypassing time-consuming induced pluripotent stem cell (iPSC) generation [113, 114]. This year, five groups reported the generation of iNSPCs from fibroblasts [115–118] 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 generated integration-free iNSPCs, demonstrating that iNSPC multipotency can be maintained without persistent transgene expression.

Combinations of neural transcription factors also can be used to reprogram iNSPCs from fibroblasts. Overexpression of *SOX2, BRN2, NR2E1, BMI1, HES1, HES5* 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.

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 [122–124]. 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 [125], mediated in part by depolarizing GABA signaling [126]. 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 [127]. 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, [128–130] and it has been hypothesized that the surplus of neurons in the prefrontal cortex in ASD[131] 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 [132, 133]. When MEF2 was removed from NSPCs using a Nestin-Cre-floxed-MEF2C, though the conditional knockout mice had normal NSPC proliferation and survival, neurons were abnormally organized and showed immature electrophysiological properties [134]. 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 protein (α-SYN) 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 [135]. 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 [135]. 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 [136]. Knockdown of Presenilin (PS1) promotes increased differentiation in the adult SGZ, reducing neurogenesis [137]. Finally, over-expression of Amyloid precursor protein (APP) causes reduced survival and proliferation of SGZ NSPCs [138]. These findings imply that dysregulation of neurogenesis may play a role in neurodegenerative disease pathology [139].

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 [140]. 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*) [141]. 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). Though fetal ventral mesencephalic transplants into PD patients have demonstrated that transplanted cells survive and integrate into existing circuits [142, 143], 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 [121]. 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.

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 [144]. Furthermore, we demonstrated the ability of a clinically utilized antipsychotic, loxapine, to improve the neural connectivity of schizophrenia iPSC-derived neurons in culture [145] and Israel and colleagues demonstrated that treatment of AD iPSC neurons with β-secretase inhibitors led to significant reductions in phospho-Tau levels [146]. A major technical issue is the scalability of both neural differentiation and phenotypic screening to permit testing of thousands of compounds. Though 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 [147]. 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, though 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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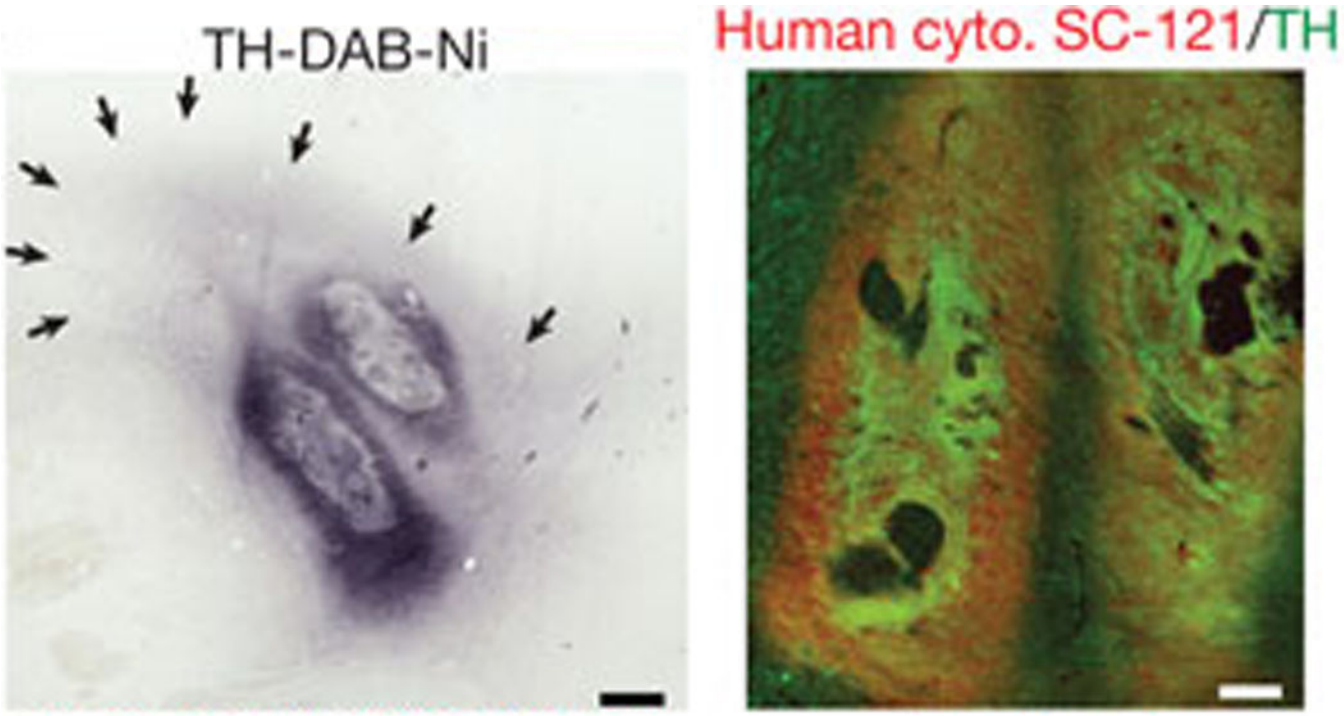

Figure 1.

Successful cell transplantation of human ESC-derived dopaminergic neurons into a monkey model of PD (MPTP lesioned rhesus monkeys). Left, representative graft 1 month after transplantation, showing expression of the dopaminergic neuron marker Tyrosine Hydroxylase (TH), with surrounding TH+ fibers (arrows). Right, co-expression of human specific cytoplasm marker SC-121 (red) and TH (green) in graft.

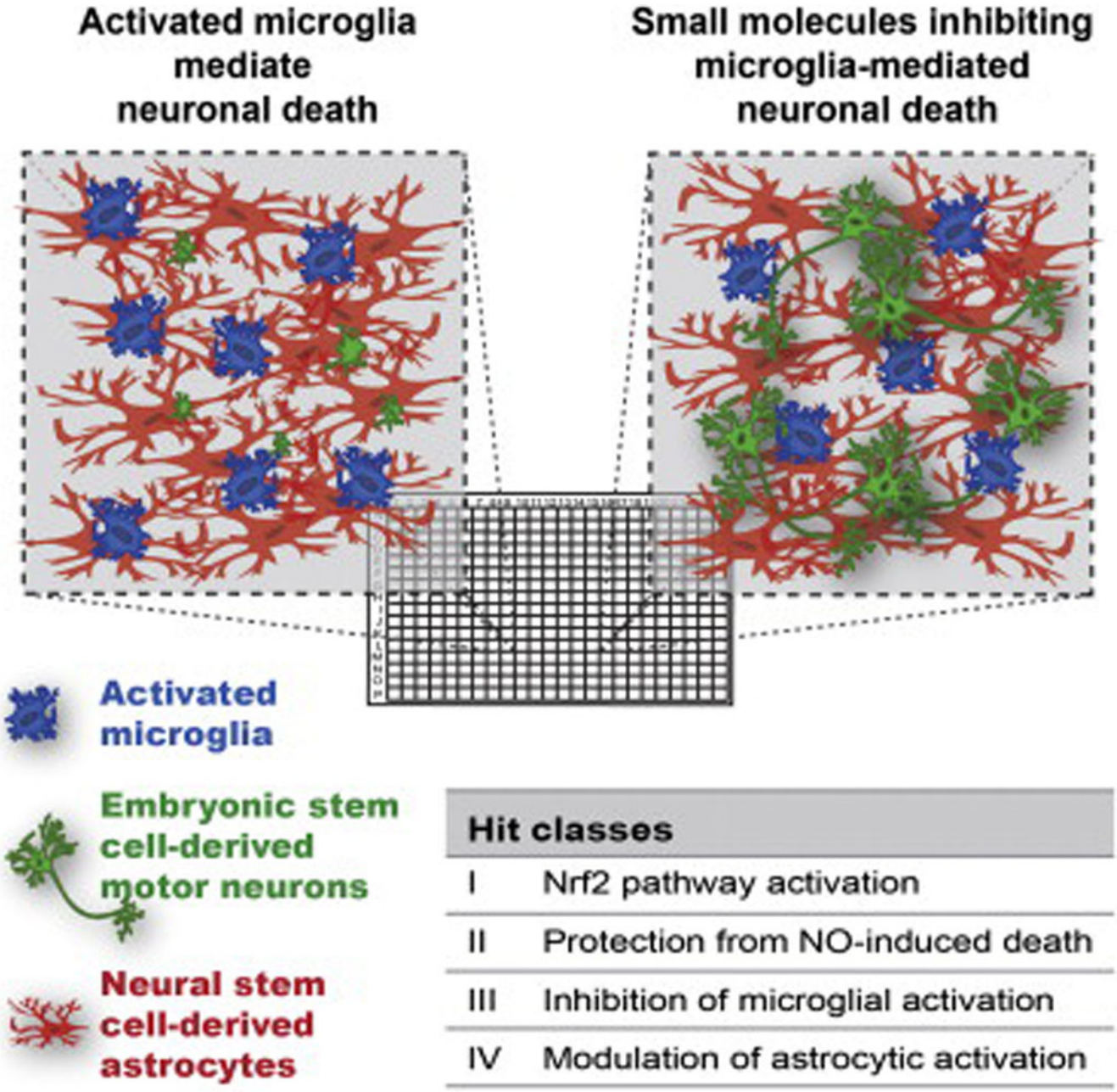

Figure 2.

Paradigm of a high-throughput screen for small-molecules capable of neural protection of ESC-derived motor neurons and astrocytes in the presence of activated microglia. After screening more than 10,000 small molecules, the 0.3% hit rate included compounds acting in the activation of the Nrf2 pathway in microglia and astrocytes, direct protection of neurons from nitric-oxide-induced degeneration and inhibition of nitric oxide production by microglia. (Adapted from Hoing *et al*, 2011)

Table 1

Partial list of NSPC markers helpful in distinguishing NSPC subpopulations

NSPC Population	Marker	References
Embryonic NC	MSX1/2, PAX3/7, ZIC1, SNAI1, FOXD3, SOX9/10, ID3, p75, AP2	Hong *et al*, 2007 [13]; Light *et al*, 2005 [14]; Bellmeyer *et al*, 2003 [15]; Mori *et al*, 1990 [16]; Stemple *et al*, 1992 [17]; de Croze *et al*, 2011 [18]
Embryonic Cortical	DLX1/2, NKX2.1, TBR2	Basak *et al*, 2007 [25]; Mizutani *et al*, 2007 [24]; Englund *et al*, 2005 [26]
Adult SVZ	Type B: GFAP Type A: DCX, PSA-NCAM, DLX2 Type C: MASH1	Doetsch *et al*, 1996 [32]; (Reviewed by Ming *et al*, 2011 [1] and Zhao *et al*, 2008 [2].)
Adult SGZ	RGC (type 1): GFAP, BLBP Progenitor/Neuroblast (type 2): TBR2, MCM2, DCX	Garcia *et al*, 2004 [39]; Suh *et al*, 2007 [40]; (Reviewed by Ming *et al*, 2011 [1] and Zhao *et al*, 2008 [2].)

Table 2

Partial list of extrinsic and intracellular factors regulating NSPCs. (Adapted from and reprinted with permission from Zhao *et al*, 2008.)

Factors	Proliferation	Differentiation	Survival	References
Extrinsic factors				
Growth factors & neurotrophins				
BDNF	+ (SVZ)		+(SGZ)	Henry *et al*, 2007 [83]; Scharfman *et al*, 2005 [84]
EGF	+			Doetsch *et al*, 2002 [37]; Aguirre *et al*, 2010 [62]
FGF	+			Westermann *et al*, 1990 [43]; Jin *et al*, 2003 [44]; Zhao *et al*, 2007 [45]
PDGF	+	+ (astrocyte)		Jackson *et al*, 2006 [85]
VEGF	+	+ (neuron)		Leventhal *et al*, 1999 [46]; Cao *et al*, 2004 [47]
Morphogens				
BMP		+ (astrocyte)		Nakashima *et al*, 1999 [48]; Yanagisawa *et al*, 2001 [49]
Noggin		+ (neuron)		Lim *et al*, 2000 [50]
Notch	+	- (neuron) - (glia)		Mizutani *et al*, 2007 [24]; Basak *et al* 2007 [25]; Aguirre *et al*, 2010 [62]
SHH	+			Ahn and Joyner, 2005 [86]; Banerjee *et al*, 2005 [87]; Machold *et al*, 2003 [88]; Palma *et al*, 2005 [51]
WNT3	+			Lie *et al*, 2005 [54]; Song *et al*, 2002 [53]
Intracellular pathways				
Transcription factors				
SOX2	+			Graham *et al*, 2003 [3]; Thomas *et al*, 2008 [4]
TLX	+			Shi *et al*, 2004 [69]; Sun *et al*, 2007 [70]
PAX6		+ (neuron)		Ericson *et al*, 1997 [8]; Zhang *et al* 2010 [7]
Olig2		+ (oligodendrocytes)		Zhou *et al*, 2001 [89]
Epigenetic regulators				
MBD1		+ (neuron)		Zhao *et al*, 2003 [81]; Li *et al*, 2008 [82]
Histone deacetylase	+ (with TLX)			Hsieh *et al*, 2004 [90]; Sun *et al*, 2007 [70]
Cell cycle regulation				
p16INK4a	-			Molofsky *et al*, 2006 [64]
p21	-			Kippin *et al*, 2005 [66]; Marques-Torrejon *et al*, 2013 [63]
p53	-			Gil-Perotin *et al*, 2006 [65]
microRNAs				
miR9	-	+ (neuron)		Krichevsky *et al*, 2006 [71]; Yoo *et al*, 2009 [76]
miR124	-			Yoo *et al*, 2009 [76]
miR137	-	+		Sun *et al*, 2011 [77]; Smrt *et al*, 2010 [78]; Szulwach *et al*, 2010 [79].
miR184	+	- (neuron)		Liu *et al*, 2010 [80]

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Table 3

Diseases implicated in aberrant regulation of NSPCs.

	Genes/ Pathways involved	Induced mutation	Cellular Phenotype	Behavioral phenotype	Reference
Developmental Disorders:					
ASD/Rett Syndrome	MEF2C	MEF2C Conditional knockout: Nestin-Cre	Embryonic: neurons compacted Postnatal: disorganization of cortical plate, immature electrophysiology	Abnormal anxiety-like behaviors, decreased cognitive function, and marked paw clasping stereotypy	Li *et al*, 2008 [134]
Schizophrenia	GABA-DISC1, AKT-mTOR	Retroviral shRNA injection into postnatal DGh	shRNA-DISC1: Accelerated dendritic growth, soma hypertrophy, ectopic primary dendrites; shRNA-NK1: Aberrant positioning, decreased dendritic length	Neither alone show differences in behavior; synergistic effect in DISC1-KD when exposed to stress	Kim *et al*, 2009 [125]; Kim *et al*, 2012 [126]
DiGeorge Syndrome/ Schizophrenia	22q11 genes: *Ranbp1*, *Cdc451*, *Hira*, *Ufd1l*, *Sept5*	Large deletion of chromosome 22q11	Embryonic: Reduced BP proliferation, aberrant neuronal connectivity	N/A	Meechan *et al*, 2009 [127]
Degenerative Disorders					
Parkinson's Disease	α-*synuclein*, *p53*, *Notch1*	Lentiviral vectors, siRNA	Reduced tubulin expression, neurite length, neuronal differentiation and survival, increased apoptosis.	N/A	Desplats *et al*, 2012 [135]
Alzheimer's Disease	*APOE4*, *PS1*, *APP*	ApoE Loss-of-function: Nestin-GFP	Increased proliferation of early NPCs in DG	Decreased water maze performance	Mu and Gage, 2011 [139]
Cancer:					
Medulloblastoma	miRNA	N/A	N/A	N/A	Genovesi *et al*, 2011 [141]