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Stem Cells in the Nervous System

ABSTRACT

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Given their capacity to regenerate cells lost through injury or disease, stem cells offer new vistas into possible treatments for degenerative diseases and their underlying causes. As such, stem cell biology is emerging as a driving force behind many studies in regenerative medicine. This review focuses on the current understanding of the applications of stem cells in treating ailments of the human brain, with an emphasis on neurodegenerative diseases. Two types of neural stem cells are discussed: endogenous neural stem cells residing within the adult brain and pluripotent stem cells capable of forming neural cells in culture. Endogenous neural stem cells give rise to neurons throughout life, but they are restricted to specialized regions in the brain. Elucidating the molecular mechanisms regulating these cells is key in determining their therapeutic potential as well as finding mechanisms to activate dormant stem cells outside these specialized microdomains. In parallel, patient-derived stem cells can be used to generate neural cells in culture, providing new tools for disease modeling, drug testing, and cell-based therapies. Turning these technologies into viable treatments will require the integration of basic science with clinical skills in rehabilitation.

Key Words: Stem Cells, Regenerative Medicine, Amyotrophic Lateral Sclerosis, Parkinson Disease

OVERVIEW

Recent developments in stem cell biology have contributed significantly to the understanding of brain development and maintenance. In this overview, the ways in which they also show promise for rehabilitation and regenerative medicine are summarized.

This review focuses on two distinct populations of stem cells: endogenous neural stem cells (NSCs) in the adult brain and pluripotent stem cell lines that can be differentiated into neural cells in culture. Dysfunction of endogenous stem cells or their niche—the specialized environment in which they grow—may underlie aspects of brain disease and aging. On the other hand, the ability to create new neurons and glia from patient-derived stem cells offers new hope for disease modeling, drug testing, and cell-based therapy. In order for these insights to generate concrete advances in regenerative medicine, a partnership must be built between those performing rigorous mechanistic biology, others using

human and animal models for preclinical studies, and physicians-scientists with a deep knowledge of patients' needs and relevant outcome measures.

During development, pluripotent embryonic stem cells (ESCs) give rise to all brain cell types, often via multipotent precursor populations of more limited potential. Although, in the adult brain, generation of new cells is reduced compared with many other tissues, adult NSCs persist in two main areas: the ventricular-subventricular zone (V-SVZ), where NSCs give rise to olfactory neurons, and the hippocampus, where new neurons involved in cognitive processes are generated. In both regions, the stem cells that give rise to neurons are specialized populations of astrocytes that maintain close interactions with the brain vasculature and can be activated by behavioral and pharmacologic stimuli. Given the ability of NSCs to migrate to sites of injury, amplification of their capacity to generate neurons has therapeutic potential. The expected benefits of modulating endogenous NSCs would be even more widespread if astrocytes from other brain regions could be induced to adopt stem cell properties. Much research is therefore focused on the mechanisms underlying NSC differentiation and on the cellular and molecular characteristics of their niche.

To everyone's knowledge, no drug has ever been tested for its effects on sick human neurons before the initiation of clinical trials for neurodegenerative diseases such as Alzheimer disease, Parkinson disease (PD), or amyotrophic lateral sclerosis (ALS). The recent technology for creating induced pluripotent stem cells (iPSCs) from patient tissues has allowed the possibility to directly evaluate emerging drugs in cultured human disease-specific cells. It is now possible to generate multiple classes of neurons and glia from human ESCs (hESCs) or patient-derived iPSCs and to establish "disease in the culture dish" models that shed light on human disease mechanisms and allow for drug testing in vitro. Moreover, although replacement of neuronal circuits remains a distant goal, the grafting of stem cell–derived support cells to slow neuronal degeneration in specific regions of the brain or spinal cord has shown promise in animal models and is being tested in early human trials.

Despite its promise, NSC biology still needs to cross several hurdles before its full clinical impact is realized. Potential implications of NSC discoveries in rehabilitation medicine for the coming decades may include improved treatments for neurodegenerative disease and stroke, among others. Taken together with the very active stem cell and bioengineering research being performed on the bone, connective tissue, and muscle, these avenues may lead to a radical change in the approach to patient rehabilitation and provide hope for significant clinical benefit.

STEM CELL BASICS

Stem cells have two essential properties: (1) They divide to give rise to another stem cell (self-renewal), and (2) they undergo differentiation to form diverse cell types (Fig. 1*A*). Stem cells play a central role both during development and in the adult. During embryogenesis, they give rise to all the different cell types that compose the human body. In addition, many (but not all) adult tissues retain pools of endogenous stem cells charged with replenishing cells lost through turnover (homeostasis), as well as regenerating the tissue after lesions. The property of self-renewal ensures that the stem cell pool is maintained throughout life. Stem cells often give rise to differentiated progeny via short-lived rapidly dividing intermediate progenitors, thereby increasing the output of cells derived from a single stem cell (Fig. 1*A*). Many adult stem cells are still capable of giving rise to different cell types albeit to a lesser extent than their embryonic counterpart.[1]

Stem cells reside within specialized cellular environments, called niches, which modulate many aspects of their biology (Fig. 1*B*). Elements within the niche provide positional cues important in determining which daughter cell retains stem cell identity and which daughter cell progresses down the lineage to form more differentiated progeny. In addition, cells within the niche provide factors important for the survival of the stem cells and the differentiation of their progeny. Properties of both stem cells and their niche vary during development and according to the tissue in which they are found. Moreover, both are affected in human disease and aging. As one example, dysregulation of either stem cells or their niche can lead to cancer.[2–5] In the following sections, stem cells and their niche in the adult nervous system, how they are affected in disease, and how they might contribute to central nervous system (CNS) regeneration are reviewed.

Endogenous Adult NSCs and Their Niche

The CNS is composed of neurons, astrocytes, and oligodendrocytes as well as other nonneural cell types. Neurons, the main effector cells of the CNS, process the information entering and leaving the CNS. Astrocytes, a very diverse class of cells, are the main support cells of the CNS, with functions that range from regulating which molecules in the blood enter the brain to phagocytosing cellular

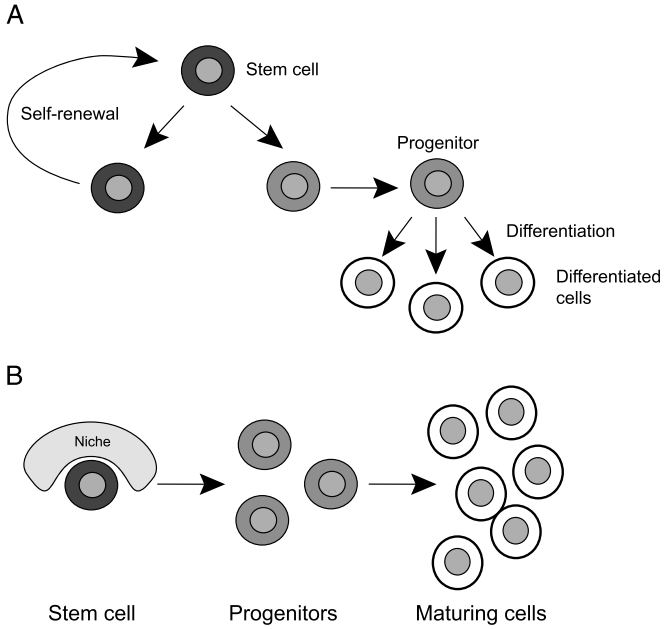

FIGURE 1 *Stem cells self-renew and give rise to differentiated progeny. A, Stem cells (black) divide to form another stem cell (self-renewal) and a progenitor cell (light gray). Progenitor cells divide to amplify their number and, in turn, give rise to more differentiated progeny (white). B, Stem cells reside in specialized niches, which provide important positional cues and regulatory elements that influence stem cell behavior. When a stem cell divides, the daughter cell that retains a stem cell identity is kept within the boundaries of the niche, whereas the other daughter cell loses the constraint on its phenotype. This second daughter cell now forms undifferentiated progenitors, which in turn give rise to more differentiated progeny.*

debris in the parenchyma and maintaining brain homeostasis.[6,7] Oligodendrocytes form myelinating sheaths along axons, allowing the rapid propagation of action potentials by neurons. During embryonic brain development, all three of these cell types arise from the same pool of stem cells: radial glial cells.[8]

Most neurogenesis ends around birth. However, it is now clear that new neurons are continuously generated by stem cells in restricted brain regions of the adult mammalian brain throughout life. Adult neurogenesis occurs in two regions, the V-SVZ of the lateral ventricles, which generates olfactory bulb neurons, and the subgranular zone (SGZ) in the hippocampal formation[9] (Fig. 2*A*). Some oligodendrocytes are also formed in both regions. Strikingly, in both adult neurogenic niches, the NSCs are specialized astrocytes, raising the question of whether astrocytes in other brain regions might also retain latent stem cell capacity.

Most of the knowledge about adult neurogenesis comes from studies in rodents. The V-SVZ extends along the length of the lateral ventricles and is the largest germinal region in the adult brain. Quiescent NSCs in the V-SVZ become activated to divide and generate intermediate transit-amplifying progenitors, which in turn give rise to new neurons that migrate to the olfactory bulb[10,11] (Figs. 2*A*, *B*). NSCs in the V-SVZ have a polarized morphology and span different compartments of the stem cell niche.[12] NSCs extend a thin process between ependymal cells, which line the ventricles, and are thereby continuously bathed by cerebrospinal fluid[13–15] (Fig. 2*B*). On the basal side, NSCs extend a long process to contact blood vessels within the V-SVZ niche. In recent years, the blood vessels in the V-SVZ have emerged as an important proliferative compartment of the niche.[16–18] Notably, the vasculature in this region of the brain has unique features. Dividing stem cells and their transit-amplifying progeny frequently contact blood vessels directly at specialized sites lacking astrocyte end-feet and pericyte coverage.[16] Moreover, signals in the blood are able to directly access the V-SVZ.[16] Thus, stem cells in this region are uniquely exposed to both contact-mediated and diffusible signals from the vasculature as well as systemic signals in the circulation. Interestingly, long distance and local neurons also innervate the V-SVZ, suggesting a role for circuit regulation within the niche.

In the SGZ, NSCs also extend a radial process and generate new neurons via a short-lived intermediate progenitor (Fig. 2*B*). The newly generated neurons travel a short distance into the granule cell layer, where they integrate into the local circuitry.

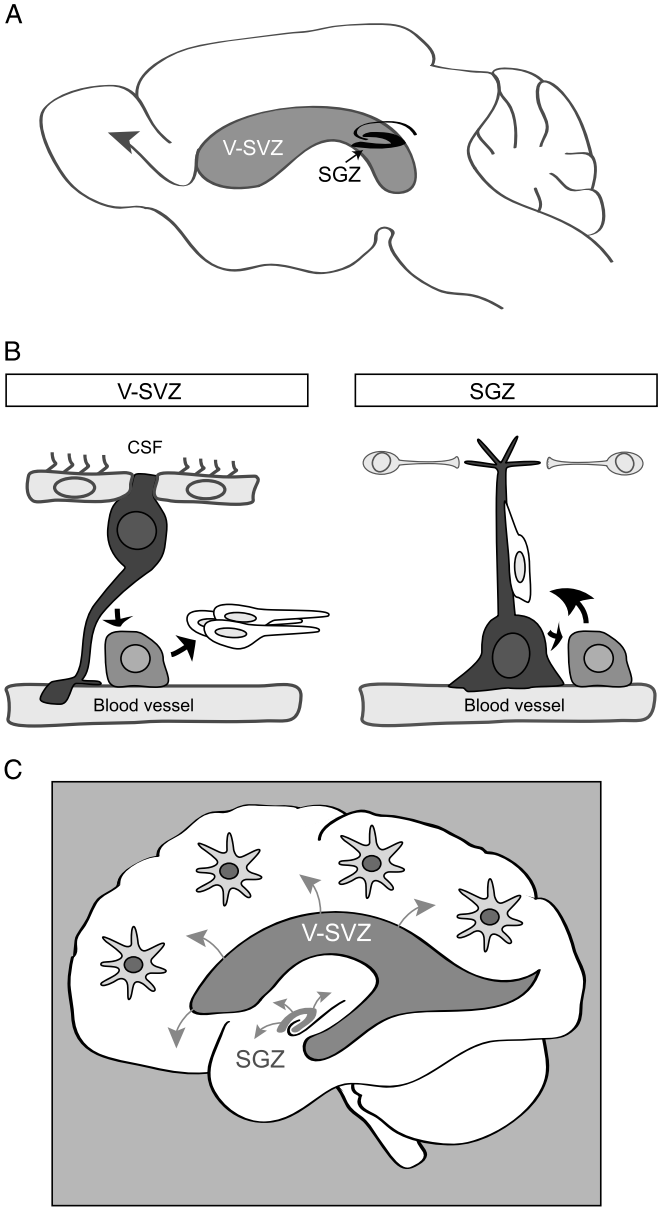

FIGURE 2 *Endogenous adult NSCs and their niche. A, Schema of a sagittal section of an adult mouse brain showing the V-SVZ adjacent to the lateral ventricles and SGZ of the hippocampal formation. New neurons born in the V-SVZ migrate a long distance to their final destination in the olfactory bulb (arrow). In contrast, adult-born neurons in the SGZ integrate locally into the circuitry. B, Schema of cell types and the anatomy of adult neurogenic niches. The V-SVZ niche (left) is composed of astrocyte NSCs (black), which contact the ventricular lumen between multiciliated ependymal cells and extend a radial process that contacts blood vessels. These cells give rise to amplifying progenitors (light gray), which in turn differentiate to migrating immature neurons (white). These immature neurons migrate to the olfactory bulbs, where they mature into interneurons. NSCs and progenitors often directly contact blood vessels at specialized sites that lack astrocyte end feet. The SGZ niche (right) is also composed of astrocyte NSCs (black) in contact with blood vessels, but these cells do not contact the ventricular lumen. SGZ NSCs also give rise to immature neurons through progenitors, and these neurons travel along the radial processes of the NSCs to integrate within the local circuitry. Local interneurons regulate SGZ NSCs. C, Schema showing adult NSC niches in the human brain. Future mechanistic studies on the biology of both regions will provide important insight into how to harness these endogenous NSCs and exploit their therapeutic potential. By modulating molecular pathways that regulate adult NSCs, it may eventually be possible to stimulate astrocytes elsewhere in the brain to become NSCs.*

The vasculature is also an important niche compartment in the SGZ. However, there are interesting differences. Unlike the V-SVZ, the vasculature is angiogenic in the SGZ, with neurogenesis occurring near the angiogenic foci.[19] The activity of local interneurons plays an important role in mediating

NSC quiescence in the SGZ[20] (Fig. 2*B*). Importantly, alterations in natural physiologic states can have potent effects on ongoing stem cell proliferation and adult neurogenesis. Exercise, pregnancy, and enriched environments stimulate different aspects of NSC proliferation and survival of newly generated neurons. In contrast, stress and aging inhibit proliferation and neurogenesis.[21] As the molecular underpinnings mediating these effects are uncovered, it may be feasible to stimulate neurogenesis and oligodendrocyte formation.

In adult humans, NSCs are also present throughout life in both the V-SVZ and the SGZ as specialized astrocytes.[22] Whereas active neurogenesis occurs in the SGZ in humans,[23] the levels of neurogenesis in the V-SVZ declines dramatically after infancy.[24] Interestingly, infants also possess a second migratory route to the prefrontal cortex not observed in nonhuman mammals. Thus, in the adult human V-SVZ, NSCs are largely in a dormant state. However, as outlined later, they divide under pathologic stimulation. As such, illuminating the pathways underlying stem cell quiescence and activation in rodents may shed light on how these stem cells are recruited upon injury or to form oligodendrocytes under baseline conditions.

Endogenous NSCs During Disease and Regeneration

Adult neurogenic niches harbor a pool of endogenous NSCs that could potentially be exploited for therapeutic purposes. Using fluorescence-activated cell sorting, adult NSCs can be directly harvested from tissue samples. Indeed, evidence that adult NSCs persist into adulthood in humans comes from studies where these cells have been isolated from surgical specimens of brain tissue and grown in cell cultures.[22,25] Isolating these cells allows for the careful dissection of their molecular characteristics in vitro, providing insight into their regulation and potential for regeneration. In addition, the ability to grow these cells in isolation allows the elucidation of the mechanisms required for neurogenesis to successfully occur.

Changes to NSCs After Disease and Injury

NSCs react strongly to pathologic conditions in the adult brain. In the V-SVZ niche, there is increased proliferation after ischemic stroke in the adjacent striatum or overlying cortex, including in humans.[26–28] In mouse models of ischemic stroke, newly formed neuroblasts migrate to the sites of ischemic lesion and form synaptic connections with neurons in the vicinity.[28] In animal models of multiple sclerosis, there is an increased influx of V-SVZ–derived cells into sclerotic lesions,[29,30] and there is evidence of this occurring in humans as well.[31,32] Determining whether these mechanisms contribute to healing, and whether they are conserved in humans, will be important in the search for better therapeutic strategies for these and other conditions.

Neurogenesis in the SGZ is associated with learning and memory, mood regulation, and pattern separation. Changes in neurogenesis may be one pathophysiologic cause of various affective disorders.[33,34] Moreover, certain psychological states (i.e., anxiety and stress) decrease the amount of hippocampal neurogenesis. Experimentally decreasing hippocampal neurogenesis in rodent models leads to behavioral effects similar to those seen in models of anxiety.[35]

Another important NSC-related change is that both V-SVZ and SGZ show a decrease in neurogenesis with aging.[36,37] It remains to be determined whether this is caused by a loss of cells or a shift to a dormant (nonproliferative) state. In contrast, SGZ NSCs in humans seem to increase their rate of neurogenesis during chronic neurodegenerative diseases such as Alzheimer disease.[38]

Therapeutic Strategies Based on Endogenous NSCs

The ability of NSCs to generate new neurons in certain niches provides hope that, if this process could be amplified and/or extended, it could serve as the basis for regenerative strategies in both neurovascular and neurodegenerative diseases. Amplification of NSCs may already be part of standard clinical practice. Intriguingly, increased neurogenesis in the SGZ has been implicated in the therapeutic effects of selective serotonin reuptake inhibitor antidepressants, potentially explaining the time needed for these drugs to take full effect.[33,34] The degree to which this can be generalized to human patients and other antidepressants remains to be determined. Importantly, physical activity increases neurogenesis in both V-SVZ and SGZ,[21] suggesting that nonpharmacologic approaches could also be used to target these cells. More generally, expanding the relatively limited potential of NSCs to generate other cell types could be important. Another still speculative therapeutic possibility is that some of the molecular mechanisms involved in the differentiation of NSCs could be used to activate astroglial cells elsewhere in the brain (Fig. 2*C*). Indeed, after traumatic brain injuries, glial cells in the vicinity of the lesion proliferate and form a glial scar. This glial

scar has been proposed to create a negative environment for neurogenesis and proper wound healing. By further understanding the niche elements in the V-SVZ and SGZ that permit such abundant neurogenesis to occur, it might be possible to find ways to turn the environment in these glial scars, and elsewhere in the brain, into a more permissive one. Elucidating this will be a key in understanding the therapeutic potential of these cells and other astroglial cells in the brain.

Making Neurons From Stem Cells in the Culture Dish

The preceding sections focused on the production of neurons from stem cells in situ and how this might be modulated in the therapeutic context. This is a rapidly developing field, but more emphasis still is being put on the potential uses of neurons and glia generated from stem cells in the laboratory, either to model diseases in vitro as a basis for drug testing or for direct cell replacement strategies.

Human Stem Cell–Derived Neurons Open New Avenues

Both aspects of this approach rely on the ability to generate neurons from human stem cells, through methods to be discussed in more detail later. This possibility is bringing about a sea change in the approaches to many neurologic and psychiatric diseases and, in particular, to neurodegenerative diseases. As one example, in patients with ALS, degeneration and death of cortical and spinal motor neurons lead to progressive muscle paralysis, often starting in the distal limbs and progressing to the respiratory muscles.[39] However, the sole Food and Drug Administration-approved drug for ALS, riluzole, confers only modest clinical benefit.[40] There is therefore a pressing need for disease-modifying treatments. One major obstacle to a successful therapy for ALS is the near-absence of validated targets, molecular events in the disease pathway whose inhibition would slow onset or progression. Genes such as superoxide dismutase 1 whose mutation can lead to ALS may be considered to be validated targets, but familial forms of the disease collectively represent only 10% of all cases. Therapeutic targets applicable to the 90% of sporadic cases would likely be genes acting early in the disease pathway. If such targets could be identified, they would provide a solid foundation for targeted drug discovery programs.

Most studies on the mechanisms of ALS have focused on mouse models expressing disease-triggering mutant forms of superoxide dismutase 1.[41] These mice develop a disease that strikingly mimics not only familial but also sporadic ALS, including selective resistance of oculomotor and slow spinal motor neurons.[42] However, even in this model, there are few validated targets other than the superoxide dismutase 1 gene itself. Moreover, concerns have been raised that results obtained in mutant superoxide dismutase 1 mice may not translate well to the clinic. Although this may in part reflect underpowered mouse preclinical studies, there is a need for human models to discover and validate novel candidate disease modifiers. Strikingly, none of the many drugs that have undergone clinical trials in ALS were ever first tested on the cells affected in this disease: human motor neurons. The best strategy would seem to be to evaluate candidate treatments in two systems in parallel: human motor neurons from familial and sporadic patients in vitro and the mouse neuromuscular system in vivo.

Making Motor Neurons from Pluripotent Stem Cells

For this reason, the authors and others have begun to use motor neurons and other cell types generated from human iPSCs to model ALS in the culture dish. The first step was to devise protocols by which stem cells can be coaxed into losing their stem cell properties and adopting those of differentiated, postmitotic neurons. This was first achieved using mouse ESCs, by carefully mimicking the stepwise process in the embryo through which external factors guide stem cells through a series of intermediate stages to become motor neurons[43] (Fig. 3). This process is very robust and generates billions of cells that are "real" motor neurons by many molecular and functional criteria.

The authors and others subsequently used adaptations of the same protocol to generate motor neurons from hESCs.[44–46] This approach made living human motor neurons widely available for the first time. However, hESC-derived motor neurons have at least one significant drawback for disease modeling: Existing lines were derived from embryos representative of healthy controls. There are essentially two ways to overcome this. First, it is possible to introduce into hESCs copies of mutant genes known to cause familial forms of ALS or other neurodegenerative diseases and to study their effect on motor neuron survival and regeneration. Second, a more far-reaching change occurred when Yamanaka and his colleagues demonstrated the possibility of reprogramming differentiated cells to iPSCs.[47] They showed

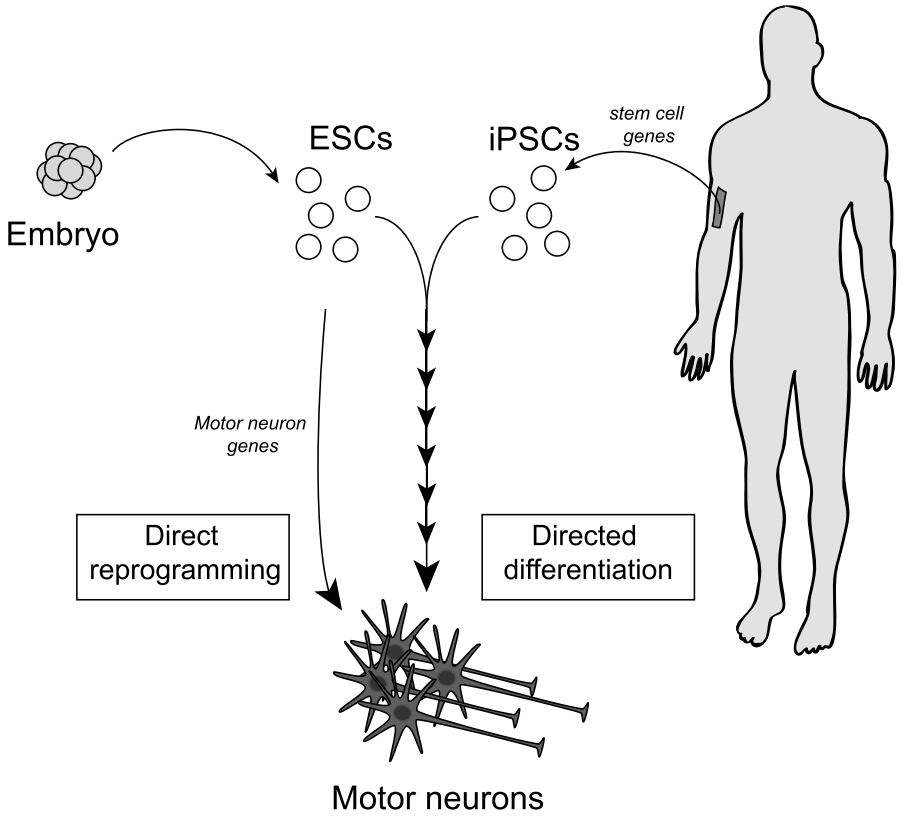

FIGURE 3 *Making motor neurons from stem cells. ESCs are generated from early human embryos, whereas differentiated skin cells can be turned into iPSCs by up-regulating specific stem cell genes. Both ESCs and iPSCs can be directed to differentiate into motor neurons through a multistep process by culturing them for weeks in the presence of factors that mimic normal neuronal development (directed differentiation). A more rapid process (days) is the direct reprogramming of ESCs (or fibroblasts, not shown) into motor neurons directly by forced expression of motor neuron genes.*

that, after the introduction of 3–4 stem cell genes into skin fibroblasts cultured after biopsy, not only do the cells adopt a stem cell phenotype, they nearly completely "forget" their skin cell origin. iPSCs from human ALS patients and controls were used to generate patient-specific motor neurons with high yield[45,48] (Fig. 3). Although some differences between these and the gold-standard hESCs certainly exist, they are relatively minor in most cases, and iPSCs have generated enormous excitement because of their potential applications in disease modeling and regenerative therapy. Directed differentiation of iPSCs provides the first patient-specific access to living preparations of many tissue types. By capturing patient-specific genetic background, iPSCs enable modeling of poorly understood complex genetic disorders, creation of humanized disease models that may be used to study disease modifiers and other correlations with clinical data, and immunologically matched tissue for eventual cell-replacement therapy.

Despite these many advantages, the derivation of iPS lines and their subsequent differentiation can be a lengthy process. More recently, different groups have shown that it is possible to take a shortcut by direct reprogramming skin fibroblasts or ESCs into motor neurons (Fig. 3). In this case, it is motor neuron genes, not stem cell genes, that are introduced as part of the reprogramming process.[49–51] This type of approach allows mouse motor neurons to be generated in as little as 2 days from ESCs, but both techniques have their advantages and will likely coexist.

Disease Modeling Using Human iPSC-Derived Neurons

The past years have seen the development of a number of hiPSC-based neurodegenerative and neurologic disease models. The basic "disease in the culture dish" paradigm of these models involves producing iPSCs from groups of patients; differentiating them into disease-relevant cell types; and assessing the derivatives for changes in gene expression, survival, protein localization/aggregation, sensitivity to exogenous stressors, or physiologic activity. Disease-related phenotypes have been observed

in iPS-derived neurons from patients with schizophrenia, Huntington disease, PD, familial dysautonomia, Rett syndrome, spinal muscular atrophy, and ALS.[52–57] Mechanistic characterization of such phenotypes is nascent but promising. Another critical feature needed for rigorous analysis is to show that any "clinical phenotype" observed in the culture dish is truly caused by the disease gene and not by interindividual differences in genetic background between the patient and control samples. The best way of doing this is to "correct" the mutation in a given iPSC line so as to create a gene-corrected (isogenic) control line, which only differs from the patient-derived line by this single change.[58] However, this has been performed in very few published studies. Some examples of diseases modeled using iPSCs will be given later.

Rett Syndrome

iPSCs derived from female patients with Rett syndrome, another neurodevelopmental disorder, can be efficiently differentiated to neurons. However, Rett iPS neurons exhibit decreased synaptic connectivity, spine density, and soma size as well as reduced spontaneous action potential firing in culture.[54] These deficits are consistent across multiple Rett iPSC lines and can be rescued by repletion of mutant protein or treatment with insulin-like growth factor-1. However, even in this system, genetic and epigenetic instabilities have proven to be concerns.[59]

Schizophrenia

iPS neurons derived from adult-onset schizophrenic patients also display decreased synaptic connectivity, spine density, and soma size in culture but show no apparent differences in spontaneous action potential.[55] Expression profiling reveals a large number of differences between schizophrenia neurons and controls, some of which have been observed in previous work performed on post-mortem patient samples. Treatment with Loxapine, but not other antipsychotic drugs, was able to reverse some putative schizophrenia iPS disease phenotypes, including select expression changes.[55]

Parkinson Disease

Sensitivity to environmental stressors has also been observed in disease-specific iPS neurons. Dopaminergic neurons derived from a single PD patient were more sensitive to 6-hydroxydopamine–induced cell death than those derived from a control iPS or hES line.[57] Although mixed cultures of dopaminergic neurons derived from a PD patient were also more sensitive to the oxidative stressor hydrogen peroxide, the tyrosine hydroxylase-positive dopaminergic neurons themselves were not. More recently, iPS models of PD have been used to address a potential drawback of such culture systems for studying late-onset adult diseases. By expressing progerin, a gene associated with premature aging, it was possible to enhance the PD-related phenotypes in the culture model.[60]

Spinal Muscular Atrophy

Spinal muscular atrophy patients show a characteristic loss of spinal motor neurons. Spinal muscular atrophy iPS-derived motor neurons exhibit a dearth of nuclear survival motor neuron protein (SMN) aggregates, a pathologic hallmark of the disease.[52] This effect can be partially rescued by treatment with drugs known to increase SMN in other tissues. Furthermore, spinal muscular atrophy iPS-motor neurons (MNs) are less abundant in culture than control iPS-MNs after 6 wks of differentiation, despite being present at similar levels 2 wks before this time point.[52] The numbers of motor neurons can be increased either by genetic correction of the deficit[61] or by blocking apoptosis.[62]

Amyotrophic Lateral Sclerosis

ALS was the first disease to be modeled using patient-derived iPSCs.[48] Since then, multiple attempts have been made to generate a model of "ALS in the culture dish" that is clearly relevant to the human disease. Perhaps, unsurprisingly, for a late-onset disease such as this, motor neurons from ALS patients do not show spontaneous degeneration in the few weeks they can be maintained in culture. Instead, in several cases, iPS-MNs derived from familial forms of the disease do exhibit some of the molecular hallmarks of human pathology.[56] Motor neurons derived from patients with the most frequent familial mutation, in C9ORF72, show characteristic accumulations (foci) of mutated RNA.[63,64] Even this is not alone sufficient to trigger neurodegeneration, and it is necessary to stress the ALS neurons with high levels of glutamate to observe exacerbated cell death. This vulnerability can be reversed using DNA antisense oligonucleotides targeting the disease gene.[63]

In addition to artificial aging or exposure to stressors, another approach to disease modeling is to more fully recreate the cellular environment of motor neurons within the spinal cord. Astrocytes normally provide a supportive niche for motor neurons within the adult CNS. However, in both mouse and human models, it has been reported that ALS

astrocytes instead become toxic for motor neurons, secreting factors that can trigger neuronal death.[65–67] The availability of stem cell–derived motor neurons and astrocytes,[68] in some cases in combination with astrocytes harvested from post-mortem CNS with familial or even sporadic forms of the disease,[69] has made it possible to generate coculture systems in which this toxic non–cell-autonomous interaction leads to spontaneous motor neuron degeneration in the culture dish. The immediate goal will be to create a fully humanized model of ALS in the culture dish that shows spontaneous neurodegeneration using astrocytes of both familial and sporadic ALS origins (Fig. 4).

The emergence of humanized disease models of ALS has opened the door to their use for screening collections of small molecules for neuroprotective drug candidates. For technical reasons, it is not yet feasible to perform screening on human models on a large scale. However, arguably, all candidate disease treatments should now be evaluated in such systems, hopefully increasing the predictive value of preclinical studies.

Cell-Based Therapy in the Nervous System

In parallel with attempts to slow neurodegeneration using drugs developed with the help of stem cell models, a future strategy would be to replace lost neurons. In reality, this is an extraordinarily challenging goal. In a disease such as ALS, it would be necessary not only to get grafted motor neurons to establish themselves in the spinal cord and send out axons to distant target muscles but also for the new motor neurons to become integrated in the circuits involved in motor control. At present, this prospect must be considered distant. Even for the dopaminergic neurons lost focally in PD patients, no robust technology yet exists to generate neurons in sufficient quantity and quality,

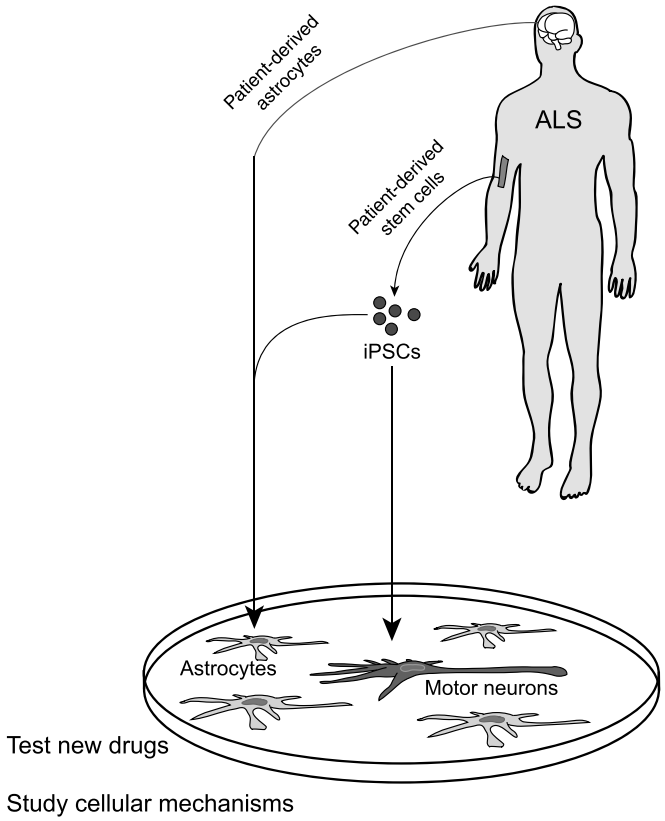

FIGURE 4 *Human ALS in the culture dish. To date, human ALS astrocytes have been shown to be toxic for mouse ESC-derived motor neurons, as are mouse ALS astrocytes for hESC-derived motor neurons. The ideal system would be a completely humanized model as depicted here, in which astrocytes (light gray) are derived from post-mortem brain or ALS ESCs/iPSCs and motor neurons (dark gray) are generated from ESCs/iPSCs. If human astrocytes lead to spontaneous motor neuron degeneration in this simplified culture system, it should be possible to test drugs for their efficacy in preventing ALS-related motor neuron cell death as well as study the cellular and molecular mechanisms underpinning disease progression.*

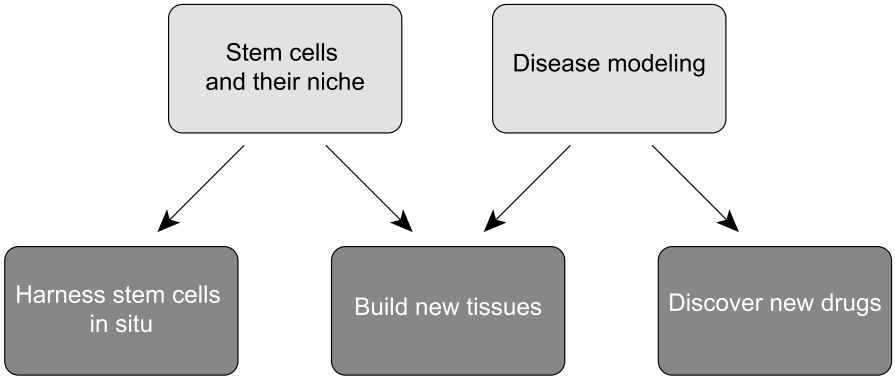

FIGURE 5 *Tapping the potential of stem cells for human health. A parallel focus on the two main areas of research outlined in this review—endogenous stem cells and their niche and disease modeling—provides a basis for three novel areas of progress toward clinical application and regenerative medicine.*

but this will likely be the neurologic disease where the first neuronal replacement trials are carried out.

An alternative, parallel approach involves the development of paracrine therapies. One possibility, as in models of epilepsy or neuropathic pain, is to reintroduce local interneurons that can correct the properties of dysfunctional neural circuits.[70,71] Stem cells and their glial derivatives have the potential to serve as local "factories" of growth factors that support the repair of nearby tissues.[72] Thus, placement of stem cells in a damaged brain may facilitate repair through these paracrine effects, rather than relying on direct replacement of missing cells. The cell populations evaluated for this type of approach range from mesenchymal stem cells to more targeted glial precursor populations.[73] Cells may be administered either locally, using cutting-edge neurosurgery, or more systemically.[74] The former approach is more invasive, but the latter poses problems of specificity and quantity of cells delivered. Perhaps, the most promising approaches are those that use stem cell derivatives not only as supportive cells in their own right but also as "Trojan horses" for the delivery of recombinant proteins with known neuroprotective activity.[75] Collectively, these approaches certainly deserve more development but need to surmount the hurdles of relatively weak efficacy in preclinical models (where evaluated), the quantity and quality of cells for human administration, and the paucity of relevant outcome measures during the early phases of clinical trial.

Putting Stem Cell Biology to Work in a Rehabilitation Department

How will stem cell biology and regenerative medicine impact rehabilitation for the coming decade? There are multiple challenges ahead, including that of managing excessive expectations. The strategy that was adopted within the Department of Rehabilitation and Regenerative Medicine at Columbia University Medical Center is a long-term one, based on supporting cutting-edge basic research in close and frequent contact with the physicians who understand patients' problems and can help to define areas that present the most promising opportunities for future intervention. The Columbia Stem Cell Initiative (external link, http://www.ColumbiaStemCell.org) has its home within the Department of Rehabilitation and Regenerative Medicine, while extending to more than 100 laboratories in all corners of the two campuses. To enhance stem cell focus within the department, a Division of Regenerative Medicine was created, which houses stem cell scientists in its own laboratory space, and research meetings and collaborations involving both basic and clinical faculty were organized.

The topic of this review—stem cells in the nervous system—encompasses only a fraction of the approaches that will be needed for successful rehabilitation. Strategically, therefore, it will be critical to integrate the authors' approaches across the whole range of pathologies encountered in rehabilitation medicine. The authors' plans are to do this by implementing the two main research areas outlined in this text—the biology of stem cells and their niche and disease modeling—and to use these as a solid basis for new therapeutic approaches based on pharmacologic interventions, bioengineering, and cell-based therapy (Fig. 5).

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