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Stem Cell Science:

Overviews of Selected Disease Areas

Stem Cells and Neurodegenerative Disease

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The Harvard Stem Cell Institute (HSCI) is a scientific collaborative established in 2004 to fulfill the promise of stem cell biology as the basis for the cure and treatment of a wide range of chronic diseases and medical conditions. HSCI's unique effort unites experts across the disciplines, schools and departments of Harvard University and all its affiliated research hospitals.

HSCI also sponsors public education programs concerning the scientific, legal and ethical implications of stem cell research, conducts a summer research program for college students, and helps educate high school teachers about stem cell science. HSCI depends upon the vision and generosity of private individuals, foundation and corporate donors to carry on its work, due to current U.S. restrictions on federal funding of embryonic stem cell research.

Introduction

The following scientific overview focuses on the use of stem cells-both in research and potential therapeutic applications - to address one of the most challenging, life-changing diseases and conditions of our time. This overview along with its companion pieces has two educational objectives: to make clear the opportunities and promise inherent in the basic science and to provide you with a picture of the research avenues that must be pursued to reach clinical applications. The overviews point to the areas where fundamental questions exist, where therapies need improvement, and where funding for research is urgently required.

Immense hope and scientific effort at institutions like the Harvard Stem Cell Institute have been invested in stem cells. Researchers seek the fullest understanding of how cells' fates are determined. They want to know how embryonic stem (ESC) cells recognize and respond to the signals that move them to become the full range of mature cells in an animal. They want to know how adult stem cells, whose fates are more restricted, contribute to the repair and regeneration of organs and tissues. Studying both types of cells in parallel will provide us information pertinent to developing stem cell therapies. With each experiment and clinical trial, researchers and clinicians move closer to these goals. This set of papers summarizes the current state of stem cell science in several key disease areas.

Stem cell research adds its own unique challenges to those faced by any early stage science. In fact, there are many cells, along with their behavior, that are still being discovered. In some cases the benefit, whether detailed fundamental knowledge, development of new drugs, or transplantable cells, is likely to be far in the future; in other cases, progress will come amazingly quickly. In stem cell research, as in few other contemporary scientific enterprises, success depends on supportive collaboration among diverse constituents: scientists and clinicians; local, state, and federal governments; universities and industry; and patients and their advocates. As a cross-institutional collaborative research and educational organization, HSCI is committed to meeting this challenge. We hope these disease overviews will help you better understand the research areas that matter to you.

I would welcome your thoughts about these overviews, your questions, and concerns. Contact me at brock_reeve@harvard.edu.

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Stem Cells and Neurodegenerative Disease

Introduction

It is difficult to speak too highly of the nervous system. It controls your breathing, your voluntary and involuntary motions, and your perception of the color blue. It contains memories of your mother, directions home, your aversion to spiders and all aspects of your personality, both conscious and subconscious. You *are* your nervous system. It is for this very reason that diseases of the nervous system are so distressing; at the least, they diminish what control we have over the world; at the worst, they diminish our ability to know the world or to recognize the loss.

The idea of treating neurodegenerative diseases with some form of cellular therapy is not a new one, but recent advances in both neurobiology and stem cell research have encouraged scientists to begin seriously contemplating the practicalities of such an approach. The problem is obviously complex, and it is worth understanding, if only to make sense of current scientific research and develop realistic expectations for its pace. To appreciate why stem cells hold promise to treat myriad neurodegenerative diseases, it is first necessary to become familiar with a few basic properties of the nervous system.

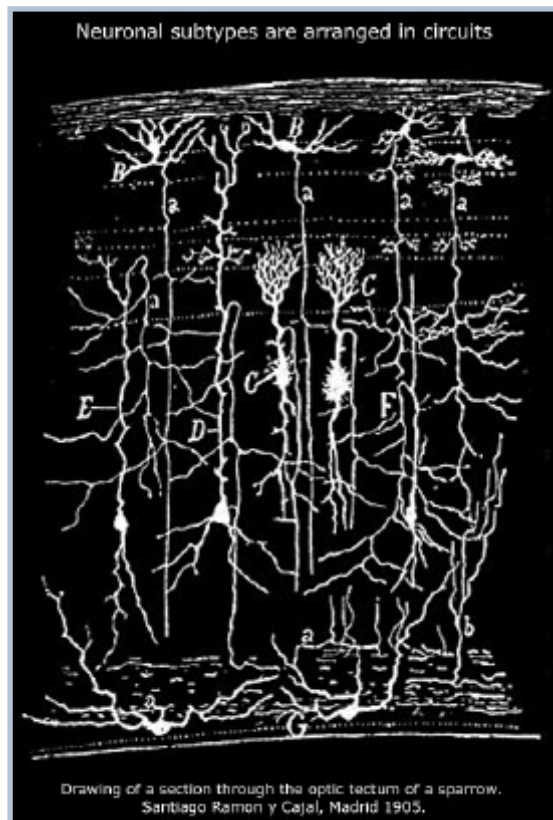
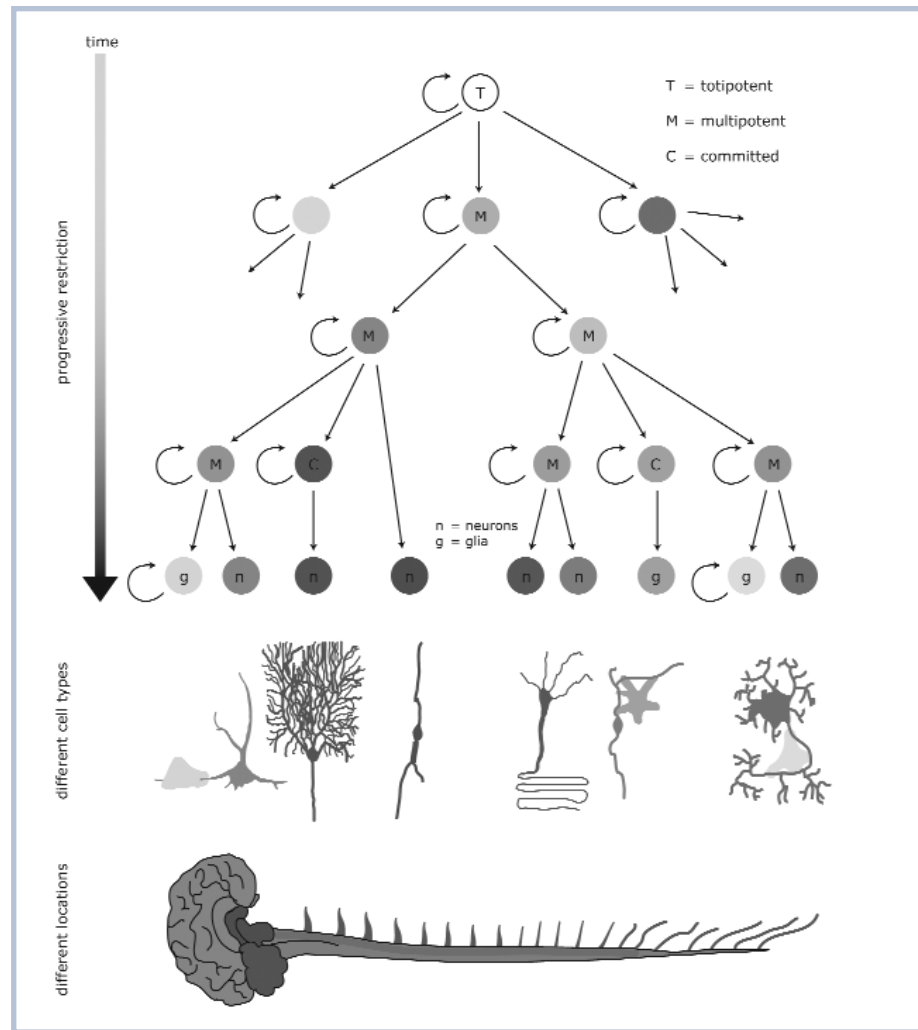


FIGURE 1
Neuronal subtypes are arranged in circuits. Courtesy of Amy Greenwood, PhD

The Nervous System

A thorough description of the structure, function and development of the nervous system would fill a thousand pages, but the most pertinent facts can be summarized easily. First, the nervous system contains two general cell types: neurons and glia. Neurons extend long projections that conduct electrochemical signals, like wires. Glia wrap around these projections to protect them and support their electrical function, much like the plastic casing around wires that provides insulation.

FIGURE 2:
The nervous system. Courtesy
of Amy Greenwood, PhD



Second, it is important to know that there are thousands of subtypes of neurons that differ with respect to their length, shape, connectivity, electrical properties and requirements for survival. That is, the components of the nervous system are not all equivalent. Finally, the function of any aspect of the nervous system, from a simple knee-jerk reflex to a complex abstract intention, depends critically on the integrity of the wiring: The circuit must be complete, accurate and robust.

The nervous system develops much the same way that all organs develop but with some added flourishes. Once a neural progenitor cell has made a final decision about its identity, it stops dividing and extends long projections in an attempt to find appropriate targets, such as muscle cells, specialized sensory cells or other neurons. During development, a neuron sends its projections through the embryonic body following a series of pathfinding cues, and when the projections reach suitable targets, they stop and make synapses (electrochemical connections between cells). Although the distances traveled by the projections are long, most connections are formed during embryogenesis when this distance is still short. As the embryo grows larger, the neuron merely extends its projection, much like an angler giving line to a hooked fish.

By adulthood, the target cell and the neuronal cell body are sometimes more than a meter apart and the projection stretches between the two. The fishing analogy is apt and emphasizes a challenge for the development of new neurons in the adult: It is one thing to catch a fish close to the boat and let it swim 100 meters away, but it is quite another to cast out 100 meters and catch the same fish.

The ultimate survival of a neuron depends on whether it has made appropriate and strong connections and whether it has integrated into a functional circuit. In fact, it is common for neurons to die during development because they are not part of a strong circuit. This property ensures that only active circuits are maintained and extraneous “wires” are not left lying around to divert energy or short the system, a condition necessary for the effective function of such a complicated network. All neurodegenerative diseases result from this caveat; they differ only in the cause of neuronal dysfunction and the subtype of neurons that are affected.

Finding Sources of New Neurons

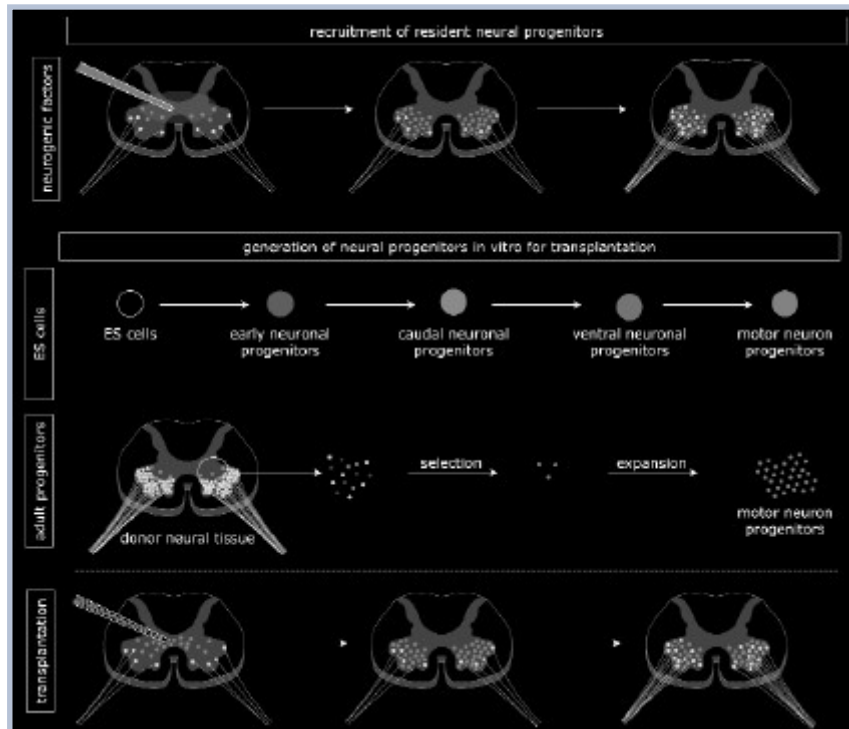
Stem cells can contribute to therapies for neurodegenerative diseases by simply providing material with which to study. For example, an abundant source of tissue is required for effective drug screening that will show scientists aspects of a disease in vitro (in the laboratory). Given that most neurodegenerative disorders involve multiple genes, multiple cell types and possibly environmental contributions, it is not possible in many cases to model a disease using cells other than those that are actually affected. However, diseased neurons are, by definition, in very short supply. Stem cell biology can provide a solution to this problem if the cell types

[In mouse studies] the most promising sources for new neurons are the resident neural progenitors in the nervous system and progenitors derived from embryonic stem cells.

in question can be produced by directed differentiation. To obtain diseased material for drug screening, embryonic stem cells (ESCs) that carry a particular disease could be derived by nuclear transfer from afflicted patients. Another option is to genetically engineer such cells to express certain disease genes and then differentiate them into the relevant neuronal subtype. These neurons could then be used to screen for compounds that have neuroprotective effects.

The ability to use stem cells to fix or model the nervous system is shaped by the basic properties of both (see Figure 3). In general, stem cells are being considered for three jobs: to replace neurons or glia that have been destroyed, to serve as delivery vectors for specific proteins or support functions, and to generate material for drug screens.

FIGURE 3:
Cell replacement therapies
for neurodegenerative disease.
 Courtesy of Amy Greenwood, PhD



Although the manipulation of the neuronal environment is an important hurdle for treating neurodegenerative disease, the optimal strategy for delivery of bioactive proteins has yet to be developed.

Replacement therapy is one option for diseases that result from the absence of defined neuronal or glial cell types, but it requires the identification of an appropriate source of progenitor cells. In cases where they exist, it may be possible to rouse endogenous (resident) adult stem cells from their slumber and encourage them to integrate into the damaged tissue. This is the ideal scenario because the progenitor cells at any particular location in the nervous system are already primed to produce the right kind of neurons for that location. Recent experiments have suggested that dormant neural stem cells might be more common in adult tissues than we have previously appreciated and that it is the molecular environment that dictates whether neurogenesis can occur. Therefore, the identification of factors that promote neurogenesis in the adult is currently of great scientific interest.

However, some areas of the nervous system may not contain enough suitable progenitor cells. Additional cells could be obtained by the expansion of adult neural progenitor cells or by the directed maturation (differentiation) of ESCs in vitro. Since both these strategies carry the risk of tumor formation, an effective therapy will need to incorporate methods to remove unwanted or aberrantly proliferative cells. Moreover, if cells are to be transplanted into the nervous system from a petri dish, they must be obtained in high quantity, with great purity, and at the appropriate stage of differentiation.

Despite the initial excitement that stem cells from sources such as bone marrow or cord blood could differentiate into neurons and glia, the veracity of the scientific studies that supported this enthusiasm have not withstood the test of time. Unfortunately, artifacts such as cell fusion have explained some of the original observations, and other studies producing results like these have not yet been replicated. At present, the most promising sources for new neurons are the resident neural progenitors in the nervous system and progenitors derived from ESCs; the competence of mouse ESCs to generate neurons has been proven.

In addition to a direct replacement strategy, it may be useful to add cells to the nervous system that have been engineered to affect the neuronal environment. For example, glial progenitors could be engineered to express high levels of growth factors that would support the differentiation or survival of new neurons. Although many such factors have been identified based on their effects in vitro or during development, few of these have shown any clinical effect when administered into the spinal canal. Although the manipulation of the neuronal environment is certainly an important hurdle for treating neurodegenerative disease, the optimal strategy for delivery of supportive proteins has yet to be developed.

Focus on Specific Diseases as Targets

While neurodegenerative diseases share many important commonalities, the specific attributes of each disease afford different opportunities and challenges for stem cell intervention. Intervention strategies will vary depending on whether the primary defect of a disease

is cell autonomous (caused by a defect in the neurons themselves) or cell nonautonomous (caused by a defect in the neuronal environment), whether single or multiple cell types must be replaced, whether it will be necessary to manipulate the local neuronal environment, and how well we understand the molecular mechanisms that drive disease progression. Some diseases that illustrate these points and that have been proposed as targets for stem cell-based therapies are discussed below.

Motor neuron disorders

Spinal muscular atrophy (SMA) is a neurodegenerative disorder that is characterized by the loss of motor neurons in the spinal cord and abnormal nerve morphology. This disorder ranges in severity but often afflicts young children and results in early lethality. Most patients with SMA harbor a mutation in the gene SMN1 (survival of motor neurons 1). Although the precise function of SMN1 is unknown, the deficit is thought to affect motor neuron cells autonomously (directly) and result in their degeneration. Thus, cell therapy for SMA would aim to replace the lost motor neurons with those that express normal levels of SMN1. One strategy for replacing lost motor neurons is with ESCs that have been differentiated into neural stem cells in vitro.

Indeed, when embryonic stem cell-derived neuronal progenitors are placed into the spinal cords of animal recipients, they can send axons to the periphery and make functional connections. It is unclear, however, if these circuits are appropriate: Motor neurons themselves come in different varieties, and it is unknown if the newly added circuits are sufficient to ameliorate SMA. Additionally, it will be necessary to replace motor neurons at many segments along the length of the spinal cord as neural progenitors are not particularly migratory and would not be expected to spread much from the injection site. Such a transplantation technique has not yet been mastered.

A similar disorder, amyotrophic lateral sclerosis (ALS), affects corticospinal motor neurons in both the spinal cord and the brain, and is typically diagnosed during adulthood. Unlike SMA, ALS is usually a sporadic disease that has both multigenic and environmental components. Even in familial cases where the genetic cause is known, it is still debated which cell population is the main focus of the disease. Recently, animal models of ALS have demonstrated that abnormal glia play a large role in disease progression; it appears that glia from mutant mice with ALS can cause degeneration of neurons from normal mice. Thus, an attractive strategy for a cell-based treatment for ALS is the transplantation of normally functioning or specially engineered glial progenitors. Like motor neuron progenitors, glial progenitors can be derived in vitro and are known to differentiate appropriately and remyelinate (wrap around to insulate) axons in some injury or disease settings in animal models. In addition, glial progenitors are migratory and may be easier to deliver. Although it may be necessary to replace motor neurons in ALS, any treatment that helps maintain even a few neurons in these pathways can mean the difference between movement that is merely impaired and complete paralysis or death.

An attractive strategy for a cell-based treatment for ALS is the transplantation of normally functioning or specially engineered glial progenitors.

There are currently no effective drug treatments for either SMA or ALS. However, since both motor neurons and glia can be differentiated from ESCs, embryonic stem-derived tissue can be used in chemical screens to identify compounds that inhibit neuronal degeneration in vitro, regardless of which cell type is responsible. ALS- or SMA-specific ESC lines can be generated either by engineering or by making disease-specific ESC lines from patients through therapeutic cloning. Indeed, it has already been shown that motor neurons derived from human ESCs behave in similar fashion to diseased cells when exposed to damaged glia in a mouse model of ALS and degenerate. Chemical screens directed at these cells would aim to identify compounds that prevent this process. Screening is also under way for compounds that positively regulate SMN1, the gene lost in SMA.

Parkinson's disease

Although the cause of Parkinson's disease is unknown, the pathology is due to the progressive degeneration of dopaminergic neurons, the main source of dopamine in mammals, that are located in the substantia nigra of the brain. Dopamine is important for voluntary movement and various behavioral processes including mood, addiction, and stress. Thus, the absence of dopaminergic input results in the improper control of the motor cortex, the region of the brain that controls movement: Parkinson's patients suffer from tremors; muscle rigidity; and slow, difficult movement. Current drug treatments aim to increase the levels of dopamine. These drugs often yield major improvements in motor function, but given the widespread effect of dopamine on the nervous system, they also have many side effects and can become less effective over time.

Parkinson's disease is a prime target for replacement cell therapy because the loss of neurons is limited to a small, selective subpopulation in the brain. In fact, dopaminergic fetal cells have been delivered to the brains of Parkinson's patients for many decades; the current procedure involves the transfer of fetal midbrain progenitors to the striatum, the area of the brain that normally receives the dopamine signals. The cells are not placed in the substantia nigra because it lies at a significant distance from its target. Despite many years of transplantation attempts, however, the efficacy of involving the striatum is still highly variable. Although many studies have cited significant improvement after transplant of fetal midbrain progenitors, two recent double-blind placebo-controlled clinical trials showed only limited efficacy for the procedure in a subset of patients. At the very least, these studies point to the absolute necessity of properly conducted trials. In addition, the use of fetal tissue is problematic for many reasons. On a technical level, its availability is limited, and the transplanted cells are heterogeneous (a combination of different cell types).

The transplant of neural tissue to Parkinson's patients has revealed some important information and points to the challenges that remain. For example, it is well established that many transplanted cells can survive in the brain for years, an important prerequisite for a

cell-based therapy. However, the lack of strong benefit provided by these cells, coupled with the side effects they sometimes induce, indicates that current techniques do not allow for the necessary circuitry to be formed. Scientists need more information about molecular cues that could induce neurogenesis in the adult substantia nigra and guide the dopaminergic axons to their targets.

The recent progress in directing the differentiation of human ESCs to dopaminergic neurons will serve to drive the field forward. Embryonic stem-derived dopaminergic neural progenitors can be produced in great abundance, providing scientists enough material to test for clinical application. Furthermore, ESCs can be engineered to express additional proteins that might assist with survival, differentiation, or pathfinding of the new neural cells. Finally, the relatively new capability to derive ESC lines from Parkinson's patients may help scientists uncover clues about the primary cause of the disease and ways to protect the neurons before they are lost.

Spinal cord injuries

Although issues surrounding the treatment of spinal cord injuries largely overlap with those relevant to motor neuron diseases, there are a few additional complications. Traumatic spinal cord injury results in the death of multiple cell types at the injury site: motor neurons, interneurons, and glial populations. Just as important, spinal cord injuries tend to sever the connection of the spinal cord to the brain, compromising the survival of the cord and the survival of all neurons below the injury site. Therefore, an effective intervention would both restore depleted cell types and rebuild the road for neuronal projections, in particular, the local injury site.

Current efforts to ameliorate spinal cord injuries are largely focused on adding cells or biomaterials that will sustain neuronal projection outgrowth across the gap created by the injury because shortly after injury, spinal cord neurons often attempt to reextend their projections. Unfortunately, many efforts fail, as the projections encounter a gap, scar tissue, or simply the wrong environmental signals.

Adding replacement cells to the spinal cord has met with modest success in animal models. It has been reported that transplanting neural stem cells to an injury site leads to increased behavioral improvement in rats, although it may also have unintended side effects due to the addition of inappropriate circuitry. In the end, it may be necessary to transplant populations of more restricted progenitors and to exert control over the local environment by adding growth factors that support survival and differentiation of neurons. Additionally, inflammation due to spinal cord injury can destroy the myelin, a protective fatty material that forms a sheath around a nerve fiber. Demyelination itself causes further neuronal loss, and it is thought that the addition of oligodendrocyte precursors (myelinating glia) could possibly rescue some neurons from death.

It has been demonstrated in animal models that certain cells, tissues or biodegradable gels can act as bridges for these projections under some circumstances. In addition to simply providing a mechanical scaffolding, such bridges could be engineered to express molecules to promote the extension of neural fibers and the survival of neurons in the area. Currently, one of the main impediments to this approach is the difficulty of preventing scar tissue from forming at the site of the transplant, a development that hinders the growth of neural projections. The fact that spinal cord injuries do not normally heal by themselves indicates that it is essential we understand and develop measures to control the local environment after an injury.

Sensory system disorders

Sensory disorders, such as blindness and deafness, often result from the loss of a small subset of neurons from the eye or ear. There are multiple causes for the loss of such neurons, but recent studies have shown that it may be possible to replace cells in these organs. Two important research challenges are the identification of the exact stage of progenitor cells to use for transplantation and the development of an appropriate delivery method.

Seeing. Many forms of blindness involve the loss of photoreceptors from the retina. Theoretically, such a deficit should be exceedingly amenable to treatment because the downstream circuitry of the visual system is initially left intact and because of the relative ease of accessing the eye. Recently, it has been shown in rodents that stem cells of a particular stage are the optimal cell type for transplantation. When placed into the subretinal space, these progenitor cells can incorporate into the adult retina and form functional circuits that provide visual information. The major hurdle to translating this finding to humans involves understanding how to produce progenitors of the appropriate stage *in vitro*. It will also be important to identify the molecular characteristics that confer the ability to integrate into the retina.

Hearing. The cells in the ear that transduce sound are called hair cells and are located in the cochlea. These cells do not typically regenerate, so when they are damaged, permanent hearing loss occurs. However, it was recently found that stem cells with the ability to differentiate into new hair cells do exist in the adult inner ear, and much attention has been focused on how to use these for therapeutic application. Additionally, the ability to generate hair cells *in vitro* from neural progenitors or ESCs is an encouraging step forward for the prospects of cell-based therapy for deafness. At present, however, it is unclear which cells would be optimal to transplant or how well they would integrate into the complex architecture and circuitry of the ear.

Glial disorders cause neurodegenerative disease because they compromise the functional support that glia normally provide the nervous system...These fall into two categories: demyelinating disorders such as multiple sclerosis... and metabolic disorders such as leucodystrophies.

Glial disorders

Glial disorders cause neurodegenerative disease because they compromise the functional support that glia normally provide the nervous system and weaken neuronal circuits. These diseases tend to fall into two categories: demyelinating disorders, such as multiple sclerosis, which result from an absence of glia, and metabolic disorders, such as leucodystrophies, which result from the impaired function of glia.

Myelination is crucial to nerve fiber health. It is provided by a subtype of glia called oligodendrocytes, and the development of these cells has been well studied. In vitro conditions have been defined under which oligodendrocytes can develop from adult or fetal neural stem cells as well as from ESCs. When oligodendrocyte precursors are delivered to the nervous system, they migrate readily and can myelinate neuronal projections in several disease models. Such progenitors could obviously be useful for treating demyelinating disorders, although the underlying cause for glial destruction would also need to be well understood.

Next Steps Toward Therapy and Repair

Although there is currently no stem cell–based treatment for diseases of the nervous system, the field has moved much closer to making this goal a reality. Progress has been driven by major jumps in our understanding of how neuronal subtypes and glia develop in vivo (in the body) and in vitro. Further research will inform our ability to coax endogenous stem cells in the adult nervous system to respond to injury and to transplant the correct type of cell to ameliorate different types of diseases. Just as important, the ability to differentiate subtypes of neurons and glia from ESCs will allow drug screens to identify neuroprotective drugs, which are currently unavailable.

The types of nervous system diseases that represent the best targets for stem cell–based therapies are those that would be improved by the transplant or induced replacement of a limited number of cell types. Parkinson’s disease, sensory disorders, and glial diseases fall into this category and could potentially be cured by a cell-replacement therapy. Motor system disorders and spinal cord injuries are more complex, but given their severity and lack of current treatment options, it can be argued that any improvement in function would be of great benefit.

There are nervous system disorders that do not currently make good targets for cell replacement therapies because the associated neurodegeneration is too widespread and diffuse. Alzheimer’s disease is one example, and it is unlikely to be ameliorated by adding more cells to the system. However, Alzheimer’s research could benefit enormously from disease-specific ESC lines that could be used to study the degeneration of neurons in vitro.

The prospect of using stem cells to intervene in neurodegenerative disease is promising, but given the complexities of the nervous system, progress will likely continue in measured

steps. To move the research toward useful therapies, it is critical that the efficacy of any experiment involving human subjects, and even experiments involving animal procedures, be performed using a double-blind placebo-controlled method. Certainly this increases the cost and the labor of an experiment, but the cost of doing research that is merely tantalizing and not conclusive, or worse, research that raises false hopes, is much higher. When there is a lack of rigorous controls, it is easy to falsely attribute an observed improvement to an incorrect cause. Finally, it is important to have scientific meetings to communicate both positive and negative results, as well as successes and cautionary tales. Despite the molecular differences between neurodegenerative diseases, their eventual stem cell therapies will likely share many features; Information gleaned in one field can drive forward progress in the others.

For more information about the HSCI Nervous System Diseases Program, visit the HSCI Web site at www.hsci.harvard.edu.

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