Navigating Cellular Repair for The Central Nervous System

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The central nervous system is subject to a number of age-dependent maladies which have no known cause. In the majority of cases, the disorder is the result of cell loss from specific areas of the brain (e.g., dopaminergic neurons from the substantia nigra pars compacta in Parkinson's disease, striatal neurons in Huntington's disease, and cortical and hippocampal neurons in Alzheimer's disease). Treatment is difficult because the causes of cell loss in these diseases are unknown. However, potential avenues to treat the diseases include either rescuing the dying cells or replacing them. The current explosion in stem cell research is focused on developing ways to fulfill these potential avenues.

Stem cells are undifferentiated, self-renewing cells with the potential to (trans)differentiate into a number of different types of cells (i.e., multipotent cells). They come in a variety of different guises, depending on their source tissue, both in the sense of age and location. The most controversial for ethical and political reasons are those sourced from embryonic and fetal tissue, which are believed to be the most plastic in the different types of cells they can become (i.e., pluripotent). *Figure 16.1* demonstrates how embryonic stem cells can become neural cells during development. Stem cells have also been identified in a number of adult tissues, including the brain, blood, and bone marrow.

It was originally thought that the central nervous system was the only organ that could not replenish itself in the adult. Consequently, neural stem cells would need to be obtained from either the embryo or fetal tissue or derived from stem cells obtained from other sources. However, this idea was proven to be incorrect by the identification of neural stem cells within the subventricular zone surrounding the lateral ventricles and the subgranular zone of the hippocampus of adult mammals.[13] The benefit of finding and being able to use these cells is to avoid the "ethical quagmire" that currently restricts stem cell research using embryonic and fetal sources, particularly in the United States. Under disease conditions, the endogenous cells do not appear to be able to fend off neurodegeneration or catastrophic injury, so use of these cells from an exogenous source may provide the help and/or sufficient numbers that these cells need to be effective. One possible explanation for the inability of the endogenous cells to reverse or control neurodegeneration is the observed decline in the generation of new neurons with age, particularly because many of the neurodegenerative disorders have an age-dependent component (reviewed in Bernal and Peterson[4]). Also, whatever is killing cells to cause the disease in the first place may also be affecting the stem cells.

Before the "stem cell explosion," fetal tissue transplants were tried in the treatment of Parkinson's disease and other disorders. In general, these transplants were effective in animal models but these techniques had limited and variable success when translated to patients, partly as a result of a limited availability of tissue as well as the induction of side effects such as dyskinesias (results of treatments for Parkinson's disease, including fetal tissue transplants reviewed by Goetz et al.[15]). Stem cells have the potential to theoretically provide a more homogenous group of cells for transplantation than used previously, which could help to enhance the benefit of these transplants.

The generation of stem cells can be a limiting factor and therefore several different methods can be applied to immortalize precursor cells. For instance, ReNeuron, a biotechnological company based in Guildford, U.K., have derived several immortalized cell lines from fetal brain tissue by transfection of a conditional immortalizing gene, which they are testing for clinical use in a number of disorders.[8,38] Scientists from ReNeuron have also published a detailed review of the necessary criteria that a cell line must meet before it can be used clinically.[18] Kobayashi[24] also reviews a number of methods of immortalizing cell lines in a recent review. These cells can also be manipulated in other ways to promote cell survival such as transfection.

Probably one of the most well-known precursor cells are those derived from the clonal NTERA-2 (NT2) cell line, which has been well used in animal studies. These cells were originally derived from a teratocarcinoma and can be made to differentiate into neural cells by incubation with retinoic acid (and are then frequently known as NT2-N cells). Transplantation of undifferentiated cells into animals resulted in tumor

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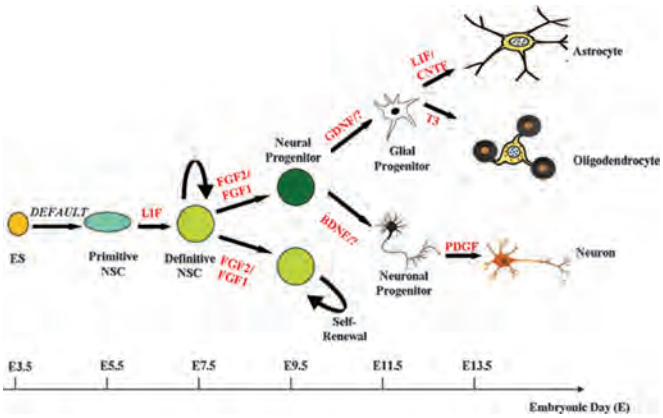

FIGURE 16.1. The ontogeny and regulation of neural stem cells (NSCs) from embryonic stem (ES) cells. ES cells will become primitive NSCs (pNSCs) by a default mechanism and then in the presence of, or absence of, specific growth factors, differentiation, or continued proliferation can occur as depicted. LIF, leukemia inhibitory factor; FGF2, fibroblast growth factors; BDNF, brain-derived neurotrophic factor; GDNF, glial cell line-derived neurotrophic factor; PDGF, platelet-derived growth factor; CNTF, ciliary neurotrophic factor; T3, triiodothyronine. Reprinted with permission from Hsu YC, Lee DC, Chiu IM: Neural stem cells, neural progenitors, and neurotrophic factors. **Cell Transplant** 16:133–150, 2007.

growth; this is also a concern with stem cells as a result of their ability to continuously proliferate. However, striatal transplantation of the neurally differentiated form of these cells (NT2-N cells) into a patient with stroke did not result in tumors and in fact the cells survived for over 2 years.[29] Additional human studies have revealed long-term survival of these cells in 14 patients with stroke with improvement of some of the stroke-induced neurological signs within the patients themselves (although not to levels in normal patients[25]). Striatal transplantation of genetically modified NT cells, so that they expressed nerve growth factor, led to long-term incorporation of these cells and improved cognitive function in nude mice subjected to traumatic brain injury.[48] As mentioned earlier, one of the concerns with transplanted precursor cells is the potential for tumorigenesis. This is discussed, along with the potential benefits of these cells, in a recent paper by Newman et al.[33] A recent study comparing transplantation of two different populations of dopaminergic NT2-N cells into denervated rat striatum revealed long-term survival but loss of the dopaminergic phenotype.[31] This suggests that cotransplantation of these cells with different factors may be necessary to enable the desired phenotype to be maintained. NT2 neurons have also been transplanted into a transgenic mouse model of amyotrophic lateral sclerosis, in which they delayed the onset of motor deficits,[14] demonstrating that these cells have a potential use in the treatment of a number of neurodegenerative disorders and this may be by a different method than cellular replacement. Of interest is a study in which the survival of NT2 cells was prolonged by the presence of umbilical cord blood cells (reviewed in El-Badri et al.[10]), suggesting that cotransplantation of both cell types could have a beneficial synergistic effect, possibly by the release of prosurvival factors.

Several studies using neural stem cells have been published recently. For instance, earlier this last year, Redmond et al.[40] published a study in which neural stem cells were transplanted into Parkinsonian primates and improved behavior outcomes were observed. The significance of this study is twofold; first, the transplanted cells were undifferentiated and did not become tumorigenic, and, second, the majority of the cells became astrocytes rather than neurons. This highlights the point mentioned earlier, that stem cells may not integrate into the cellular network replacing the dying cells, but instead may act in a supporting role, possibly "resuscitating" impaired cells or providing trophic support to reduce the likelihood that any additional cells die. This is further discussed in the commentary accompanying the Redmond et al. paper[42] (*Fig. 16.2*).

Many studies for Parkinson's disease have looked at stem cells as a source of dopaminergic cells for transplantation,[23,35] but the previously mentioned paper suggests that this may not be necessary and it may be more beneficial to transplant stem cells "as is" and allow the microenvironment to decide what the cells become rather than differentiating the cells to a specific type in vitro before transplantation. However, additional studies are required so that one can be sure that the cells do not become tumorigenic under these conditions.

The role of neurotrophic and angiogenic factors in the beneficial effects of stem cell transplants is becoming increasingly relevant as more studies demonstrate that the cells are not replacing the dying cells, but instead are in some way providing support. Zhang et al.[50] recently published a study in which neural stem cells were transfected with the neurotrophin-3 (NT-3) gene and transplanted into mice with spinal cord injury. Thirty days after transplantation, marked functional recovery was apparent, demonstrating that stem cells may not only be beneficial in their own right, but can be genetically modified to exert a greater effect and demonstrating the importance of neurotrophic factors.

The Redmond and Zhang studies[40,50] provide more evidence of how the beneficial effect of stem cell transplants may relate to neurotrophic factor secretions rather than cellular replacement. It is therefore important to know which factors these cells release into the tissue to garner benefit. Of particular relevance are likely to be the neurotrophic growth factors such as NT-3 (as demonstrated previously), bone-derived growth factor,[26] nerve growth factor,[21] glial-derived growth factor,[36,45] and the angiogenic vascular endothelial growth factor.[1] These factors have a variety of effects on cells in culture and exert some benefit in vivo also. Bull and Bartlett[7] found that bone-derived growth factor was essential

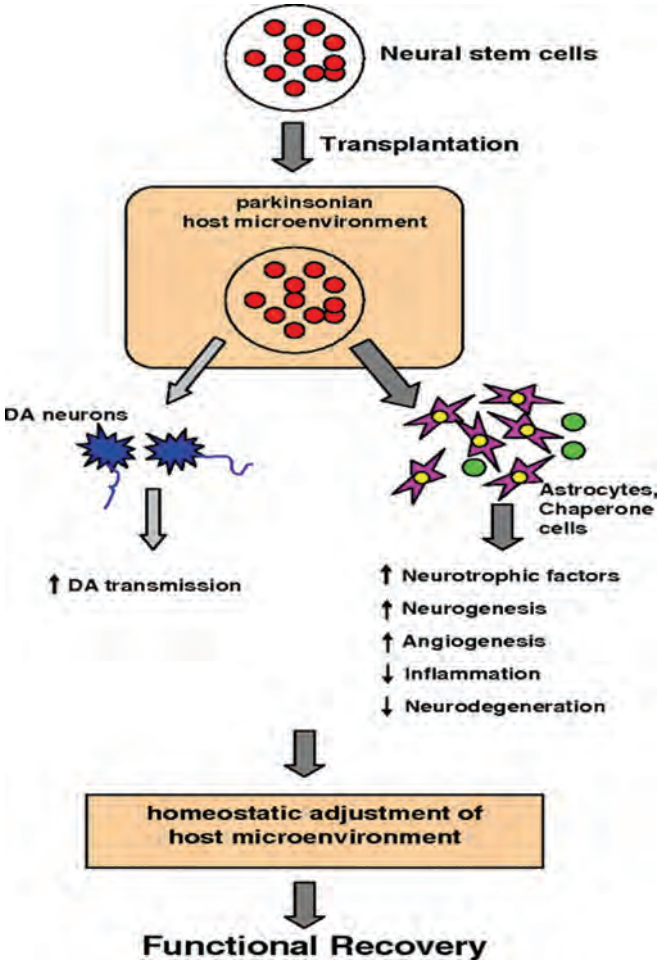

FIGURE 16.2. Multimodal hypothesis of undifferentiated human fetal neural stem cells transplanted in Parkinson's disease MPTP primates. Reprinted with permission from Sanberg PR: Neural stem cells for Parkinson's disease: To protect and repair. **Proc Natl Acad Sci U S A** 104:11869–11870, 2007.

for the formation of neurons from hippocampal progenitors, whereas glial-derived growth factor has been shown to be important in maintaining the survival of neurons at different stages of their development.[9,17] Vascular endothelial growth factor promotes the formation of new blood vessels, thus ensuring that an adequate food supply is provided for newly growing neural cells. Further details can be found in a review by Hsu et al.[19] The benefit of these factors is highlighted by studies in which the cells are encapsulated and therefore do not integrate directly but can only exert their influence through factors that they secrete. There is a considerable body of work from Emerich et al. which studies encapsulated choroid plexus cells as a treatment for Huntington's disease.[11,12] Further support of the importance of neurotrophic and angiogenic factors is provided in studies whereby the transplanted stem cells are induced to express these factors in the treatment of nervous disorders[5] or are shown to express these factors under normal conditions.[3] Interestingly, this later paper showed that embryonic stem cells would secrete greater quantities of neurotrophic factors when incubated with cortical brain extract obtained from fluid percussion-injured rats. This suggests that "injured" tissue may release a stimulus to activate stem cells. Identification of this stimulus could prove to be very fruitful in priming cells to work better in transplants. Also, a recent paper by Pisati et al.[37] demonstrated that a small number of mesenchymal stem cells after transplantation into nude mice differentiated into astroglial cells that secreted some of the aforementioned neurotrophic growth factors. As well as these growth factors, a recent report by Robertson et al,[41] suggests that sonic hedgehog is also an important factor promoting the survival of transplanted cells. Consequently, the survival of transplanted cells and possibly the endogenous cells could also be bolstered by a cocktail of factors, which the transplanted cells could be induced to secrete or alternatively by the use of cografting with additional cells, that secrete the necessary factors.[16] Cotransplantation with the factors themselves[20] is clearly an avenue of important research worth pursuing. A recent report by Shamekh et al.[43] demonstrated enhanced survival of ventromedial tissue, particularly the dopaminergic tyrosine hydroxylase-expressing neurons from rat fetuses after growth with Sertoli cells, as well as following transplantation.[44] These cells secrete a variety of neurotrophic factors and therefore this helps to demonstrate the importance of a source of neurotrophic factors in the survival and healthy growth of neurons.

The origin of adult stem cells is currently unknown, but if they originate from embryonic cells, then we would expect there to be many more adult cells than are observed. Bowie et al.[6] have identified what appears to be a "master switch" whereby changes from embryonic to adult stem cells occur, suggesting that the source could be the same. Identification of this switch and also the factor(s) that "revives" the stem cells that are said to be in a state of quiescence in the adult, possibly as a result of replication blockade,[28] could lead to additional methods of treatment for disorders, whereby the endogenous stem cells are made to proliferate and release the factors necessary to promote recovery. It is possible that transplantation of stem cells could be beneficial as a result of a ramping up of endogenous stem cells.

The cells that could provide potential benefit for neurological disorders are not restricted to just the use of neural stem cells. Embryonic stem cells are pluripotent and can form neural cells and there is increasing evidence that "adult" stem cells from the bone marrow or blood (particularly umbilical cord blood) also have the capacity to become neural cells. Also, because there is increasing evidence that these cells do not necessarily replace the dead or dying cells, but may act in a more supportive role, it is possible that the cells may not need to exhibit neural characteristics, but are just required to

release the right growth factors. However, there remains some controversy over how "plastic" adult stem cells such as mesenchymal stem cells actually are. Raedt et al.[39] propose particular criteria that stem cells need to meet before they can truly be classified as pluripotent. This does not necessarily cast doubt on their effectiveness in treatments, because, as has already been stated, replacement of dying or lost cells seems to be a minor part of the benefit that can be achieved by transplantation of these cells. An additional potential explanation for the effectiveness of these cells other than transdifferentiation and factor release would be cell fusion. This can be beneficial from a treatment point of view as demonstrated by Bae et al.,[2] who show that fusion of bone marrow stem cells with Purkinje neurons resulted in fully functioning Purkinje neurons and in this sense could improve the health of compromised cells.

The bone marrow or mesenchymal stem cells have been used in a variety of different animal models of neurological disorders with some degree of success. This has been reviewed in a number of papers aimed at spinal cord injury[27] and stroke.[46]

Stroke studies are primarily focused on a treatment that works within days of the diagnosis of a stroke. An example of a major source of cells that could be used to treat stroke in this fashion is umbilical cord blood, because there is a narrow time window (approximately 2 days) when these cells are most effective.[30] These cells have been shown to exert considerable benefit in a nonreplacement manner against the neurological and behavioral defects that result in the middle cerebral artery occlusion model of stroke.[32,33,49] Newman et al.[34] demonstrated that these cells readily secrete a number of cytokines and growth factors, which can exert a beneficial effect on surrounding cells, whereas in a recent commentary, Willing et al.[49] discuss how these cells may influence the immune response and rejection, which is frequently a concern with transplantation. Because the cells in the umbilical cord blood tend to be immature and the killer cell count is extremely low, little adverse immune response or rejection is seen. It is possible that these cells not only secrete cytokines and growth factors to promote cell survival, but also factors that reduce the likelihood of an immune response, because an anti-inflammatory response is apparent after transplantation.[47]

Umbilical cord blood cells also may be beneficial in the treatment of spinal cord injuries as a result of the release of neurotrophic factors and vascular endothelial growth factor,[22] which has been confirmed in vitro by Newman et al.[34]

Additional stroke studies have attempted to determine if there are other treatment windows of opportunity. For instance, the release of neurotrophic factors by NT2 cells transplanted 14 days after stroke in animal models has also been shown to be therapeutically beneficial.[17] ReNeuron's immortalized stem cell lines are also being tested 30 days after stroke with some degree of success.[38]

The study of stem cells, and the factors that they secrete with respect to the repair of the central nervous system, therefore has considerable potential with a number of different cell types and neurotrophic factors that could prove effective alone or more likely in combination for the treatment of neurodegenerative disorders. We are on the cusp of some groundbreaking discoveries that could lead to several beneficial treatments and/or greater understanding of the diseases themselves.

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