Stem Cells: New Opportunities to Treat Eye Diseases

Iqbal Ahmad

The recent identification and characterization of neural progenitors with stem cell properties has opened new avenues that may be useful for treating functional impairments caused by the death of specific neural cell populations.1,2 Neuronal degeneration is the cause of debilitating visual impairment associated with prevalent ocular diseases, such as retinitis pigmentosa (RP), age-related macular degeneration (AMD), retinal detachment, and glaucoma. Neural stem cells may help to restore vision in patients who have these diseases, by repopulating the damaged retina and/or by rescuing retinal neurons from further degeneration. In addition, understanding the molecular and cellular biology of neural stem cells will shed light on developmental mechanisms that regulate their maintenance, differentiation, and survival. Insights into these developmental mechanisms are likely to provide additional therapeutic approaches. This review provides an overview of the progress made in the area of neural stem cell research with emphasis on the eye. First, neural stem cells are defined and described. Second, their location and characterization in the mammalian eye is outlined. Third, progress in therapeutic usage of neural stem cell is discussed. This review does not include corneal stem cells; the reader is referred to an excellent review by Schwab and Isserhoff3 on the therapeutic uses of corneal stem cells.

DEFINING NEURAL PROGENITORS AS STEM CELLS

The generation of cellular diversity in the brain is widely believed to be a multistep process. The process is believed to involve multipotent progenitors whose proliferative and differentiation potentials are progressively restricted during development (Fig. 1). The progressive restriction of the developmental potential of neural progenitors, regulated by intricate cell–cell interactions, ultimately directs their differentiation into either neurons or glia. Neural progenitors have been isolated from different regions of both embryonic and adult brain, and their proliferation and maintenance in vitro depend on the presence of high concentrations of mitogens, such as epidermal growth factor (EGF) and/or basic fibroblast growth (FGF2).1,2,4 The potential of these progenitors to give rise to different cell types is demonstrated either by withdrawing mitogens or by exposing them to differentiation-promoting factor(s).

DEFINING NEURAL PROGENITORS AS STEM CELLS

The recent identification and characterization of neural progenitors with stem cell properties has opened new avenues that may be useful for treating functional impairments caused by the death of specific neural cell populations.1,2 Neuronal degeneration is the cause of debilitating visual impairment associated with prevalent ocular diseases, such as retinitis pigmentosa (RP), age-related macular degeneration (AMD), retinal detachment, and glaucoma. Neural stem cells may help to restore vision in patients who have these diseases, by repopulating the damaged retina and/or by rescuing retinal neurons from further degeneration. In addition, understanding the molecular and cellular biology of neural stem cells will shed light on developmental mechanisms that regulate their maintenance, differentiation, and survival. Insights into these developmental mechanisms are likely to provide additional therapeutic approaches. This review provides an overview of the progress made in the area of neural stem cell research with emphasis on the eye. First, neural stem cells are defined and described. Second, their location and characterization in the mammalian eye is outlined. Third, progress in therapeutic usage of neural stem cell is discussed. This review does not include corneal stem cells; the reader is referred to an excellent review by Schwab and Isserhoff3 on the therapeutic uses of corneal stem cells.

DEFINING NEURAL PROGENITORS AS STEM CELLS

The generation of cellular diversity in the brain is widely believed to be a multistep process. The process is believed to involve multipotent progenitors whose proliferative and differentiation potentials are progressively restricted during development (Fig. 1). The progressive restriction of the developmental potential of neural progenitors, regulated by intricate cell–cell interactions, ultimately directs their differentiation into either neurons or glia. Neural progenitors have been isolated from different regions of both embryonic and adult brain, and their proliferation and maintenance in vitro depend on the presence of high concentrations of mitogens, such as epidermal growth factor (EGF) and/or basic fibroblast growth (FGF2).1,2,4 The potential of these progenitors to give rise to different cell types is demonstrated either by withdrawing mitogens or by exposing them to differentiation-promoting factor(s).

DEFINING NEURAL PROGENITORS AS STEM CELLS

The generation of cellular diversity in the brain is widely believed to be a multistep process. The process is believed to involve multipotent progenitors whose proliferative and differentiation potentials are progressively restricted during development (Fig. 1). The progressive restriction of the developmental potential of neural progenitors, regulated by intricate cell–cell interactions, ultimately directs their differentiation into either neurons or glia. Neural progenitors have been isolated from different regions of both embryonic and adult brain, and their proliferation and maintenance in vitro depend on the presence of high concentrations of mitogens, such as epidermal growth factor (EGF) and/or basic fibroblast growth (FGF2).1,2,4 The potential of these progenitors to give rise to different cell types is demonstrated either by withdrawing mitogens or by exposing them to differentiation-promoting factor(s).

DEFINING NEURAL PROGENITORS AS STEM CELLS

The generation of cellular diversity in the brain is widely believed to be a multistep process. The process is believed to involve multipotent progenitors whose proliferative and differentiation potentials are progressively restricted during development (Fig. 1). The progressive restriction of the developmental potential of neural progenitors, regulated by intricate cell–cell interactions, ultimately directs their differentiation into either neurons or glia. Neural progenitors have been isolated from different regions of both embryonic and adult brain, and their proliferation and maintenance in vitro depend on the presence of high concentrations of mitogens, such as epidermal growth factor (EGF) and/or basic fibroblast growth (FGF2).1,2,4 The potential of these progenitors to give rise to different cell types is demonstrated either by withdrawing mitogens or by exposing them to differentiation-promoting factor(s).

DEFINING NEURAL PROGENITORS AS STEM CELLS

The generation of cellular diversity in the brain is widely believed to be a multistep process. The process is believed to involve multipotent progenitors whose proliferative and differentiation potentials are progressively restricted during development (Fig. 1). The progressive restriction of the developmental potential of neural progenitors, regulated by intricate cell–cell interactions, ultimately directs their differentiation into either neurons or glia. Neural progenitors have been isolated from different regions of both embryonic and adult brain, and their proliferation and maintenance in vitro depend on the presence of high concentrations of mitogens, such as epidermal growth factor (EGF) and/or basic fibroblast growth (FGF2).1,2,4 The potential of these progenitors to give rise to different cell types is demonstrated either by withdrawing mitogens or by exposing them to differentiation-promoting factor(s).

DEFINING NEURAL PROGENITORS AS STEM CELLS

The generation of cellular diversity in the brain is widely believed to be a multistep process. The process is believed to involve multipotent progenitors whose proliferative and differentiation potentials are progressively restricted during development (Fig. 1). The progressive restriction of the developmental potential of neural progenitors, regulated by intricate cell–cell interactions, ultimately directs their differentiation into either neurons or glia. Neural progenitors have been isolated from different regions of both embryonic and adult brain, and their proliferation and maintenance in vitro depend on the presence of high concentrations of mitogens, such as epidermal growth factor (EGF) and/or basic fibroblast growth (FGF2).1,2,4 The potential of these progenitors to give rise to different cell types is demonstrated either by withdrawing mitogens or by exposing them to differentiation-promoting factor(s).
pendent current profiles characteristic of neurons and glia. In addition to their ability to differentiate into basic neural cell types, these progenitors can also differentiate into retinal neurons when cocultured with embryonic or neonatal retinal cells.

However, despite the demonstration of multipotentiality, these cells cannot be defined as stem cells, because attempts to serially clone them have not been successful—that is, they do not appear to self-renew. The clonal generation of retinal progenitors has been achieved only by culturing retinal progenitors obtained from embryos expressing the antiapoptotic factor bcl2, in the presence of a mixture of several growth factors. Even then, the proportion of clones that contained more than two cells was only 14%. This suggests two possibilities: The proliferating cells isolated from E17 embryos are not stem cells, but rather are neural progenitors with a limited self-renewal property, or these cells are indeed stem cells, but conditions have not been identified that promote their self-renewal in vitro.

Adult

The question of whether the adult derivatives of mammalian retinal neuroepithelium–harbored cells with stem cell properties was recently addressed. Two laboratories showed that the ciliary epithelium and not the neural retina in the adult mammalian eye contains neural progenitors. The hypothesis that neural stem cells-progenitors are present in the adult ciliary epithelium was based on a well-known observation that an analogous region in adult fish and frogs, called the ciliary marginal zone (CMZ), harbors neural progenitors. Neural progenitors have also been identified in the CMZ of postnatal chickens. In addition to progenitors in the CMZ, there is evidence that two separate progenitor populations are present in the neural retina of adult fish and that these progenitors participate in normal growth and/or regeneration of the retina in response to lesion. In vivo labeling analysis with bromodeoxyuridine (BrdU) shows that the pigmented portion of ciliary body of adult rats contains cells with proliferative potential. Indeed, when cultured in the presence of EGF and/or FGF2, these cells proliferate and give rise to neurospheres containing nestin-positive cells resembling those generated by embryonic

![Figure 1. Schematic representation of relationships between stem cells, progenitors, precursors, and differentiated neural cell types. Evidence suggests that separate neuronal and glial progenitors may not be present in the retina. The reverse broken lines indicating that stem cell-progenitor precursors can be reprogrammed, are based on recent observations of Clarke et al. and Kondo and Raff.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932904/)

![Figure 2. Neural stem cells-progenitors in the mammalian eye. Neural progenitors are present both in the embryonic retina (A–C) and adult ciliary body (D–F). These cells can be isolated and maintained in the culture in the presence of EGF and/or FGF2. In the presence of mitogens, these cells generate neurospheres containing proliferating cells (as shown by BrdU incorporation in B and E) that express the neuroectodermal stem cells marker, nestin (C, F).](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932904/)

retinal progenitors (Figs. 2D–F). These cells are multipotent and can differentiate along neuronal and glial lines. Unlike embryonic retinal progenitors, these cells can self-renew, because they clonally generate neurospheres. Therefore, they fulfill the basic criteria of stem cells. These cells express the retinal progenitor markers Chlx10, Rx, and Pax6, which suggests that they possess retina-specific properties and they can differentiate into retinal cells when exposed to conducive environment. The ability of these cells to self-renew, their plasticity, and their potential to express retinal phenotypes suggests a possibility that they represent a residual population of retinal stem cells. However, because these cells are derived from pigmented ciliary epithelium, there is likelihood that they acquire stem cell properties in vitro by reprogramming or dedifferentiation. Similar mechanisms have been invoked to explain the conversion of oligodendrocyte progenitors into neural stem cells. Investigation of these possibilities will be helped by prospective identification of neural stem cells, instead of characterizing them in response to mitogens.

**Therapeutic Uses of Neural Stem Cells-Progenitors**

Neural stem cells-progenitors can be used in two different but complementary ways to treat degenerative diseases. Although these approaches have not yet been used in ocular diseases, studies in a number of animal models of neurodegeneration suggest that they may be helpful in treating degenerative changes in the retina. In addition, in vitro models of retinal differentiation consisting of ocular neural stem cells-progenitors, in combination with DNA microarrays, may provide a powerful means of identifying differentiation and survival-promoting genes that can serve as potential targets to treat retinal degeneration (Fig. 3).

**Cell-Replacement Therapy**

Cell-replacement therapy exploits the plasticity of stem cells-progenitors to replace cells and repair tissues damaged by disease or injury. There are two approaches to cell replacement therapy: replacement of damaged cells with cultured stem cells-progenitors and regeneration or replacement of damaged cells with endogenous stem cells-progenitors.

The concept that neural stem cells-progenitors can be used to repopulate damaged brain areas is supported by the remarkable survival and differentiation potential of these cells when used in heterotopic transplantation. For example, hippocampal neural stem cells-progenitors transplanted in the rostral migratory zone not only survive but also migrate to the olfactory bulb where they differentiate into site-specific neurons. These properties of neural stem cells-progenitors may be useful for brain repair, based on early evidence emerging from transplantation studies performed in animal models of neurodegeneration. For example, transplanted neural progenitors have been shown to substitute efficiently for dysfunctional oligodendrocytes by myelinating axons in animal models of myelin dysfunction. Similarly, it has been demonstrated that a subset of neural progenitors differentiate into cells with dopaminergic properties and cause modest behavioral recovery when grafted into the striatum of an animal model of Parkinson’s disease.

Similar cell replacement therapy may work to reverse photoreceptor degeneration in ocular diseases if at least two prerequisites are met. First, the transplanted neural stem cells-progenitors must differentiate into photoreceptors. Second, the differentiated cells must establish contact with the second-order neurons. The first positive evidence of the viability of this approach was reported by Takahashi et al. and Young et al., who observed that adult hippocampal stem cells-progenitors, when transplanted in the vitreous of neonatal or adult eyes, survive and incorporate into the laminar structure of the host retina. Results in a more recent study have suggested that some of the incorporated adult hippocampal stem cells-progenitors can establish synaptic contact with the host’s retinal cells. However, in each of these studies, despite the transplanted cells’ incorporation within the host’s retina and their morphologic similarities to various retinal cell types, they failed to express any retina-specific markers. At least, two explanations are available for this failure. First, hippocampal neural stem cells may be intrinsically different from retinal stem cells and may not have the plasticity to differentiate into retina-specific neurons. This intrinsic difference between region-specific neural stem cells is probably due to pattern formation in the developing nervous system. Alternatively, hippocampal stem cells are plastic, but the host retina may not have the necessary cues to induce their differentiation into retinal neurons.

A more promising approach is to use ocular stem cells-progenitors that are known to have the capacity to generate retinal neurons. Neural stem cells-progenitors isolated from either embryonic retina or the adult ciliary body possess retina-specific properties and can differentiate preferentially into cells expressing photoreceptor-specific markers when co-cultured with neonatal retinal cells. As expected, when cultured retinal stem cells-progenitors were transplanted into the subretinal space of normal rat, the grafted cells not only survived but expressed photoreceptor-specific markers suggesting their differentiation into cells of photoreceptor lineage (Fig. 4). These findings suggest that these cells may be able to replace degenerating photoreceptors in animal models of ocular degenerative diseases. However, this approach also has limitations. Although the expression of photoreceptor-specific markers by the grafted cells is encouraging, these cells do not show migration and integration into host retina comparable to that observed in the hippocampal stem cells-progenitors. Therefore, conditions must be defined that promote both the structural and functional integration of retinal stem cells-progenitors within the retinal circuitry. One of the likely cues that mobilizes the transplanted cells to migrate and integrate into host tissue is injury. For example, the widespread migration and incorporation of adult hippocampal neural stem cells-progenitors was observed only in the adult retina that were either diseased or traumatized. This premise therefore suggests that degenerating retina may offer a more conducive environment for differentiation as well as efficient integration of transplanted retinal stem cells-progenitors.

The identification of stem cells in the adult brain, spinal cord, and eye has opened tantalizing possibilities of regeneration by mobilizing endogenous stem cell populations to respond to disease and injury. Regeneration in response to lesion has been studied in the retina of adult goldfish. It has been observed that continuous photoreceptors ablated by argon laser can be selectively regenerated from a pool of proliferating progenitors resident in the retina. The identification of a quiescent population of neural stem cells-progenitors in the adult ciliary body also opens an interesting possibility that these cells can be mobilized in vivo and recruited to replace degenerated retinal cells. The concept of healing the brain or eye from within was lent further credence by an elegant experiment in which a subset of pyramidal neurons in rat cortex was damaged by chromophore-induced apoptosis. These cells project to the thalamus and form the circuitry involved in cortical function. Neural stem cells-progenitors resident either in the cortex or in the subventricular zone were activated in response to the lesion and were recruited to replace some of the degenerated pyramidal neurons. In addition, the newly differentiated
Pyramidal neurons extended processes to the thalamus, suggesting that these cells partially restored the damaged circuitry. Evidence suggests that neural stem cells—progenitors may not be the only source of neural regeneration. Cells of glial lineage may also participate in the regeneration process in response to disease or injury by providing an alternative source of neural progenitors. This notion is supported by observations that glial precursors can be reprogrammed to become multipotential neural progenitors and that neural regeneration in injured adult goldfish and postnatal chicken retina may be supported by Müller glia.

**Ex Vivo Gene Therapy**

The ability to maintain and manipulate stem cells—progenitors in culture suggests that genetically engineered neural stem cells can be used to target gene products to sites of degeneration. These gene products can include survival-promoting factors to rescue degenerating neurons, factors that can act in an autocrine manner to promote survival and differentiation of grafted cells into site-specific neurons or to deliver neurotransmitter(s) to permit functional recovery. Proof of principle of ex vivo gene therapy has been provided by a study in which intrastitial transplantation of stem cells genetically modified to secrete nerve growth factor (NGF) provided protection to a vulnerable population of striatal neurons from excitotoxic degeneration in an animal model of Huntington’s disease. Similarly, ex vivo gene therapy could be used effectively as a neuroprotective strategy to prevent retinal cell loss in RP, AMD, and glaucoma and in diseases that cause retinal detachment. This notion is supported by observations emerging from
Barriers to the Therapeutic Use of Stem Cells-Progenitors

Although the studies described herein support the therapeutic application of neural stem cells-progenitors, there are outstanding issues and concerns that currently constitute barriers to the practical and successful use of neural stem cells in the clinical realm. First is the issue of availability of neural stem cells-progenitors in sufficient quantity for therapeutic purposes. This is a significant roadblock because, contrary to expectations, neural stem cells-progenitors have not been cultured in “unlimited” quantities. Identification of conditions that will allow the generation of multipotent progenitors over multiple passages would solve this problem. The use of embryonic stem (ES) cells is another potential solution, because it is generally believed that embryonic cells have a greater proliferative potential than adult stem cells. However, the use of ES cells requires caution, because they tend to differentiate spontaneously and in some instances have displayed oncogenic potential. Second, the issue of the effects of extended exposure to mitogens is important from the viewpoint of maintaining the inherent characteristics of primitive stem cells-progenitors from one generation to the next. It is important to know whether cells that are exposed to high concentrations of mitogens over several generations undergo genetic changes and therefore acquire potentials different from their parents. This requires the identification of a panel of markers of neural stem cells-progenitors so that the primitive characteristics of these cells can be followed over several generations. Third, knowledge of the fidelity, efficiency, and consistency of the differentiation of grafted stem cells-progenitors into tissue-specific cell types is critical in predicting the functional outcome of cell replacement therapy. Although, there is evidence that transplanted cells can differentiate into site-specific cells, details regarding the proportions of grafted cells that remain undifferentiated or that differentiate into some other cell types remain incomplete. This information is particularly critical for cell replacement therapy in highly ordered and laminated sensory structures such as the retina, where the presence of undesirable glia or neurons may exacerbate rather than solve problems. Lastly, the issue of the therapeutic potential of stem cells-progenitors derived from embryonic versus adult brain, is not only important from the viewpoint of stem cell biology, but also because of ethical concerns associated with the origin and use of fetal tissues. Although adult neural stem cells-progenitors have shown significant plasticity in the variety of cell types they can generate, whether they have potential similar to embryonic neural stem cells-progenitors in their utility as transplantation reagents is unknown.

Summary

Emerging evidence suggests that multipotential stem cells-progenitors, isolated from the brain, spinal cord, and eye hold considerable promise to elucidate fundamental issues of brain development and treat neurodegenerative diseases. Therapeutic applications of neural stem cells have special appeal for the treatment of otherwise intractable degenerative diseases of the eye. However, the field of neural stem cell research is relatively nascent. Many issues related to the therapeutic use of neural stem cells-progenitors have not yet been addressed. Strategies must be developed to identify and enrich neural stem cells-progenitors in a practical way. In addition, identification of optimal conditions for their maintenance, storage and differentiation into desirable cell types for cell-replacement therapy is critical. Therefore, the true potential of these cells in brain repair can only be realized with more information about mechanisms that regulate their proliferation and differentiation and by development of techniques that allow their prospective identification and enrichment.

Acknowledgments

The author thanks Pamela Raymond, Colin Barnstable, Carl Cameras, Angie Rizzino, Anne Kessinger, and Graham Sharp for critical reading of the manuscript and for insights and suggestions; Dave Chacko and Wally Thoreson for constructive criticisms; and Justin Madson for graphics.

References


Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932904/ on 06/25/2017

New Developments in Vision Research

Written for a broad audience, the articles in this column succinctly and provocatively review a rapidly changing area of visual science that shows progress and holds potential. Authors and topics are chosen by the Editor-in-Chief in collaboration with the Editorial Board.

To avoid bias, the Editor-in-Chief subjects these articles to the same rigorous peer review process to which all other IOVS articles are subjected. Space and reference limitations are imposed on the authors.

The purpose of this series is not the recognition of individual scientists, nor exhaustive review of a subject, but the stimulation of interest in a new research area.

Editor-in-Chief