NEURAL STEM CELLS:
Development and Transplantation

Cover illustration is a micrograph of a differentiating cortical neurosphere immunostained with antibodies for nestin (green) and propidium iodide (red); see Chapter 3 by Nakano and Kornblum.

NEURAL STEM CELLS:
Development and Transplantation

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PREFACE

During development of the central nervous system, multiple types of neurons and glial cells ultimately arise from self-renewing pluripotent embryonic stem cells. Little is known about the regulation of their differentiation into multipotent neural stem cells and their subsequent progeny. Neural stem cells are a topic of intense interest at the moment for two major reasons. First, they provide models for neural development that are easily manipulated and analyzed in vitro. Second, they are candidates for cellular and gene therapy of many intractable neurological disorders, e.g., Parkinson's disease, multiple sclerosis, spinal cord injury, and others. The availability of human neural stem cells is bringing us even closer to achieving the goal of effective cellular and molecular therapy for focal as well as disseminated neurological syndromes. Although there are numerous technical and ethical/legal problems yet to resolve, the enormous potential of this field of research has driven its exponential growth. This volume will be particularly useful for students, basic scientists, and clinicians in the academic or industrial sectors who have an interest in understanding neural development and/or its application to repairing the nervous system. In addition, it will provide vital information to those interested in the ethical/legal issues.

The current work on neural stem cells was preceded by studies of embryonal carcinoma (or teratocarcinoma) cells. This earlier work showed that these stem cells of the blastocyst stage could produce derivatives of all three germ layers (ectoderm, mesoderm and endoderm; Martin & Evans, 1975; McBurney, 1976) and could produce neuronal cells in vitro (Darmon et al., 1981). The latter study was the first to use serum-free N2 medium (Bottenstein and Sato, 1979) to generate large numbers of neurons from these pluripotent stem cells and to show a default mechanism of neural specification without a feeder layer, formation of embryoid bodies, or the presence of inducers, e.g., retinoic acid. These and other findings suggested that understanding some aspects of early neural development could indeed be derived from studying stem cells in vitro. It is interesting to review the comments of an NIH study section that evaluated a grant application I submitted in 1983 in which I proposed using the clonal 1003 mouse embryonal carcinoma cell line we described in Darmon et al. (1981) to isolate a neural stem cell line, generate monoclonal antibodies against different stages of differentiation to produce additional lineage markers, and determine the environmental signals that would induce neurotransmitter phenotypes other than cholinergic. I provided data showing I could obtain >95% postmitotic neurons that synthesized high levels of acetylcholine (but

not tyrosine hydroxylase, serotonin, or glutamic acid decarboxylase) and exhibited both regenerative responses and delayed rectification using patch clamp techniques (Bottenstein, 1985). No GFAP-positive astrocytes were generated. The critique stated that "one's confidence in such studies is shaken by the ease (amply demonstrated by experiments carried out by the principal investigator) with which neuronal characteristics can be changed by various manipulations of the culture environment" and "there was some skepticism whether this kind of phenomenology in culture can provide basic insight into the problem of differentiation." These statements were not prescient of where this field is now. There was also resistance during this time to using these cells as models of normal development due to their tumorigenic origin, even though it had been shown that transplanted teratocarcinoma cells could participate in normal development and integrate into the host (Brinster, 1974; Mintz & Illmensee, 1975) and 1003 cell cultures, after neural differentiation occurs, contain no undifferentiated stem cells and are unable to form tumors in nude mice (Darmon et al., 1982).

The use of serum-free N2 medium (Bottenstein and Sato, 1979) has been of great benefit in identifying neural stem/progenitor cells in vitro and permitting their differentiation. In addition to its widespread use for neural cultures in general, it made possible our initial findings with the 1003 pluripotent stem cells, the discovery of oligodendrocyte progenitor cells by Raff et al. (1983), and is extensively used by investigators in the neural stem cell field. The addition of epidermal growth factor to N2 medium permitted the expansion and detection of mouse embryonic and adult neural stem cells first described by Reynolds et al. (1992) and Reynolds and Weiss (1992), respectively.

A seminal discovery was the identification of neural stem cells in the adult mouse (Reynolds and Weiss, 1992), which suggested that generation of new neurons and oligodendrocytes in vivo might be possible after development was complete, contrary to the extant view that this did not occur. The activation and generation of new astrocytes at injury sites is well known and can inhibit the repair process. This needs to be considered in transplant studies. Current studies are only now addressing the issue of stimulation of endogenous stem cells to produce the desired neural progeny to participate in the repair process.

It is now clear that both embryonic and neural stem cell lines as well as embryonic and adult sources of neural stem cells provide an expandable source of neurons and glia that can be used for studies of neural development and for cellular transplants that may be able to affect repair of nervous system injuries or disorders. Five major issues require further study in this field. First is the absence of a library of stage-specific markers to

identify the lineage position of embryonic and neural stem cells more precisely. Some markers have been described but many more need to be identified. Second is the need to better understand the differentiation potential of cells derived from different sites in the nervous system and at different stages of neural development. Third is the need to discover additional regulators of differentiation into specific phenotypes, e.g., notably cholinergic neurons and myelinating oligodendrocytes, and their molecular mechanisms of action. Fourth is the problem of immunological rejection of transplants. One solution is the use of autologous transplants. The fifth is to formulate standardized culture methods for maintaining and handling neural stem cells before and during experiments and transplantations. This will require optimization of and consensus on a variety of parameters, including different culture media (basal medium and supplements) for proliferation and differentiation protocols, passage technique, and acceptable passage numbers for specific purposes. Standardization is essential for replicating the findings of various investigators in this field, for comparing data from different investigators, and in order to consistently produce desired differentiated phenotypes. Currently, there are multiple methods being used and this complicates analysis of experimental data and can result in variability in the repertoire of differentiated progeny.

The range of topics covered in this volume is wide and the authors were carefully selected for their expertise in the various subfields. I asked them to share their view of the "state of the art", its present limitations, and future perspectives. The book begins with a chapter on stem cells as models for neural development and neurological disorders to provide a context for the subsequent chapters. This is followed by a discussion of stem cell lineage and fate determination and subsequently a related chapter on stage-specific and cell fate markers. Traditional sources and properties of embryonic and neural stem cells are described as well as alternative transdifferentiated ones. Methods of purification of neural stem cells from heterogeneous tissue sources and their clonal analyses are included. The generation and properties of rodent and human embryonic and neural stem cell lines and their use in research and repair paradigms is covered. The following chapters discuss the regulation of survival, proliferation, and differentiation of neural stem cells and specifics regarding culture methods. The final five chapters address the use of neural stem cells for cellular and gene therapy. Two of these review the various animal transplantation studies and one discusses the exciting new topic of stimulation of endogenous neural stem cells. This is followed by a discussion of cellular therapy in humans directed at repairing injuries and diseases in the central nervous system. The final chapter reviews methods of regulating and modifying

neural stem cell/progenitor gene expression for multiple purposes that include human gene therapy.

In summary, although we may be at the early stages of understanding neural stem cell lineage, differentiation, and transplant potential, the goals of this area of research are clear and the interest level is very high. The new fields of bioinformatics, genomics, and proteomics and their associated techniques should provide further insights that will result in exciting new information about early neural development and its clinical application to humans with developmental, metabolic, immunological, degenerative, aging, traumatic, or ischemic disorders of genetic or epigenetic origin. While the intractability of many neurological disorders drives the clinical side of this field, caution is imperative and success will depend on the findings of the basic scientists and their wise application by clinicians.

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