神经干细胞和中枢神经系统发育

Stem Cells and CNS Development

Mahendra S.Rao

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Totowa,New Jersey

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Preface

Neural differentiation is an early embryonic event that occurs soon after germ layer specification from the blastula. The early formed ectoderm undergoes further patterning to separate into two identifiable components, the presumptive neural ectoderm and the presumptive epidermis. Neural tissue segregates as a clearly demarcated epithelium termed the neuroepithelium (or neuroectoderm). This neuroepithelium generates the central nervous system (CNS), whereas cells at the margins of the neuroepithelium will generate the peripheral nervous system (PNS). A variety of evidence has been accumulated to show that the process of neural differentiation involves a sequential restriction in differentiation potential.

A fundamental breakthrough in our understanding of nervous system development was the identification of multipotent neural stem cells (neurospheres) about 10 years ago. Dr. Samuel Weiss and colleagues showed that EGF (epidermal growth factor)-dependent stem cells could be harvested from different brain regions at different developmental stages and that these could be maintained over multiple passages in vitro. The original finding that EGF-dependent neural stem cells exist has been replicated and extended by many investigators, and there has been a veritable explosion of research on stem cells, their role in normal development, and their potential therapeutic uses. Different classes of neural stem cells have been identified, new markers described, cell lines generated, and factors that regulate the differentiation process characterized. Other investigators have shown that these pluripotent stem cells likely generate CNS and PNS derivatives via the generation of intermediate lineage restricted precursors that differ from each other and from multipotent stem cells. The therapeutic implications of accessing a virtually unlimited population of homogenous progenitor cells to treat CNS disorders or for gene and drug discovery has not escaped investigators, and several companies have been formed to exploit stem cell technology and several research institutions have initiated transplant studies. This rapid transition from a basic discovery to clinical trials is both surprising and unprecedented.

In *Stem Cells and CNS Development*, I have invited some of the leading authorities in the field of neural stem cell biology to summarize their findings and describe how these results may lead to novel therapies. The first part of the book surveys the various kinds of stem cells, progenitor cells, and precursors that have been described, while the second half describes how these cells are beginning to be used for therapeutic purposes. It is my hope that this book will serve as a valuable compendium of practical information on the current state of the field for all those engaged in this research.

Mahendra Rao

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Defining Neural Stem Cells and Their Role in Normal Development of the Nervous System

Sally Temple

INTRODUCTION

Stem cells are key players in the development and maintenance of specific tissues in most animals. The discovery of stem cells in the central and peripheral nervous systems (CNS and PNS) is a relatively recent event. First, continued neurogenesis (neuron generation) in the adult pointed to a long-lived progenitor cell *(1)*. Isolation of stem-like cells from the embryonic CNS, including basal forebrain *(2,3)*, cerebral cortex *(4)*, hippocampus *(5)*, spinal cord *(6)*, and the PNS *(7)* as well as evidence for multipotent, stem-like progenitors in vivo *(8–10)*, have indicated that they are important components of the developing nervous system (Fig. 1). Much excitement surrounded the isolation of adult stem cells from known neurogenic (neuron-generating) zones (the subventricular zone and hippocampal dentate gyrus) in rat, primate, and human (reviewed in ref. *11*). More recent evidence for continued neurogenesis in areas not previously considered to be neurogenic, such as the spinal cord *(12,13)* and neocortex *(14,15)*, suggests that stem cells may be a more widespread feature of the adult nervous system than previously imagined (Fig. 1).

Current research is focused on identifying the characteristics and functions of neural stem cells, in both developing and adult systems, to reveal their place in CNS biology and to facilitate the harnessing of these remarkable cells for repairing damaged nervous systems. To help us understand more about neural stem cells, we can explore a wealth of knowledge concerning stem cells in other systems and organisms, looking for common themes that might explain the essential stem cell state, as well as differences that might reveal the uniqueness of neural stem cells.

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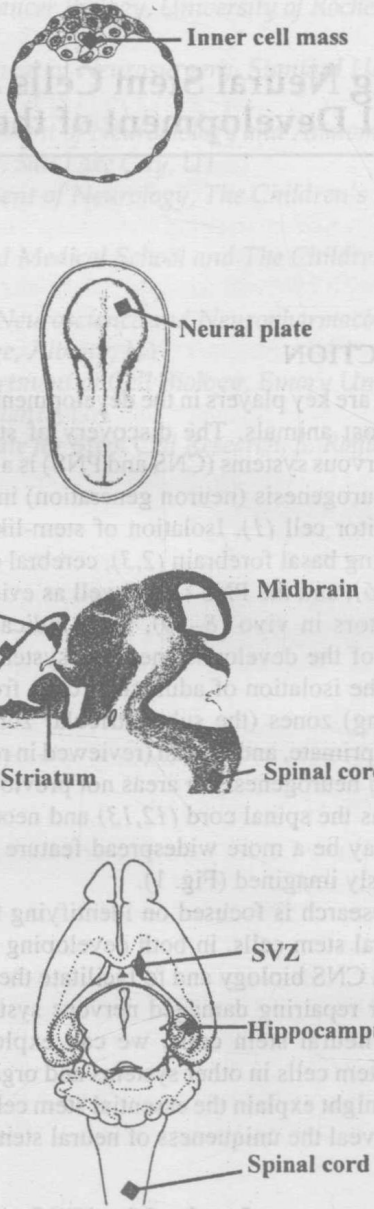

Fig. 1. Multipotent stem cells generating neurons and glia have been isolated from the early embryo through to adult.

The term *stem cell* has a number of different meanings to different people — depending on the system being analyzed and the perspective of the researcher using the term. A general definition is "a cell that is capable of both self-renewal and differentiation." Most researchers in the stem cell field would agree with this baseline definition. In this chapter, progenitor is used as a blanket term to describe any dividing cell that can generate differentiated progeny, whether or not it can self-renew, and the term precursor is used to describe a cell that is committed to a specific fate.

Besides the two fundamental features of self-renewal and differentiation, other specific characteristics have been attributed to stem cells, some of which apply only to stem cells in particular systems, rather than being a general feature of all stem cells, as illustrated in a recent review *(16)*. This chapter discusses some of the general terminology used to describe and define stem cells, focusing on the terms that might apply to neural stem cells, especially during normal neural development.

DOES THE NERVOUS SYSTEM ARISE FROM A SINGLE STEM CELL TYPE?

The *potency* of a progenitor cell represents the range of cell types it can generate. In a general model, stem cells are the basic cell type from which all others emanate through restriction of potency. The most primitive cells are considered *totipotent* — able to generate the entire organism or the entire tissue that is being considered. Subsequent restriction of potency can occur within a stem lineage, so that stem cells may be *pluripotent* or *multipotent* (making many different cell types, but perhaps not all), *oligopotent* (having a few choices), or *unipotent* (making one type of progeny). The epidermal stem cell in the adult skin is thought of as unipotent, as it appears to generate solely keratinocytes. Stem cells may also release multipotent or oligopotent or unipotent restricted progenitor cells, which serve as transit populations to expand the stem cell progeny prior to terminal differentiation. Formation of blood is believed to follow this general model, in which a primitive hemopoietic stem cell proceeds via restriction of potency within its stem and progenitor progeny toward final hemopoietic cell fates *(17)*.

It is not clear at this point whether there is a stem cell type that can give rise to the entire nervous system with its wealth of neuronal and glial cell types. Embryonic stem cells (ES), which are considered totipotent themselves, can generate neural stem-like cells *(18)*. In tissue culture, or on transplantation into the embryo, the neural derivatives of ES cells can integrate and generate a wide variety of cell types *(19–22)*. Whether they can generate the entire range of neural cell types remains to be resolved, but

these cells may emerge as the most plastic neural stem cells. A stem cell that can generate both PNS and CNS derivatives exists in the early embryo, suggesting it has a broad range of potency *(23)*. Multipotent stem cells have been isolated from various regions of the developing and adult CNS (reviewed in refs. *11* and *24–29*) and from the neural crest, which gives rise to the PNS *(30–32)*. Dissociated embryonic neural progenitor populations that include some stem cells can be transplanted from one region of the developing CNS into other regions and show remarkable properties of integration *(33)*, producing cells that resemble those endogenous to the transplant site.

The contribution of stem cells versus other progenitor cells to the differentiated cells in the new site has not yet been determined, but these data suggest that the stem cell may be able to respond to regional information and differentiate accordingly. As with the ES cells, it is not yet clear whether nervous system-derived stem cells can generate the entire range of neural cell types. The results of some studies indicate that their potency may be limited. After transplantation, differentiating cells may acquire the morphology typical of their new location but do not always express its characteristic molecular markers, suggesting a lack of complete integration. For example, telencephalic cells grafted into the embryonic diencephalon or mesencephalon continue to express telencephalic markers, even into adulthood *(34)*. Similarly, hippocampus-derived stem cells on transplantation into the adult retina expressed appropriate neuronal and glial morphologies but not end-stage markers of retinal differentiation *(35)*. Furthermore, cells from one CNS region may incorporate more successfully in some regions than others. In one study, mouse progenitor cells from the lateral ganglionic eminence (LGE) or from the ventral mesencephalon (VM) were dissociated and injected into the lateral ventricles of embryonic rats at a similar stage of development. The LGE cells preferentially incorporated into the striatum, whereas the VM cells preferentially incorporated into the hypothalamus and midbrain. None of these cells, derived from basal CNS regions, incorporated efficiently into dorsal structures such as the cerebral cortex and hippocampus *(36)*.

These data may indicate specific regional restrictions in potency. Progenitor cells from embryonic ferret cerebral cortex transplanted into an older cortex can produce age-appropriate cells, but cells from an older cortex, upon transplantation into a younger cortex, are unable to make age-appropriate cell types *(37)*. Similarly, mid-hindbrain progenitors show a wider degree of regional incorporation at embryonic day (E)10.5 than at E13.5 *(38)*. These data indicate a temporal restriction in potency; this has particular significance when we consider the range of potency of adult neural stem cells. They can generate neurons, astrocytes, and oligodendrocytes,

but the types of neurons and glia generated may be limited. In vivo, adult neural stem cells are primed to generate interneurons, and this appears to be their behavior after transplantation to adult neurogenic zones *(39,40)*. When placed in developing nervous system areas, adult stem cells can generate more cell types than they can after transplantation into the adult (for example, as in the retina; *35*). It is important, however, to establish whether they are capable of generating the major projection neurons in the CNS, most of which arise early in embryonic development. Furthermore, adult neural stem cells cultured for long periods may become increasingly biased toward production of glial cells, in some cases eventually losing neurogenic potential *(41)*.

Besides generating a wide variety of neural cells, stem cells derived from the nervous system may also be capable of producing cells of other tissues. In one remarkable study, it has been shown that a stem cell derived from the adult CNS could generate blood cells after transplantation into the bone marrow of an irradiated host *(42)*. Although we marvel at the plasticity of the stem cell involved, this experiment does not speak to its potency in generating *neural* cell types. Also, it was not clear what the characteristics of the starting cell were. Was it a neural stem cell that acquired the features of a hemopoietic stem cell through transdifferentiation or dedifferentiation? Or could there be a small population of totipotent stem cells in the brain, perhaps even derivatives of migratory germ cells that did not reach the germinal ridges *(43)*, that was responsible for blood cell production in this experiment? If the cell was indeed a neural stem cell, is its remarkable plasticity a reflection of its normal biology, or could it be the result of growing for long periods in tissue culture prior to transplantation? Undoubtedly answers to these questions will be found soon and will help us understand the types of stem cells present in the adult brain, their normal potency, and how long-term cell culture might alter them.

Although the presence of a neural stem cell capable of generating all neural tissue has not yet been documented, the idea that normal development might proceed through gradual restriction of potency, as occurs in the blood system, is supported by studies of developing nervous system stem cells. In the CNS and PNS, multipotent progenitors generate restricted progenitors for neurons and glial cells *(28)*. The heterochronic cortical transplantation studies mentioned previously also support this model. How might restriction of potency occur within the stem cell? It has been suggested for stem cells in a number of systems that the more primitive stem cells express a wide variety of transcripts at a low level, perhaps maintaining genes in an "open" chromatin configuration that is poised for transcription. Restriction

of potency would proceed by turning off some genes and enhancing expression of others *(44,45)*. For the nervous system, this might explain why fetal glutamatergic and γ-aminobutyric acid (GABA)ergic cortical neurons both express glutamic acid decarboxylase (GAD) transcripts *(46)*, or why neural progenitor cells in the spinal cord express genes characteristic of both interneurons and motorneurons before selecting one or the other phenotype *(47,48)*. Restriction of potency might involve a hierarchy of transcription factors that drive the cell toward a particular fate. In *Drosophila*, proneural genes, e.g., achaete, scute, and atonal, confer competence for neural differentiation, via a chain of transcription factor activation *(49)*.

Homologs of these genes may operate similarly in vertebrates. For example, Mash-1, a mouse homolog of *Drosophila* achaete/scute, stimulates expression of the transcription factor Phox2a, which in turn stimulates expression of panneuronal properties and of the receptor c-RET, specifying subtypes of autonomic lineage cells *(30,50,51)*. Mash-1 initiates a cascade with different components in the olfactory system *(52)*. It also appears important for generating neurons in the ventral embryonic forebrain, perhaps via influence on Notch signaling *(53,54)*. Given the prevalence of achaete/scute and atonal homologs as well as other members of the bHLH transcription factor family, in the developing vertebrate nervous system, there is undoubtedly much to be learned about how these factors might interact within stem cell lineages to generate diverse neural cell fates.

STEM CELL POTENCY AND REGIONAL IDENTITY WITHIN THE EMERGING NERVOUS SYSTEM

It is important to note that potency is empirically determined — a cell is challenged with specific environmental signals, and we examine what types of cells it can generate. Hence, a cell that develops in a normal context may not show its full range of potency. A cell present in the cerebellum may be capable of making motor neurons if transplanted into the spinal cord — this reveals its plasticity, but not its normal developmental biology. Furthermore, it cannot be concluded from this experiment that early neural stem cells are undifferentiated and do not possess regional information, only that the information that they might have can be changed. In fact, it is likely that stem cells normally acquire regional information very early. If progenitor cells are removed from different regions of the early embryonic nervous system and placed in tissue culture, they develop into cell types characteristic of the region from which they were derived. Thus embryonic retina progenitors give retinal cells, embryonic cerebellar progenitors produce cerebellar cells, and embryonic neural crest progenitors generate typical PNS derivatives.