Cellular signaling in neural stem cells: implications for restorative neurosurgery

Yvette D. Marquez, M.S., Michael Y. Wang, M.D., and Charles Y. Liu, M.D., Ph.D.

Department of Neurological Surgery, Keck School of Medicine, University of Southern California, Los Angeles; and Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California

Over the course of the past few decades, it has become apparent that in contrast to previously held beliefs, the adult central nervous system (CNS) may have the capability of regeneration and repair. This greatly expands the possibilities for the future treatment of CNS disorders, with the potential strategies of treatment targeting the entire scope of neurological diseases. Indeed, there is now ample evidence that stem cells exist in the CNS throughout life, and the progeny of these stem cells may have the ability to assume the functional role of neural cells that have been lost. The existence of stem cells is no longer in dispute. In addition, once transplanted, stem cells have been shown to survive, migrate, and differentiate. Nevertheless, the clinical utility of stem cell therapy for neurorestoration remains elusive. Without question, the control of the behavior of stem cells for therapeutic advantage poses considerable challenges. In this paper, the authors discuss the cellular signaling processes that influence the behavior of stem cells. These signaling processes take place in the microenvironment of the stem cell known as the niche. Also considered are the implications attending the replication and manipulation of elements of the stem cell niche to restore function in the CNS by using stem cell therapy.

Key Words • neural stem cell • stem cell fate • cell signaling • stem cell niche

OVERVIEW

The treatment of CNS disorders has traditionally been limited by the belief that, unlike other tissue such as the skin or liver, the CNS is not capable of repair and regeneration. Over the course of the past few decades, however, it has become apparent that the adult CNS may in fact have the capability of repair and regeneration. The concept of neurorestoration refers to the replacement of cellular and structural elements that have been lost, and consequent restoration of function.

The disease processes that represent potential targets for this mode of therapy span the scope of neurological disorders. For example, neurodegenerative disorders such as Parkinson disease have been the focus of tremendous attention. Huntington disease is another potential target. Patients with white matter and demyelinating diseases such as multiple sclerosis could also benefit from the replacement of the cellular elements that contribute the myelin sheaths of axon tracts. Suppression of seizures in patients with epilepsy and recovery of function after stroke have also been identified as potential goals. Furthermore, pediatric patients suffering from abnormal neurodevelopment and victims of traumatic injury to the brain, spinal cord, and peripheral nerves could benefit from this treatment, with possible restoration of normal function.

Cellular transplantation therapy is one strategy that plays a central role in neurorestoration. In the past, efforts to increase the levels of dopamine in the basal ganglia to treat Parkinson disease led workers to transplant adrenal medul- lary grafts. In addition, progenitors harvested from fetal tissue have been used as a source of transplantable neural precursor cells. Unfortunately, the transplantation of primary tissue would require the preparation of graft material from multiple fetuses for each patient. Clearly, the limited availability of fetal tissue and the moral and ethical objections to its use present serious social and political barriers to its further exploration and development, and most certainly to its future widespread clinical application.

In the search for another source of tissue for transplantation, stem cells have received a tremendous amount of attention, both in the scientific and in the popular literature. Broadly defined, stem cells are multipotent entities that are capable of self-renewal and proliferation into the differentiated cells of tissues and organs. In the nervous system, the NSCs would differentiate into all the cellular elements of the CNS, including neuronal subtypes, oligodendroglia, astrocytes, Schwann cells, and neural crest derivatives such as smooth-muscle cells.

Abbreviations used in this paper: CNS = central nervous system; DSL = Delta, Serrate, LAG-2; NSC = neural stem cell.
Two general categories of stem cells (embryonic and adult) have been identified as potentially capable of generating adequate quantities of graft material for practical utility. Embryonic stem cells that are derived from the inner cell mass of the embryonic blastula could be clonogenically expanded to yield large quantities of tissue to treat multiple patients. Furthermore, consistent with the initial findings, stem cells have been identified in certain areas of the adult brain. Therefore, it appears that neurogenesis persists well into adulthood, and that these adult stem cells could potentially be mobilized to migrate and differentiate to replace cells that have been lost. This could be accomplished either in vivo, directly from the natural niches of these stem cells in the brain, or after in vitro modification or clonogenic expansion.

Little question remains about the existence of NSCs. Furthermore, there is now little doubt that stem cells can be harvested and transplanted, after which they survive, migrate, differentiate, and in some animal models even appear to ameliorate “neurological deficits.” In addition, there is even evidence that NSCs can be induced to “activate” in response to insults. Nevertheless, the clinical utility of stem cell therapy for neurorestoration remains elusive. Without question, the control of the behavior of stem cells for therapeutic advantage poses considerable challenges. In this paper, we discuss the cellular signaling processes that influence the behavior of stem cells. These signaling processes take place in the microenvironment of the stem cell known as the niche. Our ultimate ability to use stem cells effectively for therapeutic purposes may hinge on our understanding and manipulation of these signaling processes.

CELLULAR SIGNALING AND NSC BEHAVIOR

During the normal process of CNS development, multipotent neural precursors determine cell fate and migrate to form the familiar and appropriate layers and patterns. These choices are determined by a combination of intrinsic and extrinsic signals. Intrinsic signals can be regarded as pre-programmed subroutines in the genetic program of the precursor cells. These subroutines are activated and modulated by a sequential pattern of spatially and temporally organized extrinsic signals. The identity and temporal and spatial order of these intrinsic and extrinsic signals has been the subject of extremely active investigation in the field of neuroembryology, and many signaling paradigms have already been elucidated.

Several general signaling modalities exist. In the process of inductive signaling, adjacent cells acquire different fates through their selective exposure to locally acting extrinsic signals. In a slight modification, gradient signaling refers to the dose-dependent response to extrinsic signals by adjacent cells, with more proximal cells experiencing a higher signal concentration and thus choosing a fate different from that of cells more distal to the signal source. With a higher degree of complexity, the cells providing the signal to the neural precursors may themselves be subject to an antagonist signal provided by yet another cell. In combinatorial signaling, precursor cells choose fates in response to two separate signals. Finally, in the contact-mediated modality of lateral signaling, small relative differences between signals provided by interacting cells are amplified in a feedback mechanism to cause dramatic differences in the fates chosen by the signaling cells.

Similar to the processes known to exist in normal neuroembryological development, intrinsic and extrinsic signals are important in stem cell differentiation and migration. In response to local environmental cues, decisions are made regarding fate. Stem cells exist in niches in which extrinsic signals modulate the intrinsic signals that drive self-renewal and determination of cell fate. The extrinsic signals found in the niche can be soluble signals from either a distant or a local source. Examples of soluble signals include stem cell mitogens such as fibroblast growth factor–2, a glycosylated form of the cysteine protease inhibitor cystatin C, epidermal growth factor, neuregulin-1, bone morphogenetic proteins, and the transforming growth factor–β and Wnt families of signaling proteins. In addition to soluble factors, contact-mediated factors such as the Notch signaling system can regulate cell fate (Fig. 1C). Finally, proteins such as β1 integrins found in the extracellular matrix (Fig. 1D) are another important modality of contact-mediated signaling in stem cell niches. In the presence of multiple cues, the cell integrates the signals (Fig. 1E) and chooses self-renewal or a pathway of differentiation.

Consideration of the Notch signaling system demonstrates some of the elements of signaling through integral membrane proteins. Notch is a very strong extrinsic signaling modality that has been shown to be an important determinant of cell fate during development in a wide spectrum of tissue types, from the hematopoietic system to the CNS, and it has been evolutionarily conserved across species. Neighboring cells in developing tissues communicate through Notch signals to direct cell fate decisions. Neighbors may be equivalent or biased in response to other signals so that one cell is the signaler and the other is the receiver. This process segregates specific cell lineages from clusters and helps define borders. It is also important in the maintenance of the differentiated state and has been implicated in neoplastic processes such as leukemia and cervical cancer. Furthermore, defects in its ligand and receptor are known to be important in the Alagille and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy congenital syndromes, respectively. Most importantly for neurorestoration, however, it is the strongest known signal for gliogenesis, and appears to be of paramount importance in the choice of fates between neurons and glia.

The Notch receptor was first characterized in Drosophila melanogaster and is known to be a 300-kD single-pass transmembrane receptor. The extracellular domain contains 36 tandem epidermal growth factor–like repeats and three cysteine-rich LIN-12/Notch repeats. The intracellular domain consists of six tandem ankyrin repeats, a glutamine-rich domain (opa), and a PEST sequence. The intracellular and extracellular domains of the Notch receptor are noncovalently linked, as indicated in the schematic drawing (Fig. 1). Similar receptors have been identified across species, including the nematode Caenorhabditis elegans, sea urchins, and vertebrates, including rodents and humans.

The ligands to the Notch receptor belong to the DSL family of transmembrane proteins. This ligand family is defined by the unique DSL domain near the amino terminus.

Y. D. Marquez, M. Y. Wang, and C. Y. Liu

Neurosurg. Focus / Volume 19 / September, 2005
Cellular signaling in stem cell niche

Fig. 1. Schematic representation of a stem cell niche. The stem cell has a preprogrammed set of intrinsic signals that determine its choice of fate and pattern formation. This is modulated by extrinsic signals in the form of soluble and contact-mediated modalities, which may interact with receptors on the surface of the stem cell. Soluble factors may come from distant sources (A) or be locally secreted (B). Contact-mediated signals may be presented by a neighboring cell (C) or by the extracellular matrix ([ECM], D). The stem cell integrates the signals and modifies its intrinsically determined fate and patterning decision (E).

of the proteins. In addition to the DSL domain, these ligands also contain tandem epidermal growth factor repeats of varying numbers, a cysteine-rich region, a transmembrane domain, and a nonfunctional intracellular domain. Similar to the Notch receptor, DSL ligands have been identified in organisms that span the phylogenetic scale, including humans. Known DSL ligands include Delta and Serrate in D. melanogaster, LAG-2 and APX-1 in C. elegans, xDelta1 in Xenopus spp., mDelta1ike1 and mSerrate1 in mice, and rJagged in rats. In addition, hDelta1, hJagged1, and hJagged2 have been identified in humans. Comparisons of DSL proteins across species show remarkable conservation, indicating an important role that has been persistently over time.10

IMPLICATIONS FOR RESTORATIVE NEUROSURGERY

With the therapeutic application of NSCs for neurorestoration in mind, a clearer picture is emerging. Both in normal neurodevelopment and stem cell biology, the precursor cells display preprogrammed behavior modified by cues from the local environment. The fundamental assumption is that differentiation and predictable behavior of NSCs can be achieved if the appropriate cocktail of soluble/diffusible or contact-mediated signals is present. In addition, several corollary considerations are quickly evident. For example, can we use NSCs from different sources in an equivalent fashion? The answer to this important question requires that we understand the developmental potential of all the types of NSCs.

This understanding may not be achievable with the methods currently available for the study and isolation of NSCs. After NSCs are harvested and identified, they are clonogenically expanded in floating cultures outside of their natural niches. Stem cells are known to change and dedifferentiate over time in the absence of normal environmental cues. Therefore, their developmental potential may be hopelessly obscured outside of their niches. In addition, even if the stem cells maintain their developmental potential when eventually transplanted, their long-term fate and thus therapeutic efficacy may depend on the environmental signals present in the transplantation site. The stem cells may need to be modified in vitro prior to transplantation and deliberately programmed to differentiate along certain lines. Alternatively, after transplantation, the neighboring cells in the transplantation site and eventual integration sites may need to train the new stem cells, and the efficacy of the therapy may depend on the effectiveness of the training. Furthermore, in the normal embryological process, the extrinsic signal that determines appropriate development is organized not only temporally but also spatially, with a three-dimensional matrix of graded positional signals that is obviously absent in current in vitro systems, and it is perhaps also absent in vivo at the target site of therapy.

CONCLUSIONS

Given these considerations, it would appear that our ability to use NSCs effectively for therapeutic purposes may be critically dependent on our ability to manipulate the signals that determine stem cell behavior in a temporal and spatially appropriate fashion, both at the treatment target site and during the in vitro processing before transplantation. Fortunately, this is an area of extremely active investigation, with new signaling modalities and ways to manipulate them being elucidated. The promise of NSCs may ultimately be realized not merely by their existence, but also by our ability to control their behavior. For neurosurgeons, this may mean that the microenvironment into which stem cells are transplanted may be as important as the cells themselves and the anatomical target.

References

9. Garcia-Alonso L, Romani S, Jimenez F: The EGF and FGF re-

Y. D. Marquez, M. Y. Wang, and C. Y. Liu

Manuscript received August 11, 2005. Accepted in final form August 25, 2005. Address reprint requests to: Charles Y. Liu, M.D., Ph.D., 1200 North State Street #5046, Los Angeles, California 90033. email: