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Global Gene and Cell Replacement Strategies via Stem Cells

Kook In Park, MD, PhD

Department of Pediatrics & Pharmacology, Yonsei University College of Medicine

Cell-based therapies such as neural transplantation have, until recently, been reserved for focal or regionally restricted neurologic diseases. These are best exemplified by Parkinson's disease where the goal has been the engraftment and enhanced survival of dopamine-producing cells within the striatum, or by forestalling degeneration of dopaminergic neurons within the substantia nigra. However, the pathologic lesions of most neurogenetic diseases are usually widely disseminated in the brain and spinal cord and have not typically been regarded as within the purview of neural transplantation. Such diseases include not only the inherited neurodegenerative diseases of the pediatric age group (e.g., lysosomal storage diseases, leukodystrophies, inborn errors of metabolism, hypoxic-ischemic encephalopathy), but also such adult maladies as Alzheimers disease, Huntingtons disease, multi-infarct dementia, multiple sclerosis, amyotrophic lateral sclerosis, and brain tumors (especially glioblastomas). Therapeutic approaches for such global problems have typically depended on pharmacologic or genetic interventions; they have been regarded as beyond the purview of cellular-mediated approaches.

Multipotent neural stem cells (NSCs) are operationally defined by their ability to self-renew, to differentiate into cells of all glial and neuronal lineages throughout the neuraxis, and to populate developing or degenerating CNS regions. Thus their use as graft material can be considered analogous to hematopoietic stem cell-mediated reconstitution and gene transfer. The recognition that NSCs propagated in culture could be reimplanted into mammalian brain, where they might integrate appropriately *throughout* the mammalian CNS and stably express foreign genes, has unveiled a new role for neural transplantation and gene therapy and a possible strategy for addressing the CNS manifestations of diseases that heretofore had been refractory to intervention.

Numerous subsequent studies over the past decade reaffirmed that neural progenitors from many regions and developmental stages could be maintained, perpetuated, and passaged *in vitro* by a number of epigenetic and genetic methods. Examples include the transduction of genes interacting with cell cycle proteins (e.g., v*myc*) and by mitogen stimulation (e.g., EGF and/or bFGF). Some o these methods may operate through common cellular mechanisms. This speculation is supported by the observation that many progenitor cell lines behave similarly in their ability to reintegrate into the CNS despite the fact that they were generated by different methods, obtained from various locations, and reimplanted into various CNS regions. Some of these NSC lines appear sufficiently plastic to participate in normal CNS development from germinal zones of multiple regions along the neuraxis and at multiple stages of development from embryo to old age. They appear, as well, to model the *in vitro* and *in vivo* behavior of some primary fetal and adult neural cells, suggesting that insights gleaned from these NSC lines may legitimately reflect the potential of CNS progenito or stem cells.

Some of the inherent biologic properties of NSCs may circumvent some of the limitations of other techniques for treating metabolic, degenerative, or other widespread lesions in the brain. They are easy to administer (often directly into the cerebral ventricles), are readily engraftable, and circumvent the blood-brain-barrier. A preconditioning regime is not required prior to administration as is required for bone marrow transplantation. One important property of NSCs is

their apparent ability to develop into integral cytoarchitectural components of many regions throughout the host brain as neurons, astrocytes, oligodendrocytes, and even incompletely differentiated but quiescent progenitors. Therefore, they may be able to replace a range of missing or dysfunctional neural cell types. A given NSC clone can give rise to multiple cell types within the same region. This is important in the likely situation where return of function may require the reconstitution of the whole milieu of a given region -- e.g., not just the neurons but also the glia and support cells required to nurture, detoxify, and/or myelinate the neurons. They appear to respond *in vivo* to neurogenic signals not only when they occur appropriately during development, but even when induced at later stages by certain neurodegenerative processes, e.g. during apoptosis. NSCs may be attracted to regions of neurodegeneration in the young as well as in the aged.

NSCs also appear to accommodate to the region of engraftment, perhaps obviating the necessity for obtaining donor cells from many specific CNS regions or the imperative for precise targeting during reimplantation. The cells might express certain genes of interest intrinsically (e.g., many neurotrophic factors), or they can be engineered *ex vivo* to do so since they are readily transduced by gene transfer vectors. These gene products can be delivered to the host CNS in a direct, immediate, and stable manner. While NSCs can migrate and integrate widely throughout the brain particularly well when implanted into *germinal* zones, allowing reconstitution of enzyme or cellular deficiencies in a global manner, this extensive migratory ability is present even in the parenchyma of the diseased adult and aged brain. Despite their extensive plasticity, NSCs never give rise to cell types inappropriate to the brain (e.g., muscle, bone, teeth) or yield neoplasms.