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Gene Therapy and Cell Transplantation for Alzheimer's Disease and Spinal Cord Injury

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The targeted delivery of genes and the transplantation of suitable cell types into the adult nervous system have received considerable interest over the last years. The development of improved vector systems for *in vivo* gene delivery and the discovery of neural stem cells in the adult nervous system have opened new venues for potential therapeutic intervention in progressive neurodegenerative disease and nervous system injury. Thus, strategies have evolved for the delivery of potentially neuroprotective molecules, such as neurotrophic factors, and the replacement of cells and tissue lost due to CNS injury and degeneration.

Key Words: Gene therapy, alzheimer's disease, spinal cord injury

Neuroprotective strategies are especially interesting in neurodegenerative diseases such as Alzheimer's disease (AD), where neurons in the central nervous system undergo widespread degeneration. One of the systems severely affected in AD are the cholinergic neurons in the basal forebrain. Loss of cholinergic function is closely correlated with AD pathology and decline of cognitive function and the only partially effective treatment for AD targets the cholinergic system (using anti-cholinesterase compounds).

In the mid 1980's, several groups discovered that nerve growth factor (NGF), the first neurotrophic factor cloned, can rescue cholinergic neurons in the medial septum from lesion-induced degeneration in adult rodents.[1,2] Subsequent studies demonstrated the same neuroprotective effects of intracerebroventricular infusions of NGF in the non-human primate brain.[3-5] These results raised the possibility that NGF might be able to ameliorate cholinergic deficits in Alzheimer's disease. This was further supported by rodent studies that showed that infusions of NGF prevent the degeneration of cholinergic neurons in the nucleus basalis after excitotoxic lesions,[6] and that NGF can reverse age-related declines in cholinergic neuronal morphology and prevent age-associated deficits in memory function in aged rats.[7,8]

As a result, clinical trials with a few patients were conducted that had to be discontinued due to the adverse effects observed following NGF infusions into the ventricles. These adverse effects include pain syndromes, migration and proliferation of Schwann cells and hypophagia, and result from effects of NGF on non-targeted structures in the central and peripheral nervous system.[9] For NGF to be therapeutically useful it needs to be delivered in a well-targeted, spatially restricted manner. As an alternative to ICV infusions *ex vivo* gene delivery of NGF was tested in several studies demonstrating that cellularly-delivered NGF was efficient in preventing the degeneration of basal forebrain cholinergic neurons,[10] and could also ameliorate behavioral deficits in aged memory-impaired rats.[11,12] Subsequent studies in monkeys determined that NGF delivered by genetically modified cells prevents the lesion

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induced loss of medial septal cholinergic neurons similar as previous NGF infusions.[13,14] Furthermore, NGF delivery by genetically modified primary fibroblasts reversed the age-related decline in the number of neurons in the nucleus basalis labeled for the low-affinity nerve growth factor receptor p75, a marker for basal forebrain cholinergic neurons.[15] In addition, cholinergic innervation in the cortex was restored to levels of young animals.[16]

Thus, evidence from rodent and primate studies suggested that NGF delivered by genetically modified fibroblasts in a well-targeted, spatially restricted and intraparenchymal manner to target neurons in the basal forebrain might be effective in slowing or preventing the degeneration of cholinergic neurons in Alzheimer's disease.

To establish a safety and toxicity profile of NGF delivery by genetically modified fibroblasts additional monkeys received autologous fibroblast grafts expressing NGF. Over a wide range of cell volumes signs of toxicity such as weight loss, pathological responses of Schwann cells and general indices of pain were not observed. NGF could not be detected in the cerebrospinal fluid, cellular grafts did not form tumors and cells did not migrate from the injection site.

Based on the extensive preclinical data on the efficacy and safety of NGF delivery by genetically modified cells, a phase I clinical trial of NGF gene delivery for Alzheimer's disease has been initiated at the University of California, San Diego. Eight patients with early stage Alzheimer's disease have been enrolled in the study. Patients received increasing doses of NGF secreting autologous fibroblasts implanted in the region of the nucleus basalis of Meynert. The first subject underwent cell implantation in April 2001, the study was completed in November 2003 with a one-year follow-up of the last implanted subject. Data obtained from this study are currently evaluated to address the efficiency and safety of NGF gene therapy in AD patients.

Studies described above focused on *ex vivo* gene therapy rather than *in vivo* gene therapy as *ex vivo* gene delivery was much further developed than *in vivo* gene delivery at the time these studies were initiated. *In vivo* gene delivery is much simpler than the implantation of genetically modified cells. Animal studies conducted with AAV and lentiviral vectors support the fact that *in vivo* NGF gene delivery is equally effective in preventing the degeneration of cholinergic neurons [17,18] (and unpublished data). Additional safety and toxicity studies are needed for future clinical studies of *in vivo* NGF gene therapy. In addition, it would be desirable to be able to regulate the expression of therapeutic genes after *in vivo* gene transfer. Systems such as the tetracycline, rapamycine or ecdysone-regulated system are potential candidates, but need further development before entering clinical trials.

In contrast to experimental therapies for neurodegenerative diseases that aim to prevent or slow neuronal degeneration, experimental therapies for spinal cord injury are mostly aimed at augmenting the regeneration and growth of injured axons. Inhibitors of axonal growth present in CNS myelin,[19] and potentially inhibitory, extracellular matrix molecules up-regulated at the injury site,[20] contribute to the failure of axonal regeneration in the adult mammalian spinal cord. Cystic degeneration at the injury site further requires a suitable axonal growth substrate to allow axonal growth through a lesion site into the distal host spinal cord, and injured axons might require a growth-stimulating signal at the lesion site. Axon growth promoting effects of neurotrophic factors have therefore been investigated in some detail. Genetically modified fibroblasts expressing neurotrophic factors such as BDNF, NT-3, NT-4/5, GDNF and LIF have been grafted to a spinal cord lesion site in adult rodents, providing a suitable growth substrate for injured axons, and inducing axonal growth responses of specific neuronal populations and in some instances partial functional recovery.[21-26] Although axonal growth is robust into neurotrophin expressing cellular grafts, limited axonal growth is observed distal to the lesion site. The continued expression of neurotrophic factors at the lesion site and the lack of a neurotrophin gradient distal to the lesion site might contribute to the lack of long-distance axonal growth. Regulatable systems for the controlled expression of neurotrophic factors at the lesion site[27] and *in vivo*

neurotrophin gene transfer distal to the lesion site might be able to induce more extended axonal growth. Other cell types such as neural progenitor cells,[28] bone marrow stromal cells, Schwann cells[29] and olfactory ensheathing cells[30] are potential candidates for a cellular growth substrates at the lesion site.

Activation of cellular regeneration programs by cyclic nucleotides or neurotrophin delivery at the cell soma might further enhance axonal regeneration. Successful regeneration is likely to require a combinatorial approach of growth enhancing agents and neutralization of inhibitory molecules at and beyond the lesion site.

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