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Genetically Modified Astrocytes Secreting Beta-Nerve Growth Factor (β-NGF) Support Adrenal Chromaffin Cells Grafted into the Striatum

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Intracerebral transplantation of genetically engineered cells is a useful method for delivering recombinant growth factors to the brain. Despite the clear experimental and therapeutic potential of this technique, relatively few cell types have been studied as targets for genetic manipulation and transplantation. Our interest has been to determine whether primary type I astrocytes, the normal support cell of the CNS, are suitable for this purpose.

For these studies, type I astrocytes purified from newborn rat cerebral cortex were genetically modified in culture to constitutively express a mouse β-NGF transgene, by infection with a replication-defective retroviral vector as previously described (Cunningham et al., Brain Res 561: 192-202). Infected astrocytes were found to release NGF into the culture medium at a rate that is >10-fold higher than that of sister cultures of uninfected astrocytes (9.3±2.9 pg/10^5 cells/h vs. <0.8 pg NGF/10^5 cells/h).

To evaluate the potential of these transgenic astrocytes to provide trophic support to brain grafts, their effects on the survival and neuronal transdifferentiation of intrastriatal adrenal chromaffin cell co-grafts were evaluated in the unilateral 6-hydroxydopamine-lesioned rat (an animal model of Parkinson's disease). NGF-producing transgenic astrocytes (AsN.8) were fluorescently labeled with DiI, mixed 1:1 with suspensions of postnatal day 12 adrenal chromaffin cells (AC; 80,000 cells of each type) and stereotaxically implanted into the dopamine-denervated striatum of adult Fischer 344 rats (n=7). Chromaffin cells grafted alone (AC, n=4) or with normal astrocytes (AC + As, n=4) served as controls. Host rats were sacrificed ten weeks post-grafting. Astrocytes and chromaffin cells were identified by fluorescence microscopy and tyrosine hydroxylase immunoreactivity (TH-IR), respectively. When co-grafted with NGF-producing transgenic astrocytes, chromaffin cell survival was enhanced 5-12 fold over controls (1168±143 vs. 221±128 and 99±5 TH-IR cells; AC + AsN.8 vs. Ac and AC + As grafts, respectively, $p<0.01$). Furthermore, 36% of the chromaffin cells co-grafted with the genetically modified astrocytes displayed a phenotype characteristic of neuronally differentiated sympathetic neurons, i.e., large soma (30-40 μm in diameter), TH-IR neuronal processes and extensive process elongation. These processes were also immunoreactive for the low affinity NGF receptor, but not for choline acetyltransferase, indicating that the NGF-producing grafts do not stimulate aberrant sprouting by host brain NGF-responsive cholinergic neurons. The effects of the transgenic astrocytes on chromaffin cell survival and morphology were paralleled by a 40% reduction in apomorphine-induced rotation. The results of this study demonstrate that genetically engineered astrocytes are an effective vehicle for delivering long-term β-NGF trophic support.

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