BEHAVIOUR OF EGFP-LABELLED NS CELLS TRANSPLANTED IN THE ADULT RODENT BRAIN

Reitano Erika, Luciano Conti, Donato Goffredo, and Elena Cattaneo

Department of Pharmacological Sciences and Center of Excellence on Neurodegenerative Diseases, University of Milano, Via Balzaretti 9, 20133 Milan, Italy

Significant progress has yielded the discovery of an homogeneous neural stem cell population, named NS cells, that undergoes sustained symmetrical self-renewal with complete suppression of differentiation in adherent culture. These cells can be derived from different sources (ES cells, foetal and adult CNS material), are stable and exhibit high neurogenic potential after in vitro extensive passages. Our previous studies indicate that ES-derived NS cells survive and undergo neuronal and glial differentiation in vitro. In order to enhance the in vitro differentiation of NS cells, we developed an optimize procedure that results in highly efficient generation f fully mature neurons. The procedure consists of the sequential exposure of the NS to defined media. In these conditions the survival rate, after 2 weeks is about 90% and decrease to 80% after three weeks and at this latter time point 80% of cells express neuronal markers (MAP2 and NeuN).
We have also analyzed the properties of NS cells after intracerebral transplantation. eGFP-labelled NS cells were injected into the adult mouse brain (striatum and hippocampus) and their survival and differentiation properties were analysed over time by observation of cell morphology and expression of specific phenotypic markers. We observed no histological evidence of dysregulated proliferation or tumor formation. Grafted cells appeared capable to migrate, incorporate and differentiate within the host tissue developing mature neuronal morphologies. Our immunohistochemical analyses revealed co-expression of eGFP with the neuronal markers TuJ, NeuN, Smi32. Notably, no glial phenotypes were observed. Analyses of neuronal differentiation revealed that the grafted cell acquire region-specific mature neuronal morphologies and phenotypes. Donor cells injected into the hippocampus generated granular neurons in the dentate gyrus and pyramidal neurons in the CA1 and CA3 fields. Similarly, we observed graft-derived multipolar neuronal cells in striatum and pyramidal neurons in the cortex. On the whole, these results provide the first evidence about the potentiality of long-term expanded NS to efficiently generate mature neurons in vitro and in vivo.