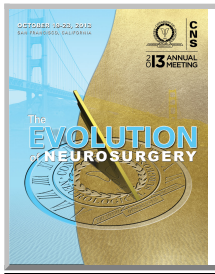

Grafts of CHR2-Expressing Human Neural Stem Cells in Experimental Stroke Model

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Introduction

Cell therapy is a promising approach for brain repair. Grafted neural stem cell progeny may provide neurotrophic support and replace cells lost to injury or disease. Currently the potency of the grafted cells to modulate local circuits and whether stimulatory input to the grafted cells will influence cellular differentiation, integration and motor performances in animal model of ischemic brain injury are undetermined. This study explores whether chronic optogenetically-controlled depolarization of the grafted cells will modulate neural differentiation and integration into the local circuitry.

Methods

We isolated multipotent NSCs from human embryonic stem cells. The NSCs were transduced with lentiviral vectors carrying the channelrodopsin-2 (ChR2) gene fused with enhanced yellow fluorescent protein (ChR2–EYFP) under the EF1alpha promoter. The ChR2 expression and response of the NSC progeny to blue light stimulation (470 nm) was confirmed in vitro. To test the function of these cells in stroke model, Sprague Dawley rats were subjected to middle cerebral artery occlusion (65 minutes). One week later, immunosuppressed rats received transplantation of NSCs (2 x 105) into the ischemic boundary zone in the striatum. Chronic optogenetic stimulation of the grafted cells was carried out daily after transplantation for 1 month. Five animal groups were established: vehicle, vehicle with stimulation, NSC transplant with chronic stimulation, NSC transplant without chronic stimulation and NSC with control (non-ChR2) vector.

Results

To characterize the function of the ChR2, hNSCs were differentiated in vitro and subjected to optogenetic stimulation. Voltage-clamp recordings showed that 475 nm blue light-induced inward photocurrents

in neural stem cells stably expressing ChR2.

The animals that received the CHR2-engineered NSC grafts with chronic stimulation showed an enhanced sensorimotor performance. Histopathological analysis demonstrated that ChR2-expressing hNSCs engrafted into the stroke-damaged brain, expressed human specific antigens and differentiated into neurons.

Conclusions

ChR2-expressing hNSCs are engraftable in infarct -damaged brain tissue.

Optical stimulation of the grafts favored the use of stroke-disabled forelimb.

Excitatory neurotransmissions offer beneficial effects in experimental stroke model.

Learning Objectives

1. Neural transplantation and optogenetic stimulation as a potential therapeutic intervention for stroke.

2. Neural stem cell engineering for biological and therapeutic use.

3. Differentiate between various mechanisms by which neural stem cell grafts promote functional recovery