Combining MRI and Optogenetics: Expression of Channelrhodopsin-2 in stem cells

Jenny Kressel[1,2], Benedikt Berninger[3], Vasilis Ntziachristos[2], Claus Zimmer[1], and Albrecht Stroh[1, 4]

[1] Department of Neuroradiology, Klinikum rechts der Isar, Technical University Munich, Munich, Germany
[2] Institute for Biological and Medical Imaging, Helmholtz Zentrum München, Munich, Germany
[3] Institute for Stem Cell Research, Helmholtz Zentrum München, Munich, Germany
[4] Institute of Neuroscience, Klinikum rechts der Isar, Technical University Munich, Munich, Germany

Magnetic resonance imaging (MRI) is a unique non-invasive technique to monitor *in vivo* procedures with high-resolution and a non-limited depth penetration. It requires a cellular magnetic labeling, we used a non-genetic targeting with very small superparamagnetic iron oxide particles (VSOPs).
To allow for specific stimulation of labeled stem cells we aim at combining this technique with optogenetics. Therefore, we introduced the genetically encoded optogenetic construct Channelrhodopsin-2 (ChR2) into labeled stem cells. Using a lentiviral gene transfer we transduced both embryonic stem cells as well as adult SVZ neural stem cell. Following illumination with blue light (470nm), the membrane protein ChR2 leads to a high-speed depolarization, fast enough to drive precisely timed light-evoked spikes in stem cell derived neurons.

STEM CELL CULTURING

Embryonic stem cells (ESCs, CRL-1934, ATCC, Manassas, USA), were co-cultured as described in [1].

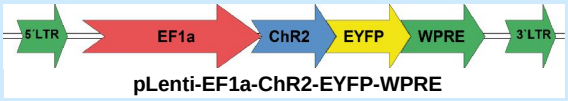
Neural precursor cells from the adult mouse subventricular zone (SVZ), were isolated and cultured as neurospheres.

Neuronal differentiation of ESCs was induced via embryoid body formation and LIF removal. Nestin-positive cells were selected and expanded. After 10 days of expansion, progenitors were differentiated into neurons by removing N2 supplement and bFGF, and adding NGF and B-27 supplement for 14 days.

MAGNETIC LABELING

Stem cells were magnetically labeled using very small superpara-magnetic iron oxide particles (VSOPs, C200, Ferropharm, Teltow, Germany). These sterile VSOPs consist of a 5 nm iron oxide core coated by monomer citrate yielding a diameter of 9 nm. The uptake of VSOPs was evaluated using Prussian blue staining and phase contrast microscopy [2]. For stable labeling, a concentration of 0.5 mM Fe^{2+}/Fe^{3+} in the Opti-MEM incubation media for 4 hours is sufficient.

OPTOGENETIC CONSTRUCT



pLenti-EF1a-ChR2-EYFP-WPRE

Figure 1: For detection, the microbial opsin ChR2 was fused to EYFP [3].The fusion protein is under the control of the ubiquitious elongation factor 1α promotor (EF1 α). This promotor guarantees ChR2 synthesis in all cell types.
The optogenetic construct (10.898bp) was delivered into the mammalian host cells using a lentivirus (Retroviridae). Leading to a stable, strong and cell cycle phase undependent ChR2 expression.

ChR2-EXPRESSION IN LABELED ESCs

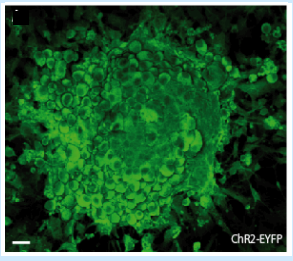


Figure 2:
Emryonic stem cells cultured as a embroid body. The cells were labeled with 0.5mM VSOPs and lentivirally transduced with ChR2. Confocal microscopy demonstrated a strong membrane-bound expression of ChR2-EYFP. Scale bar 10µm.

TRANSCRIPTIONAL ANALYSIS OF ChR2⁺-ESCs

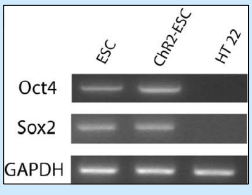


Figure 3:
RT-PCR analysis of pluripotency marker expression of both ChR2-ESCs as well as native ESCs revealed strong expression of Oct4 and Sox2, compared to mouse hippocampal cells HT22 (negative control).

NEURONAL DIFFERENTIATION OF LABELED ChR2⁺-ESCs

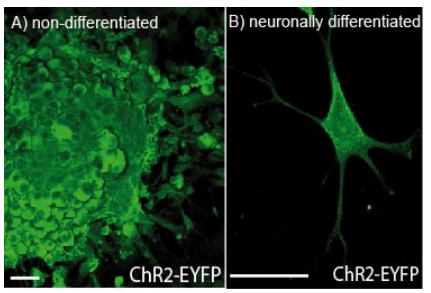


Figure 4: ESCs were labeled with 0.5 mM VSOPs and lentivirally transduced with ChR2. Subsequent neuronal differentiation resulted in a bipolar, neuronal phenotype. Fluorescence microscopy indicates strong membranous expression of ChR2 in ESCs and mature neurons. Scale bar 10µm. A) non-differentiated ESCs, B) ESCs derived neurons.

ChR2-EXPRESSION IN ADULT NEURAL PRECURSOR CELLS

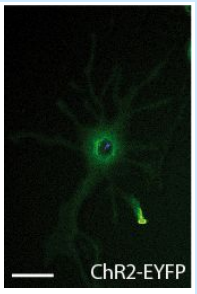


Figure 5:
Neural precursor cells from the adult mouse subventricular zone (SVZ), were isolated and lentivirally transduced with ChR2.

CONCLUSIONS

Magnetically labelled ESCs, as well as neural precursor cells from the adult mouse SVZ, were transduced with a lentiviral ChR2-EYFP construct. Confocal, fluorescence microscopy indicates membranous expression of ChR2. Transcriptional analysis (RT-PCR) of the pluripotency markers Oct4 and Sox2 revealed no impact on stemness. Subsequent neuronal differentiation resulted in a neuronal phenotype.
Lentiviral integration of ChR2-EYFP into embryonic and adult stem cells has the perspective of non-invasive control via illumination with blue light. Both *in vitro* as well as after transplantation. Even in non-excitable cells, optogenetic stimulation may result in alterations in gene expression due to activation of voltage gated calcium channels.

[1] Bibel M. *et al., Nat. Neurosci.*, 2004.
[2] Stroh A. *et al., Mol. Imaging*, 2009.
[3] Boyden ES. *et al., Nat. Neurosci.*, 2005.

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