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High-efficiency transfection and survival rates of embryonic and adult mouse neural stem cells achieved by electroporation

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ABSTRACT

Cells of the central nervous system are notoriously difficult to transfect. This is not only true for neurons and glial cells but also for dividing neural stem and progenitor cells (NSCs). About ten years ago a major advance was provided by introduction of the nucleofection technology that allowed for transfection of approximately half of the exposed NSCs. However, limitations were encountered with the need for large numbers of NSCs for a single transfection and compromised survival rates with typically only one-third of the cells surviving the pulse conditions. Here, we report the establishment of a pulse protocol that targets NSCs with high efficiency and twofold higher NSC survival rates using the 4D Nucleofector device. We demonstrate that the established protocol not only provides a clear and significant improvement over existing protocols with transfection rates above 80% and two-thirds of the NSCs surviving for at least 48 h, but also their unaltered differentiation along neuronal and glial lineages. This improved protocol for the transfection of sensitive mouse central nervous system derived cells will provide an important step forward for studies of gene function by overexpression or knock-down of genes in cultured NSCs.

1. Introduction

The mouse as an animal model displays an irreplaceable and widely used system to investigate the relevance of genes with respect to development, physiology and disease. In particular the development of the central nervous system (CNS), the most complex tissue in mammals, raises the question of the influence of genes that control proliferation and differentiation of neural stem and progenitor cells (NSCs). NSCs undergo extensive proliferation before they give rise to all three major cell types of the CNS in a highly orchestrated fashion. Neurons are generated first during embryonic development followed by gliogenesis of astrocytes and oligodendrocytes around birth and early postnatally (Kriegstein and Alvarez-Buylla, 2009). However, many of the intrinsic cues that influence NSC behaviour in a spatial and temporal manner remain unknown or need to be studied in vitro to understand their molecular mechanisms. Currently, a variety of culture methods are used to gain information about the functional importance of genes that control the behaviour of cortical NSCs (Britanova et al., 2008; Hand et al., 2005). One approach to investigate these issues is the transfection of NSCs in order to overexpress or knock-down genes of interest. Electroporation is the favoured non-viral method for this purpose in vivo (De Vry et al., 2010) and in vitro (Gresch and Altrogge, 2012). The application of an electrical pulse induces the transient opening of pores in the plasma membrane enabling to introduce DNA and/or RNA molecules to enter the cytoplasm and nucleus. Nucleofection became available through Amaxa and represents a feasible technique with respect to transfection of mouse NSCs (Moritz et al., 2008; von Holst et al., 2007). However, the need of high cell numbers in combination with comparatively low cell viability and still limited transfection rates of cortical NSCs have impaired the usefulness of this method to a certain degree.

Here, we have established an optimized pulse protocol using the new Lonza 4D Nucleofector device that strongly improved the electroporation efficiency and survival of mouse cortical NSCs. In addition, we provide evidence that one order of magnitude fewer NSCs can be employed for each single transfection.

Abbreviations: BrdU, 5-bromo-2-desoxyuridine; BSA, bovine serum albumin; CNS, central nervous system; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; FGF-2, fibroblast growth factor 2; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; NSC, neural stem cell; KRH, Krebs–Ringer Hepes buffer; PBS, phosphate buffered saline; SEZ, subependymal zone.

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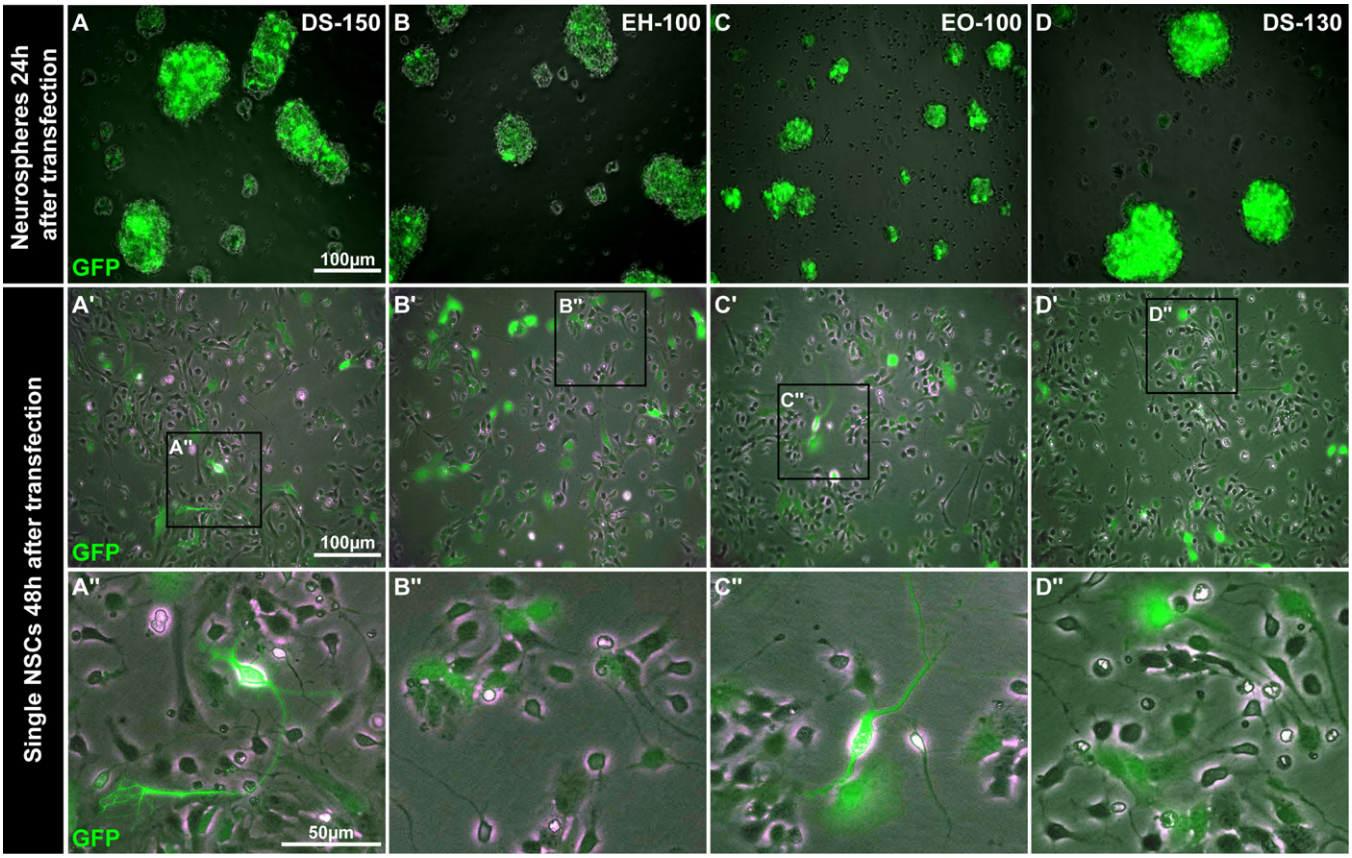

Fig. 1. Nucleofected NSCs in neurosphere cultures and as single cells. Merged pictures of phase contrast and immunofluorescent EGFP images (green) of live cortical neurospheres cultures 24 h after transfection of mouse cortical NSCs with the plasmid pEGFP-N1 and the pulses DS-150, EH-100, EO-100 or DS-130 as indicated above in the images are shown (A–D). Merged pictures of phase contrast and immunofluorescent EGFP images (green) of nucleofected, dissociated neurosphere-derived single cell cultures 24 h after seeding (A′–D′). Pictures A″–D″ show higher magnifications of nucleofected single NSC cultures that are boxed in the middle panel. Scale bars are 100 μm (A, A′–D, D′) and 50 μm (A″–D″). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2. Materials and methods

2.1. Isolation and expansion of mouse NSC

Cortical cells were isolated from E13.5 mouse forebrains and digested gently with 30 U/ml Papain (Worthington) as described previously (Sirko et al., 2010; von Holst et al., 2006). Single cells grew in chemically defined neurosphere medium containing F12/DMEM 1:1 (Sigma), B27 2%, Pen/Strep 1% (both Gibco, Life Technologies) and L-glutamine 1% (Fluka) supplemented with 20 ng/ml EGF and FGF2 (Peprotech) as growth factors at 37 °C and 6% CO_2 in a humidified atmosphere. Under these conditions NSCs grow as free-floating cellular aggregates called neurospheres as published earlier (Akita et al., 2008; Reynolds and Weiss, 1996; Sirko et al., 2010; von Holst et al., 2006). After 5 days in vitro neurospheres were trypsinized for 5 min at room temperature and gently triturated to obtain single NSC cell suspensions that were either expanded as secondary neurospheres under the same conditions as above or directly used for transfection.

2.2. Isolation and expansion of adult mouse NSCs

Cells from the adult subependymal zone (SEZ) of the lateral ventricles were isolated according to the protocol of Ortega et al. (2011). After isolation of the SEZ the cells were dissociated and cultured as above.

2.3. Vectors

Our routinely used antibody against GFP (Millipore) excluded to use the maxGFP Vector (Lonza) that is provided with the P3 Primary Cell 4D Nucleofector Kit due to its low detection ability. In order to determine the transfection rates as accurately as possible we used the pEGFP-N1 plasmid (Clontech) instead.

2.4. 4D nucleofection

For lower cell numbers we used 5×10^5 neurosphere-derived NSCs per sample and resuspended them in 20 μl P3 primary cell solution containing 0.5 μg plasmid DNA. Cells were transferred into the nucleofector cuvette (20 μl) according to the manufacturer's protocol (Lonza). After the pulse application, 180 μl prewarmed neurosphere medium was added to the electroporated NSCs in the cuvette. NSCs were gently resuspended in the cuvette and transferred into a sterile 1.5 ml tube. After centrifugation with $80 \times g$ for 5 min at room temperature, the supernatant was discarded and the cell pellet was resuspended in 500 μl neurosphere medium containing EGF and FGF2 (20 ng/ml). For higher cell numbers ($2.5–5 \times 10^6$) the provided Lonza container was used for transfection and the neurosphere-derived NSCs were resuspended in 100 μl P3 primary cell solution with 5 μg plasmid DNA. The transfected NSCs were resuspended in 500 μl prewarmed neurosphere medium, transferred to a 15 ml Falcon tube and centrifuged also at $80 \times g$ for 5 min at room temperature.