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Research Report

Electroporation-based gene transfer for efficient transfection of neural precursor cells

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Abstract

Transplantation of neural precursor cells (NPCs) is a potential tool to replace dysfunctional or degenerated neuronal or glial cell types in the central nervous system. Furthermore, transplantation of genetically engineered neural precursor cells might provide a strategy to target therapeutic gene products to the diseased nervous system. Here, we describe a novel and highly efficient electroporation-based transfection protocol for mitogen-expanded mouse NPCs. Transfection of NPCs with the reporter gene enhanced green fluorescent protein (EGFP) or the neural adhesion molecule L1 revealed transfection efficacies of more than 70% as estimated by the number of EGFP-positive or L1-immunoreactive cells 1 day after transfection in vitro. The percentage of EGFP- or L1-positive cells decreased with increasing time in culture. Positive cells were detectable for up to 3 weeks after transfection. When EGFP- or L1-transfected NPCs were grafted into the retina of adult wild-type or L1-deficient mice, they differentiated into glial cells some of which expressed EGFP and L1 for up to 2 and 3 weeks, respectively, the longest post-transplantation periods investigated.

Theme: Development and regeneration
Topic: Transplantation

Keywords: Ex vivo gene therapy; Non-viral transfection; Neural precursor cell; Retina

1. Introduction

The mammalian central nervous system (CNS) lacks effective endogenous regenerative potential for most disorders or after injury, mainly because the mature CNS has a limited potential to generate new neurons or to initiate functional recovery of damaged axons. A promising treatment for neurodegenerative disorders might be the transplantation of neural cells which are able to replace lost or dysfunctional cells or which protect affected endogenous cells by expressing therapeutic gene products. Neural precursor cells (NPCs) are among the candidate cells for such cell-based therapeutic treatments of the diseased CNS [8,27,34,44]. NPCs have been isolated from several regions of the developing and adult CNS of diverse mammalian species, including humans. They can also be derived from pluripotent embryonic stem cells. In culture, NPCs can be maintained and expanded in an undifferentiated state in monolayer cultures or as floating neurospheres. Upon induction of differentiation, they give rise to the three principle cell types of the CNS, neurons, astrocytes, and oligodendrocytes, depending on distinct epigenetic signals which exert a significant influence on fate decisions of NPCs [16,35]. Indeed, transplantation

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experiments have shown that grafted NPCs integrate and migrate in the host tissue without disrupting its cytoarchitecture, and eventually undergo region-specific differentiation without signs of tumor formation, underlining their therapeutic potential [8,34]. However, most regions of the mature mammalian CNS do not express instructive signals that direct differentiation of grafted NPCs into distinct nerve cell types, with the exception of two neurogenic areas of the adult brain, the subventricular zone and the dentate gyrus. Rather, multipotent NPCs primarily differentiate into astrocytes and oligodendrocytes when transplanted into non-neurogenic areas of the normal or diseased CNS [3,39,51]. Cell replacement strategies might thus require differentiation of NPCs into specific neural cell types prior to transplantation. The transfer of regulatory genes that control fate decisions of NPCs [17] is among the potential strategies to derive distinct neural cell types from neural precursor cells.

In addition to cell replacement strategies, NPCs are also candidate cells to establish ex vivo gene therapies for a variety of neurological disorders [32,34,38]. Mitogen-expanded or immortalized NPCs genetically engineered to express therapeutic gene products and grafted into the diseased or injured brain have indeed been shown to ameliorate morphological and eventually functional deficits [1,4,5,7,22,31,33,45]. NPCs can be efficiently transduced with viral vectors. However, virus production and titration is time consuming and associated with safety concerns. Non-viral transfection methods such as liposome-based methods, calcium phosphate co-precipitation, or standard electroporation techniques, on the other hand, usually result in only low transfection efficiencies of primary cells, including mitogen-expanded NPCs [9,14,21].

Here we describe an efficient non-viral, electroporation-based transfection technique that has been optimized for NPCs isolated from the spinal cord of embryonic mice. Using this technique, we were able to successfully transfect more than 70% of NPCs with the reporter gene "enhanced green fluorescent protein" (EGFP). A similar percentage of cells could be transfected with L1, a cell recognition molecule of the immunoglobulin superfamily that supports migration and survival of nerve cells, elongation and fasciculation of axons, and when offered as a substrate, influences proliferation and differentiation of NPCs [10,11,43]. The percentage of EGFP- and L1-positive cells decreased with increasing time after transfection, but some positive cells were detectable for up to 3 weeks after transfection in vitro and for similar time periods after intraretinal transplantation in vivo. This novel transfection technique might be used as a rapid screening method to study effects of ectopically expressed genes on fate decisions of NPCs, and might also stimulate investigations of the therapeutic potential of genetically engineered NPCs for the treatment of a variety of neurological disorders.

2. Materials and methods

2.1. Animals

NPCs were isolated from spinal cords of 14-day-old mouse embryos. C57BL/6J wild-type mice, transgenic mice ubiquitously expressing EGFP under control of a chicken β-actin promoter [37], or mutant mice deficient in the neural adhesion molecule L1 [10,43] were used as donors. EGFP-transgenic mice were maintained on a C57BL/6J genetic background and their genotype was determined by analyzing tail biopsies for the presence of EGFP fluorescence. L1-deficient mice were genotyped using polymerase chain reaction [43]. NPCs were transplanted into the retina of adult (i.e., at least 2-month-old) wild-type or L1-deficient mice.

2.2. Cultivation and transfection of NPCs

Spinal cords were removed and placed into a defined medium composed of a 1:1 mixture of Dulbecco's Modified Eagle's Medium and F-12 supplemented with glucose (0.6%), sodium bicarbonate (3 mM), B27 (2%; Gibco, New York, USA), glutamine (2 mM), HEPES buffer (5 mM), epidermal growth factor (EGF; 10 ng/ml; TEBU, Offenbach, Germany), and fibroblast growth factor-2 (FGF-2; 10 ng/ml; TEBU). The tissue was mechanically dissociated using a fire-polished Pasteur pipette and cells were plated in uncoated tissue culture flasks at a density of 100,000 cells/ml. Cultures were passaged every fifth day.

For expression of the neural adhesion molecule L1 under control of the CMV promoter, a 4050-bp *Eco*RI fragment containing the full-length coding sequence of L1 [47] was ligated into vector pcDNA3 (Invitrogen, Karlsruhe, Germany). For expression of EGFP under control of the CMV promoter, vector pEGFP-N1 (Clontech, Heidelberg, Germany) was used. For transfection of NPCs, neurospheres from the third to fifth passage were mechanically dissociated into single cells and centrifuged for 5 min at 800 rpm and 4 °C. The culture medium was removed and about 5×10^6 cells were resuspended in 100 μl of the nucleofector solution for mouse neurons (Amaxa biosystems, Cologne, Germany). DNA (5 or 10 μg) was added, cells and DNA were gently mixed and cells were electroporated using the Nucleofector™ device (Amaxa biosystems). Transfections were performed with a program (A33) that was selected in a series of pilot transfection experiments with the EGFP reporter gene and proven to result in the highest transfection efficacies. Directly after transfection, NPCs were placed into (i) culture medium containing EGF and FGF-2, (ii) culture medium containing 1% fetal calf serum (FCS), or (iii) culture medium lacking serum and growth factors.

2.3. Analysis of transfected cells in vitro

NPCs from wild-type mice and L1-deficient mouse mutants were transfected with EGFP and L1, respectively.