Neuroscience Letters 558 (2014) 154–158

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Morphological analysis of embryonic cerebellar grafts in SCA2 mice

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HIGHLIGHTS

- Embryonic cerebellar graft survives for 12 weeks in both SCA2 and control mice.
- The grafts contained numerous Purkinje cells.
- Long distance graft-to-host axonal connections were rarely seen.
- Size of the cerebella and density of PCs did not seem to be reduced in SCA2 mice.

ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form 9 November 2013
Accepted 14 November 2013

Keywords:
Cerebellar degeneration
Neurotransplantation
Purkinje cells

ABSTRACT

SCA2 transgenic mice are thought to be a useful model of human spinocerebellar ataxia type 2. There is no effective therapy for cerebellar degenerative disorders, therefore neurotransplantation could offer hope. The aim of this work was to assess the survival and morphology of embryonic cerebellar grafts transplanted into the cerebellum of adult SCA2 mice. Four month-old homozygous SCA2 and negative control mice were treated with bilateral intracerebellar injections of an enhanced green fluorescent protein-positive embryonic cerebellar cell suspension. Graft survival and morphology were examined three months later. Graft-derived Purkinje cells and the presence of astrocytes in the graft were detected immunohistochemically. Nissl and hematoxylin–eosin techniques were used to visualize the histological structure of the graft and surrounding host tissue. Grafts survived in all experimental mice; no differences in graft structure, between SCA2 homozygous and negative mice, were found. The grafts contained numerous Purkinje cells but long distance graft-to-host axonal connections to the deep cerebellar nuclei were rarely seen. Relatively few astrocytes were found in the center of the graft. No signs of inflammation or tissue destruction were seen in the area around the grafts. Despite good graft survival and the presence of graft-derived Purkinje cells, the structure of the graft did not seem to promise any significant specific functional effects. We have shown that the graft is available for long-term experiments. Nevertheless, it would be beneficial to search for ways of enhancement of connections between the graft and host.

1. Introduction

The wide spectrum of human hereditary cerebellar degenerative diseases [1] is also reflected by the large number of mouse models of these diseases used for investigation of cerebellar functions and pathology [2]. Human autosomal dominant spinocerebellar ataxias (SCA) SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 are caused by an enlarged region of CAG trinucleotide repeats in the gene, resulting in an expansion of the polyglutamine tract (poly-Q) in the corresponding protein [1].

Transgenic mice carrying the human ataxin 2 gene, with enlarged CAG repeat sequence, are used as a model of human spinocerebellar ataxia 2 (SCA2) [3–6]. SCA2 mice suffer from a

Abbreviations: ATXN2^{Q127}, ataxin 2 with 127 glutamine repeats; DNA, Deoxyribonucleic acid; EGFP, enhanced green fluorescent protein; GAFP, glial fibrillary acidic protein; PC, Purkinje cell; pcd, Purkinje cell degeneration; PCR, polymerase chain reaction; SCA, spinocerebellar ataxia.

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http://dx.doi.org/10.1016/j.neulet.2013.11.020

reduction in Purkinje cell (PC) number and show progressive motor deficits [3–5]. In the most frequently used natural mutant mouse models of hereditary cerebellar degeneration, such as pcd and Lurcher mice, functional deficits appear early on [7–9], while in SCA2 mice, it develops later and the onset varies based on: (1) the number of glutamine repeats, (2) line of mice and also differs between (3) homozygotes and heterozygotes [4,5]. In the SCA2 mouse line, expressing Q58 ataxin 2 (Q58-11 line), motor deficits on the rotarod test become evident at the age of 26 weeks in heterozygotes and at 16 weeks in homozygotes and the number of PCs have declined by 50% by the age of 6 months [5]. SCA2 mice, in the line expressing ataxin 2 with 127 glutamine repeats ($ATXN2^{Q127}$), have a more obvious pathological phenotype with an earlier onset [4].

Currently, therapy for hereditary cerebellar degenerations is ineffective; however, cerebellar transplantation could be promising. Several studies have shown long-term graft survival [10] and improvement of motor performance due to treatment with various neurotransplantation methods [11–14] in mouse models of cerebellar degeneration. Particularly, in C57BL/6J SCA2 transgenic mice Chang et al. [15] found that intravenous injection of human mesenchymal stem cells increased the survival of host PCs, delayed onset of disease and improved motor function. Despite the abundant research, there are no studies regarding the fate of embryonic cerebellar grafts in SCA2 mice.

The aim of this work was to assess the survival and morphology of embryonic cerebellar cell suspension grafts transplanted into the cerebellum of adult SCA2 mice.

2. Materials and methods

2.1. Animals

Cerebellar transplantation was studied in SCA2 transgenic mice of the B6D2-Tg(Pcp2SCA2)11Plt/J strain [5]. The C57BL/6-Tg(ACTB-EGFP)1Osb/J male mice were used to get graft donor embryos expressing enhanced green fluorescent protein (EGFP). Mice of both strains were purchased from The Jackson Laboratory (Bar Harbor, USA) and the colonies were then maintained in the animal facility at the Faculty of Medicine, Pilsen, CZ. The mice were kept under standard conditions with a 12:12 h light:dark cycle and water and food available *ad libitum*.

All experiments described in this article were conducted in full compliance with EU Guidelines for scientific experimentation on animals and with the permission of the Ethics Commission of the Faculty of Medicine, Pilsen, CZ. All efforts were made to minimize the number of animals used and their suffering.

2.2. Genotyping

The genotyping of SCA2 transgenic mice was performed on the basis of The Jackson Laboratory Genotyping protocols database (web page: http://www.jax.org). The Jackson Laboratory Genotyping protocol (Tg(Pcp2SCA2)11Plt-alternate1) has been designed as end point polymerase chain reaction (PCR) with the result transgene is present or not. This protocol was modified for quantitative assessment and the mice were classified into three subgroups "negative", "heterozygote", and "homozygote" according to amplification Ct. We are aware of the limitations of this approach relative to the unknown number of transgene copies inserted.

Deoxyribonucleic acid (DNA) was isolated from a piece of the mouse tail using DNeasy Blood & Tissue Kit (QIAGEN). DNA concentration was determined, and all DNA samples were diluted to a concentration of 60 ng/μl. The sequence of primers used for quantification of the transgene was as follows: transgene forward primer 13867 5′- AAT ACC TAT GAC GCC CAT GC -3′; transgene reverse primer 13868 5′- ATG AGC CCC GTA CTG AGT TG -3′. For the reference gene (internal positive control Reverse) forward primer oIMR7338 5′- CTA GGC CAC AGA ATT GAA AGA TCT-3′ and reverse primer oIMR7339 5′- GTA GGT GGA AAT TCT AGC ATC ATC C -3′ synthesized by GeneriBiotech (Hradec Kralove, Czech Republic) were used. DNA amplification was monitored with 0.5x Sybr-Green I (Molecular Probes, USA). The results of genotyping ("negative", "heterozygote", and "homozygote") were obtained according to ΔCt values (Ct for transgene minus Ct for reference gene).

For the experiment, only mice showing the maximal number of copies of the transgene, which were considered to be "homozygous" for SCA2 ($n = 15$), and negative ($n = 23$) individuals (both males and females) were used. The study was performed as a blinded-study since at the time of transplantation and histological examination the genotype of the mice was not known.

2.3. Transplantation

Embryonic cerebellar tissue was obtained from day 12 (E12, embryonic day 12) EGFP-expressing mouse embryos, which were obtained from the cross-breeding of females from the host strain with EGFP-expressing males. Donor females, with conception-timed pregnancies, were deeply anesthetized with Thiopental. Embryos were removed from the uterus, the embryonic cerebella were dissected, treated with trypsin for 10 min and mechanically suspended in the vehicle (0.9% NaCl and 0.6% glucose). The suspension concentration was adjusted to 50,000 cells/μl.

Four-months-old host mice were anaesthetized with Ketamine (100 mg/kg of the body weight) and Xylazine (16 mg/kg of the body

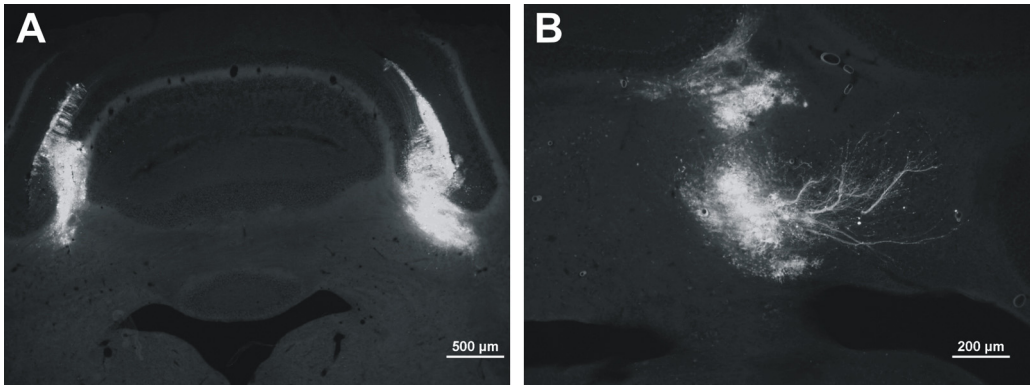

Fig. 1. A host cerebellum with the bilateral graft; the graft is located in the white matter close to the deep cerebellar nuclei and in the host cerebellar cortex; EGFP-positive fibers sprout from the right graft through the molecular layer of the cerebellar cortex and EGFP-positive Purkinje-like cells are distributed along the fissure (A; objective 4×). A subcortical graft with EGFP-positive fibers growing toward the deep cerebellar nuclei area (B; objective 10×). Native specimens, EGFP-fluorescence.