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Original Research Article

Fate of two types of cerebellar graft in wild type and cerebellar mutant mice☆

Miroslava Čedíková [a,b], Zbyněk Houdek [c,d], Václav Babuška [e], Vlastimil Kulda [e], František Vožeh [b,c], Nicolas Zech [f], Lenka Černá [g], Kristýna Krakorová [g], Milena Králíčková [a,b,], Jan Cendelín [b,c]*

[a] Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University in Prague, Plzeň, Czech Republic
[b] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University in Prague, Plzeň, Czech Republic
[c] Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University in Prague, Plzeň, Czech Republic
[d] Centre of Biology, Geosciences and Environmental Education, Faculty of Education, University of West Bohemia in Plzen, Plzeň, Czech Republic
[e] Department of Medical Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University in Prague, Plzeň, Czech Republic
[f] IVF Centers Prof. Zech – Pilsen, Plzeň, Czech Republic
[g] Department of Neurology, Faculty of Medicine in Pilsen, Charles University in Prague, Plzeň, Czech Republic

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ABSTRACT

The possibility of regeneration in a damaged central nervous system (CNS) is limited and therapy of CNS diseases accompanied with neuronal loss is problematic.

Lurcher (Lc) mutant mice represent a model for olivocerebellar degeneration.

Heterozygotes suffer from a complete loss of cerebellar Purkinje cells and a secondary reduction of granule cells and inferior olive neuron number.

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Introduction

Stem cell therapy is a potential treatment for various central nervous system (CNS) diseases and different stem cell (SC) types have already been grafted into animal models as well as humans. Due to inconsistent results, it is still a relevant question which stem cell type will prove to be most therapeutically effective. The impact of SC therapy has to be followed in a complex manner as long as the stem cells might

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* *Corresponding author at*: Department of Histology and Embryology, Faculty of Medicine, Charles University, Karlovarská 48, 301 66 Plzeň, Czech Republic. Tel.: +420 377 105 229; fax: +420 377 105 290.
E-mail address: milena.kralickova@lfp.cuni.cz (M. Králíčková).

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not only replace missing or malfunctioning cells and support inner regenerative processes but also provoke post transplantation innate immune events (De Vocht et al., 2012).

Any regeneration in the damaged central nervous system is limited and therapy of CNS diseases accompanied with neuronal and glia loss is problematic. Even though neurons and glia have been generated successfully from SC cultures and many advances from the laboratory have already been translated into clinically useful therapies, there are still many inconsistencies and hence questions remaining.

A number of stem and progenitor cell types ranging from embryonic SC (for review see Björklund and Lindvall, 2000), neural SC (Svendsen et al., 1996, 1997), bone marrow derived SC (Azizi et al., 1998, for review see Chopp and Li, 2002) to embryonic carcinoma SC (Borlongan et al., 1998) have been proposed as therapy for neurological diseases. Satisfactory results have been obtained by transplanting adult SC or embryonic SC into the brain of animal models of Alzheimer's disease, Huntington's disease, Parkinson's disease, stroke or spinal cord injuries (Baker et al., 2000; for review see Kondziolka et al., 2004; Sykova et al., 2006; Orlacchio et al., 2010).

Carcinoma SC are considered dangerous because of the risk of donor-derived malignant tumour development in the host. On the other hand, it has been shown that, these cells, once differentiated, never reacquire a tumorigenic capacity (Garbuzova-Davis et al., 2002) and they have been shown to be beneficial in animal models of several neurological diseases. Nevertheless, carcinoma SC have never been tested in animal models of the cerebellar degeneration.

Lurcher mutant mice are one of the natural models of hereditary olivocerebellar degeneration. Lurchers are heterozygotes (+/Lc) carrying a mutation in the glutamate receptor δ2 – subunit gene, which is expressed predominantly by Purkinje cells (Araki et al., 1993). Purkinje cells become extinct during the first 3 months of postnatal life. The mechanism of their death is excitotoxic apoptosis (Zuo et al., 1997). The extinction of Purkinje cells, a primary effect of the mutation, is followed by a secondary degeneration of granule cells and inferior olive neurons. The degeneration is complete at postnatal day 90 when there are virtually no Purkinje cells left, 10% of granule cells and 30% of inferior olive neurons survive (Caddy et al., 1977, Caddy and Biscoe, 1979). The unaffected homozygous littermates of these mutants – wild type mice (+/+) – are healthy and which can be used as ideal controls. Affected homozygotes (Lc/Lc) are not viable due to a neuronal loss during intrauterine development (Cheng and Heintz, 1997; Resibois et al., 1997). Lurcher mice are a valuable tool for neurotransplantation research (for review see Triarhou, 1996; Cendelín et al., 2012). For a review, see Cendelín and Vozeh (2013).

How stem cells and their progeny integrate into recipient cerebellum and how they interact with host cells is as yet not well understood.

The aim of the study was to analyze the survival potential and appearance of the P19 carcinoma SC-derived neuroprogenitors or embryonic cerebellar cells grafted on Lurcher mutant and wild type mice 2 and 6 months after the transplantation.

We have compared two types of cell suspensions – less defined but more homogenous population of P19 carcinoma CS – derived neuroprogenitors and more determined less homogenous population of embryonic cerebellar cells obtained from the cerebella of mouse embryos.

Material and methods

Animals

Lurcher mutant ($n = 49$) and wild type ($n = 52$) mice (both males and females) of the B6CBA strain were used. Their age at the time of transplantation was 90–120 days. The mice were reared in standard conditions with 12:12 h light:dark cycle (6 am–6 pm), at the temperature of 22–24 °C. Food and water were available ad libitum.

All experiments reported here were conducted in full compliance with the EU Guidelines for scientific experimentation on animals and with the permission of the Ethical Commission of the Faculty of Medicine in Pilsen.

Graft preparation

Stem-cell culture and differentiation

Mouse P19 embryonic carcinoma SC line was purchased from the European Collection of Cell Culture, Wiltshire, UK. Embryonic carcinoma stem cells (ECSC) were isolated from teratocarcinoma induced in the C3H/He strain of mice (McBurney and Rogers, 1982). The cells had been genetically modified to express the green fluorescent protein (GFP). A permanent transfection of P19 cells was performed using GFP plasmid (Clontech Laboratories, Takara Bio Inc., Mountain View, CA) according to the CalPhos™ Mammalian Transfection Kit (Clontech Laboratories, Takara Bio Inc., Mountain View, CA). Neuroprogenitors were obtained from P19 cells after exposure to retinoic acid for 2 days.

The ECSC were cultured in a serum – free medium; Dulbecco's modified Eagle's medium (DMEM)/F12 1:1 with L-glutamine, antibiotics and ITS (insulin, transferrin, selenium). To induce neurogenesis, the cells were treated with retinoic acid (RA, $c = 5 \times 10^{-7}$ mol/l) for two days and on the third day they were cultured in a serum – free medium without RA (Pacherník et al., 2005a,b, 2007). Their phenotype was confirmed using the methods described in our previous papers (Babuška et al., 2010; Houdek et al., 2011, 2012).

Embryonic cerebellar graft preparation

Embryonic cerebellar tissue was gained from 12 days old GFP expressing mouse embryos obtained with the cross-breeding of GFP males [C57BL/6-Tg(ACTB-EGFP)1Osb/J strain] and wild type B6CBA females. Donor females were overdosed with thiopental. Embryos were removed from the uterus and pooled in a cold solution (0.9% NaCl and 0.6% glucose). The cerebella were then dissected and pooled in a solution of the same composition. Then they were treated with trypsin and mechanically suspended.

Transplantation

The transplantation was performed under general anaesthesia with the combination of ketamine (100 mg/kg b.w.) and xylazine (16 mg/kg b.w.) i.p.

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