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Brain Research 1051 (2005) 137 – 144
BRAIN RESEARCH
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Research Report

Neural cograft stimulates the survival and differentiation of embryonic stem cells in the adult mammalian auditory system

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Accepted 5 June 2005
Available online 5 July 2005

Abstract

Mouse embryonic stem (ES) cells were transplanted into the cochlea of adult guinea pigs in order to explore their survival, differentiation, and possible integration with the host tissue. With the purpose of investigating the possible effect of manipulating the local embryonic microenvironment, ES cells were transplanted into the cochlea with or without an embryonic neuronal cograft consisting of dorsal root ganglion (DRG) tissue. To detect the survival and differentiation of ES cells, cells expressing green fluorescent protein (GFP) were used in combination with immunohistochemical detection of a neuronal marker, neural class III β-tubulin (TUJ1 antibody). At 4 weeks following transplantation implanted ES cells were found close both to the sensory epithelium, and the spiral ganglion neurons (SGNs) with their peripheral dendritic processes projecting to the organ of Corti. There was a significant difference in the number of surviving TUJ1 (+) ES cells between the DRG cograft group and the non-cograft group ($P < 0.01$, ANOVA). Neurite-like projections were also identified between TUJ1-positive ES cells and the peripheral dendritic processes from SGNs. The results suggest that an embryonic neuronal microenvironment may be one of the key factors in the survival and differentiation of ES cells in the adult auditory system.

Theme: Development and regeneration
Topic: Sensory systems

Keywords: Hair cell; Hearing loss; Dorsal root ganglion; Embryonic stem cell; Organ of Corti; Spiral ganglion; Transplantation

1. Introduction

Throughout life, some cells, e.g., blood cells and skin cells, are continuously replaced. These cells have a relatively short lifespan compared to non-renewing tissues such as the auditory epithelium and neurons, which are thought to last for the lifetime of the individual. However, acoustic over-stimulation and toxic insults are potentially deleterious to the cells of the inner ear, and may result in permanent hearing impairment or deafness. Recent observations demonstrate that the adult utricular sensory epithelium contains pluri-potent cells [20]. The situation in the cochlea is complex and it has been suggested that cochlear precursor cells cannot be reactivated to differentiate into sensorineural cells, i.e., spiral ganglion neurons and hair cells, in adult mammals [6,9]. An alternative strategy for replacing degenerated or missing spiral ganglion neurons would be to apply a substitution therapy based on exogenous stem cells [14].

We have previously shown survival and differentiation of adult neural stem cells implanted into the scala tympani of the cochlea [14]. However, the number of surviving cells was relatively low and it was thus of interest to explore more efficient alternatives. It has been demonstrated that cells derived from ES cells, e.g., neurons and neural precursors [21,22], cardiomyocytes [17], and mast cells [30] survive well when implanted to appropriate sites in the

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doi:10.1016/j.brainres.2005.06.016

host. The cells integrate and to some measurable extent function within the host tissue. The therapeutic potential is further indicated by experiments where transplantation of ES cell-derived neural cells have resulted in differentiation into dopaminergic neurons in rodents with Parkinson's disease [3,16,19], or into neural cells in the animal model of spinal cord injury [21,22]. The survival of undifferentiated ES cells was also observed along the rat auditory nerve fibers [11]. In the present study, undifferentiated ES cells were transplanted into the cochlea in order to explore their potential use in a cell replacement therapy in the inner ear.

A key issue when transplanting ES cells to adult tissue is to ascertain that the cells eventually develop into relevant cell types. It has been shown that stem cells can differentiate into site-specific lineage under the influence of the microenvironment [4,8,25,28,29,31]. In embryos, the death and removal of a cell will normally result in a compensatory change of fate of neighboring cells. Thus, the differentiation of embryonic cells is determined primarily by its neighbors and not by its ancestry [7]. Consequently, a specific microenvironment may need to be established if implanted ES cells are to differentiate into sensorineural cells in the inner ear. A recent study has shown that medium from embryonic rat retina contained factors that induced neuronal differentiation of stem cells [15]. Moreover, neuronal differentiation of stem cells transplanted into rat retina explants was more efficient when using embryonic retinas as compared to postnatal host tissue [2]. Here, by using an undifferentiated ES cell line, we tested the hypothesis that embryonic neuronal cografts (dorsal root ganglions, DRGs) transplanted into the scala tympani of the cochlea could provide an embryonic microenvironment enhancing the survival and differentiation of simultaneously implanted ES cells.

2. Materials and methods

Cografting DRG tissue with ES cells was tested in pigmented guinea pigs (250–350 g) using both normal animals ($n = 24$) and animals treated with neomycin in order to damage the inner ear sensory epithelium ($n = 24$). The four experimental groups (each containing 12 animals) were as follows:

Group A: Normal hearing animals transplanted with ES cells only.
Group B: Neomycin-treated animals transplanted with ES cells only.
Group C: Normal hearing animals simultaneously transplanted with ES cells and a DRG cograft.
Group D: Neomycin-treated animals simultaneously transplanted with ES cells and a DRG cograft.

The animals were sacrificed at 2 weeks or 4 weeks after implantation ($n = 6$ for each survival period) and the inner ears processed for histology. The care and use of animals and all experimental procedures were approved by the regional ethical committee (approval nos. 283a-d/02 and 464/03).

2.1. Preparation of GFP expressing ES cells for transplantation

The procedures for preparing of ES cells have been described previously [8]. Briefly, mouse ES cells of the GSI-1 line were grown on primary mouse fibroblasts (mitotically inactivated by 35 Gy γ irradiation) in DMEM (Gibco-BRL) supplemented with 15% fetal calf serum (HyClone), 1000 U/ml of mouse recombinant leukemia inhibitory factor (LIF, Invitrogen), 0.1 mM 2-mercaptoethanol (Sigma), 0.1 mM non-essential amino acids, 2 mM glutamine, 100 U/ml of penicillin, and 100 mg/ml streptomycin (all obtained from Gibco-BRL). Transfection of the ES cells was performed by electroporation with a plasmid coding for GFP under control of the phosphoglycerate kinase promoter (QBiogen). G418 (Invitrogen) was used to select for clones that had incorporated the transfected genes and clones with high GFP expression were manually picked using fluorescence microscopy. The cell density of GFP expressing ES cells was adjusted to 10^4/μl in fresh DMEM and the ES cells were kept in 4 °C for transplantation.

2.2. Preparation of DRG tissue

Donor DRGs were obtained from mouse fetuses (C57BL/6, B&K, Sweden) at embryonic days 13–14 (E13–14) [12]. Under aseptic conditions and deep anesthesia (ketamine, 4 mg/100 g body weight, and xylazine, 1 mg/100 g body weight, i.m.), the abdomen and uterus of the pregnant mouse were exposed. The embryos were transferred to tissue culture medium (DMEM, Gibco). The DRG tissues were dissected out and kept in the same culture medium at 4 °C until transplantation.

2.3. Inner ear damaging procedures

In order to destroy the sensory epithelium [18,27], a 10% neomycin solution was injected into the left middle ear cavity through the tympanic membrane of guinea pigs following anesthesia (same drugs as above, body weight-adjusted dosage) [12]. After the injection, the head of the animal was maintained in the appropriate orientation for about 15 min so that the neomycin could reach the inner ear by diffusion through the round window membrane. This procedure generally causes severe loss of the sensory cells and a subsequent and progressive loss of spiral ganglion neurons. Two days later these neomycin-treated animals were ready for transplantation.

2.4. Transplantation of ES cells and DRGs

After establishing deep anesthesia (as above), the left post-auricular region of the animal was shaved and cleaned