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Research Report

Preferential differentiation of neural progenitor cells into the glial lineage through gp130 signaling in *N*-methyl-D-aspartate-treated retinas

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Abstract

The purpose of this study was to investigate the differentiation of neural progenitor cells (NPCs) following retinal transplantation in *N*-methyl-D-aspartate (NMDA)-treated eyes. NMDA was injected into the vitreous cavity of adult rat eyes. NPCs were prepared from telencephalic neuroepithelium of enhanced green fluorescence protein (EGFP) transgenic mice on embryonic day 14.5. A cell suspension was injected into the vitreous cavity in experimental eyes. Immunohistochemistry was conducted at 1, 2 or 4 weeks after transplantation of NPCs in an effort to determine the survival and differentiation of transplanted NPCs. Similar experiments were conducted using glycoprotein (gp)130-null (−/−) mice. Examination of retinal sections revealed that transplanted NPCs could survive for at least 4 weeks in NMDA-treated retinas. Immunohistochemical studies for specific cell-type markers revealed that, among the transplanted NPCs at 2 weeks after transplantation, the mean percentage (±standard deviation) of glial fibrillary acidic protein (GFAP)-positive (glial) cells was 63.5 ± 7.4%, demonstrating the differentiation of transplanted NPCs with a preference for the glial lineage. Furthermore, the mean percentage of βIII-tubulin-positive (mature neuronal) cells was 18.8 ± 4.5%. Following transplantation of NPCs isolated from gp130−/− mice into NMDA-treated retinas, the mean percentage of GFAP-positive cells (17.6 ± 7.0%), was significantly lower than that in NPCs isolated from wild-type mice (59.1 ± 6.0%, $P = 0.04$, Mann–Whitney U test). Preferential differentiation of NPCs into the glial lineage is induced through gp130 signaling in NMDA-treated eyes.

Theme: Development and regeneration
Topic: Cell differentiation and migration

Keywords: Neural progenitor cell; Transplantation; NMDA; Retina; Gp130; Differentiation

1. Introduction

In efforts to restore visual function in patients with significant visual impairment, substantial research effort has recently been expended towards the development of retinal regenerative therapy [6,12,15,18,21,28,31,35]. However, our knowledge of the regulatory mechanisms underlying the differentiation, migration and integration of transplanted progenitors in diseased retinas remains far from satisfactory. Experiments utilizing transplantation of progenitor cells into the retina can assist our understanding of conditions in the host retina that affect the fate of transplanted cells.

Common progenitor cells are known to differentiate into neuronal and glial lineages [30]. The molecular mechanisms associated with lineage determination for progenitor cells in retinas are thought to involve stimulation by a series of

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intrinsic and extrinsic factors [14]. Glycoprotein (gp)130-stimulating cytokines, such as ciliary neurotrophic factor (CTNF), interleukin (IL)-6 and leukemia inhibitory factor (LIF) are regarded as important factors for neural differentiation in the developing central nervous system [2,29] and in the retina [20,22]. On the other hand, *N*-methyl-D-aspartate (NMDA) induces glutamate receptor-related neurotoxicity in the retina [13], and NMDA-treated eyes have been used as a model for retinal diseases and glaucoma [1,8,10]. In diseased eyes following NMDA-treatment [8], as well as in ischemic retinas [9] or in conditions following mechanical injury [3,32], the up-regulated expression of ciliary neurotrophic factor (CNTF), a gp130-stimulating cytokine, has been demonstrated. Taken together, following transplantation into diseased (NMDA-treated) eyes, the differentiation of transplanted NPCs may be altered by the up-regulated expression of gp130-stimulating cytokines.

Herein, we will report that, in NMDA-treated retinas, transplanted NPCs preferentially differentiate into the glial lineage, and that gp-130 signaling plays an important role in the differentiation after transplantation.

2. Materials and methods

2.1. Culture of neural progenitor cells (NPCs)

NPCs were prepared from the telencephalic neuroepithelium of enhanced green fluorescence protein (EGFP) transgenic mice [19] on embryonic day (E) 14.5. NPCs have the potential to differentiate into neural or glial lineages [2,17]. The isolation and cultivation of NPCs were performed as previously described [17]. In brief, dissociated cells were cultured on poly-L-ornithine (Sigma, St. Louis, MO)/bovine fibronectin (Nitta Gelatin, Osaka, Japan)-coated dishes using a 1:1 mixture of Dulbecco's Modified Eagle's Medium/Ham's F12 (DMEM/F12; Gibco/BRL, Rockville, MD) supplemented with N2 (Sigma) and 10 ng/ml basic fibroblast growth factor (bFGF; R&D System, Minneapolis, MN) for 4 days. Cells were then detached in Hank's balanced salt solution (HBSS), reseeded into culture vessels and grown in the aforementioned medium containing 10 ng/ml bFGF and 10 ng/ml epidermal growth factor (EGF; R&D System) on hydroxyethyl methacrylate (Hema)-coated dishes. Neurospheres formed within 3–5 days.

2.2. Host animals (retinal damage models)

Experiments were performed on adult male Sprague–Drawley rats (6-weeks-old, weight 180–200 g), which were housed at room temperature on a 12-h light/dark cycle while given water and food ad libitum. The retinal damage model was created in a manner similar to that previously described by Morizane et al. [16]. Briefly, rats were anesthetized by intramuscular injection of xylazine (10 mg/kg) and ketamine (20 mg/kg). Pupils were dilated with drops of phenylephrine hydrochloride and tropicamide and a single dose of 5 μl of 4 mM NMDA (total 20 nmol; Sigma) was injected into the vitreous cavity using a microsyringe fitted with a 33-gauge needle. All studies were conducted in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

2.3. Transplantation of NPCs into NMDA-treated eyes

Neurospheres of NPCs were trypsinized, and suspended at 1×10^5 cells/μl in DMEM/F12 supplemented with N2. At 1 day after NMDA injection, 5.0 μl of cell suspension (containing 5×10^5 cells) was slowly injected into the vitreous cavity of the host using a microsyringe fitted with a 33-gauge needle. For immunosuppression, 10 mg/kg of cylosporine A (Wako, Osaka, Japan) was injected subcutaneously every day.

2.4. Tissue sectioning

Animals were sacrificed by an intraperitoneal overdose injection of pentobarbital at 1, 2 and 4 weeks after transplantation. Eyes were enucleated and eyecups were directly immersed in 4% paraformaldehyde for 1 h. The anterior segment and lens were subsequently removed. Posterior eyecups were immersed in the same fixative for 24 h at 4 °C and then in 15%, 20% and 25% sucrose-PBS for cryoprotection. Specimens were embedded in optimal cutting temperature (OCT) compound (Sakura, Tokyo, Japan), and 20-μm frozen sections were made using a cryostat (Leica, Heidelberg, Germany).

2.5. Immunohistochemistry

Specimens were washed with 0.1 M PBS, then incubated in 0.1 M PBS containing 5% skim milk (Nacalai Tesque, Kyoto, Japan) and 2% goat serum (Jackson Immuno Research, West Grove, PA) for 30 min to block nonspecific antibody binding. Samples were then incubated with primary antibodies diluted in 0.1 M PBS containing 5% skim milk for 24 h at 4 °C in a humid chamber. A description of the primary antibodies used follows. Mouse monoclonal anti-nestin (1:200; BD Pharmingen, San Diego, CA) was used as an immature undifferentiated cell marker. Mouse monoclonal anti-GFAP (1:500; Sigma) and rabbit polyclonal anti-GFAP (1:500; Dako, Glostrup, Denmark) were used as markers for astrocytes and reactive Müller glia of the retina. Mouse monoclonal anti-β-tubulin isotype III (1:100; Sigma) was used as a marker for mature neural cells, ganglion cells and amacrine cells of the retina. Mouse monoclonal anti-syntaxin (1:200; Sigma) was used as a marker for amacrine cells of the retina. Mouse monoclonal anti-glutamine synthetase (1:200; Chemicon, Temecula, CA) was used as a marker for Müller glia and