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Transplantation with retinal progenitor cells repairs visual function in rats with retinal ischemia–reperfusion injury

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HIGHLIGHTS

- Retinal progenitor cells (RPCs) transplantation is effective for RIR therapy.
- Subretinal space and the superior colliculus are suitable sites for grafting.
- Subretinal space transplantation could significantly improve the ERG response.
- Superior colliculus transplantation could significantly improve the VEP response.

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ABSTRACT

The retinal ischemia–reperfusion injury (RIR) is a common pathological process that leads to progressive visual loss and blindness in many retinal diseases such as retinal vascular occlusion disease, diabetic retinopathy, and acute glaucoma. Currently, there has been no effective therapy. The purpose of this study was to investigate the effects of transplantation of retinal progenitor cells (RPCs) into the subretinal space (SRS) and the superior colliculus (SC) in a rat model of RIR injury. We used cultured postnatal day 1 rat RPCs transfected with adeno-associated virus containing the cDNA encoding enhanced green fluorescence protein (EGFP) for transplantation. RIR injury was induced by increases in the intraocular pressure to 110 mmHg for 60 min. The effects of transplantation were evaluated by immunohistochemistry, electroretinography (ERG), and visual evoked potentials (VEP). We found that in rats with RIR injury, RPCs transplanted into the SRS and the SC survived for at least 8 weeks, migrated into surrounding tissues, and improved the ERG and VEP responses. Cells transplanted into the SC improved the VEP response more than those transplanted into the SRS. Our data suggest that transplantation of RPCs into the SRS and the SC may be a possible method for cell replacement therapy for retinal diseases.

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1. Introduction

Retinal diseases such as glaucoma, diabetic retinopathy, age-related macular degeneration, and vascular occlusion disease affect millions of people. Loss of retinal cells is regarded to be the irreversible cause of blindness in these retinal diseases. Currently, there are no effective treatments available for these retinal diseases. Recent studies have indicated that stem cells transplanted into the retina can survive and integrate into the host [25], suggesting that the development of stem cell therapy to replace missing retinal cells for the treatment of retinal diseases.

Retinal ischemia is a common cause of visual loss in humans [14]. An animal model of retinal ischemia–reperfusion (RIR) injury, which mimics clinical situations such as retinal vascular occlusion disease and acute glaucoma, is widely used to study retinal neuronal cell damage after ischemic insult [19]. RIR injury mainly causes retinal ganglion cell (RGC) loss or dysfunction. Replacement of missing RGCs via stem cell therapy is a promising treatment to restore vision loss in these retinal diseases. Several studies have shown that stem cells transplanted into the retina integrate into the host retina and restore visual function in RIR models [2,3], suggesting that stem cell therapy may be useful for the treatment of RIR.

For the past few decades, several types of cells have been used for the treatment of retinal diseases, including embryonic stem cells [23], brain-derived precursor cells [22], bone marrow-derived hematopoietic stem cells [12], and retinal progenitor cells (RPCs) [11]. Compared with poor integration of embryonic

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cells into the host retina, retinal precursors derived from postnatal day 1–7 (P1–P7) retinas show robust integration into the host retina [11], suggesting that retinal precursors derived from P1–P7 retinas may be suitable for transplantation. Because allogeneic transplants appear to be tolerated in the SRS without need for immunosuppression [1], many investigators choose the SRS as the site of transplantation [9,10]. Superior colliculus (SC), receiving approximately 65% of the contralateral RGC projections, represents the primary retinal projection area in rats [21]. RGCs can be retrogradely labeled by FluoroGold injection into the SC [15], and retinal injury can induce early onset and long-lasting neuronal damage in the SC [4,13]. Retinal precursors transplanted into the SC may result in migration of retinal precursors into the retina, and subsequent differentiation into RGCs.

In this study, we aimed to explore the role of transplantation of RPCs derived from P1 retinas in repair of retinal function in a rat model of RIR injury. SRS and SC were selected as the sites for transplantation.

2. Materials and methods

2.1. Animals

Seventy adult male Sprague-Dawley rats (weighing 200–250 g) and 3 P1 rats were used in this study. Rats were supplied by the Center of Experimental Animals, Xi'an Jiaotong University School of Medicine. Principles of laboratory animal care were followed and all procedures were conducted according to guidelines established by the National Institutes of Health.

Rats were randomly assigned into 7 groups: control group (rats without any treatment), sham group (sham-operated group), RIR group (retinal ischemia–reperfusion injury group), RIR + SC-C group (RIR rats with transplantation of RPCs into the superior colliculus), RIR + SC-PBS group (RIR rats with transplantation of PBS into the superior colliculus), RIR + R-C group (RIR rats with transplantation of RPCs into the SRS), and RIR + R-PBS group (RIR with transplantation of RPCs into the SRS). Animals were sacrificed at eight weeks after the transplantation.

2.2. Rat retinal progenitor cell preparation

Retinal progenitor cell from P1 neural retinas were isolated and cultured in serum-free medium containing N2, B27, EGF and bFGF. Neurospheres were formed after 2–3 days of culture, and were then passaged every 7–10 days by mechanical dissociation. Rat retinal progenitor cells (RPCs) were passaged 2 times before transplantation.

For adeno-associated virus (AAV) transfection of stem cells, rat RPC spheres were dissociated, and the cells were seeded at a density of 1.0×10^5 cells per well in 1 ml of fresh serum-free culture media. Cells were transfected with AAV containing the cDNA encoding enhanced green fluorescent protein (AAV-EGFP) at a multiplicity of infection of 100 for 72 h at 37 °C in 5% CO_2. The spheres that arose were visualized with a fluorescent microscope, and only green spheres were used for the transplantation experiment.

2.3. RIR injury model

Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (3.5–4 ml/kg). Pupils were dilated with1% tropicamide eye drops. The middle of the anterior chamber of the left eye was cannulated with a steel 30-gauge infusion needle connected to a pressure device. The intraocular pressure was elevated to 110 mmHg for 60 min. In the sham-operated control group, the middle of the anterior chamber of the left eye was cannulated with a steel 30-gauge infusion needle without the elevation of the intraocular pressure. Bacitracin ointment was applied to the eye after surgery.

2.4. Subretinal and superior colliculus Transplantation

RPCs were transplanted into the SRS and SC immediately after RIR injury. After the superior temporal pole of the sclera was exposed, the sclera and choroid were penetrated by a 30-gauge syringe needle. A 1 μl cell suspension (approximately 1×10^4 cells) was slowly injected into the SRS with a microinjector. The needle was allowed to remain in place for 30 s and was then very slowly withdrawn to minimize efflux of the transplanted cell suspension. For SC transplantation, the guide cannula was tied in the SC according to the following coordinates: 6 mm posterior to the bregma, 1.5 mm right to the midline, and 6 mm ventral to the skull surface. 1 μl of the cell suspension was injected into the right SC. For the control RIR + R-PBS and RIR + SC-PBS groups, 1 μl PBS was injected into the SRS and SC, respectively. No evidence of damage to the SC was found from histologic examination of SC sections performed at eight weeks after transplantation.

2.5. ERG and VEP recording

Full-field ERGs and VEP were recorded on the left eye for each rat eight weeks after the cell therapy. Animals were dark-adapted overnight and prepared for recording under dim red light. The responses were analyzed using RetiScan RetiPort electrophysiology equipment (Roland Consult, Germany). A silver wire loop was placed directly on the center of the rat cornea, and subcutaneous needle electrodes in the forehead and tail served as the reference and ground electrodes, respectively. For VEP, the recording electrode was inserted into the skin over the visual cortex. Bright-flash stimuli were given by a contact LED stimulator at a distance of 15 cm. VEP response was averaged from 100 sweeps.

2.6. Immunohistochemistry

Animals were sacrificed at eight weeks after the transplantation. Enucleated and fixed the left eyes and brain then cut in 12-μm-thick sections using a cryostat microtome. After blocking with 10% goat serum for 30 minutes, sections were incubated with primary antibodies anti-rhodopsin antibody (mouse IgG, 1:200; Abcam, MA, USA), anti-Pax6 antibody (rabbit IgG,1:200; Abcam, MA, USA), anti-Thy-1 antibody (H-110, rabbit IgG,1:200; santa cruz biotechnology, CA, USA), anti-protein kinas C alpha (C-term) (rabbit IgG,1:200; Epitomics, CA, USA), and anti-nestin antibody (mouse IgG,1:200; Abcam, MA, USA) at 4 °C overnight, followed by incubation with fluorescent-labeled secondary antibody FITC (anti-mouse IgG, 1:200; Abcam, UK) or Cy3 (Goat Anti-Rabbit IgG or Rabbit Anti-mouse IgG, 1:200; Beijing CoWin Biosciences Co., Beijing, China). Sections were examined under a fluorescence microscope.

2.7. Statistical analysis

The data are presented as mean ± SD. One-way ANOVA with subsequent post hoc tests was used to compare differences among groups. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Characterization of cultured cells for transplantation

The cultured cells, expressing pax6 (Fig. 1A) and Nestin (Fig. 1B), also expressed antigens specific to RGCs (Thy1.1; Fig. 1C), photoreceptors (rhodopsin; Fig. 1D), and bipolar cells (PKCα; Fig. 1E). These results suggested that the colony-forming cells from the neural

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