Transplanted Embryonic Retinal Stem Cells have the Potential to Repair the Injured Retina in Mice

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Research article

Keywords: Retinal stem cell, Retinal injury mice, Transplantation, Intravitreal injection

Posted Date: September 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-42741/v1

Version of Record: A version of this preprint was published on January 9th, 2021. See the published version at https://doi.org/10.1186/s12886-020-01795-1.

Abstract

Background: Stem cell transplantation has been reported as one of the promising strategies to treat retinal degenerative diseases, but this field is so complicated and still relatively new. This study investigated the potential role of transplantation of the embryo-derived retina stem cells (RSCs) into the vitreous cavity in repairing the damaged retina in mice.

Methods: RSCs were isolated from Kunming mice E17 embryonic retina and ciliary body tissues. RSCs were cultured, differentiated, and labeled with 5-bromo-2'-deoxyuridin (BrdU). Retinal injury was induced in left eyes in male Kunming mice by ring clamping of the optic nerve. The 6^{th}-generation of BrdU-labeled RSCs were transplanted into the damaged retina by the intravitreal injection, and saline injected eyes were used as the control. Hematoxylin and eosin histological staining, and BrdU, Nestin and Pax6 immunostaining were performed.

Results: Embryo-derived RSCs were identified by the positive stains of Pax6 and Nestin. BrdU incorporation was detected in most of RSCs. The damaged retina showed diffuse edema, disordered and loose-arranged morphological changes in all retinal layers. In the damaged retina with RSCs transplantation, the positive staining for BrdU, Pax6 and Nestin were revealed on the retinal surface. Notably, a small amount of RSCs migrated into the retinal ganglion cell layer and inner nuclear.

Conclusions: Embryonic RSCs have similar characteristics to neural stem cells. Transplantation of RSCs by intravitreal injection would be able to repair the damaged retina.

Background

Blindness eye diseases traditionally include cataract, corneal infection, and refractive stromal lesions. With the development of the basic research and clinical technologies, many of the oculopathy are becoming treatable. However, the spectrum for blindness eye disease is changing remarkably. Currently, retinal disease is a leading cause of vision loss worldwidely [1]. Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are two representative retinal diseases [1]. Photoreceptors (PRs) is responsible for conversion of the light into electrical signals for further process and integration. Anatomically, PRs are in contact with the retinal pigment epithelium (RPE). RPE involves the transport of nutrients, the recycling of proteins, and the elimination of photoreceptor debris. RPE also secretes some growth factors [2]. Therefore, dysfunctions or death of RPE cells can damage and/or induce loss of PRs, leading to damage of vision and in some cases ultimately causing blindness. Of note, RPE are not able to endogenously regenerate [3]. Stem cells have the key ability to self-renew and differentiate to various types of cells. It has been shown that stem cells are becoming an attractive source of cell therapy in replacing or repairing damaged RPE and PRs [4]. Retinal stem cell therapy is one of the promising therapeutic alternatives to recover vision in patients with retinal disease [2, 5].

Three classes of stem or progenitor cells are utilized for cell therapy, including pluripotent stem cells (PSCs), fetal cells, and postnatal/adult cells. Cell-based therapies for retinal diseases that are currently

under investigation usually use PSCs either embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) [3, 4, 6–9]. To date, a lot of studies have shown that stem cells or stem-cell-derived cells improve the survival and function of host cells, not by producing the missing cells, but by secreting growth factors [10]. The transplanted cells can migrate and integrate into the various layers of the retina, potentially induced by neural differentiation stimulating factors [11–14]. iPSCs are similar to ESCs, but derived by de-differentiating fully differentiated adult somatic cells (such as skin fibroblasts or white blood cells) into stem cells and then re-differentiating them into target cells [4]. Evidently, there are theoretical ethical advantages in using iPSCs for transplantation; but potential risks such as development of malignancy are present due to unknown mechanism of transformation and the transcription factors [15, 16]. As such, RSCs derived from ESCs seem to have more benefits in treating retinal disease. Therapeutic cells can be delivered to the intravitreal space or the subretinal space. However, subretinal injection posed a greater risk, which can result in retinal detachment [6].

In this study, we aimed to discuss the current stem cell transplantation approaches, and investigated the feasibility and significance of embryonic RSCs transplantation by intravitreal delivery in a retinal damaged mice model. We found that embryo-derived RSCs have similar characteristics to neural stem cells (NSCs), which is evidenced by Pax6 and Nestin expression. Transplanted RSCs were observed in all layers of the retina, suggesting that they have potential to function to repair the damaged retina. Accordingly, these findings here provide theory evidence for further study of RSCs transplantation in the treatment of retinal disease.

Methods

Animals

Healthy Kunming mice aged 5–6 weeks (n = 6; 3 pregnant and 6 non-pregnant) were purchased from Beijing HuaFuKang Biotechnology Co., LTD (Beijing, China), and maintained on a regular diurnal lighting cycle (12:12 light:dark) with ad libitum access to food and water under specific-pathogen-free conditions at the Central Animal Care Services of Shandong University. After procedure, mice were euthanized by introduction of 100% carbon dioxide into a bedding-free cage initially containing room air with the lid closed at a rate sufficient to induce rapid anesthesia, with death occurring within 2.5 minutes. This study was approved by the Shandong Provincial ENT Hospital (Shandong Provincial Western Hospital) Ethical Committee (Project No: XYK-20200701), and all procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Preparation of retinal stem cells

At the gestational age of 17 days, pregnant Kunming mice (n = 3) were euthanized, and embryos were immediately excised. As described previously [17–19], both the retina and ciliary body tissue including the pigmented layer-the ciliary marginal zone (CMZ) were cut into small pieces under a dissecting microscope for RSCs isolation (Fig. 1). The resulting tissues were digested using 100 U/mL collagenase (cat. no. C0130, Sigma-Aldrich, Shanghai, China) for 1 h, followed by 0.25% trypsin (cat. no. 15050057,

Invitrogen, Shanghai, China) for 15 min. Single cells were obtained by passing the suspension through a stainless steel 50 μm porous filter. Subsequently, the cells were transferred to a 25 mL culture flask at an inoculum density of 5×10^4 /mL in a 1:1 nutrient mixture of Dulbecco's modified Eagle's medium (DMEM) and F-12 (cat. no. 11330057, Gibco, Gaithersburg, MD, USA), supplemented with 1% B-27 (cat. no. 17504044, Gibco) and 20 ng/ml bFGF (cat. no. PHG0368, Gibco). Cells were cultured at 37 °C under 5% CO_2. After 8 days of culture, the primary suspension of cell clusters was passaged in a 1:2 ratio by 1 h digestion of collagenase, followed by disruption as a result of being forced through a sterile syringe for 5 times. The process was repeated every 5–7 days. After passaging, the sixth-generation cells were identified for RSCs using indirect immunofluorescence staining for Nestin and Pax6.

5-Bromo-2'-deoxyuridine (BrdU) labeling of RSCs

RSCs from the sixth-generation cultures were added to Poly-L-lysine packaged sterilized culture dishes and cultured for 3 days. Thereafter, 200 μg/ml of BrdU solution (cat no. 19–160, Sigma-Aldrich) was added to the cells, and incubated for 2 days in the presence of BrdU. Incorporation of BrdU was assessed using immunohistochemical staining.

Retinal injury model

Six male Kunming mice were anesthetized with 2% pentobarbital sodium by intraperitoneal injection. This allowed us to drop tetracaine solution (cat. no. 4512, Sigma Pharmaceuticals, North Liberty, IA, USA) into the left eyes for surface anesthesia, and circumclasp optic nerve with a micro-artery clamp for 15 s at 2 mm behind the globe. The right eyes without any treatment were used as the controls. Retinal slicing and hematoxylin and eosin (H&E) histological staining was performed 1 week after procedure.

Retinal stem cell transplantation

The standard intravitreal injection for RSCs transplantation was used as described previously [20]. Six Kunming male mice with left injured retina were randomly divided into RSCs injection group (n = 3) and PBS injection group (n = 3) in order to investigate the role of transplanted RSCs. Mice were anesthetized with 2% pentobarbital sodium by intraperitoneal injection, and the limbs and head were fixed well to allow access to the left (operative) eyes. Under a surgical microscope, a 10 μl micro-syringe connected to a 30G needle was inserted into the vitreous cavity from the corneoscleral limbus of the left injured eye in 3 mice, and 2 μl cell suspension of RSCs (3×10^4 cells/μl) from the 6th -generation cultures was carefully injected into the vitreous cavity. All injections were successful, which was verified by observing for 30 seconds without bleeding. The left injured retina in the other 3 mice were injected with 2 μl of sterile PBS in the same manner, serving as the controls. After two weeks, mice were euthanized, and eyeballs were removed for immunohistochemical staining. Of note, the operator was aware of the group allocation and disclosed it until results were obtained and analyzed.

H&E staining

H&E staining was performed on paraffin-embedded tissue sections according to the standard methods.

Immunohistochemistry staining

The paraffin-embedded tissue sections were initially placed into a temperature-controlled chamber at 60 °C for 30 min, followed by 10 min-deparaffinization in xylene and a series of gradient ethanol solutions: 100% ethanol for 1 min, 95% for 1 min, and 70% for 1 min. After hydration in distilled water for 2 min, the slices were immersed into 3% hydrogen peroxide for 15 min at room temperature to block endogenous peroxidase activity. For antigen retrieval, the slices were immersed into 2 mol/L HCl for 1 h and then dried at room temperature, followed by 0.1 mol/L NaOH for 2 s and rinse with PBS for 2 min. This step was repeated three times.

Subsequently, sections were stained with mouse anti-BrdU antibody (1:100, cat. no. ab8152, Abcam, Cambridge, United Kingdom) overnight at 4 °C. After rinsed three times with PBS, sections were incubated with sheep anti-mouse IgG solution (1:50, cat. no. ab6710, Abcam) at room temperature for 30 min. 3,3 diaminobenzidine (DAB) coloration was performed under microscope, and hematoxylin was used to stain the nuclei for 3 min. The specimens were then rinsed with water, dehydrated, and sealed for observation and imaging. Representative images were taken with optical microscope (Leica DM2500, Will and International Trade Co., LTD, Hong Kong).

Indirect immunofluorescence staining

Indirect immunofluorescence staining of Nestin and Pax6 were performed on cold acetone-fixed frozen tissue sections. After 3 washes with PBS, the sections were blocked in 10% goat serum for 1 h to decrease background signal. Rabbit anti-Pax6 (1:100, cat. no. ab195045, Abcam) and mouse anti-Nestin (1:100, cat. no. ab6320, Abcam) were added and incubated overnight at 4 ℃ in a wet box. After 5 washes with PBS, Alex488 and 594 conjugated goat anti-rabbit or mouse IgG (1:2,000, cat. no. A-11034, cat. no. A-11032, Invitrogen) were applied at 37^{o}C for 1 h in the dark room. Notably, the staining when the primary antibodies were omitted and only the secondary antibodies were applied were used as the blank controls. After 5 washes with PBS, DAPI was added and incubated in dark for 2 min. The specimens were then sealed with glycerin and immediately observed and photographed under a fluorescence microscope (BD-YG500, Shenzhen Boshida Optical Instrument Co., Ltd., Shenzhen, China).

Results

Culture and identification of retinal stem cells

Mouse retinal and ciliary bodies from E17 embryos were used to isolate RSCs. Under phase contrast microscope, most of the primary cultured cells showed fusiform or round, and a few were long and thin strip cells at day 4 after culture (Fig. 2A). The cell vitality was 40–50%. For the 4th -generation cells, 3 days after culture, cell density was increased, and most of the cells were round (Fig. 2B). After extended to the sixth generation, the cells grew well, and round cell density increased (Fig. 2C), indicative of having morphological characteristics of RSCs.

The sixth-generation cells were cultured, and after 3 days, Pax6 and Nestin were stained using indirect immunofluorescence staining for identification of RSCs. It has been demonstrated that Pax6 is required

for the multipotent state of retinal progenitor cells [21]. Nestin, a cytoskeletal intermediate filament, is initially characterized in neural stem cells. However, current extensive evidence suggested that Nestin plays an essential role in stem cell functions, including self-renewal, differentiation and migration [22]. Therefore, Pax6 and Nestin are commonly used as the markers of retinal stem cells. Our results show that Nestin was predominantly localized in cytoplasm, and Pax6 was mainly observed in nuclei (Fig. 3), suggesting that the 6th -generation cells are retinal stem cells.

Retinal histological alterations in the retinal injury model

Retinal injury was induced by circumclasp optic nerve. One week after the operation, retinal structure was checked using H&E staining. The normal/control eyes retina exhibited a typical retinal structure with three cell layers which were arranged in rows: retinal neurosensory layer (RNL), retinal pigment epithelium (RPE), and choroid layer (CL). Nevertheless, diffuse edema was observed in the retinal tissues of the operated/injured eyes, showing a disordered and loose-arranged morphology of all the three cell layers (Fig. 4). Representative images from one of the retinal injured mice were provided.

Retinal stem cells transplantation for repair of the damaged retina

The 6th -passage RSCs labeled with BrdU were used for transplantation. Since it can be incorporated into the newly synthesized DNA of replicating cells, BrdU is frequently used in analysis of neural stem cell biology, in particular to label and to fate-map dividing cells [23]. Firstly, incorporation of BrdU was assessed on the fixed cells. Immunohistochemical staining show that approximately 90% of RSCs were labeled with BrdU, evidenced by brownish staining in nuclei (Fig. 5).

RSC suspensions were then transplanted into the damaged retina using the intravitreal injection method. 2 weeks post-operation, pathological sections were stained for BrdU, Pax6 and Nestin. In the transplanted retina with RSCs, nuclear staining of BrdU and Pax6, and cytoplasmic staining of Nestin, were revealed on the surface of retina, predominantly between the retinal ganglion layer and inner nuclear layer (Fig. 6). Notably, a few of positive stains were also detected in the outer nuclear layer in the damaged eyes that accepted transplantation of RSCs (Fig. 6). In the control eyes, all layers of the retina were not positively stained. Representative images from one of the retinal injured mice with RSCs or PBS injections were provided.

Discussion

Stem cell engineering has opened a new avenue for repairing damaged nervous tissues. Following transplantation of cultured stem cells into eyes, they further integrate into the retinal microenvironment, and then proliferate and differentiate into target cells, to regenerate damaged neurons [6]. This offers recovery and reconstruction of the retinal function, with opportunities to treat irreversible blindness ophthalmopathy.

Previous work by Amirpour N et al. has demonstrated that the injection of ESCs into the subretinal space of rats effectively alleviated photoreceptor cell degeneration and death [24]. However, it is difficult to obtain ESCs because *in vitro* culturing is limited [25], posing a bottleneck for transplantation. Neural stem cells (NSCs) have been found in the embryonic nervous system and in certain parts of the adult brain. Due to continuous self-renewal and proliferative ability, these cells can be differentiated into specific neurons and glial cells. Recently, it has been reported that these cells were successfully integrated into the various layers of the retina [26–28]. The major challenge is how the NSCs differentiate into mature retinal cells. Some studies have shown that differentiation is associated with the growth environment of the cell [29–31]. In the present study, we isolated, cultured, and propagated mouse RSCs from E17 embryos. After extended to the 4th generation, the cells presented with phenotype of RSC. To further verify if these cells are stem cell and have characteristics of NSCs, we stained the stem cell marker Pax6 and NSC-specific marker Nestin [21, 22]. Cultured RSCs not only presented stem cell morphologies under phase contrast microscope, but also highly expressed Pax6 and Nestin, indicating that the cultured RSCs belong to the NSC family. Therefore, embryonic RSCs might be becoming prospective cells for retinal transplantation.

Considering the RSC as the most suitable seed cell, we further examined the feasibility of RSC transplantation for treatment of damaged retina. In general, there are currently two types of transplantation methods: subretinal space injection and vitreous cavity injection [6, 32]. Maintaining intraocular structure and preventing immune rejection is crucial for the survival of transplanted cells. In the case of subretinal space injection, it is often difficult to avoid blood retinal barrier disruption within the eyeball, which can cause severe swelling as well as degeneration and/or death of transplanted cells [32]. The intravitreal injection is a simplest feasible way in clinical practice for intraocular medication, which allows the eyeball to remain intact and reduces the occurrence of damage to the retina barrier. Moreover, this method offers a clear field of view during operation [6, 32, 33]. Although both of these grafting methods are capable of integrating seed cells into the retina, some studies recently compared the two methods, showing that trauma induced by the subretinal injection is considerably greater leading to retinal detachment [17]. Therefore, the intravitreal injection method is commonly preferred for delivery of medication or stem cell. In our study, we delivered BrdU-labeled RSCs into vitreous cavity for cell transplantation. Upon examination of the retina post-operation till 2 weeks no infections or bleeding were observed. Close observation of sectioned specimens revealed a complete intraocular structure with apparent anatomic arrangement. These findings suggest that intravitreal injection is a safe delivery way with less trauma for transplantation.

In order to gain an insight into the cell arrangement, we investigated the effect of transplanted RSCs on the retinal neuron composition. Retinal neurons are typically divided into three layers: ganglion cells, bipolar cells, and photoreceptor cells, from the inner to outermost layer, respectively [34]. Photoreceptor cells convert light stimuli into nerve impulses, the bipolar cells transfer nerve impulses to ganglion cells, and the nerve impulses transfer through the ganglion cells nerve fiber to the optical center, which produces the visual [34]. As the retinal ganglion cells are associated to nerve fibers, any mechanical damage to the optic nerve can block axoplasmic transport of the ganglion cells, leading to direct

impairment of ganglion cell nutrition [34]. The pathology of such retinal damage and visual function lesions are mimicked by ring clamping of the optic nerve [35].

Here, we firstly performed retinal damaged mice model by ring clamping of the optic nerve. 1 week after operation, H&E histological analysis showed diffuse edema in the retinal tissues, along with disordered and loose-arranged morphological changes of all layers. We then attempted to transplant the BrdU-labeled RSCs in close proximity to the ganglion cell layer, which allowed us to assess the effect of damaged optic nerves directly. Notably, the percentage of the BrdU-positive RSCs was greater than 90%, indicating that almost all the RSCs were labeled. Characterization of transplanted tissues was thereafter carried out after 2 weeks. Our results showed that the BrdU-positive cells were present in vitreous cavity, the retinal surface and the ganglion cell layer, indicating a successful transplantation of the RSCs and their entry into the damaged retina. This is further demonstrated by the presence of Pax6 and Nestin-positive cells in the retina, predominantly between the retinal ganglion layer and inner nuclear layer. Morphological evaluation also suggests that the transplanted RSCs were integrated into the host retina, implying its potential to substitute for the damaged cells. This observation is consistent with previous reports [36, 37]. However, differentiation of the transplanted RSCs was not distinguishable through light microscopy.

Several studies have shown that transplanted neural stem cells in retinas can partially differentiate into neurons [38–40], but generally depend on microenvironment of the host [41, 42]. In addition, it has been reported that this differentiation is also related to the host age. Li N et al. showed that the integration ability of stem cells transplanted into the vitreous cavity of rats decreased with the increase of host age [43]. Nevertheless, this phenomenon may be associated with a range of other factors such as the existence of a large number of undifferentiated cells in the neonatal host, an imperfect barrier function, and some growth factors promoting cell migration [44]. Furthermore, it has been shown that when the graft contains more mature cells it easily forms aggregates of cells or rosettes, which disrupt the integration of graft and host and thus prevent the transplanted cells from reconstructing the retinal function [45]. However, embryonic cells or stem cells are not easy to form a rosette even with a high number [46]. As such, it is evident that the purity of transplanted cells is extremely important to allow differentiation into functional target cells even if ESCs or embryonic RSCs are used.

Conclusions

Our findings suggest that embryo-derived RSCs exhibited similar properties to those of NSCs, and that transplantation of RSCs had potential to repair the damaged retina. Additionally, intravitreal transplantation of RSCs is a simplest feasible delivery way for treatment of retinal disease. However, many problems about RSC transplantation still require further extensive investigation, such as the key factors for transplanted RSCs survival, the role of the transplanted RSCs-produced cytokines in repairment of damaged retina, and the factors that can promote the differentiation of the transplanted RSCs into mature retinal functional cells.

Abbreviations

AMD: age-related macular degeneration; RP: retinitis pigmentosa; PR: Photoreceptors; RPE: retinal pigment epithelium; PSCs: pluripotent stem cells; ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; RSCs: retinal stem cells; CMZ: ciliary marginal zone; DMEM: Dulbecco's modified Eagle's medium; BrdU: 5-bromo-2'-deoxyuridine; PLL: Poly-L-lysine; H&E: hematoxylin and eosin; RNL: retinal neurosensory layer; RPE: retinal pigment epithelium; CL: choroid layer; NSCs: neural stem cells; RGL: retinal ganglion layer; INL: inner nuclear layer; ONL: outer nuclear layer.

Declarations

Ethics approval and consent to participate

This study was approved by Shandong Provincial ENT Hospital (Shandong Provincial Western Hospital) Ethical Committee (Project No: XYK-20200701), and all procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Shandong Province Technology Government Agency (No. 013GSF31804) and Shandong Province Hospital and Research Institute of EENT.

Author's contribution

Involved in the design of the study (HW); conduct of the study (XF, PC, XZ and JW); collection, management, and analysis of the data (XF and PC); preparation of the manuscript (XF); and critical revision of the manuscript (XF and HW). All authors have read and approved the final manuscript.

Acknowledgements

We thank Drs. Hu Huang and Jing Ji for technical support in cell cultures.

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Figures

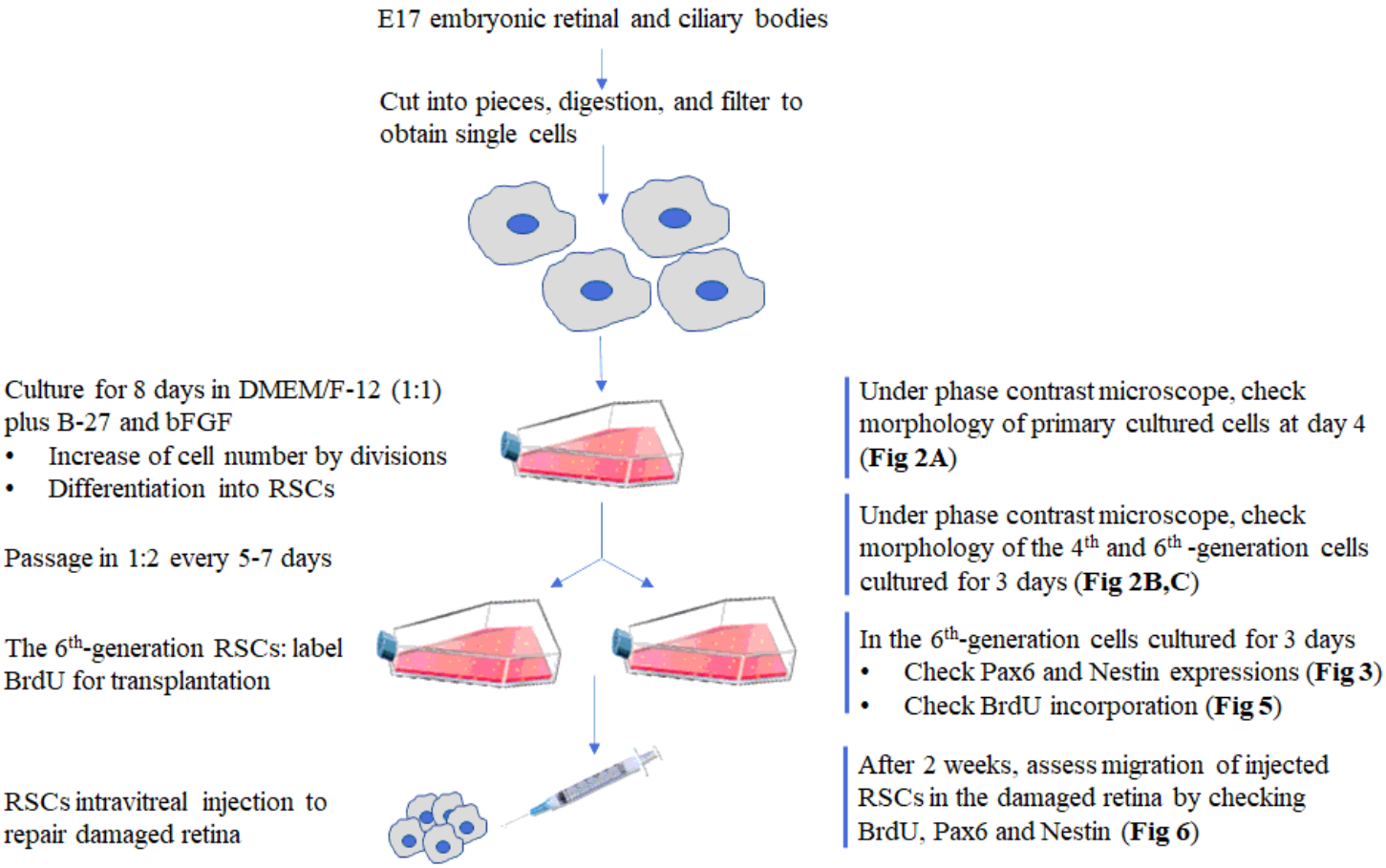

Figure 1

Schematic of the preparation of retinal stem cell

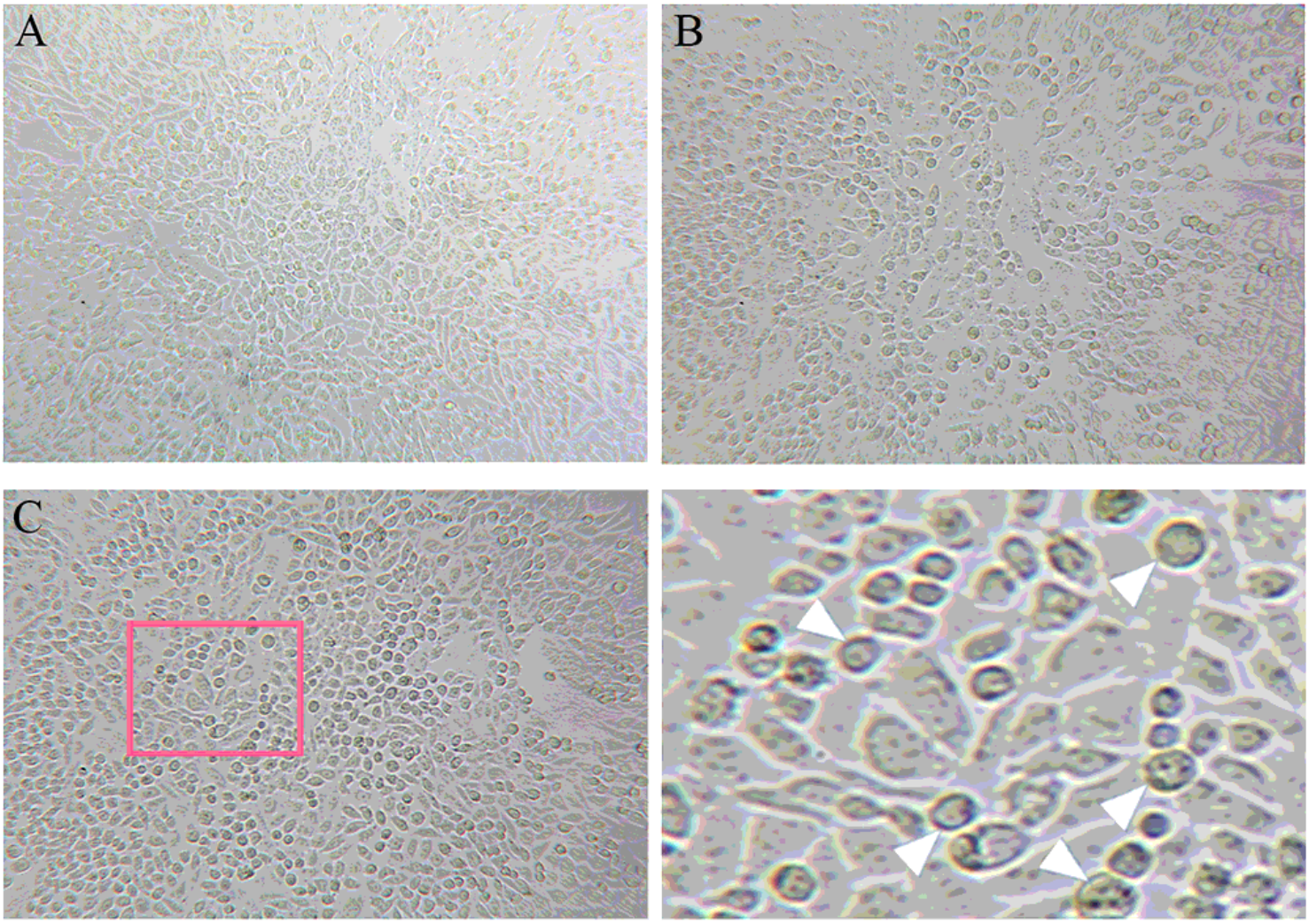

Figure 2

Generation of mouse retinal stem cells Retinal stem cells were isolated from embryonic E17 retinal and ciliary bodies. A. Phase contrast imaging of primary cultured cells for 4 days. Most of the cells showed fusiform or round, and a few were long and thin strip cells. B. 3 days after extended to the 4th generation, cell density increased, and mostly were round cells. C. After extended to the 6th generation, the cells grew well, and round cell density increased as indicated by the arrowhead. Magnification: ×200 for all images. A high-power view of the selected area was presented on the right.

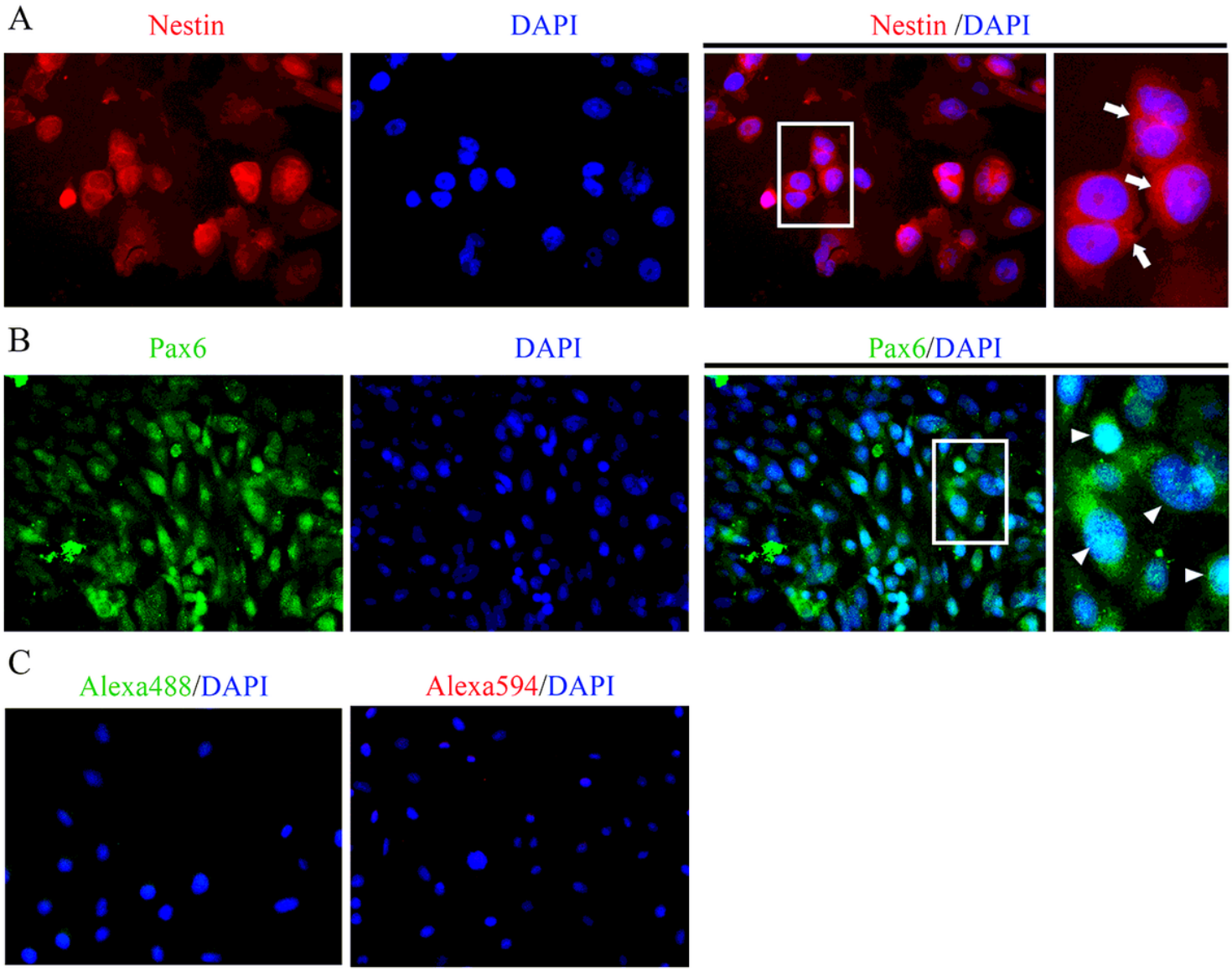

Figure 3

Immunofluorescence staining of Pax6 and Nestin on retinal stem cells Indirect immunofluorescence confocal microscopy was used to image Pax6 and Nestin on the sixth-passage retinal stem cells cultured for 3 days. Nuclei were labeled with DAPI. A. Nestin is predominantly localized in cytoplasm (arrow). B. Pax6 is observed mainly in nuclei (arrowhead). C. Primary antibodies were omitted and only the secondary antibodies Alexa488 or 594 conjugated goat anti-rabbit or mouse IgG were applied for the blank controls. Magnification: x200 for Pax6, x640 for Nestin. A high-power view of the selected area was presented on the right.

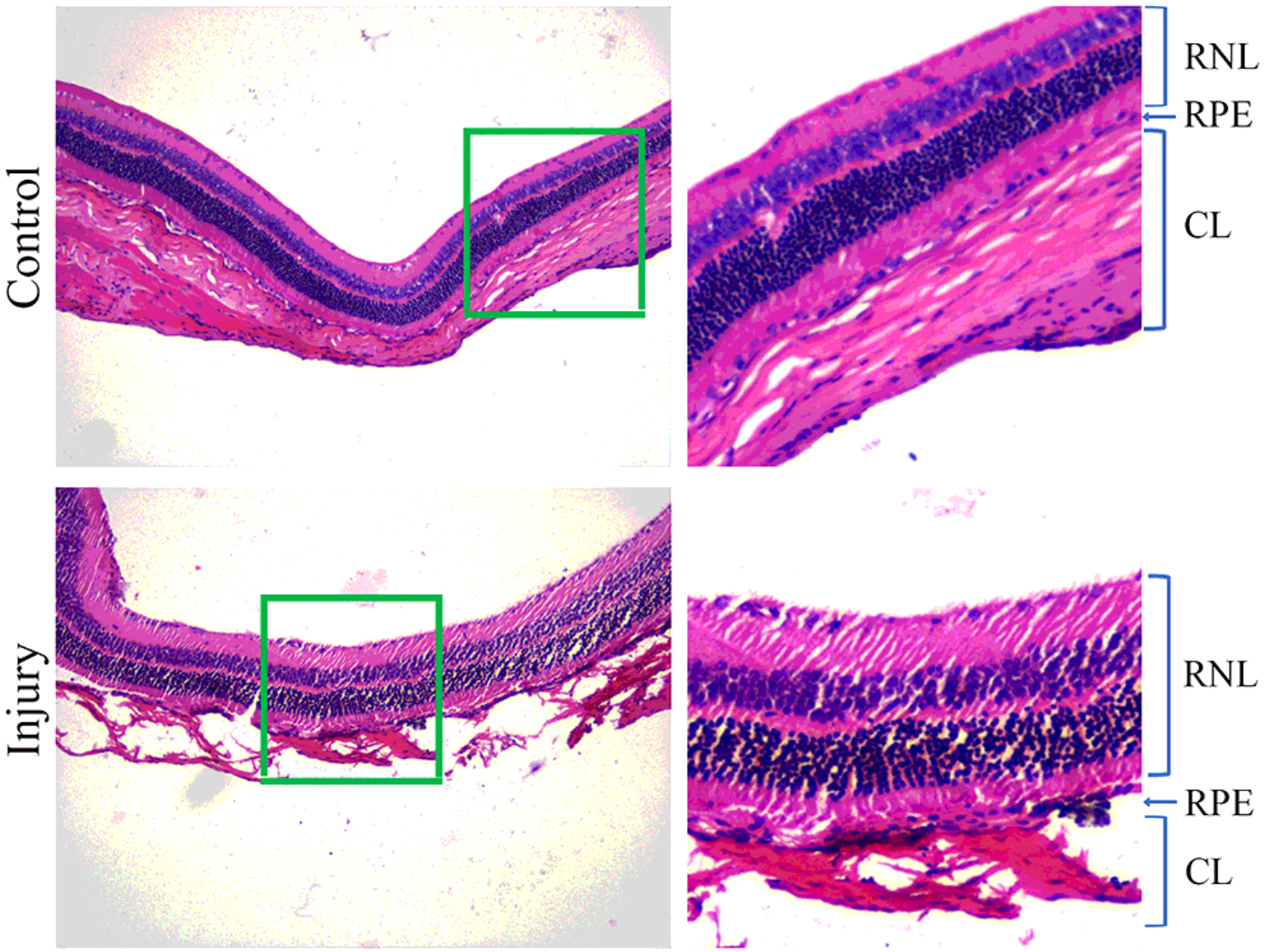

Figure 4

Histological staining of retinal morphology in retinal injured model Retinal injury model was performed by ring clamping of the optic nerve as described in the Methods part. Retinal structure was revealed using Hematoxylin and Eosin (H&E) staining 1 week after injury. Retina of the control eyes show well-arranged three layers: retinal neurosensory layer (RNL), retinal pigment epithelium (RPE), and choroid layer (CL). Retina of the operated/injured eyes shows full-thickness edema, loosening and disordered structures of all the three layers. Representative images from one of the retinal injured mice were provided. Magnification: x100. A high-power view of the selected area was presented on the right.

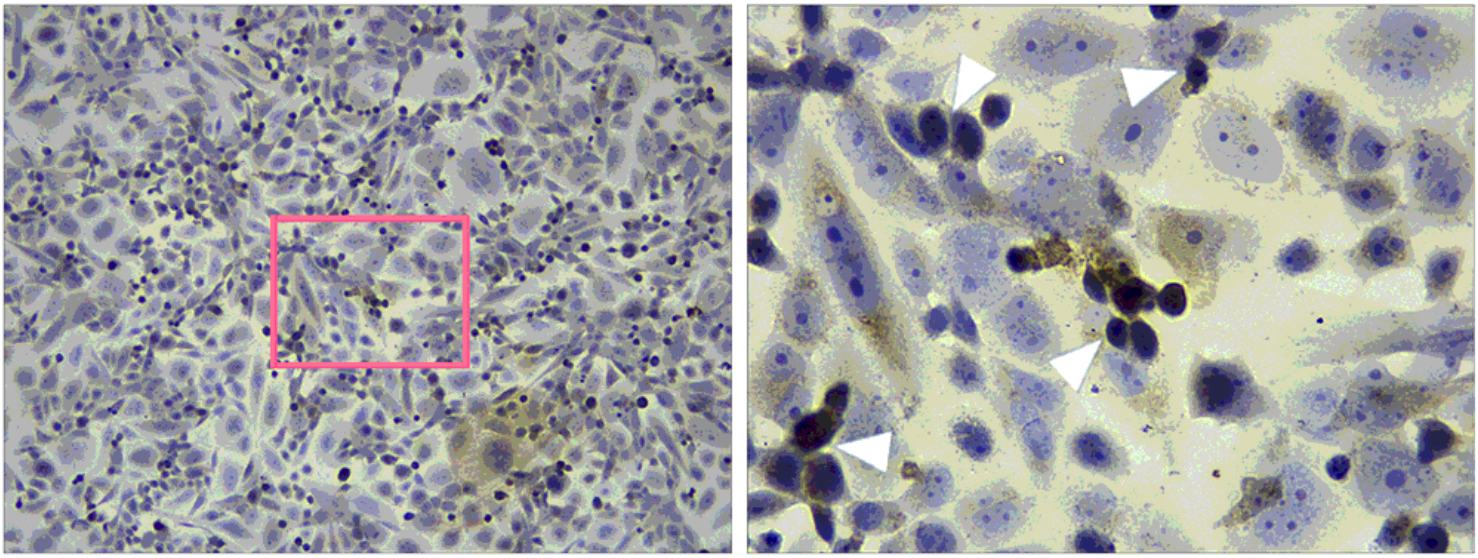

Figure 5

Immunohistochemical staining of BrdU on retinal stem cells The sixth-generation retinal stem cells were cultured for 3 days after passaging. Immunohistochemical staining of BrdU were then performed on fixed cells. BrdU was incorporated into most of retinal stem cells, evidenced by yellow-brown nuclei (arrowhead). Magnification: ×200. A high-power view of the selected area was presented on the right.

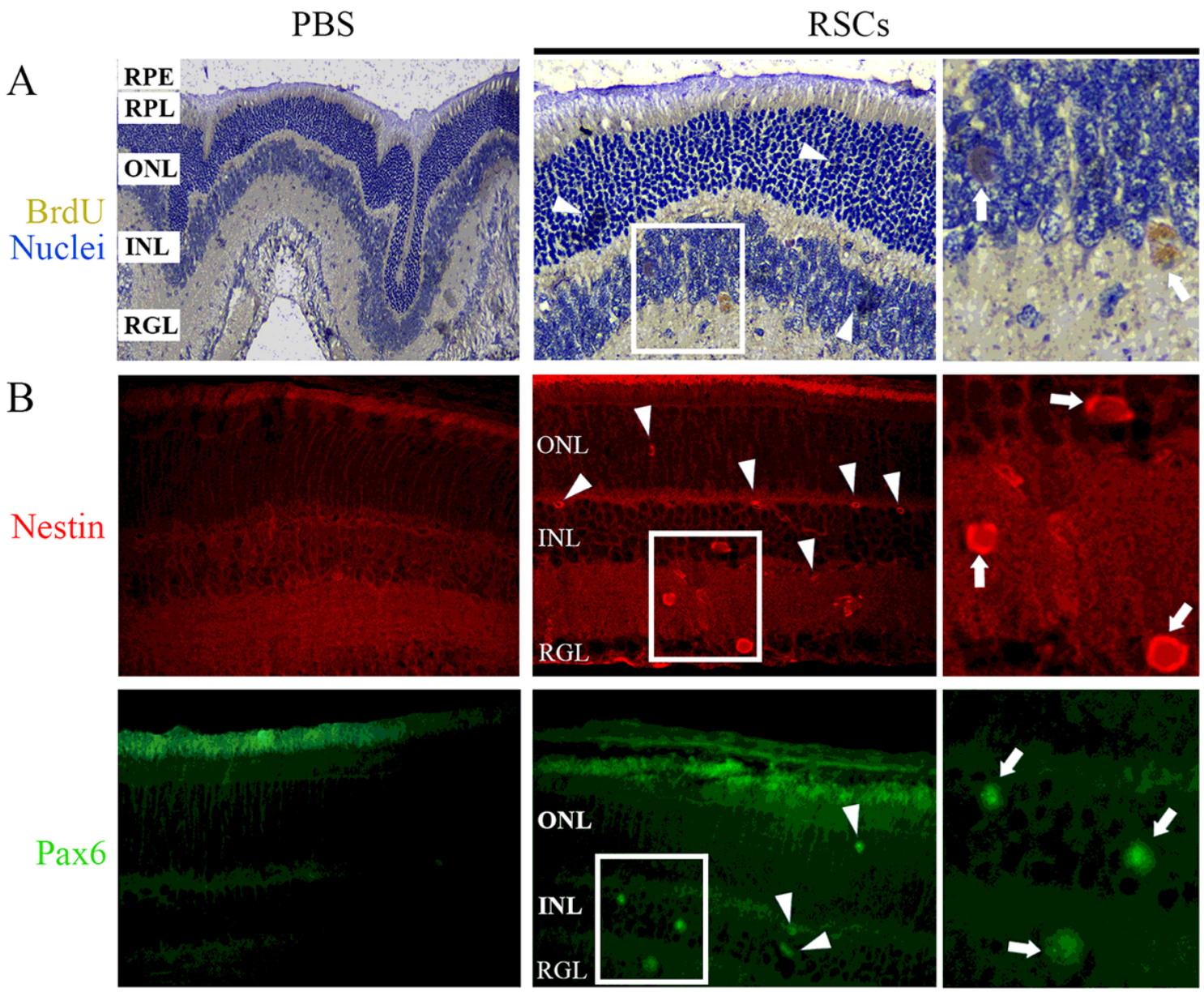

Figure 6

Transplanted retinal stem cells were detected in retinal injury model The sixth-passage retinal stem cells (RSCs) labeled with BrdU were transplanted to the operated/injured eyes for 2 weeks as described in the Methods part. PBS injected eyes were used as the controls. A. Localization of BrdU-retaining cells by immunohistochemistry staining. B. Immunofluorescence staining of Nestin and Pax6. In retinal injured eyes transplanted with RSCs, nuclear staining of BrdU and Pax6, and cytoplasmic staining of Nestin, were revealed on the surface of retina, predominantly between the RGL and INL (arrow). A few of positive stains for BrdU, Pax6, and Nestin were also detected in the ONL (arrowhead). In the PBS injected eyes, all layer of the retina remained unstained. Representative images from one of the retinal injured mice with RSCs or PBS injection were provided. Magnification: x400 for all images. A high-power view of the selected area was presented on the right. RGL: retinal ganglion layer (ganglion cell), INL: inner nuclear

layer (bipolar cell, Müller glia, and horizontal cell), ONL: outer nuclear layer (Rod and cone cell), RPL: retinal photoreceptor layer, RPE: retinal pigment epithelium.

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