

RESEARCH

Open Access



Protection of GAP-43 gene–modified bone marrow mesenchymal stem cells on retinitis pigmentosa

Yuxi He¹, Yuxiao Guo² and Guanfang Su^{1*}

Abstract

Background To observe the therapeutic effects of growth associated protein-43 (GAP 43) gene modified rat bone marrow mesenchymal stem cells (BMSCs) on experimental retinal degeneration.

Methods A total of 80 male Royal College of Surgeons (RCS) rats were divided into three groups at postnatal 21 (P21) randomly. A cell suspension of 5×10^4 BMSCs modified with GAP-43 in 2 μ l PBS was injected into the subretinal space of BMSCs + GAP-43 group rats, BMSCs group rats received 5×10^4 BMSCs in 2 μ l PBS and PBS group rats received 2 μ l PBS. Photoreceptor necrosis and apoptosis was assessed by HE staining and Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling (TUNEL) detection. The expression of GAP-43, Glutamine synthetase (GS), rhodopsin (RHO), Caspase-8 and Caspase-9 was analyzed by immunofluorescence, western blot.

Results Transplantation of GAP-43-modified BMSCs into the subretinal cavity of RCS rats revealed that: retinal GAP-43 expression was significantly elevated ($P < 0.05$); Rhodopsin (Rho) expression, a photoreceptor marker, was upregulated 1.5-fold ($P < 0.05$), and the thickness of the outer nuclear layer increased to $79.78 \pm 6.83 \mu\text{m}$ (control $29.92 \pm 4.17 \mu\text{m}$, $P < 0.01$); The apoptosis rate decreased to 41.9% (control 63.8%, $P < 0.01$), and Caspase-8/9 activation was inhibited; Müller cell proliferation was reduced (GS decreased, $P < 0.05$). suggesting that GAP-43-modified BMSCs delayed retinal degeneration through anti-apoptosis and pro-photoreceptor survival.

Conclusions The results suggest that GAP-43 gene modified BMSCs has the therapeutic effect on the early stage of retinal degeneration, which might make more photoreceptors preserved.

Highlights

- GAP-43–modified bone marrow mesenchymal stem cells (GAP-43 + BMSCs) were transplanted into the subretinal space of RCS rats.
- GAP-43 + BMSCs preserved outer nuclear layer thickness and rhodopsin-positive outer segments better than unmodified BMSCs and PBS.
- GAP-43 + BMSCs reduced TUNEL-positive photoreceptors and downregulated Caspase-8 and Caspase-9 activation in the degenerating retina.

*Correspondence:
Guanfang Su
sgf@jlu.edu.cn

Full list of author information is available at the end of the article

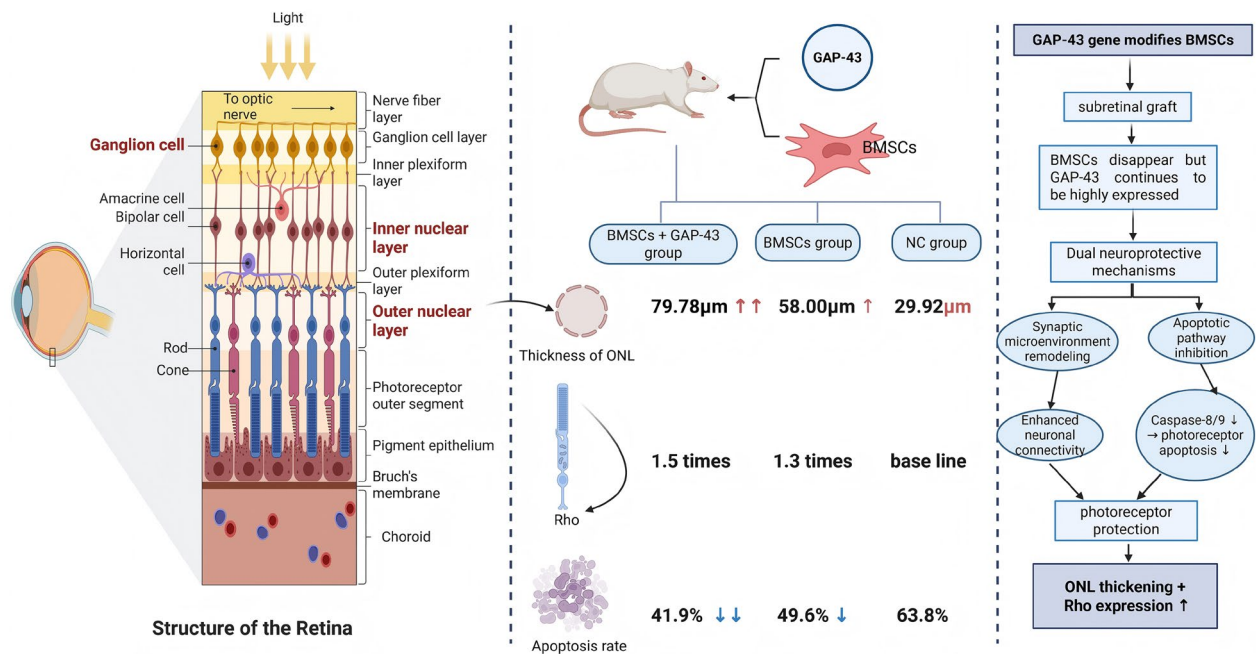


© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

- GAP-43 overexpression broadened retinal GAP-43 distribution and modulated Müller glial activation, supporting a neuroprotective microenvironment.
- GAP-43 gene modification enhanced the structural and molecular neuroprotective effects of BMSC transplantation in early-stage retinal degeneration.

Keywords Bone marrow mesenchymal stem cells, Cell apoptosis, Cell transplantation, Growth associated protein-43, Retinitis pigmentosa

Graphical Abstract



Introduction

Retinitis Pigmentosa (RP) encompasses a group of inherited retinal disorders characterized by dysfunction of retinal pigment epithelial (RPE) cells and progressive degeneration of photoreceptors [1]. In recent years, cell transplantation therapy for RP has emerged as a promising therapeutic strategy. Studies indicate that transplanted cells may either act as substitutes for degenerated photoreceptors [2] or secrete neurotrophic factors to enhance retinal cell survival, growth, and functionality [3].

However, the clinical translation of cell-based therapies faces significant challenges, including the limited availability of pluripotent retinal stem cells [4] and immune rejection in allogeneic or xenogeneic transplantation [5]. Consequently, bone marrow mesenchymal stem cells (BMSCs) have gained prominence as a preferred candidate for transplantation therapy due to their immunomodulatory properties and accessibility.

Our previous experiments revealed that induced retinal ganglion-like cells express the GAP-43 gene, whereas undifferentiated primary BMSCs did not express the GAP-43 gene, as confirmed by suppression subtractive

hybridization (SSH). This suggests that GAP-43 may act as a critical regulator activated during BMSCs differentiation or a key component of intracellular signaling pathways governing neural differentiation. Although the precise mechanism underlying BMSCs differentiation into neural cells remains unclear, we further demonstrated that GAP-43 promotes BMSC differentiation into neuron-like cells and that intravitreal injection of GAP-43-transduced BMSCs enhances neural repair in a rat model of traumatic optic neuropathy [6].

In this study, GAP-43 was transduced into BMSCs using lentiviral vectors, and the modified cells were delivered into the subretinal space of RCS rats. We aimed to investigate the differentiation potential of GAP-43-modified BMSCs within the retinal degeneration microenvironment of RP and to evaluate the morphological and molecular outcomes of subretinal BMSC transplantation in vivo, including outer nuclear layer thickness, rhodopsin expression, Müller cell activation and apoptotic signaling.

Retinitis pigmentosa (RP) is a progressive retinal degenerative disorder characterized by the loss of photoreceptors and subsequent vision impairment. Despite

advances in gene and cell therapies, effective strategies to preserve photoreceptor function and inhibit retinal cell apoptosis remain limited. Bone marrow mesenchymal stem cells (BMSCs) have shown promise due to their immunomodulatory properties and capacity to promote retinal repair; however, their therapeutic efficacy is constrained by insufficient neuronal differentiation and survival in the hostile retinal microenvironment. Growth-associated protein-43 (GAP-43), a key regulator of axonal growth and synaptic plasticity, may enhance the neuroprotective capacity of BMSCs when genetically modified.

Based on this rationale, we hypothesize that GAP-43 gene modification of BMSCs will promote photoreceptor survival and inhibit apoptosis by modulating intrinsic and extrinsic apoptotic pathways and by creating a synaptic microenvironment favorable for retinal neuron connectivity. This mechanism-based study aims to evaluate the effects of GAP-43-modified BMSCs on retinal degeneration in an established rat model of RP, providing insights into the underlying neuroprotective mechanisms and exploring a translational approach for cell-based retinal therapies.

Materials and methods

Isolation, culture, and identification of BMSCs

Wistar rats were obtained from the Animal Center of Jilin University. RCS rats (NBRP Rat No: 0011) were procured from the Graduate School of Medicine at Kyoto University (Japan) and housed in a specific pathogen-free (SPF)-grade animal facility at the Animal Service Center of Jilin University. Animals were maintained under controlled temperature (22–25 °C) and humidity (50–60%) with a 12-hour light/dark cycle. All experimental procedures involving rats were approved by the Experimental Animal Ethics Committee of Jilin University (Permit No: KT201903043).

For BMSC isolation, 8-day-old neonatal female Wistar rats were sterilized in 75% ethanol for 5 min. BMSCs were harvested from the femurs of adult female Wistar rats by inserting a 21-gauge needle into the bone shaft and flushing with 5 mL Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12; HyClone) repeatedly. The rinsed solution was gently poured into a centrifuge tube containing an equal volume of pre-cooled 1.073 g/L Percoll separation liquid (Sigma-Aldrich) and centrifuged at $2500 \times g$ for 20 min. The intermediate mononuclear cell layer was collected, washed twice with phosphate-buffered saline (PBS), and resuspended in DMEM/F12 supplemented with 5% fetal bovine serum (FBS; Gibco). Cells were maintained in a humidified incubator (SANYO, Japan) at 37 °C with 5% CO₂. After 24 h, non-adherent cells were removed via medium replacement, and the culture medium was refreshed

every 3 days. Upon reaching ~90% confluence, cells were passaged to sustain exponential growth. Third-passage BMSCs were utilized for subsequent experiments.

After three passages, the cultured cells exhibited the typical morphological characteristics of bone marrow-derived mesenchymal stem cells. To further confirm their identity, cells from the third passage were collected and analyzed by flow cytometry using antibodies against CD90, CD44, CD11b, and CD45 (BD Biosciences, USA). **Detection of Surface Markers on BMSCs Using Flow Cytometry.**

Specific experimental steps are as follows: (1) Indirect fluorescent labeling for BMSC surface markers CD11b and CD44: Passage cells to the third generation, digest and collect cells, wash three times with PBS. Add diluted primary antibodies CD11b and CD44 (1:200). Use PBS as a control for the primary antibody. Incubate at 4 °C for 1 h. Add diluted FITC-labeled secondary antibody and incubate at 4 °C in the dark for 30 min. Wash three times with PBS (5 min per wash). Detect expression of surface markers CD11b and CD44 using flow cytometry. (2) Direct Fluorescent Labeling for BMSC Surface Markers CD90 and CD45 At the third passage, cells were digested and collected, washed three times with PBS, and incubated with diluted CD90-FITC and CD45-FITC antibodies at 4 °C for 1 h. Wash cells three times with PBS (5 min each wash). Detect expression of surface markers CD90 and CD45 using flow cytometry.

Construction of lentiviral-mediated GAP-43 gene expression vector and its control viral vector

The lentiviral-mediated GAP-43 gene expression vector (LV5-GAP-43) and control viral vector (LV5-NC) lentiviral vectors were synthesized by Shanghai Sangon Biotech. Third-passage BMSCs were seeded into 96-well plates at a density of 5×10^3 cells/mL. After 24 h, the cells were transduced with LV5-GAP-43 or LV5-NC lentiviruses at multiplicities of infection (MOIs) of 5, 10, and 20. Transduction efficiency was assessed 48 h post-transfection using fluorescence microscopy; successful viral integration was confirmed by the presence of green fluorescent protein signals. The optimal MOI was selected based on fluorescence intensity and cell viability. Subsequently, BMSCs were transduced with LV5-GAP-43 or LV5-NC at the optimal MOI to generate stably modified BMSCs-LV5-GAP-43 and BMSCs-LV5-NC cell lines.

Animal experiments

A total of 80 male RCS rats at postnatal day 21 (P21) were used in this study. Animals were housed under specific pathogen-free conditions and were randomly assigned to three groups: (1) GAP-43 + BMSCs group, (2) BMSCs group, and (3) NC group. Only the right eye of each rat was used for subretinal injection and subsequent

analysis. Due to pilot experiments for optimizing the subretinal injection procedure and exclusion of animals with severe surgical complications, not all enrolled rats were included in every quantitative analysis; the exact number of eyes used for each assay and time point is specified below and in the corresponding figure legends.

Based on a previous subretinal transplantation study in RCS rats, in which 50,000 bone marrow-derived mesenchymal stem cells were injected per eye and shown to delay retinal degeneration, and on our own pilot surgical experience, we selected a dose of 5×10^4 BMSCs per eye. In our hands, suspending this cell number in 2 μ L PBS reproducibly generated a localized subretinal bleb while avoiding extensive retinal detachment or reflux into the vitreous.

In the GAP-43 + BMSCs group, a suspension of 5×10^4 BMSCs-LV5-GAP-43 cells in 2 μ L PBS was injected into the subretinal space of the right eye at P21. In the BMSCs group, the right eye received 5×10^4 BMSCs-LV5-NC cells in 2 μ L PBS. In the NC group, the right eye received 2 μ L PBS alone. All animal procedures were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, USA) and were approved by the Experimental Animal Ethics Committee of Jilin University (Permit No.: KT201903043).

Subretinal injections were performed under an operating microscope after pharmacologic pupillary dilation. A small corneal puncture was first made to reduce intraocular pressure and minimize reflux of cells during micropipette insertion. A glass micropipette was then introduced tangentially through the sclera into the subretinal space, and the cell suspension or PBS was slowly injected to form a shallow subretinal bleb. Immediately after injection, the fundus was examined by indirect ophthalmoscopy to assess retinal integrity, confirm bleb formation and exclude signs of vascular compromise. Retinal reattachment was confirmed on follow-up fundoscopy. Animals exhibiting massive retinal detachment, vitreous hemorrhage or lens injury were excluded from further analysis.

Rats were anesthetized via intraperitoneal injection of pentobarbital (40 mg/kg; Shanghai Lianshuo Biotechnology Co., Ltd., Shanghai, China). For donor cell tracking and dynamic analysis of retinal GAP-43 expression, rats from the GAP-43 + BMSCs group were sacrificed at 3, 7, 10 and 20 days after surgery; at each time point, 3 rats ($n=3$ eyes) were used. In these eyes, frozen retinal sections were prepared to visualize fluorescent BMSCs, and retinal tissue was collected for Western blot analysis of GAP-43.

For the main endpoint at 30 days after transplantation, rats from each treatment group were used for structural, apoptotic and protein expression analyses. For outer

nuclear layer (ONL) morphometry, 3 rats per group ($n=3$ eyes/group) were processed for hematoxylin–eosin (HE) staining, and ONL thickness was measured on sagittal sections passing through the optic nerve head. For immunofluorescence analysis of GAP-43, Rho and glutamine synthetase (GS), 3 rats per group ($n=3$ eyes/group) were processed for retinal cryosections and stained with the corresponding antibodies. For assessment of photoreceptor apoptosis, 3 rats per group ($n=3$ eyes/group) were analyzed by TUNEL assay.

In addition, another set of rats (3 rats per group, $n=3$ eyes/group) was sacrificed at 30 days after transplantation, and the injected eyes were used for Western blot analysis of retinal GAP-43, Rho, GS, Caspase-8 and Caspase-9. For each rat, only the right eye was included in the analysis. Investigators performing ONL measurements, TUNEL cell counting and Western blot densitometry were masked to group allocation.

For morphological and immunofluorescence analyses, eyes were enucleated, post-fixed in Davidson's fixative for 20 min, and subsequently infiltrated with 10%, 20% and 30% sucrose solutions for 2 h each, followed by overnight incubation in 30% sucrose at 4 °C before embedding and sectioning.

Immunofluorescence staining

Retinal tissues were embedded in optimal cutting temperature compound (OCT) compound (SAKURA Tissue-Tek® O.C.T. Compound, USA), and horizontal Sect. (10 μ m) were prepared using a cryostat. To maintain orientation, the superior pole of each eye was marked with a suture. Slides were collected for staining. One set of retinal sections was stained with hematoxylin and eosin (Nanchang Yvlu Chemical Reagent Co., Ltd., Jiangxi, China) to evaluate the injection site, while other sets were stained with specific antibodies for immunofluorescence analysis. The injection site (IN) was the primary focus of detection.

BMSCs-LV5-GAP-43 and BMSCs-LV5-NC cells grown on glass coverslips were fixed with 4% paraformaldehyde (PFA) for 15 min, washed three times with PBS, and permeabilized with PBS containing 0.5% (v/v) Triton X-100 for 10 min at room temperature. These fixation and permeabilization steps were not applied to the retinal sections. Both cells and tissues were blocked with 3% (w/v) bovine serum albumin (BSA) for 1 h at room temperature and incubated overnight at 4 °C with the following primary antibodies: rabbit anti-GAP-43 (1:500, ab16053), mouse anti-Rho (1:800, ab5417), and rabbit anti-GS (1:800, ab49873) (all from Abcam).

After washing with PBS, the samples were incubated for 30 min at room temperature with DyLight 488-conjugated secondary antibodies: Goat anti-Mouse IgG, Cross-Adsorbed (35503, Thermo Fisher Scientific) or

Goat anti-Rabbit IgG, Cross-Adsorbed. Nuclei were counterstained with DAPI. Coverslips were mounted using antifade mounting medium (Vector Laboratories, Inc., Burlingame, CA) and examined under a confocal microscope (Olympus, Japan).

Western blot

Protein was extracted from cells and tissues using a cell lysis reagent (BIOSS; Beijing Biosynthesis Biotechnology Co. Ltd.), supplemented with 1% PMSE, and incubated on ice for 30 min. The insoluble protein fraction was removed by centrifugation at 10,000 g for 10 min at 4 °C. Protein samples (100 µg) were heat-denatured, resolved by 10% SDS-PAGE, and transferred to polyvinylidene fluoride (PVDF) membranes.

After blocking the membranes with 5% (w/v) nonfat milk for 2 h at room temperature, they were incubated overnight at 4 °C with the following primary antibodies: anti-GAP-43 antibody (1:500, ab16053, Abcam), anti-RHO antibody (1:1000, ab5417, Abcam), anti-GS antibody (1:5000, ab49873, Abcam), anti-Caspase-9 antibody (1:500, ab32539, Abcam), anti-Caspase-8 antibody (1:500, 4790, Cell Signaling Technology), and β -actin mouse monoclonal antibody (1:5000, AA128, Beyotime). The membranes were washed three times with Tris-buffered saline–Tween (TBST) solution and subsequently incubated with a horse radish peroxidase-conjugated secondary antibody (1:5000; BIOSS; Beijing Biosynthesis Biotechnology Co. Ltd.) for 1 h. After washing with TBST, signal detection was performed using the Amersham enhanced chemiluminescence (ECL) Prime Western Blot Detection Reagent (Millipore, Germany) with a FluorChem HD2 system (Alpha Innotech, USA). The bands were quantified by densitometry using ImageJ software, with β -actin as the internal control.

TUNEL analysis

The site for obtaining frozen sections from each eyeball was equidistant from the optic nerve. Five random fields were selected from the injection sites in each section. Apoptotic cells were identified using the In Situ Cell Death Detection Kit, Fluorescein (11684795910, Roche), according to the manufacturer's instructions. Images were captured at 20 $\times$ magnification using a confocal microscope (Olympus, Japan). TUNEL-positive cells and DAPI-stained cells from the same fields were quantified using Image Pro software in a blinded manner to calculate the apoptosis rate.

Statistical analyses

Statistical analyses were performed using ImageJ, SPSS 27.0, and Excel. Student's *t*-test and analysis of variance (ANOVA) were used to assess the differences among variables. A *P* value of less than 0.05 was considered

statistically significant. All experiments were repeated at least three times, with data presented as the mean $\pm$ SEM. The significance levels were indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$, reflecting increasing levels of significance.

Result

Morphology and identification of BMSCs

After three passages, the cultured cells displayed the typical plastic-adherent, spindle-shaped, fibroblast-like morphology of bone marrow–derived mesenchymal stromal cells (Fig. 1A). To further confirm their identity, third-passage cells were harvested and analyzed by flow cytometry using antibodies against CD90, CD44, CD11b and CD45. As shown in Fig. 1B, the majority of cells were positive for the mesenchymal markers CD90 (94.7%) and CD44 (74.4%), whereas only a small fraction expressed the hematopoietic/monocytic markers CD11b (0.12%) and CD45 (2.4%). This CD90⁺/CD44⁺/CD11b[−]/CD45[−] immunophenotype, together with the characteristic morphology and the standard bone marrow isolation procedure, was used to define the cultured cells as BMSCs in this rat model.

GAP-43 expression in BMSCs-LV5-GAP-43

Infection of BMSCs with GAP-43 lentivirus expression vector and detection of multiplicity of infection

BMSCs were infected with the GAP-43 lentivirus vector. The third-generation BMSCs were inoculated into a 96-well culture dish at a density of 5×10^3 cells/ml. After 24 h, the corresponding numbers of LV5-GAP and LV5-NC viruses were added according to MOI values of 5, 10, and 20, respectively. Forty-eight hours post-transfection, green fluorescent BMSCs were observed under a fluorescence microscope against a black background, indicating successful virus transfection into BMSCs and determination of the optimal infection multiplicity. Cells were selected and purified using 10 ng/mL puromycin. BMSCs were infected with the GAP-43 lentivirus vector and divided into three groups: GAP-43 + BMSCs group (BMSCs-LV5-GAP-43), BMSCs group (BMSCs-LV5-NC) and NC group (untransduced BMSCs). Forty-eight hours after infection, the two lentiviruses were observed under a fluorescence microscope and photographed (Fig. 1C). In the images, A1, B1, C1 show fluorescence microscope images of BMSCs group cells infected with MOI = 5, 10, and 20, respectively. A2, B2, C2 are the corresponding bright-field images. D1, E1, F1 show fluorescence microscope images of GAP-43 + BMSCs group cells infected with MOI = 5, 10, and 20, respectively, and D2, E2, F2 are their corresponding bright-field images. As shown, the number of fluorescent cells decreased when the MOI was reduced from 10 to 5. At an MOI of 20, a large number of cells died, as observed in the bright-field images.

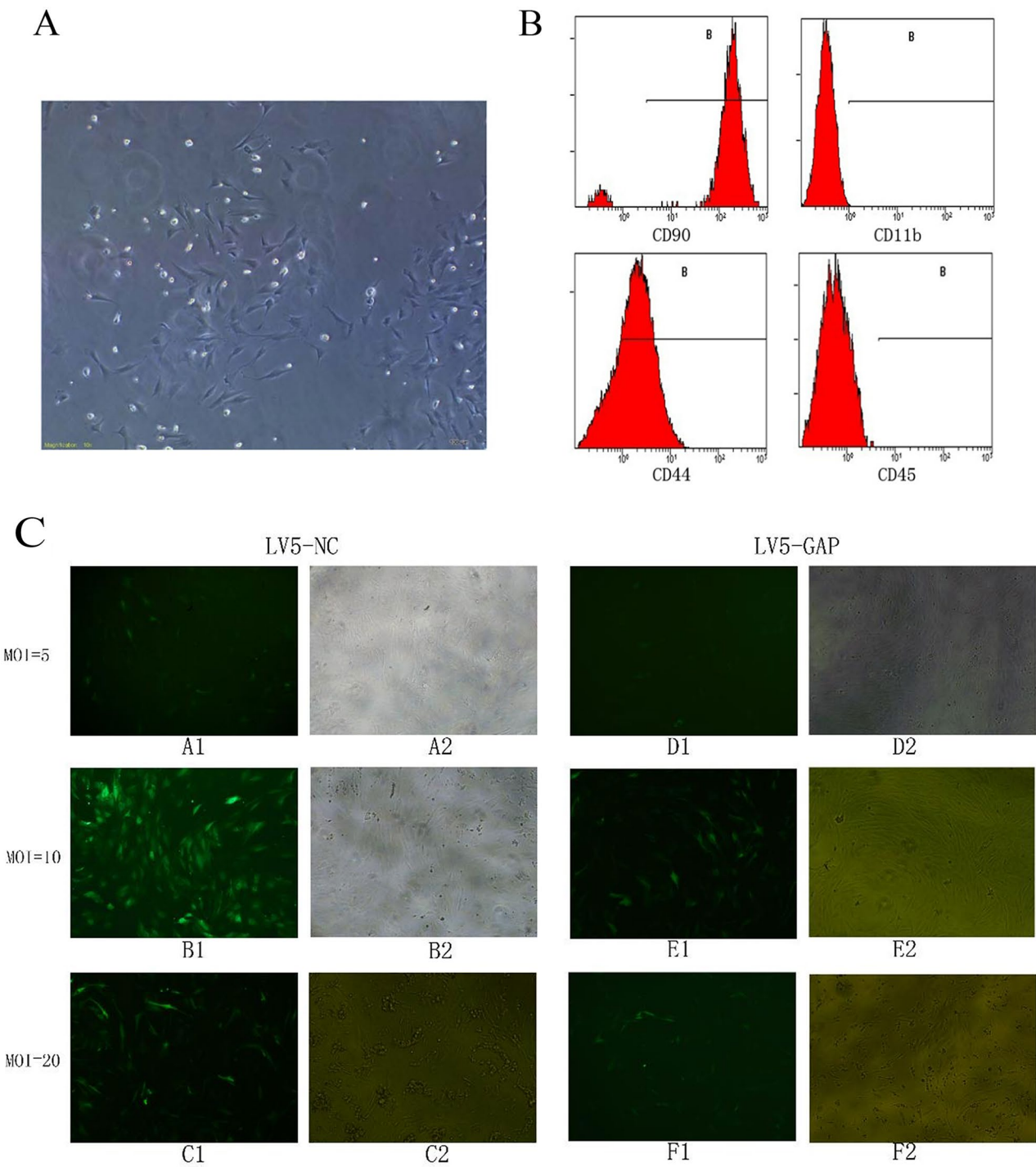


Fig. 1 Detection of BMSCs morphology and surface labeling and fluorescence and light microscopy images (100x) of lentivirus-infected BMSCs 48 h after infection. **(A)** Primary BMSCs at passage 3 exhibited a spindle-shaped, fibroblast-like morphology. **(B)** Flow cytometric analysis showing high expression of CD90 (94.7%) and CD44 (74.4%) and low expression of CD11b (0.12%) and CD45 (2.4%) in third-passaged BMSCs. **C(A1, B1, C1.)** LV5-NC infected cells with MOI=5, 10, and 20, respectively. **C(A2, B2, C2.)** Corresponding light microscopic images, respectively. **C(D1, E1, F1.)** LV5-GAP infected cells with MOI=5, 10, 20, respectively. **C(D2, E2, F2.)** Corresponding light microscopic images

Therefore, we selected MOI = 10 as the optimal infection multiplicity for further experiments. The images demonstrate that the lentivirus containing the target gene GAP-43 successfully infected BMSCs.

Detection of GAP-43 protein expression by cell immunofluorescence and Western blot

The GAP-43 + BMSCs group represents protein from BMSCs-LV5-GAP-43 cells, the BMSCs group represents protein from BMSCs-LV5-NC cells, and the NC group represents protein from untransduced BMSCs. Immunofluorescence results showed that GAP-43 protein was stained red in the GAP-43 + BMSCs group (Fig. 2A), while GAP-43 expression was not detected in the BMSCs group (Fig. 2B). Western blot results revealed that GAP-43 expression in the GAP-43 + BMSCs group was significantly higher compared to both the LV5-NC and NC groups ($P < 0.05$; Fig. 2C-D). The Western blot data were consistent with the immunofluorescence results.

BMSCs disappear after transplantation but GAP-43 upregulation promotes retinal transmurial synaptic environments

After subretinal transplantation, BMSCs gradually disappeared, and by 20 days post-transplantation, there were almost no fluorescent cells remaining (Fig. 3A). However, the expression of GAP-43 increased over time (Fig. 3C). The difference in gray values was statistically significant ($P < 0.05$) (Fig. 3D). These results indicate that BMSCs could not survive in the degenerative retinal microenvironment, but the expression of GAP-43 increased with time.

At 30 days after transplantation into the subretinal space, immunofluorescence staining showed distinct patterns of GAP-43 expression at the injection sites in the three groups (Fig. 3B). In the GAP-43 + BMSCs group, the overall GAP-43 signal was significantly higher than in the BMSCs and NC groups ($P < 0.05$), and the spatial distribution was also different. In the GAP-43 + BMSCs group, GAP-43 immunoreactivity extended throughout the entire retinal thickness, whereas in the BMSCs and NC groups GAP-43 expression was mainly confined to the retinal ganglion cell layer and inner nuclear layer. These observations indicate that although BMSCs-LV5-GAP-43

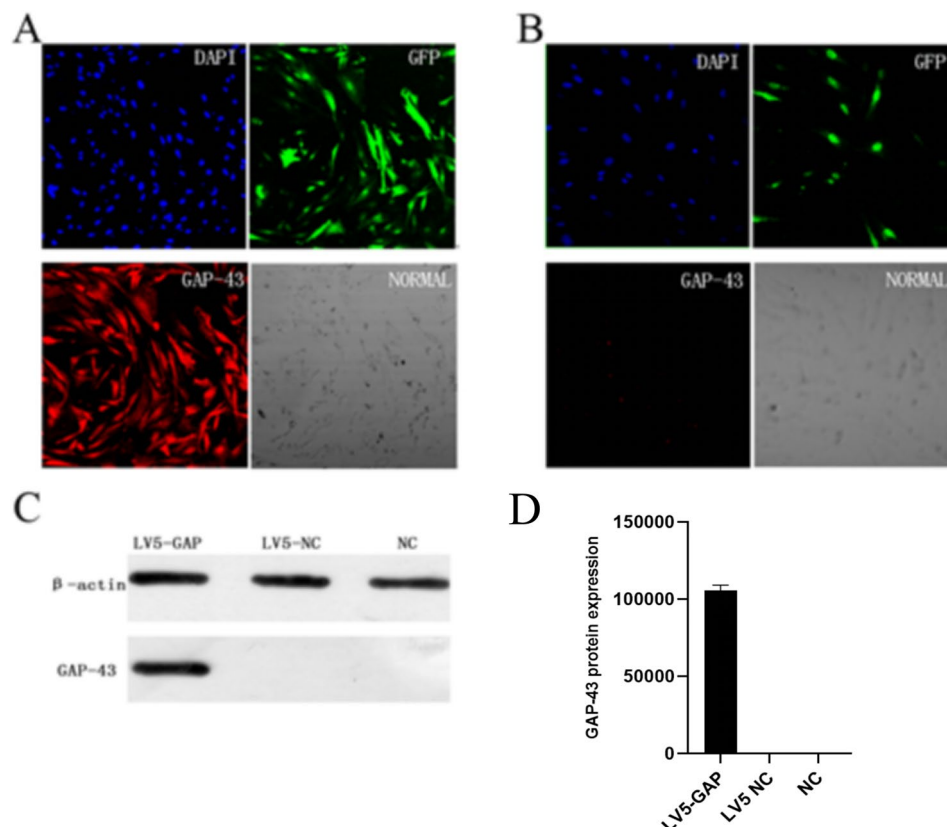


Fig. 2 Detection of GAP-43 expression. **A.** Cell immunofluorescence analysis revealed that the expression of GAP-43 in GAP-43 + BMSCs group. **B.** GAP-43 expression was not detected in BMSCs group (red-GAP-43, green-cell body, blue- cell nucleuses). **C-D.** Western blot analysis demonstrated that the protein expression of the GAP-43 was significantly increased in the GAP-43 + BMSCs group, compared with the BMSCs group and NC group ($P < 0.05$)

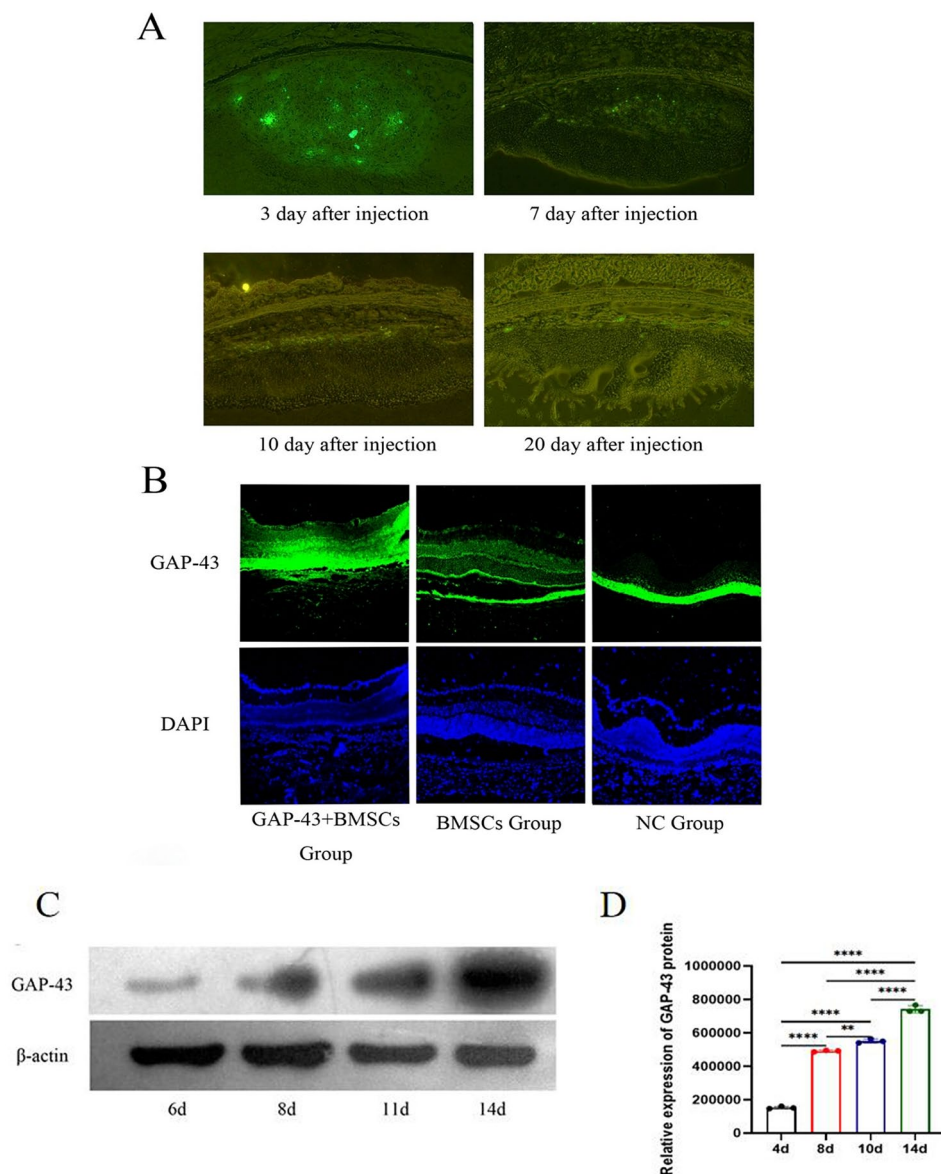


Fig. 3 The dynamic changes of GAP-43 expression after BMSCs transplantation. **A.** BMSCs gradually were disappeared after transplantation, there were almost no cells with fluorescent until 20d after transplantation. **B.** The expression of the position, in BMSCs group and NC group, GAP-43 expression in retinal ganglion cell (RGC) and inner nuclear layer (INL), in GAP-43 + BMSCs group, GAP-43 expression in whole retina. **C-D.** Western blot analysis demonstrated that the expression of GAP-43 increased with time (The data is shown as average value $\pm$ standard deviation, $n=3$, $**P<0.01$, $****p<0.0001$)

were delivered only into the subretinal space and did not maintain direct contact with neurons in the inner retina, GAP-43 upregulation was associated with a more widespread enhancement of a synaptic growth-associated marker across retinal layers. In this context, GAP-43-modified BMSCs are likely to provide trophic and microenvironmental support to inner retinal cells by promoting axonal outgrowth and synaptic remodeling between retinal neurons in different layers, thereby helping to maintain neuronal connectivity within the degenerating retina, in line with previous reports that BMSCs exert neuroprotective effects in retinal degeneration

models mainly through paracrine secretion of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF).

Subretinal transplantation of GAP-43-modified BMSCs ameliorates photoreceptor degeneration

The thickness of ONL after 30 days subretinal transplantation

At 30 days post-treatment, the outer nuclear layer (ONL) was thicker in the treated areas of the GAP-43 + BMSCs group and the BMSCs group compared to the corresponding areas in the NC group eyes (Fig. 4A; $P<0.05$). The ONL thickness in the GAP-43 + BMSCs group was

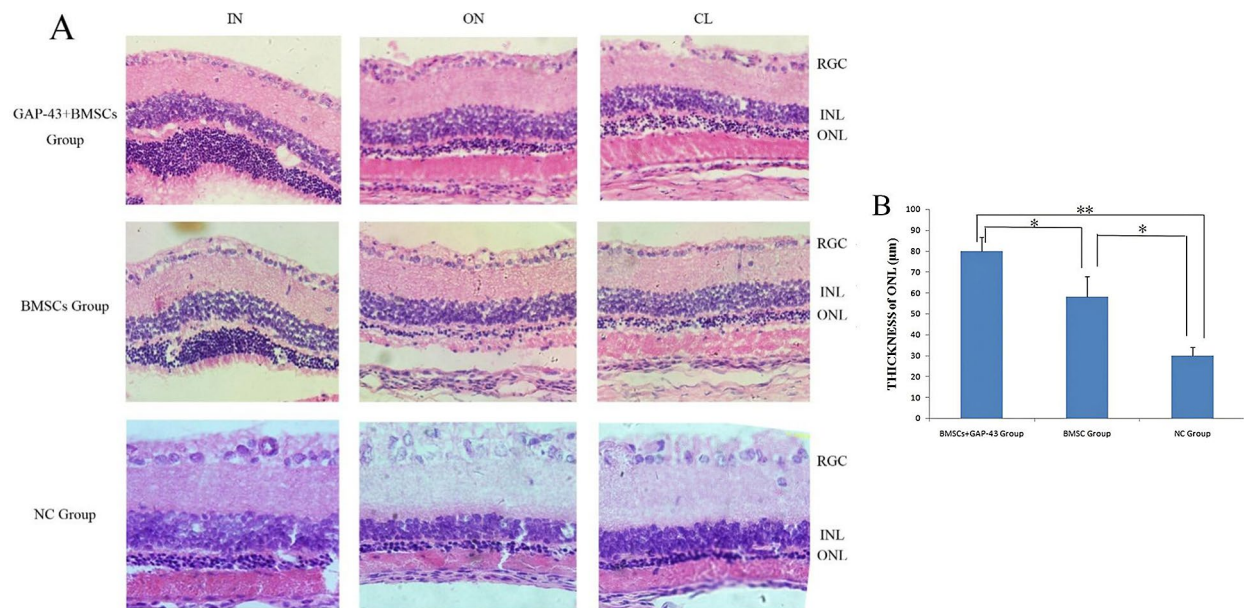


Fig. 4 Evaluation of improvement in retinopathy. **A.** HE sections revealed that pathological changes in the retina were markedly improved in the GAP-43+BMSCs group, compared with the BMSCs and NC groups after 30 days subretinal injection. **B.** Thickness of ONL GAP-43 + BMSCs group > BMSCs group > NC group. (The data is shown as average value ± standard deviation, $n = 3$, $*P < 0.05$, $**p < 0.01$)

Table 1 Thickness of ONL

ONL(μm)	GAP-43 + BMSCs group	BMSCs group	NC group
AVERAGE	79.78	58.00	29.92
STDEV	6.83	9.87	4.17

even greater than that in the BMSCs group. Quantitative analysis revealed that photoreceptors rescued by BMSCs injection had an average thickness of $79.78 \pm 6.83 \mu\text{m}$ in the GAP-43 + BMSCs group and $58.00 \pm 9.87 \mu\text{m}$ in the BMSCs group. These differences were statistically significant compared to the $29.92 \pm 4.17 \mu\text{m}$ in the NC group. No evidence of an inflammatory response was observed in any of the retinas that received cell injections. The data are summarized in Table 1. Statistical comparisons indicated that the morphological difference (i.e., the length of

the outer nuclear layer) between the non-dystrophic retina and the cell-transplanted dystrophic retina was significant, even in the best-rescued area ($P < 0.05$, Fig. 4B). There was no significant difference in ONL thickness in the contralateral retina among the three groups ($P > 0.05$), which excludes the influence of individual differences in retinal ONL thickness.

Rho position and expression in retina after 30 days subretinal transplantation

Thirty days after the cells from each group were transplanted into the retinas of RCS rats, total retinal protein was extracted from each experimental group, and the expression of Rho protein was detected by Western blot (Fig. 5D-E). The expression of Rho in the

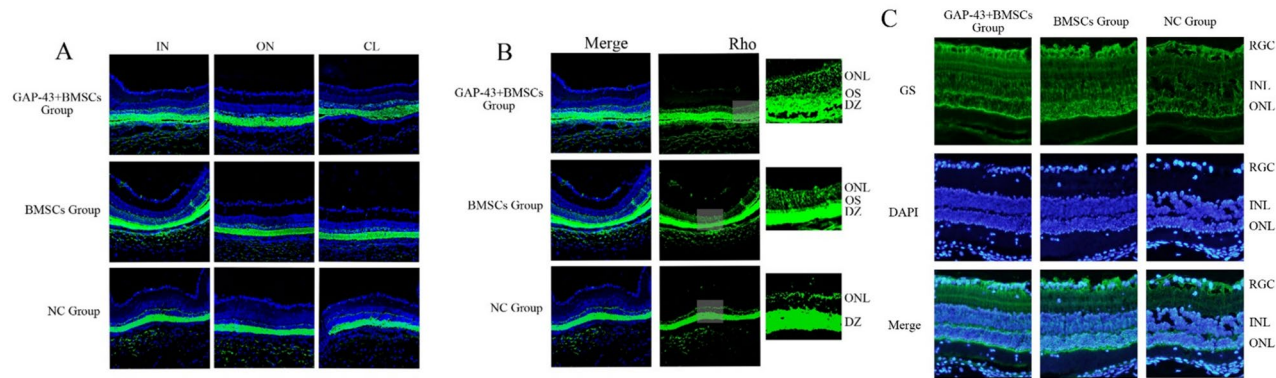


Fig. 5 Expression analysis of retinal functional markers. **A.** Major locations of Rho expression in the retina 30 days after subretinal cell transplantation. IN, ON, and CL. **B.** Detailed images of injection site DZ and OS. **C.** Immunofluorescence showed that GS expression was increased in GAP-43 + BMSCs group and BMSCs group, but GS expression in GAP-43 + BMSCs group are more clearly and increased

GAP-43 + BMSCs group and the BMSCs group was significantly higher than in the NC group ($P < 0.05$). This suggests that the injection of BMSCs into the retina of RCS rats promotes the expression of Rho in photoreceptor cells and helps protect these cells.

After 30 days of cell transplantation into the subretinal space of RCS rats, frozen sections were prepared following fixation and dehydration, and immunofluorescence was used to detect the primary expression sites in the IN, the optic nerve (ON) site, and the contralateral injection site (CL). Figure 5A-B show the primary expression sites of Rho. The following results were obtained from analysis of the immunofluorescence images for each group: (1) Rho is primarily expressed in the photoreceptor cell layer of the retina, including the outer segments (OS) and cell bodies of photoreceptor cells. (2) When comparing IN with CL, the photodetection fluorescence intensity in the GAP-43 + BMSCs group and the BMSCs group at IN was significantly higher than that at CL, while in the NC group, no significant difference in fluorescence intensity was observed between the two sites. (3) No significant difference in fluorescence intensity was found between the ON and the untransplanted cell area in any of the groups. (4) In the IN of each group, RPE cells lost the ability to phagocytize OS discs in retinal degeneration (RP), causing accumulation of the discs between the RPE and OS, which formed debris zones (DZ). In the GAP-43 + BMSCs group, the length and density of OS were significantly better than those in the other two groups, and the shape of the OS was still faintly visible in the DZ. In the BMSCs group, fluorescence in the DZ region was dense, and the structure could not be clearly distinguished. Although the OS layer was visible, its length and density were much lower than in the GAP-43 + BMSCs group. In the NC group, only a dense fluorescent band in the DZ was observed.

GS expression and position in retina after 30 days subretinal transplantation

GS-stained Müller cell bodies were observed in the retinas. GS expression was higher in the BMSCs group and the GAP-43 + BMSCs group compared to the NC group, with the BMSCs group showing a more pronounced increase (Fig. 5A-B). This pattern indicates enhanced activation and preservation of Müller glial cells at the injection site. Consistently, the density of GS-positive Müller cells was greater in both the GAP-43 + BMSCs group and BMSCs groups than in the NC group, with Müller cells in the BMSCs group appearing more clearly defined and more densely packed (Fig. 5C).

GAP-43-Modified BMSCs attenuate photoreceptor apoptosis in RCS rats via dual suppression of Caspase-8 and Caspase-9 pathways

Anti-apoptotic effect in retina after 30 days subretinal transplantation

Apoptotic cells were primarily located in the outer nuclear layer of the retina, indicating photoreceptor apoptosis. Compared to the DAPI images of the injection sites in each group, the thickness of the outer nuclear layer was greatest in the GAP-43 + BMSCs group, followed by the BMSCs group, while the NC group had the thinnest outer nuclear layer. Concurrently, the density of photoreceptor cells in the three groups decreased in the same order, consistent with the previous results (Fig. 6A). The apoptosis rates of photoreceptor cells were as follows: 0.419 ± 0.030 in the GAP-43 + BMSCs group, 0.496 ± 0.080 in the BMSCs group, and 0.638 ± 0.044 in the NC group (Table 2). Statistical comparisons of the apoptosis rates showed no significant difference between the GAP-43 + BMSCs group and the BMSCs group ($P > 0.05$), but the difference between the BMSCs group and the NC group was statistically significant ($P < 0.05$), and the difference between the GAP-43 + BMSCs group and the NC group was statistically significant ($P < 0.01$, Fig. 6B).

Therefore, the injection of BMSCs into the retina of RCS rats protects photoreceptor cells by reducing apoptosis. The apoptosis rate of photoreceptor cells in the GAP-43 + BMSCs group was lower than in the BMSCs group, and the outer nuclear layer was the thickest, suggesting that high expression of GAP-43 plays a positive role in protecting photoreceptor neurons, inhibiting photoreceptor cell apoptosis, and maintaining retinal structure integrity.

The expression of Caspase-8 and Caspase-9 after 30 days subretinal transplantation

As is well known, Caspase-8 and Caspase-9 mediate the extrinsic and intrinsic apoptotic pathways, respectively. Caspase-8 expression progressively decreased in the GAP-43 + BMSCs group, the BMSCs group, and the NC group, while Cleaved Caspase-8 expression progressively increased (Fig. 6C-D). These results indicate that Caspase-8 protein was fully activated, and the Caspase-8-mediated extrinsic pathway is involved in the apoptosis of retinal photoreceptor cells in RCS rats. However, the sequential increase in the expression of three histone cleavage forms of Caspase-8 suggests that both BMSCs and BMSCs modified by GAP-43 gene could inhibit the activation of Caspase-8, thereby reducing apoptosis. Notably, the effect of high levels of GAP-43 in the retinal environment was more pronounced in inhibiting apoptosis.

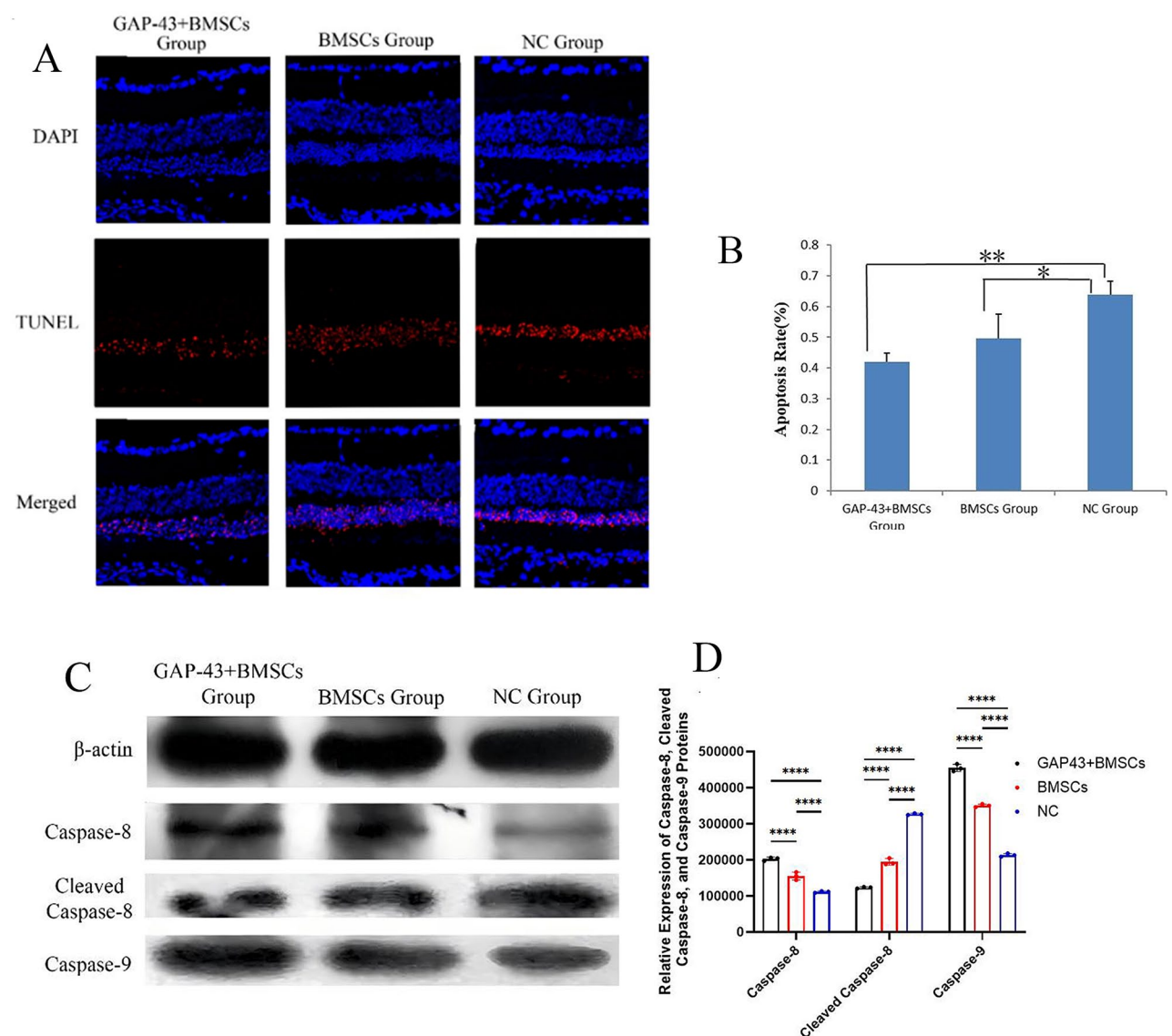


Fig. 6 Detection and analysis of retinal cell apoptosis. **A.** TUNEL shows apoptosis photoreceptor cell of retina, the TUNEL positive cells shown in red are mainly concentrated in the outer nuclear layer of the retina; Blue is retinal DAPI staining. **B.** Apoptosis Rate NC group > BMSCs group > GAP-43 + BMSCs group. **C.** Western blot showed Caspase-8 expression was decreased successively in GAP-43 + BMSCs group, BMSCs group and NC group, while Cleaved Caspase-8 expression was increased in succession. Caspase-9 expression was also decreased in succession in GAP-43 + BMSCs group, BMSCs group and NC group. (**** $p < 0.0001$)

	GAP-43 + BMSCs group	BMSCs group	NC group
Apoptosis Rate	0.419	0.496	0.638
STDEV	0.030	0.080	0.044

Similarly, Caspase-9 expression decreased progressively in the GAP-43 + BMSCs group, the BMSCs group, and the NC group (Fig. 6C-D). This suggests that the apoptosis of retinal photoreceptor cells in RCS rats is also mediated by Caspase-9. Intracellular and extracellular death signals are transmitted to the mitochondria, where

they activate the precursor Caspase-9, forming apoptotic bodies. However, the progressive decrease in the expression of three histone Caspase-9 forms indicates that the cleavage of Caspase-9 protein increased sequentially. This finding suggests that both BMSCs and BMSCs modified by the GAP-43 gene could inhibit the activation of Caspase-9 in the intrinsic pathway, with the anti-apoptosis effect being more pronounced in the BMSCs modified by GAP-43. These data indicate that GAP-43-modified BMSCs confer robust structural and molecular neuroprotection in the early stage of retinal degeneration

by inhibiting apoptosis and promoting photoreceptor survival.

Discussion

In recent years, cell transplantation therapy and gene therapy in RP have made significant progress. BMSCs are known for their low immunogenicity [7], extensive availability, and good plasticity, and the isolation and culture of BMSCs are relatively simple. As a result, BMSCs have been widely used in cell transplantation [8, 9]. BMSCs can self-renew, differentiate into multiple lineages, migrate, and exhibit anti-inflammatory and immunomodulatory properties, which together may support therapeutic effects in clinical applications [10]. BMSCs also serve as an effective vehicle; many studies have shown that after modification with genes, BMSCs can stably express proteins. Numerous studies have indicated that BMSCs promote the survival of retinal ganglion cells and axonal regeneration through various mechanisms [11–13].

In our experiment, after subretinal transplantation, the retina appeared flat, elevated, and white under the surgical microscope. The elevated area accounted for nearly a quarter of the whole retina. However, the number of BMSCs gradually decreased and eventually disappeared at the injection site after 20 days. After 30 days of cell transplantation, in the GAP-43 + BMSCs group, the expression of GAP-43 remained higher than in the other two groups, and its distribution also differed. In the BMSCs and NC groups, GAP-43 was expressed in the RGC and INL, while in the GAP-43 + BMSCs group, GAP-43 was expressed throughout the entire retina. Although we injected cells only into the subretinal space, GAP-43, a synaptic marker, was expressed in the full retina. This suggests that BMSCs-LV5-GAP-43 did not have direct contact with other neurons, but they provided nutritional support to the inner retinal cells.

Quantitative analysis revealed that the photoreceptors rescued by BMSCs injection had an average thickness of $79.78 \pm 6.83 \mu\text{m}$ in the GAP-43 + BMSCs group, $58.00 \pm 9.87 \mu\text{m}$ in the BMSCs group, and $29.92 \pm 4.17 \mu\text{m}$ in the NC group. GAP-43 has been shown to be involved in nerve differentiation and growth [14, 15]. As an intracellular protein, GAP-43 is located on the nerve cell membrane, and its expression is related to axon extension [16, 17]. According to the principles of neuronal damage and presynaptic rearrangement, the expression of GAP-43 mRNA and the distribution of protein are consistent with axon growth [18, 19]. In our experiment, the changes in GAP-43 expression corresponded to the process of synaptic axon maturation, indicating that GAP-43 plays a key role in the development of the nervous visual system.

At the mechanistic level, GAP-43 is now recognized as a prototypical growth and plasticity protein that

participates in multiple, interrelated processes rather than a single function. Recent work has shown that GAP-43 is highly enriched in growth cones, that its overexpression enhances axonal sprouting and synapse formation, and that gene deletion disrupts axonal pathfinding and is frequently lethal or associated with major circuitry defects [20]. In parallel, GAP-43 remains enriched in specific adult cortical and limbic circuits where it is tightly linked to long-term potentiation and experience-dependent synaptic plasticity [21]. Taken together, these data support a model in which GAP-43 primarily regulates axonal growth and presynaptic structural plasticity, with secondary neuroprotective effects arising from the stabilization and remodeling of neural circuits. In the visual system, genetic manipulation of GAP-43 has been shown to critically influence the establishment and refinement of retinotopic projections, indicating that GAP-43 is essential for orderly visual connectivity rather than for a single neuronal subtype [22]. In our RCS model, GAP-43 overexpression via BMSCs-LV5-GAP-43 was associated with broader GAP-43 immunoreactivity across retinal layers, preservation of ONL thickness and rhodopsin-positive outer segments, and reduced activation of apoptotic markers. We therefore interpret GAP-43 as a modulator of the retinal microenvironment and connectivity: by promoting axonal outgrowth and synaptic remodeling between surviving interneurons, Müller glia and photoreceptors, GAP-43 may help maintain or re-establish synaptic contacts, maintain glutamate handling and trophic support, and thus indirectly support photoreceptor survival within the degenerating retina. However, because the present study employed only a gain-of-function approach using BMSCs-LV5-GAP-43 without selective knockdown or inhibition of GAP-43 in specific retinal cell types, the relationship between increased GAP-43 expression and neuroprotection in our model remains correlative. Future work using cell type-specific loss- and gain-of-function strategies and pathway-level interrogation will be required to demonstrate a direct causal role for GAP-43 in mediating the observed structural and molecular protection.

RPE cells lose the ability to phagocytize OS discs in RP, causing the discs to accumulate between the RPE and OS, forming DZ. The DZ in the NC group became more prominent, with outer segments no longer visible and a strong aberrant accumulation of rhodopsin in rod cell bodies. In contrast, in the GAP-43 + BMSCs group and the BMSCs group, although debris zones were visible, Rho-stained outer segments and rod cell bodies were present in the retinas. In the GAP-43 + BMSCs group, rhodopsin-stained outer segments were more clearly defined than in the BMSCs and NC groups.

Previous studies in the RCS rat and in other experimental models of retinal degeneration have suggested

that BMSCs protect photoreceptors mainly through paracrine mechanisms. Inoue et al. showed that bone marrow-derived mesenchymal stem cell-conditioned medium delays photoreceptor apoptosis in retinal cell cultures and that subretinal MSC transplantation preserves outer nuclear layer thickness and electroretinographic responses in RCS rats, implying that MSC-secreted trophic factors promote photoreceptor survival in this model [23]. In a light-damaged rat retina model, Zhang et al. further demonstrated that subretinally transplanted BMSCs express BDNF and bFGF, with increased retinal BDNF/bFGF immunoreactivity and reduced photoreceptor apoptosis after transplantation [24]. In addition, recent reviews have emphasized that mesenchymal stem cells release a broad spectrum of growth and neurotrophic factors that support the survival and growth of retinal neurons and modulate inflammatory and apoptotic pathways in degenerating retinas [25]. Taken together, these findings support the concept that BMSC-based therapies act primarily by modulating the retinal microenvironment rather than by directly replacing lost photoreceptors. In our study, donor BMSCs in the subretinal space progressively decreased and were no longer detectable at the injection site by 20 days, whereas preservation of ONL thickness and rhodopsin-positive outer segments remained evident at 30 days; moreover, we did not observe morphological evidence of BMSC-derived photoreceptors or other retinal neurons. These observations, in combination with previous work, further support a predominantly paracrine neuroprotective mechanism rather than substantial neuronal replacement in our model. In this context, the more widespread upregulation of the synaptic growth-associated protein GAP-43 across retinal layers that we observed in the GAP-43 + BMSCs group, together with the preservation of ONL thickness and rhodopsin expression, is compatible with the idea that GAP-43-modified BMSCs provide trophic and microenvironmental support to inner retinal cells by promoting axonal outgrowth and synaptic remodeling between neurons in different layers, thereby helping to maintain neuronal connectivity within the degenerating retina.

Additionally, BMSCs mediate immune regulation, repair, and regeneration of retinal cells through paracrine extracellular vesicles. Intravitreal injection of extracellular vesicles has been proposed as a treatment for retinal diseases affecting the inner retina [26]. For example, studies have shown that exosome miR-21 secreted by mesenchymal stem cells has a therapeutic effect on photoreceptor apoptosis and retinal disorders, potentially making it a therapeutic drug for RP [27]. Similar results have been observed in the model of oxidative stress-induced retinopathy, where adipose mesenchymal stem cells migrate to damaged RPE cells and protect them in

a paracrine manner [28]. Our team's previous research demonstrated that in the model of hypoxia-induced retinal detachment, BMSCs transplantation initiated retinal autophagy, significantly reducing the death of photoreceptor cells, protecting retinal structure, and promoting the survival of retinal cells in a nutrient-restricted environment. Two weeks after transplantation, ONL thickness increased, and photoreceptors became more regularly arranged and thicker [13]. It remains unclear whether the increased number of photoreceptors in the GAP-43 + BMSCs group reflects true replacement of lost photoreceptors by donor cells or, more likely, enhanced survival of host photoreceptors mediated by trophic support from BMSCs. In the present study, we did not observe donor cells with photoreceptor morphology or perform lineage tracing. Together with accumulating evidence from RCS rats and other retinal degeneration models showing that mesenchymal stem cells exert their effects predominantly via secretion of extracellular vesicles and neurotrophic factors rather than direct differentiation into photoreceptors [18, 19, 23–25], we therefore interpret our findings as primarily reflecting paracrine, microenvironment-modifying actions of BMSCs, while a minor contribution from cell replacement cannot be completely excluded. With the additional overexpression of GAP-43, the protective effect on photoreceptors was further enhanced: GAP-43 expression increased throughout the retina, creating an environment more conducive to synaptic connectivity and signal transmission between neurons in different layers.

The combination of BMSCs and GAP-43 expression significantly protected ONL thickness, preserved rhodopsin-positive outer segments and reduced activation of caspase-8/9, thereby maintaining photoreceptor survival in the dystrophic retina. Notably, the present study focused on structural and molecular endpoints and did not include direct assessments of visual function such as electroretinography (ERG) or optokinetic/optomotor tests, which is an important limitation. Previous work in RCS rats and other retinal degeneration models has shown that preservation of ONL thickness and photoreceptor outer segments after cell- or gene-based interventions closely correlates with improved ERG b-wave amplitudes and visual acuity measured by optokinetic or optomotor responses [23, 29–32]. Future experiments with GAP-43-modified BMSCs will therefore integrate ERG and behavioral assays to determine whether the structural and molecular neuroprotection observed here translates into sustained functional visual rescue.

Previous studies in the RCS rat and other inherited retinal degeneration models have evaluated unmodified bone marrow-derived cell therapies. Tzameret et al. reported that subretinal transplantation of 2.5×10^6 human bone marrow-derived mesenchymal stem cells (hBM-MSCs)

as a thin subretinal layer in RCS rats delayed photoreceptor loss and preserved ERG responses for approximately 12–20 weeks, with photoreceptor rescue largely confined to regions in proximity to the injection site [33]. Park et al. showed that subretinal or intravitreal injection of 5×10^4 human CD34⁺ bone marrow-derived stem cells in a rat model of inherited retinal degeneration led to a transient increase in ERG b-wave amplitudes and regional preservation of the outer nuclear layer (ONL) at 2–4 weeks, although these functional effects attenuated over time [34]. In our study, we used a comparable cell dose of 5×10^4 syngeneic BMSCs per eye and found that GAP-43 overexpression enhanced the morphological and molecular benefits of BMSC transplantation at 30 days: ONL thickness reached $79.78 \pm 6.83 \mu\text{m}$ in the GAP-43 + BMSCs group compared with $58.00 \pm 9.87 \mu\text{m}$ in the BMSCs group and $29.92 \pm 4.17 \mu\text{m}$ in the NC group (Table 1), and the photoreceptor apoptosis rate decreased from 0.638 ± 0.044 in NC and 0.496 ± 0.080 in the BMSCs group to 0.419 ± 0.030 in the GAP-43 + BMSCs group (Table 2). While differences in species, cell source, injection route and outcome measures preclude strict quantitative comparison across studies, these effect sizes indicate that, within our experimental system and time frame, GAP-43 gene modification confers additional photoreceptor-sparing and anti-apoptotic effects beyond those achieved by BMSC transplantation alone.

Although Tzameret et al. and Park et al. reported photoreceptor and ERG improvements lasting approximately 12–20 weeks in RCS rats or several months in patients, the beneficial effects tended to attenuate over time as the underlying degeneration progressed [33–35]. In the present study, our outcome measurements were restricted to an early 30-day window, and we therefore cannot determine whether GAP-43-mediated photoreceptor protection and anti-apoptotic effects persist at later stages; longer-term follow-up and, potentially, repeated dosing paradigms will be required to fully define the durability of the therapeutic benefit.

Müller cells are a specialized type of glial cell in the retina. In vertebrate retinas, Müller cells account for more than half of the total retinal cells and make contact with nearly all retinal cells. They play essential roles in light conduction, maintaining retinal neuronal homeostasis, regulating neuronal metabolism, providing nutritional and antioxidant factors, and regulating the blood-retina barrier function. Müller cells also participate in synaptic activity. During neurotransmission, Müller cells can rapidly clear glutamate from the synaptic space, preventing excitotoxic damage to retinal neurons. Glutathione, an important antioxidant, neutralizes reactive oxygen species to protect neurons. When oxidative stress occurs in the retina, Müller cells can rapidly use glutamate to produce glutathione, providing protection [36, 37]. Recent

studies have shown that reprogrammed Müller cells can proliferate and differentiate into all types of retinal cells, suggesting that Müller cells possess a strong ability to regenerate the retina [38–40]. However, Müller cell proliferation can also disrupt the integrity of the retinal blood-retina barrier, block nutrient supply to neuronal cells, and promote neuronal degeneration. During RPE degeneration in RCS rats, Müller cells proliferate abnormally, migrate to the lesion site and other parts of the retina, and form a “glial block” in the subretinal space. Müller cells can be moderately activated in the early stages and inhibited in later stages to maintain the nutrient supply cycle for retinal neurons. Recent experiments have confirmed that transplantation of BMSCs-derived mitochondria significantly inhibits glial fibrillary acidic protein (GFAP)-positive glial scarring, while maintaining retinal metabolic homeostasis by modulating calcium channels and BDNF pathways [41].

In our experiment, GS-positive Müller glial cells were prominent at the injection site in both the GAP-43 + BMSCs group and the BMSCs group. Western blot analysis showed that GS expression was higher in these two groups than in the NC group, indicating enhanced activation and preservation of Müller cells in the degenerating retina. Given the central role of Müller glia in providing metabolic, neurotrophic and antioxidant support and in maintaining retinal homeostasis, such Müller cell activation may contribute to the improved photoreceptor survival and remodeling of the retinal microenvironment observed after BMSC transplantation and GAP-43 overexpression. This study demonstrates that BMSCs combined with GAP-43 gene modification can remodel the synaptic connectivity environment—GAP-43 is widely expressed throughout the retina, promoting inter-neuronal signaling. Huang X et al. also demonstrated that mitochondria derived from BMSCs further improve photoreceptor survival by enhancing the BDNF signaling pathway. This synergistic effect provides a new approach for “cell-gene” combination therapy.

From a safety standpoint, we did not observe gross retinal detachment, neovascularization, hemorrhage or mass-like lesions at the injection sites during the 30-day observation period, and GS-positive Müller glia, although activated, remained organized rather than forming dense scar-like structures in the GAP-43 + BMSCs group. Nonetheless, this study was not designed as a dedicated safety study: we did not systematically quantify GFAP-positive reactive gliosis, microglial activation or chronic inflammatory infiltrates, and our follow-up did not extend beyond 30 days. Therefore, rare or delayed adverse events such as abnormal gliosis, chronic inflammation or late-onset proliferative lesions cannot be completely excluded. Preclinical and translational studies have underscored that while MSC-based approaches can

exert neuroprotective effects in retinal diseases, inappropriate cell sources or delivery routes—particularly unregulated intravitreal injections—may trigger harmful glial and inflammatory responses [17, 41–44]. Conversely, early-phase clinical trials and meta-analyses suggest that carefully controlled MSC therapies using good manufacturing practice (GMP)-grade cells and standardized subretinal or suprachoroidal delivery can be relatively safe in patients with inherited retinal degenerations, although long-term surveillance remains crucial [8, 25, 45]. These considerations highlight the need for rigorous long-term safety monitoring and standardized manufacturing and dosing protocols when advancing GAP-43–modified BMSCs toward clinical translation.

Together with previous work demonstrating that Müller glia can re-enter the cell cycle and, in some contexts, generate multiple retinal neuronal subtypes or support neuronal regeneration [38–40], and that BMSC-derived mitochondria can attenuate GFAP-positive gliosis and restore metabolic homeostasis in degenerating retina [41], our findings suggest that GAP-43-modified BMSC transplantation not only protects photoreceptors but also engages endogenous glial responses and may potentiate intrinsic repair mechanisms. Although we did not perform lineage tracing or proliferation assays in the present study, the increased GS-positive Müller glia and widespread GAP-43 immunoreactivity across retinal layers are consistent with a scenario in which endogenous glial and neuronal cells actively participate in remodeling the degenerating retinal microenvironment in response to BMSC transplantation and GAP-43 overexpression.

We also detected the apoptotic rate in the three groups at the injection site using the TUNEL assay. Apoptotic cells were mainly concentrated in the ONL, primarily due to photoreceptor apoptosis. There are two main apoptotic signaling pathways: the extrinsic death receptor signaling pathway and the intrinsic mitochondrial apoptotic pathway [1]. In our experiment, the expression of Caspase-9 in the GAP-43 + BMSCs group, the BMSCs group, and the NC group progressively decreased. This suggests that Cleaved Caspase-9 may have increased, activating the downstream reactions in the intrinsic mitochondrial pathway. The expression of Caspase-8 also decreased progressively in these groups, while Cleaved Caspase-8 expression increased. This indicates that GAP-43 may affect the death receptor signaling pathway, reducing photoreceptor apoptosis. We found that GAP-43 expression in neuronal cells may be related to apoptosis. Shyamala et al. found that when GAP-43 cannot reach an optimal concentration in the microenvironment, it activates the apoptotic pathway [46]. In a rat model of neuropathic pain, inhibiting Caspase-3 protein also affected GAP-43 expression [47]. We conclude that GAP-43 may regulate factors in the apoptotic pathway, indirectly

influencing cell apoptosis. Beyond caspase-dependent apoptosis, mesenchymal stem cell–based therapies have been shown to modulate additional molecular pathways that are highly relevant to retinal degeneration, including oxidative stress responses, autophagy, necroptosis and inflammatory signaling. For example, Usategui-Martín et al. reported that human bone marrow MSC secretome preserves retinal morphology and exerts a neuroprotective effect by activating antioxidant machinery, modulating autophagy-related genes and proteins, and inhibiting apoptosis- and necroptosis-related pathways in an in vitro model of retinal neurodegeneration [48]. Likewise, Adak et al. summarized evidence that MSCs in experimental retinal diseases can regulate PI3K/Akt and MAPK/ERK survival pathways and enhance neurotrophic signaling such as BDNF/TrkB [49]. These observations suggest that the GAP-43–associated reduction in caspase-8/9 activation and photoreceptor apoptosis observed in our study is likely to occur in parallel with broader MSC-mediated changes in oxidative stress, autophagy and inflammatory pathways, although we did not directly analyze these cascades here. Future studies combining GAP-43 overexpression with targeted assessment of these signaling networks will be important to clarify how these molecular pathways are interconnected in the context of BMSC therapy for retinal degeneration.

From a translational perspective, our findings suggest that GAP-43 gene modification can enhance the neuroprotective effects of BMSC transplantation in early-stage retinal degeneration, but several key issues must be addressed before clinical application in human RP. In terms of delivery, subretinal injection—as used in the present study—is already applied in adeno-associated virus (AAV) gene therapy and RPE cell trials and enables targeted delivery to the photoreceptor–RPE interface, albeit with greater surgical complexity. Less invasive routes such as intravitreal or suprachoroidal injection have also been explored for stem cell delivery and may offer more favorable risk–benefit profiles in some settings, but they are associated with distinct biodistribution patterns and inflammatory risks [8, 17, 35, 45]. Immune considerations are equally important: although the eye is relatively immune-privileged, retinal gene therapy trials have shown that innate and adaptive immune responses to AAV vectors can occur in a dose- and route-dependent manner, influencing both efficacy and safety [50–52]. For a combined cell- and gene-based product such as GAP-43–modified BMSCs, additional attention must be paid to potential allo-immune reactions to donor cells and to GMP-grade manufacturing, including standardized release criteria, vector copy number control and genomic stability testing. Finally, GAP-43–modified BMSCs would be regulated as an advanced therapy medicinal product, and their clinical translation will

require robust preclinical toxicology and tumorigenicity studies followed by carefully staged early-phase clinical trials with long-term safety monitoring [5, 25, 50–52]. Nonetheless, our data support the concept that rationally engineered MSCs can beneficially modulate the degenerating retinal microenvironment and may complement gene-specific therapies for genetically heterogeneous RP.

Conclusion

Subretinal transplantation of GAP-43–modified bone marrow–derived mesenchymal stem cells (BMSCs-LV5-GAP-43) in RCS rats led to a progressive increase in retinal GAP-43 expression despite the gradual disappearance of donor cells in the degenerative environment. Compared with unmodified BMSCs and PBS, BMSCs-LV5-GAP-43 treatment preserved outer nuclear layer thickness and rhodopsin-positive outer segments, reduced TUNEL-positive photoreceptors and downregulated Caspase-8 and Caspase-9, indicating a structural and molecular neuroprotective effect on the dystrophic retina. GAP-43 overexpression was associated with a more widespread distribution of this synaptic growth–associated protein across retinal layers and with altered Müller glial activation, which may contribute to maintaining a retinal microenvironment supportive of neuronal connectivity. Together, these data suggest that GAP-43 gene modification enhances the anti-apoptotic and photoreceptor-sparing effects of BMSC transplantation in early-stage retinal degeneration. Future studies should further delineate the molecular pathways involved, incorporate electrophysiological and behavioral assessments to determine whether these structural benefits translate into rescue of visual function, and explore clinically applicable delivery strategies.

Abbreviations

AAV	Adeno-associated virus
ANOVA	analysis of variance
BDNF	Brain-derived neurotrophic factor
BMSCs	Bone marrow mesenchymal stem cells
BSA	Bovine serum albumin
CL	Contralateral injection site
DMEM/F12	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
DZ	Debris zone
ECL	Enhanced chemiluminescence
ERG	Electroretinography
GAP-43	Growth-associated protein 43
GFAP	Glial fibrillary acidic protein
GMP	Good manufacturing practice
GS	Glutamine synthetase
IACUC	Institutional Animal Care and Use Committee
IN	Injection site
INL	Inner nuclear layer
LV5-GAP-43	Lentiviral vector encoding GAP-43
LV5-NC	Lentiviral negative control vector
MOI	Multiplicity of infection
NC	Negative control
OCT	Optical cutting temperature compound
ON	Optic nerve site
ONL	Outer nuclear layer

OS	Outer segments
PVDF	Polyvinylidene fluoride
RCS	Royal College of Surgeons
RGC	Retinal ganglion cell
Rho	Rhodopsin
RP	Retinitis pigmentosa
RPE	retinal pigment epithelium
SPF	Specific pathogen-free
TBST	Tris-buffered saline with Tween-20
TUNEL	Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12967-026-07685-2>.

Supplementary Material 1

Acknowledgements

The authors gratefully acknowledge the support from the Norman Bethune Plan Project of Jilin University (No. 2025B39) and the Jilin Province Medical and Health Talent Special Project (grant no. 2024WSZXC21).

Author contributions

Project administration and supervision: Guanfang Su. Conceptualization: Guanfang Su and Yuxi He. Experimental work, data collection, visualization and original draft preparation: Yuxi He. Data curation and verification: Yuxiao Guo. Writing – review and editing: Guanfang Su. All authors read and approved the final manuscript.

Funding

This work was supported by the Norman Bethune Plan Project of Jilin University (No. 2025B39) and the Jilin Province Medical and Health Talent Special Project (grant no. 2024WSZXC21).

Data availability

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

Declarations

Ethics approval and consent to participate

This experiment was conducted in the SPF barrier facility of the Jilin University Laboratory Animal Center under the supervision of the Jilin University Animal Ethics and Welfare Committee (IACUC), in accordance with institutional and national guidelines for the ethics and welfare of experimental animals. Wistar rats and Royal College of Surgeons (RCS) rats (NBRP rat No. 0011) were purchased from the School of Medicine, Kyoto University, Japan, and housed in the Animal Service Center of Jilin University in SPF-class animal facilities. Housing and husbandry complied with GB 14925, and animals had free access to food and water. All experiments were approved by the Laboratory Animal Committee of Jilin University in 2019 (project title: “Study on the mechanism of retinal degeneration cells protection by GAP-43-modified BMSCs”, license number KT201903043).

Consent for publication

Not applicable. This study involved experimental animals only and did not include any human participants or individual person's data.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Ophthalmology, The Second Hospital of Jilin University, Changchun, Jilin Province 130041, China

²The Second Clinical Medical College of Jilin University, Changchun, Jilin Province 130012, China

Received: 4 September 2025 / Accepted: 31 December 2025

Published online: 09 January 2026

References

- Liu W, et al. Retinitis pigmentosa: progress in molecular pathology and biotherapeutic strategies. *Int J Mol Sci.* 2022;23(9):4883. Published 2022 Apr 28. <https://doi.org/10.3390/ijms23094883>.
- Akiba R, et al. Progress of iPSC cell-based transplantation therapy for retinal diseases. *Jpn J Ophthalmol.* 2023;67(2):119–28. <https://doi.org/10.1007/s10384-022-00974-5>.
- Wang S, et al. Bruch's-Mimetic nanofibrous membranes functionalized with the Integrin-Binding peptides as a promising approach for human retinal pigment epithelium cell transplantation. *Molecules.* 2022;27(4):1429. Published 2022 Feb 21. <https://doi.org/10.3390/molecules27041429>.
- Singh RK, Nasonkin IO. Limitations and promise of retinal tissue from human pluripotent stem cells for developing therapies of blindness. *Front Cell Neurosci.* 2020;14:179. Published 2020 Sep 10. <https://doi.org/10.3389/fncel.2020.00179>.
- Creber NJ, et al. Current and emerging approaches to cochlear immunosuppression with translation to human inner ear stem cell therapy: A systematic review. *PLoS ONE.* 2025;20(2):e0318165. Published 2025 Feb 13. <https://doi.org/10.1371/journal.pone.0318165>.
- Zhu Q, et al. Lentiviral-mediated growth-associated protein-43 modification of bone marrow mesenchymal stem cells improves traumatic optic neuropathy in rats. *Mol Med Rep.* 2015;12(4):5691–700. <https://doi.org/10.3892/mmr.2015.4132>.
- Yin N, et al. The mechanisms of MicroRNA 21 in premature ovarian insufficiency mice with mesenchymal stem cells transplantation: the involved molecular and immunological mechanisms. *J Ovarian Res.* 2024;17(1):75. Published 2024 Apr 4. <https://doi.org/10.1186/s13048-024-01390-8>.
- Lin H, et al. Bone marrow mesenchymal stem cells: aging and tissue engineering applications to enhance bone healing. *Biomaterials.* 2019;203:96–110. <https://doi.org/10.1016/j.biomaterials.2018.06.026>.
- Arthur A, Gronthos S. Clinical application of bone marrow mesenchymal Stem/Stromal cells to repair skeletal tissue. *Int J Mol Sci.* 2020;21(24):9759. Published 2020 Dec 21. <https://doi.org/10.3390/ijms21249759>.
- Chu DT, et al. An update on the progress of Isolation, Culture, Storage, and clinical application of human bone marrow mesenchymal Stem/Stromal cells. *Int J Mol Sci.* 2020;21(3):708. Published 2020 Jan 21. <https://doi.org/10.3390/ijms21030708>.
- Harrell CR, et al. Therapeutic potential of exosomes derived from adipose Tissue-Sourced mesenchymal stem cells in the treatment of neural and retinal diseases. *Int J Mol Sci.* 2022;23(9):4487. Published 2022 Apr 19. <https://doi.org/10.3390/ijms23094487>.
- Mead B, et al. Viral delivery of multiple MiRNAs promotes retinal ganglion cell survival and functional preservation after optic nerve crush injury. *Exp Eye Res.* 2020;197:108071. <https://doi.org/10.1016/j.exer.2020.108071>.
- Liu X, et al. Bone marrow mesenchymal stem cells enhance autophagy and help protect cells under hypoxic and retinal detachment conditions. *J Cell Mol Med.* 2020;24(6):3346–58. <https://doi.org/10.1111/jcmm.15008>.
- Wu S, et al. Advances in large gap peripheral nerve injury repair and regeneration with bridging nerve guidance conduits. *Macromol Biosci.* 2023;23(10):e2300078. <https://doi.org/10.1002/mabi.202300078>.
- Okada M, et al. Phosphorylation of GAP-43 T172 is a molecular marker of growing axons in a wide range of mammals including primates. *Mol Brain.* 2021;14(1):66. Published 2021 Apr 8. <https://doi.org/10.1186/s13041-021-00755-0>.
- Zhang L, et al. The Inhibition of miR-17-5p promotes cortical neuron neurite growth via STAT3/GAP-43 pathway. *Mol Biol Rep.* 2020;47(3):1795–802. <https://doi.org/10.1007/s11033-020-05273-1>.
- Chung D, et al. GAP-43 and BASP1 in axon regeneration: implications for the treatment of neurodegenerative diseases. *Front Cell Dev Biol.* 2020;8:567537. Published 2020 Sep 3. <https://doi.org/10.3389/fcell.2020.567537>.
- Holahan MR. A shift from a pivotal to supporting role for the Growth-Associated protein (GAP-43) in the coordination of axonal structural and functional plasticity. *Front Cell Neurosci.* 2017;11:266. Published 2017 Aug 31. <https://doi.org/10.3389/fncel.2017.00266>.
- Okada M, et al. JNK1-Dependent phosphorylation of GAP-43 Serine 142 is a novel molecular marker for axonal growth. *Neurochem Res.* 2022;47(9):2668–82. <https://doi.org/10.1007/s11064-022-03580-6>.
- Hadtstein F, Vrolijk M. Vitamin B-6-Induced neuropathy: exploring the mechanisms of pyridoxine toxicity. *Adv Nutr.* 2021;12(5):1911–29. <https://doi.org/10.1093/advances/nmab033>.
- Liu M, et al. Pathways and networks-based analysis of candidate genes associated with nicotine addiction. *PLoS ONE.* 2015;10(5):e0127438. Published 2015 May 12. <https://doi.org/10.1371/journal.pone.0127438>.
- Zhu Q, Julien JP. A key role for GAP-43 in the retinotectal topographic organization. *Exp Neurol.* 1999;155(2):228–42. <https://doi.org/10.1006/exnr.1998.6989>.
- Inoue Y, et al. Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. *Exp Eye Res.* 2007;85(2):234–41. <https://doi.org/10.1016/j.exer.2007.04.007>.
- Zhang Y, Wang W. Effects of bone marrow mesenchymal stem cell transplantation on light-damaged retina. *Invest Ophthalmol Vis Sci.* 2010;51(7):3742–8. <https://doi.org/10.1167/iovs.08-3314>.
- Holan V, Palacka K, Hermankova B. Mesenchymal stem Cell-Based therapy for retinal degenerative diseases: experimental models and clinical trials. *Cells.* 2021;10(3):588. Published 2021 Mar 7. <https://doi.org/10.3390/cells10030588>.
- Mathew B, et al. Uptake and distribution of administered bone marrow mesenchymal stem cell extracellular vesicles in retina. *Cells.* 2021;10(4):730. Published 2021 Mar 25. <https://doi.org/10.3390/cells10040730>.
- Deng CL, et al. Photoreceptor protection by mesenchymal stem cell transplantation identifies Exosomal MiR-21 as a therapeutic for retinal degeneration. *Cell Death Differ.* 2021;28(3):1041–61. <https://doi.org/10.1038/s41418-020-00636-4>.
- Barzelay A, et al. Adipose-Derived mesenchymal stem cells migrate and rescue RPE in the setting of oxidative stress. *Stem Cells Int.* 2018;2018:9682856. Published 2018 Dec 13. <https://doi.org/10.1155/2018/9682856>.
- Pinilla I, et al. Measuring the cone contribution to the ERG b-wave to assess function and predict anatomical rescue in RCS rats. *Vis Res.* 2005;45(5):635–41. <https://doi.org/10.1016/j.visres.2004.09.014>.
- Sauvé Y, Lu B, Lund RD. The relationship between full field electroretinogram and perimetry-like visual thresholds in RCS rats during photoreceptor degeneration and rescue by cell transplants. *Vis Res.* 2004;44(1):9–18. <https://doi.org/10.1016/j.visres.2003.08.015>.
- Thomas BB, et al. Optokinetic test to evaluate visual acuity of each eye independently. *J Neurosci Methods.* 2004;138(1–2):7–13. <https://doi.org/10.1016/j.jneumeth.2004.03.007>.
- Ahmed F, Rajendran Nair DS, Thomas BB. A new optokinetic testing method to measure rat vision. *J Vis Exp.* 2022;(185):10.3791/63357IF: 1.0 Q3 B4. Published 2022 Jul 27.
- Tzameret A, et al. Transplantation of human bone marrow mesenchymal stem cells as a thin subretinal layer ameliorates retinal degeneration in a rat model of retinal dystrophy. *Exp Eye Res.* 2014;118:135–44. <https://doi.org/10.1016/j.exer.2013.10.023>.
- Park UC, et al. Subretinal versus intravitreal administration of human CD34⁺ bone marrow-derived stem cells in a rat model of inherited retinal degeneration. *Ann Transl Med.* 2021;9(15):1275. <https://doi.org/10.21037/atm-20-4662>.
- Chen X, et al. Stem cell therapy for inherited retinal diseases: a systematic review and meta-analysis. *Stem Cell Res Ther.* 2023;14(1):286. Published 2023 Oct 5. <https://doi.org/10.1186/s13287-023-03526-x>.
- Lai D, et al. The role of Müller cells in diabetic macular edema. *Invest Ophthalmol Vis Sci.* 2023;64(10):8. <https://doi.org/10.1167/iovs.64.10.8>.
- Shinozaki Y, Koizumi S. Potential roles of astrocytes and Müller cells in the pathogenesis of glaucoma. *J Pharmacol Sci.* 2021;145(3):262–7. <https://doi.org/10.1016/j.jphs.2020.12.009>.
- Nagashima M, et al. Midkine-a is required for cell cycle progression of Müller glia during neuronal regeneration in the vertebrate retina. *J Neurosci.* 2020;40(6):1232–47. <https://doi.org/10.1523/JNEUROSCI.1675-19.2019>.
- Salman A, et al. Insights on the regeneration potential of Müller glia in the mammalian retina. *Cells.* 2021;10(8):1957. Published 2021 Jul 31. <https://doi.org/10.3390/cells10081957>.
- Gao H, et al. Müller Glia-Mediated retinal regeneration. *Mol Neurobiol.* 2021;58(5):2342–61. <https://doi.org/10.1007/s12035-020-02274-w>.
- Huang X, et al. Mitochondrial transfer between BMSCs and Müller promotes mitochondrial fusion and suppresses gliosis in degenerative retina. *iScience.* 2024;27(7):110309. Published 2024 Jun 20. <https://doi.org/10.1016/j.isci.2024.110309>.
- Wen YT, Tsai RK, et al. The benefits and hazards of intravitreal mesenchymal stem cell (MSC) Based-Therapies in the experimental ischemic optic neuropathy. *Int J Mol Sci.* 2021;22(4):2117. Published 2021 Feb 20. <https://doi.org/10.3390/ijms22042117>.

43. Tassoni A, et al. Molecular mechanisms mediating retinal reactive gliosis following bone marrow mesenchymal stem cell transplantation. *Stem Cells*. 2015;33(10):3006–16. <https://doi.org/10.1002/stem.2095>.
44. Chen X, et al. Mesenchymal-Stem-Cell-Based strategies for retinal diseases. *Genes (Basel)*. 2022;13(10):1901. Published 2022 Oct 19. <https://doi.org/10.3390/genes13101901>.
45. Lin Y, et al. Interaction between mesenchymal stem cells and retinal degenerative microenvironment. *Front Neurosci*. 2021;14:617377. Published 2021 Jan 21. <https://doi.org/10.3389/fnins.2020.617377>.
46. Mani S, et al. Failure to express GAP-43 during neurogenesis affects cell cycle regulation and differentiation of neural precursors and stimulates apoptosis of neurons. *Mol Cell Neurosci*. 2001;17(1):54–66. <https://doi.org/10.1006/mcn.2000.0931>.
47. Zhang H, et al. The involvement of caspases in neuroinflammation and neuronal apoptosis in chronic pain and potential therapeutic targets. *Front Pharmacol*. 2022;13:898574. Published 2022 May 3. <https://doi.org/10.3389/fphar.2022.898574>.
48. Usategui-Martín R, et al. Retinal neuroprotective effect of mesenchymal stem cells secretome through modulation of oxidative Stress, Autophagy, and programmed cell death. *Invest Ophthalmol Vis Sci*. 2022;63(4):27. <https://doi.org/10.1167/iovs.63.4.27>.
49. Adak S, Magdalene D, Deshmukh S, Das D, Jaganathan BG. A review on mesenchymal stem cells for treatment of retinal diseases. *Stem Cell Rev Rep*. 2021;17(4):1154–73. <https://doi.org/10.1007/s12015-020-10090-x>.
50. Trapani I, et al. Gene therapy of inherited retinal degenerations: prospects and challenges. *Hum Gene Ther*. 2015;26(4):193–200. <https://doi.org/10.1089/hum.2015.030>.
51. Brar AS, et al. Gene therapy for inherited retinal diseases: from laboratory bench to patient bedside and beyond. *Ophthalmol Ther*. 2024;13(1):21–50. <https://doi.org/10.1007/s40123-023-00862-2>.
52. Ma Y, Shen Y. Immune responses in retinal gene therapy: challenges, mechanisms, and future strategies. *Front Immunol*. 2025;16:1664968. Published 2025 Oct 15. <https://doi.org/10.3389/fimmu.2025.1664968>

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.