• Basic Research •

Inhibition of IGF-1Rα affects the differentiation fate of rat optic cup-derived retinal stem cells to retinal ganglion cells *in vitro*

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Abstract

● **AIM:** To explore the impact of insulin-like growth factor-1 receptor α (IGF-1Rα) on the differentiation fate of optic-cup-derived retinal stem cells (OC-RSCs) into retinal ganglion cells (RGCs) *in vitro*.

● **METHODS:** OC-RSCs were isolated from optic cups of rats on embryonic day 12.5, and high-purity OC-RSCs were obtained by conditioned culture and passage. Differentiation of OC-RSCs into RGCs under different serum concentrations was examined using flow cytometry, and the serum concentration with high interference with differentiation ratio was selected. Furthermore, the effect of blocking IGF-1Rα on the differentiation of OC-RSCs into RGCs was analyzed through immunocytochemistry and Western blotting.

● **RESULTS:** Immunohistochemical analysis revealed IGF-1Rα was highly expressed in rat embryos at day 12.5. OC-RSCs were isolated and purified, and high-purity OC-RSCs were obtained. When 2.5% serum was administered, the ratio of differentiated RGCs (Thy-1.1 positive) decreased significantly, and the results of immunoblotting also confirmed the blockade of IGF-1Rα reduced Thy-1.1 protein expression.

● **CONCLUSION:** IGF-1Rα blocking can reduce the differentiation of OC-RSCs into RGCs.

● **KEYWORDS:** insulin-like growth factor-1 receptor α; retinal ganglion cell; rat optic cup; retinal stem cells; differentiation fate

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INTRODUCTION

Retinal ganglion cells (RGCs) are the only output neurons in mammals that receive and integrate visual signals into the visual circuit and transmit them to the brain[1-4]. Damage to RGCs is an important pathological feature of various neurodegenerative diseases, such as glaucoma, the leading cause of irreversible blindness worldwide, and induced multiple risk factors such as ocular hypertension, hypertension, and genetic susceptibility[5-7]. Loss of RGCs is a major cause of irreversible blindness in glaucoma. Ganglion cells cannot regenerate after death[8-10]. In humans and mammals, this leads to the loss of RGCs function, which eventually leads to progressive degeneration of the optic nerve[11]. Aging, damage, degeneration and apoptosis of RGCs directly lead to impaired to optic function and progressive optic nerve lesions[12], such as inherited optic neuropathies. Therefore, it is very necessary to investigate a variety of research avenues to provide treatments for such diseases, such as cell replacement therapy[13] and pharmacological treatments[14].

As a group of neurons in the central nervous system, RGCs are located in the inner layer of the retina, and their axons extend to the optic nerve to form the distal end and connect with the brain[15]. Functional retinal stem cells (RSCs) can give rise to all retina cell types and have great potential value for treating eye diseases characterized by irreversible loss of cells[16-18]. However, photoreceptors pose a great challenge and current RSCs differentiation paradigms are unable to effectively capture them. The sequential nature of retinal neurogenesis may be the main reason for this lack of success.

In mammals, the generation of major retinal cell classes follows an evolutionarily conserved temporal sequence, in which RGCs appear first[19], followed by cones and amacrine cells, and horizontal cells shortly thereafter. Rods appear in the next wave of cell birth and most bipolar cells and Müller glia are generated during the last phase of retinal neurogenesis. Although RSCs can be captured at any stage of retinal development, their capacity to differentiate into a particular type of retinal cell is stage-specific[20]. Postnatal retinal RSCs can preferentially give rise to later-generated retinal cell types (*e.g.* photoreceptors) *in vitro* compared to the early generated retinal cell types (*e.g*. RGCs) derived from retinas at embryonic stages[20-22]. Interestingly, RSCs from different stages express different patterns of transcription factors, which are thought to be related to their differentiation bias[23-24]. This phenomenon is consistent with retinoneurogenesis. However, the bias of RSCs for photoreceptors, which can be cultivated at any stage of the retinal development process, should be remodeled before use in retinal transplantation; that is, inducing differentiation into RGCs or altering the differentiation fate of RGCs will be of great significance for future RGCs replacement therapies to reverse vision loss caused by optic neuropathy[25].

In our previous study, we cultivated RSCs from optic cups of rats on embryonic day 12.5 (ED12.5, tailbud stage) and named optic-derived-RSCs. The tailbud stage in retinal neurogenesis is special because retinal cell differentiation has not yet occurred at this time (ED11.5-ED12.5 in rats), and RGCs are only generated at ED13[26]. Therefore, optic-cup-derived retinal stem cells (OC-RSCs) are easily purified stem cell population that has the potential to differentiate into all types of retinal cells[27], and more inclined to differentiate into RGCs. However, the information regarding bias in the phenotypic potential of OC-RSCs for developing into RGCs and the mechanisms that influence the differentiation of RSCs into RGCs remains unknown. Therefore, we analyzed the differentiation potential of OC-RSCs following an RGCs line through techniques such as immunohistochemistry, flow cytometry, and Western blotting, as well as the differentiation fate of RGCs after blocking insulin-like growth factor-1 receptor α (IGF-1Rα). Our data showed that OC-RSCs tended to differentiate into RGCs (Thymus cell antigen 1.1, Thy-1.1 positive), which indicated that OC-RSCs could be used as a suitable cell model for the development of RGCs. In view of the high expression of IGF-1Rα in the optic cup at the tailbud stage and the differentiation bias of OC-RSCs to RGCs, this hypothesis was confirmed by the down-regulation of Thy-1.1 expression caused by blocking IGF-1Rα, which has certain guiding significance for the study of IGF-1Rα function in the future.

MATERIALS AND METHODS

Ethical Approval All animals were obtained from the Experimental Animal Center of Third Military Medical University (Army Medical University) and were maintained under pathogen-free conditions. All procedures were performed according to protocols approved by the Institutional Review Board of the Southwest Hospital of the Army Medical University and conformed to the NIH guidelines on the ethical use of animals. All experiments involving human cells and tissues were carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of the Southwest Hospital of the Army Medical University (SCXK20170002).

Embedding and Sectioning of Rat Eye Tissue and IGF-1Rα Detection Pigmented Long Evans rats (Taconic) were anesthetized and perfused at embryonic days 12 to 15 (ED12-ED15), as reported previously[28]. Briefly, the rats were anesthetized with 1% pentobarbital (150 mL/kg) and perfused with 0.9% sodium chloride. Subsequently, the eyes of the embryos were removed rapidly, and placed in 4% paraformaldehyde at 4℃. After 30min, the corneas were removed and the optic cups were fixed in 4% paraformaldehyde for 2h again. The optical cups were then transferred to 30% sucrose for dehydration overnight. The next day, following the absorption of water in the optic cups, the inside and surrounding areas were filled with Tissue-Tek O.C.T. Compound (OCT; 4583; Sakura Finetek Inc. Torrance, California, USA) embedding agent and then cooled at -80℃. Retinal tissues were cryosliced into serial sections with a thickness of 10 μm. Immunofluorescence was performed on the slices of the optic cups from ED12.5, ED13.5, and ED15.5 following previously described protocols[29]. Briefly, after the slices were rewarmed at room temperature for 2h, the slices were washed three times with 0.1 mol/L phosphate buffer saline (PBS), and permeabilized with 0.5% Triton X-100 for 10 min, and blocked with 3% bovine serum albumin (BSA) and 5% goat serum for 30min at 37℃. The slices were incubated with insulin-like growth factor-1 receptor α (IGF-1Rα) antibody overnight, followed by washing with PBS. The slices ere were incubated with fluorescent secondary antibodies for 1h at 37℃, and then the nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI; D8200; Solarbio Life Sciences, Beijing, China). Fluorescent images were acquired using a fluorescence microscope (BX51; Olympus, Japan).

Cell isolation, Culture and Identification Rat OC-RSC preparation to generate spheres was performed on ED12.5, as previously described[27,30]. In short, 75 embryos at ED 12.5 were isolated from 8 timed-pregnant Long-Evens rats, and optic cups (OCs) was isolated. The OCs were gently triturated with fire-polished glass pipettes to dissociate single cells in D-Hanks' solution (SH30031.02; Hyclone, Utah, USA). Isolated cells were cultured at 5×10^4 cells/cm^2 as

cell suspensions in uncoated flasks. The suspension culture consisted of serum-free medium containing DMEM/F-12, B27 supplement (1:50; Invitrogen Corp., Carlsbad, CA, USA), and 20 ng/mL human recombinant basic fibroblast growth factor (bFGF; E9644; Sigma-Aldrich Inc., St. Louis, MO, USA). Cells were cultured at 37℃ in a 5% CO_2 incubator. Half of the culture medium was changed every two days and after 6–8d in culture, the primary neurospheres were collected and passaged using 0.025% trypsin (SM-2004; Sigma-Aldrich Inc., St. Louis, MO, USA) combined with gentle mechanical trituration. The resulting single cells were plated at the same density and cultured in the same manner. After culturing OCs on poly-D-lysine-coated cover glass slides for 72h, the expression of retinal stem cell related proteins (Ceh Homeobox 10, CHX10 and Paired Box 6, PAX6) and proliferative capacity (Bromodeoxyuridine, BrdU) were detected after fixation with 4% paraformaldehyde.

Differentiation of OC-RSCs to RGCs For OC-RSC differentiation, bFGF was removed from the serum-free defined media and the medium was supplemented with different concentrations of fetal bovine serum (FBS; 0.5%, 2.5%, 5%, and 10%). OC-RSCs at passage 3 were plated on poly-D-lysine (P6407; Sigma-Aldrich Inc., St. Louis, MO, USA)-coated coverslips at a cell density of $(3\text{-}4)\times10^4$ cells/cm^2. Cells were allowed to remain under induction conditions for a period of 7d. The induction culture medium was changed every alternate day.

One microgram/milliliter of IGF-1Rα (sc-463; Santa Cruz Biotechnology, Texas, USA) antibody was used to verify the differentiation direction of OC-RSCs, and differentiation medium containing 2.5% FBS (without IGF-1Rα) was used as a control. OC-RSCs were differentiated for 7d, and the differentiation medium was completely changed every 2d.

Immunocytochemistry Immunochemical analysis of CHX10, PAX6, BrdU, and thymus cell antigen 1.1 (Thy-1.1) markers was performed on OC-RSCs and OC-RSCs that differentiated on the coverslip for 7d according to a previously defined protocol, respectively[28]. Briefly, sphere colonies adhering to the coverslips were collected and rinsed briefly with 0.1 mol/L PBS, fixed in 4% paraformaldehyde at 4℃, and then rinsed again. Nonspecific binding sites of differentiated cells were blocked with 3% BSA and 5% goat serum for 30min at 37℃, and cells were then incubated with primary antibody reagents in blocking solution (1% BSA) at 4℃ overnight. The next day, the differentiated cells were incubated with a fluorescent-conjugated secondary antibody for 1h. Finally, the nuclei were stained with Propidium Iodide (C0080; Solarbio Life Sciences, Beijing, China). Fluorescence was visualized using a Leica confocal microscope (DM IRE2; Leica Microsystems GmbH, Wetzlar, Germany). The primary antibody reagents used are as follows: CHX10 (M05180, Rabbit, 1:100, Boster Biological Technology Co. Itd, Wuhan, China), PAX6 (PB9768, Rabbit, 1:100, Boster Biological Technology Co. Itd, Wuhan, China), BrdU (BM0201, mouse, 1:100, Boster Biological Technology co. Itd, Wuhan, China), and Thy-1.1 (4175, mouse, 1:200, Temecula, California, USA). Fluorescent secondary antibodies (Alexa Fluor 488-conjugated Goat anti-Mouse, A-11031, 1:500, Life, USA; Alexa Fluor 488-conjugated Goat anti-Rabbit, A-11034, 1:500, Life, USA) were used for visualization. Our experiment was repeated three times independently using three different rats.

Flow Cytometry Differentiated rat-derived OC-RSCs were digested into single cells with trypsin (SH30042.01; Hyclone, Logan, Utah, USA), fixed according to the operation manual with a flow detection kit (554714; BD; New Jersey, USA), and stained with primary antibody Thy-1.1 (4175; mouse; 1:200; Temecula, California, USA) for 30min at 4℃. The fluorescein isothiocyanate-conjugated fluorescent secondary antibodies were incubated for 30min at 4℃ after the unconjugated primary antibody was washed with PBS. An isotype antibody incubated under the same conditions was used as the control group. Finally, cells were washed with PBS and analyzed by flow cytometry using a BD FACS Calibur flow cytometer (Becton Dickinson; New Jersey, USA). In total, 10 000 events were collected per sample and stored for analysis. Data were analyzed using FlowJo 7.6.1 flow cytometry software. Positive areas were gated according to the isotype group. The experiment was repeated three times.

Western Blot Analysis Cells differentiated for 7d were lysed, and cell lysates were extracted using RIPA lysis buffer (Pierce Biotechnology, Rockford, IL, USA). Protein content was quantified and normalized using the Bradford method. For SDS polyacrylamide gel electrophoresis (PAGE), a 10% gel was used and approximately 50 μg of soluble protein was loaded per lane. Polyvinylidene fluoride (PVDF) membranes containing transferred proteins were incubated at 4℃ overnight in a blocking solution (5% non-fat milk in Tris-Buffered Saline and Tween 20 Buffer) containing Thy-1.1 (202501, 1:500, BioLegend, Beijing, China) antibodies. β-actin (A5441, 1:1000, Sigma-Aldrich Inc., St. Louis, MO, USA) antibody was used to monitor the loading. Secondary antibodies conjugated to horseradish peroxidase were incubated for 1h at room temperature. The electrochemiluminescence reagent was used to visualize the bands, which were scanned using a Bio-Rad exposure system (Bio-Rad). Relative protein expression levels were quantified using Image J software (NIH), with β-actin as a control.

Statistical Analysis For comparisons between the two groups, data were analyzed using the nonparametric Mann-Whitney *U* test. Multiple comparisons were performed using one-way

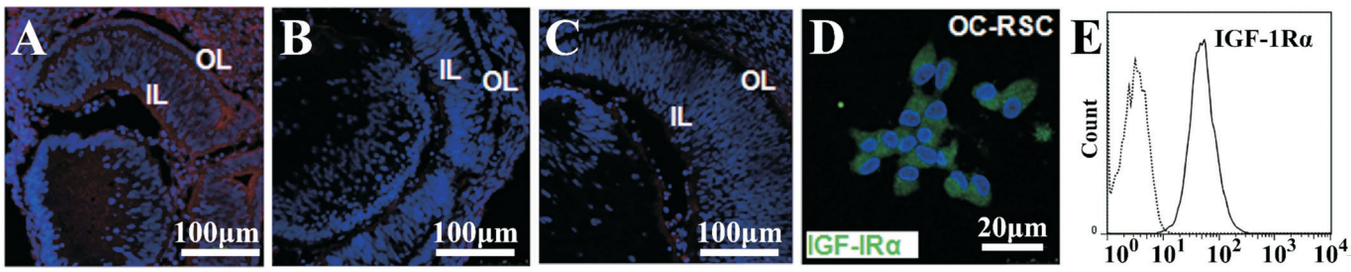

Figure 1 Expression of insulin-like growth factor-1 receptor alpha (IGF-1Rα) in the developing retina and OC-RSCs A: Embryonic day 12.5; B: Embryonic day 13.5; C: Embryonic day 15.5; D: Fluorescence detection of IGF-1Rα expression on the surface of OC-RSCs; E: FCM of IGF-1Rα expression on the surface of OC-RSCs. A-C, red, IGF-1Rα, bar=100 μm; D, green, IGF-1Rα, bar=20 μm. OL: Outer layer; IL: Inner layer; FCM: Flow CytoMetry; OC-RSC: Optic-cup-derived retinal stem cells.

ANOVA followed by Tukey's multiple comparison test. We used Statistical Product and Service Solutions (SPSS) software (version 22.0), and statistical significance was set at $P<0.05$.

RESULTS

Expression of IGF-1Rα in the Developing Retina and OC-RSCs IGF-1Rα-positive cells (red) were present in the layers of the OC at ED12.5, as shown in Figure 1A. As the rat retina developed, the positive expression of IGF-1Rα decreased (Figure 1A-1C). The percentage of IGF-1Rα-positive cells decreased significantly until ED15.5 (Figure 1C). The cellular smear of OC-RSCs revealed that most of the cells expressed IGF-1Rα (Figure 1D), and the percentage of IGF-1Rα-positive cells reached 95.6%±1.4% (n=3 experiments) at ED12.5 was also confirmed by Flow CytoMetry (FCM; Figure 1E).

Isolation, Culture, and Identification of OC-RSCs During the primary culture, OC-RSCs isolated from ED12.5 were grown in suspension in serum-free medium that was changed every 2-3d to form clonal spheres. Approximately 4d later, the clonal spheres adhered and grew, and new OC-RSCs were grown with the clone as the center (Figure 2A). The OC-RSCs were passaged when their density reached approximately 80%. Passaged OC-RSCs showed small phase-bright cell bodies and two or more protrusions (Figure 2B). Immunofluorescence identification of CHX10 and PAX6 in OC-RSCs showed that CHX10 and PAX6 were highly expressed in the nucleus.

Differentiation of OC-RSCs Toward Thy-1.1 Positive Cells To initiate the differentiation of OC-RSCs, the differentiation of OC-RSCs was induced by adding FBS while removing the bFGF and different concentrations of FBS were used to verify the differentiation efficiency. After 7d of differentiation, the expression of Thy-1.1 in OC-RSCs treated with FBS at concentrations of 0.5%, 2.5%, 5%, or 10% was detected by flow cytometry, and the percentage of each phenotype was analyzed. The proportion of Thy-1.1-positive cells was as high as 90% (89.89%±2.84%, n=6) when the FBS concentration was 0.5% (Figure 3A). The percentage of Thy-1.1 positive cells declined with increasing FBS concentration; the proportion of Thy-1.1-positive cells dropped to approximately

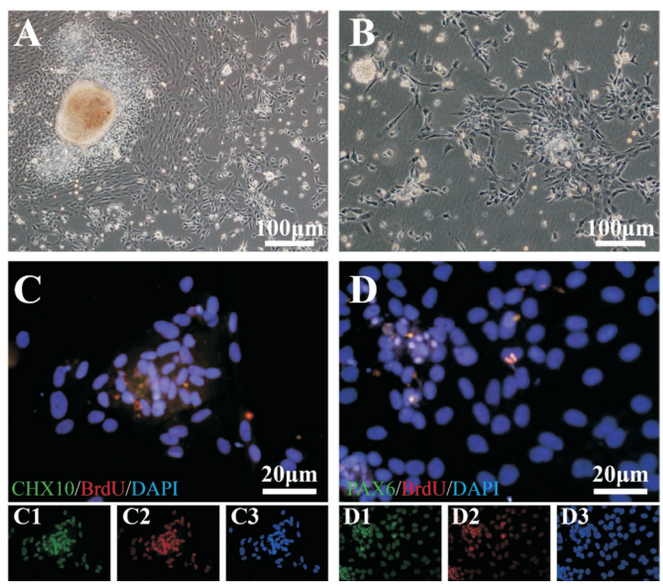

Figure 2 Morphology and identification of OC-RSCs A: Passage 0; B: Passage 3; A and B: bar=100 μm; C: Co-expression of CHX10 and BrdU markers; D: Co-expression of PAX6 and BrdU markers, C and D, bar=20 μm. CHX10: Ceh Homeobox 10; PAX6: Paired box 6; BrdU: Bromodeoxyuridine; OC-RSC: Optic-cup-derived retinal stem cells; DAPI: 4',6-diamidino-2-phenylindole.

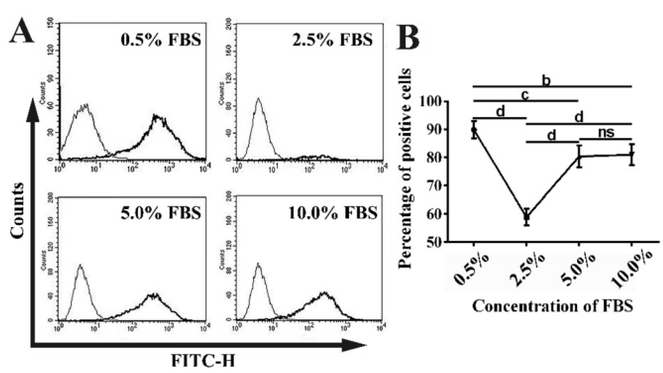

Figure 3 Differentiation of OC-RSCs into RGCs under different serum concentrations A: Flow cytometry analysis of OC-RSCs differentiation; B: Statistical chart of differentiation of OC-RSCs under different serum concentrations (n=6), $^aP<0.05$; $^bP<0.01$; $^cP<0.001$; $^dP<0.0001$; ns: No significance. FBS: Fetal bovine serum; OC-RSC: Optic-cup-derived retinal stem cells; RGCs: Retinal ganglion cells; FITC-H: Fluorescein isothiocyanate.

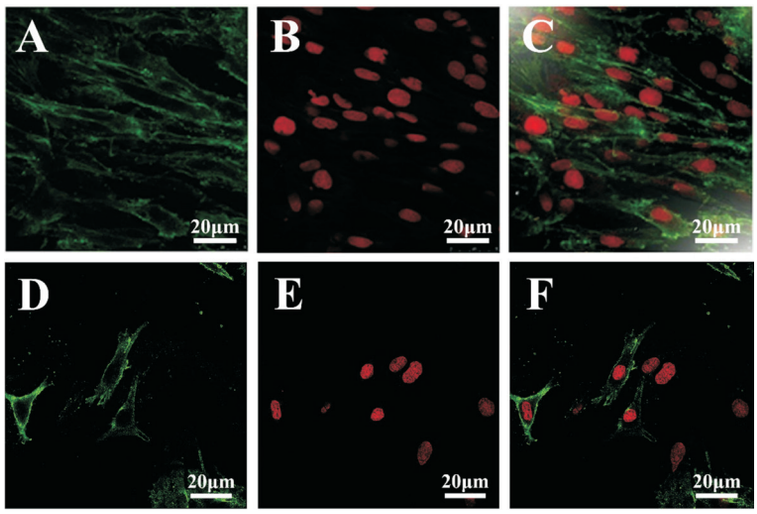

Figure 4 Immunocytochemical analysis of the IGF-1Rα antibody inhibiting the differentiation of OC-RSCs into RGCs for 7d A-C: No IGF-1Rα antibody was added at a serum concentration of 2.5%; B: IGF-1Rα antibody 1 μg/mL was added at a serum concentration of 2.5%; green, Thy 1.1; red, PI staining; bar=20 μm. IGF-1Rα: Insulin-like growth factor-1 receptor alpha; OC-RSC: Optic-cup-derived retinal stem cells; RGCs: Retinal ganglion cells; Thy 1.1: Thymus cell antigen 1.1; PI: Propidium iodide.

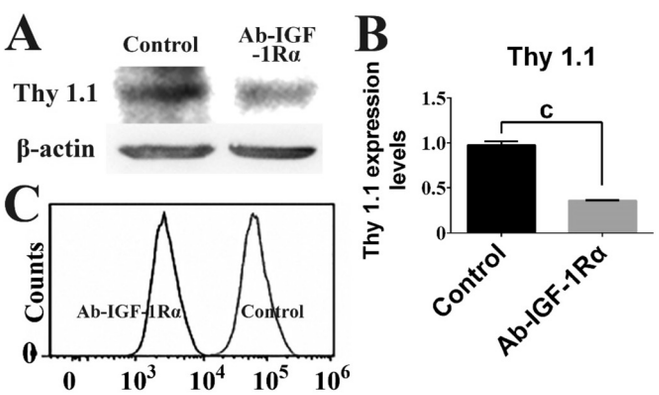

Figure 5 Western blot analysis of the IGF-1Rα antibody inhibiting the differentiation of OC-RSCs into RGCs for 7d A: Western blot analysis of the RGCs marker Thy-1.1; B: Statistical analysis of Western blot of the RGCs marker Thy-1.1 (n=3), ^cP<0.001; C: Gray value analysis of Western blot of RGCs marker Thy-1.1. IGF-1Rα: Insulin-like growth factor-1 receptor alpha; Ab-IGF-1Rα: Antibody insulin-like growth factor-1 receptor alpha; OC-RSC: Optic-cup-derived retinal stem cells; RGCs: Retinal ganglion cells; Thy 1.1: Thymus cell antigen 1.1.

60% (58.89%±2.69%, n=6) at the FBS concentration of 2.5%. The percentage of Thy-1.1-positive cells in 5% FBS (80.39%±3.60%, n=6) or 10% FBS (80.98%±3.39%, n=6) increased slightly, which was higher than that of 2.5% FBS (Figure 3B).

Antagonists of IGF-1Rα Hinder the Differentiation of OC-RSCs into RGCs Considering the relatively low proportion of 2.5% FBS inducing OC-RSCs differentiate into RGCs, the addition of IGF-1Rα antibody has a more significant effect on the differentiation of OC-RSCs into RGCs, therefore, the differentiation medium containing 1 μg/mL of the IGF-1Rα antibody and 2.5% FBS was used as the experimental group, and the normal differentiation group (containing 2.5% FBS, but not 1 μg/mL IGF-1Rα antibody) was used as the control. Immunocytochemical analysis showed that the proportion of Thy-1.1-positive cells decreased significantly after continuous differentiation for 7d (Figure 4A-4F). Similar results were obtained by flow cytometry (Figure 3A-3B). Furthermore, we verified the Thy-1.1 protein expression level by Western blotting after 7d of differentiation of OC-RSCs induced by 1 μg/mL IGF-1Rα antibody and 2.5% FBS. The results also confirmed that IGF-1Rα antibody significantly reduced the expression level of the Thy-1.1 protein (Figure 5A-5C, P<0.001).

DISCUSSION

The adult retina comprises of seven distinct cell types, including six types of neurons, such as cone and rod photoreceptors, bipolar cells, amacrine cells, horizontal cells, ganglion cells, and glial cells (Müller glial cells), which together make up the three cell layers of the retina[31]. OC-RSCs derived from the tailbud stage are common progenitor cells of retinal neurons and glial cells. However, it has been reported that RSCs have a distinct ability to give rise to stage-specific retinal cell types. For example, RSCs from the postnatal retina preferentially give rise to later-generated retinal cell types (such as rod photoreceptors) compared to those derived from retinas at the embryonic stages which give rise to earlier retinal cell types[22,32-33]. In this regard, OC-RSCs should have a greater potential for differentiation into RGCs, and this potential has been confirmed by the results of immunochemistry, flow cytometry, and Western blotting in this study. Our data confirmed that FBS promoted a high proportion of OC-RSCs differentiation into RGCs within 7d, and this preference of OC-RSCs for differentiation into RGCs indicates that OC-

RSCs can be used as a cell model for the differentiation of RSCs into RGCs.

The application of stem cells in optic nerve diseases usually relies on high-purity and high quantity of RSCs with the ability to differentiate into RGCs[34]. Our results provide further evidence for the preference of OC-RSC differentiation into RGC and also proved that RSCs have obvious stage specificity for the differentiation ability of specific retinal lineages. Previous studies have shown that RSCs follow an evolutionarily conserved time sequence in retinogenesis, but the ability of RSCs to produce any cell type(s) at the beginning of retinal production is not limited or restricted, and more than one cell type can be generated at any one time[35]. Several studies have shown that RSCs exhibit cell-to-cell variability in their gene expression patterns and cell fate potentials[35-39]. Retinal cells at the equivalent age of ED16 *in vitro* have a bias toward rod or bipolar cell differentiation, while others differentiate into amacrine or horizontal cells, thus providing good evidence for the heterogeneity of RSCs at different neurogenic stages, particularly with respect to gene expression patterns[35,37]. Neural stem cells/progenitors, enriched from early (ED14) and late (ED18/postanatal day 1) stages of histogenesis of the developing rat retina, represent two sub-populations, as they are distinct in their proliferative response to different mitogens, differentiation potential, and transcriptional profiles[40].

During retinal development, retinal cell fate specification is strictly regulated by multiple transcription factors. The membrane properties help integrate epigenetic information relayed through the environment, such as growth factors, with modulating cell-intrinsic properties. This, in turn, determines whether the stem cells/progenitors remain quiescent, proliferate, or differentiate. Several receptors are involved in retinal development, including IGF-1Rα[41]. Numerous growth factors have been evaluated for their effects on the proliferation of rod precursors, with the general consensus that insulin-like growth factor (IGF), regulated by a systemic growth hormone, controls the production of new rods in correspondence with the regulation of body growth[41-42]. IGF-1 is a known growth factor composed of 70 amino acids, which can regulate retinal cell proliferation[43-44], differentiation, metabolism and development by activating multiple signaling pathways[44-45], as well as regulating the expression of neurotrophic factors[44], and it is widely expressed in the central nervous system, including the retina, and plays a core role in embryonic development and growth, which is crucial for the growth and maturation of the retina[46-47]. In addition, IGF-1 can regulate the size and composition of the retina by stimulating the proliferation of retinal progenitor cells, and is a key module in retinal development, with significant evolutionary significance[48]. IGF-1R is a heterotetrameric glycoprotein expressed throughout the life cycle of neural cells, IGF receptors and ligands play crucial roles in the development of the central nervous system and have been found in developing and adult retina[48-49]. IGF-1Ra, as the main functional subunit of IGF-1R[45], binds to IGF-1, promoting receptor activation and phosphorylation, thereby activating downstream signaling pathways associated with it[50]. This is necessary for normal embryonic retinal development, especially in neuroretinal epithelial tissue[44]. Studies have shown that the IGF-1R signaling pathways play an important role in the nervous system, including cell proliferation and differentiation[51]. Inhibition of the IGF1 signaling pathway can increase cell lifespan and prevent neurodegeneration[52]. Wang *et al*'s[53] research suggested that IGF-1 can regulate the PI3K/Akt and MAPK/Erk pathways to promote RSC proliferation through the IGF-1 receptor (IGF-1R), and under differentiation conditions, IGF-1 can also regulate the differentiation of retinal progenitor cell (RPC) cells through IGF-1R. Research has shown that IGF-1R is downregulated with retinal development[54-55], and our results showed that the expression of IGF-1Rα at different developmental stages was different and significantly decreased in the optic cup after ED12.5. These results suggest that IGF-1Rα might be of special importance for OC-RSCs and their differentiation into RGCs. Therefore, we speculate that if IGF-1R is blocked, cell differentiation may be inhibited. According to our findings, the percentage of Thy-1.1 positive cells decreased significantly with the addition of the IGF-1Rα antibody. These results suggest that IGF-1Rα is an important factor that inhibits the differentiation of OC-RSCs into RGCs *in vitro*.

Clearly, OC-RSCs offer great potential for manipulating the differentiation of stem cells into specific retinal phenotypes. Evidently, IGF-1Rα is required for the differentiation of OC-RSCs into RGCs. With the addition of the IGF-1Rα antibody, the positive expression of Thy-1.1 changed dramatically. These results provide a novel method for manipulating OC-RSC differentiation and suggest the possibility of modifying OC-RSCs differentiation bias, and also provide a basis for studying the mechanism of IGF-1Rα regulating the differentiation of RSCs into RGCs.

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