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Biochemical and Biophysical Research Communications 363 (2007) 347–354
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Sonic hedgehog promotes stem-cell potential of Müller glia in the mammalian retina

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Received 27 August 2007
Available online 10 September 2007

Abstract

Müller glia have been demonstrated to display stem-cell properties after retinal damage. Here, we report this potential can be regulated by Sonic hedgehog (Shh) signaling. Shh can stimulate proliferation of Müller glia through its receptor and target gene expressed on them, furthermore, Shh-treated Müller glia are induced to dedifferentiate by expressing progenitor-specific markers, and then adopt cell fate of rod photoreceptor. Inhibition of signaling by cyclopamine inhibits proliferation and dedifferentiation. Intraocular injection of Shh promotes Müller glia activation in the photoreceptor-damaged retina, Shh also enhances neurogenic potential by producing more rhodopsin-positive photoreceptors from Müller glia-derived cells. Together, these results provide evidences that Müller glia act as potential stem cells in mammalian retina, Shh may have therapeutic effects on these cells for promoting the regeneration of retinal neurons.

Keywords: Sonic hedgehog; Müller glia; Retinal regeneration; Stem cell; Photoreceptor; Rhodopsin

CNS is not considered to lack of regenerative capacity, since neurogenesis has been demonstrated to exist in the adult mammalian brain. Newly born neurons are found in subventricular zone of lateral ventricle and subgranular zone of hippocampal dentate gyrus throughout the whole life [1,2]. Radial glia from developing mammalian brain can differentiate into neurons and glia [3]. As major radial glia in retina, Müller cells from birds and mammals have been reported to dedifferentiate into stem cells after retinal injury [4,5]. Müller glia can also be induced toward a neuronal lineage [6,7]. However, neurons generated from Müller glia are too few to contribute to replacement therapy for retinal degenerative disease. Recent study has shown that regeneration of adult retina could be regulated by signaling molecules [8], which suggests that Müller glia have potential to generate more required neurons under certain environment.

Sonic hedgehog (Shh) is a soluble signaling protein that plays crucial roles in developing CNS, such as regulating specification of neurons and glia [9] and the proliferation of neuronal precursors [10,11]. Shh pathway is also closely associated with retinal development. Shh promotes proliferation of retinal progenitors [12,13], and is involved in cell fate determination of retinal cells, including ganglion cells [14], photoreceptors, [12,15], and Müller glia [13].

Shh can also function in adult CNS, recent studies have shown that proliferation of adult hippocampal progenitors is regulated by Shh [16]. In regard to retina, Shh acts as a mitogen for ciliary marginal zone in chicks and retinal margin in rodents, and Shh induces these cells to regenerate into retinal neurons [17,18], which indicates that Shh pathway may be an important regulator in neurogenesis of adult mammalian eye. Based on the evidence that Patched (*ptc*), a component of Shh receptor complex, is expressed in adult Müller glia [13], we hypothesize that Müller glia are target cells and modulated by Shh signaling.

In this study, we examine the role of Shh signaling in Müller glia and adult retinal regeneration. We have determined Shh is a potent mitogen for rat Müller glia both

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0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.08.178

in vitro and in vivo, Shh also induced Müller glia to dedifferentiate and adopt the phenotype of rod photoreceptor. However, inhibition of Shh signaling by cyclopamine significantly weakened the proliferation and dedifferentiation of Müller glia. Moreover, delivery of Shh to retina led to increase in generation of rod photoreceptors from Müller glia-derived cells. These data implicate Shh signaling may act as an important regulator in adult retinal regeneration.

Materials and methods

Müller cell culture. Müller glia were obtained according to previously described [6,19]. In brief, eyes from postnatal day 7 Sprague–Dawley rats were enucleated, retinas were carefully dissected from eye cups, avoid possible contamination of retinal pigmented epithelium and ciliary epithelium. Then neural retinas were digested with 0.25% Trypsin and 0.1% type I collagenase. Dissociated retinal cells were seeded in culture dishes containing DMEM/F12 supplemented with N2, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% FBS, then incubated at 37 °C in 5% CO_2. The floating retinal aggregates and debris were removed by forcibly pipetting when Müller cells adhered to dish.

Cells were plated on poly-D-lysine and laminin coated glass coverslips in 24-well plate. SHH-N (5, 10, and 20 nM) or cyclopamine (10 μg/ml) was added to culture medium on the first day and maintained at the same concentration throughout 2-day culture period. SHH-N (R&D systems) was dissolved in saline and 0.1% BSA. Cyclopamine (Sigma) was dissolved in 45% cyclodextrin in PBS. BrdU (10 μM) was added for the final 18 h to label dividing cells. After 2 days in culture, coverslips were fixed in 4% paraformaldehyde for 10 min and processed for immunocytochemistry.

FACS analysis. The purity of Müller cell was analyzed by FACS analysis using specific antibody glutamine synthetase (GS). Briefly, 10^6 cultured Müller cells were collected, washed with ice cold PBS containing 1% BSA, and penetrated in 0.3% Triton X-100 for 10 min at 4 °C. Cells were incubated with appropriate dilution of GS at 4 °C for 1 h. GS was not added in negative control. After incubation with fluorescent second antibody at 4 °C for 1 h, cells were resuspended in PBS–BSA for FACS analysis.

Propidium iodide (PI) staining was used for analysis of DNA content and cell cycle phase distribution. Müller cells treated with SHH-N, cyclopamine or control were harvested for analysis. PI fluorescence was determined by flow cytometry using a FACScan and Cellquest software for acquisition. Modfit software (Verity Software House) was used for quantifying cell cycle phase distribution.

Real-time quantitative RT-PCR. Total RNA was isolated from Müller cells using TRIZOL reagent (Invitrogen). Single-stranded cDNA was synthesized using MMLV reverse transcriptase (Promega) and oligo-dT primers. Real-time RT-PCR was performed with SYBR green using Roto-Gene 3000 (Corbett Research). PCR primers for *ptc* were 5′AGAACCAC AGGGCTATGCTCG3′ (forward) and 5′AGCAGAACCAGTCCGTT GAGA3′ (reverse); for Gli1 were 5′GTGGCAACAGGACGGAACTT3′ (forward) and 5′CGACTGTGAGACCCTATACCC3′ (reverse); for Nrl were 5′GCCTGAGGTCCCTGGAATGAGTGT3′ (forward) and 5′TAG TGTTTGGGGCGGGGAAGAT3′ (reverse); for Crx were 5′CCTCACT ATCGGCAATGCC3′ (forward) and 5′ATGTGCCTGCCTTCCTCTT C3′ (reverse); for rhodopsin 5′CATGCAGTGTTCATGTGGGA3′ (forward) and 5′AGCAGAGGCTGGTGAGCATG3′ (reverse); for β-actin were 5′CCTCTATGCCAACACAGTGC3′ (forward) and 5′ATACTC CTGCTTGCTGATCC3′ (reverse). PCR was performed with 5 ng cDNA templates and 400 nM forward and reverse primers. Three minutes at 94 °C was followed by 40 cycles that consisted of 20 s at 94 °C, 1 min at the annealing temperature corresponding to the primers used, and 30 s at 72 °C. The final extension reaction was performed for 5 min at 72 °C. PCR products were visualized on a 2% agarose gel. Expression levels of each transcript were standardized by comparison with the amount of β-actin mRNA.

Intravitreal injection. Photoreceptor apoptosis was induced in SD rats by i.p. injection of 60 mg/kg *N*-methyl-*N*-nitrosourea (MNU) [20]. Immediately after MNU application, left eyes (control) were injected with sterile saline, 0.1% bovine serum albumin (BSA), and BrdU (30 nmol), and right eyes (treated) were injected with the same solution plus SHH-N (400 ng/injection) or cyclopamine (5000 ng/injection). Rats were injected daily for consecutive 3 days after MNU. On day 7 or 21, animals were killed, eyes were harvested and fixed in 4% paraformaldehyde. Cryostat sections were cut at 14 μm and thaw mounted onto slides for immunohistochemistry.

Immunohistochemistry. Sections were permeabilized in PBS/0.3% Triton X-100/5% serum and incubated with primary antibodies at 4 °C overnight. Sections immunolabeled for BrdU were treated in 2 N HCl for 20 min, followed by 0.1 M borate buffer incubation for 10 min, then rinsed in PBS before addition of anti-BrdU solution. When sections were performed double labeling with BrdU and other antigens, antibodies to the other specific antigen were first labeled. The primary antibodies used in this study include rabbit anti-GS (1:5000, Sigma), mouse anti-Vimentin (1:200, antibody diagostica Inc.), mouse anti-BrdU (1:400, Roche), rabbit anti-BrdU (1:200, Lab Vision), mouse anti-nestin (1:500; Chemicom), mouse anti-β-tubulin (1:500, Sigma), mouse anti-rhodopsin (1:5000; Sigma), mouse anti-Pax6 (1:500, DSHB), and mouse anti-Sox2 (1:500, DSHB). Fluorescence-conjugated secondary antibodies were Alexa Fluor 594 or 488 (Invitrogen). The sections were mounted and images were captured using a confocal microscope (Zeiss, Germany).

Cell counts and analysis. To determine the number of stained cells in cell culture, five coverslips were counted in each culture condition, and at least 3 random fields were selected for cell counting. Five sections from each animal were selected for analysis, and at least three different animals were analyzed in each condition, an average was obtained for under each condition. Data from different treated groups were compared statistically with the appropriate Student's *t*-test. Data were expressed as means ± SEM.

Results

Müller glia express components of Shh pathway

After 7 days in culture, fusiform cells were observed to extend radially from the margin of cell aggregates. Another 7 days later, these spindle-like cells reached 80% confluence (Fig. 1A). FACS analysis showed that in our monolayer culture 94.5% cells expressed antigen of Müller glia, GS (Fig. 1B and C). The purity of cells was consistent with previous report [6,19]. Cultured cells co-expressed specific markers of Müller glia, GS and Vimentin (Fig. 1D–F).

To determine the effects of Shh on Müller glia, we should confirm whether *ptc* or *gli1*, a target gene of Shh, is expressed in Müller glia. RT-PCR analysis revealed that cultured Müller glia expressed both *ptc* and *gli1* mRNA (Fig. 1G), the level of transcripts increased corresponding to addition of Shh or decreased in the presence of Shh pathway inhibitor, cyclopamine. We did real-time quantitative RT-PCR to quantify the changes of level. When adding 5, 10, and 20 nM SHH-N or cyclopamine separately into culture medium, *ptc* mRNA was 1.76-, 1.88-, 3.22-, 0.77-fold to the control; differences were significant between the control and Shh-treated groups (Fig. 1H). Under the same condition, Gli1 mRNA was 1.15-, 5.83-, 6.58-, 0.69-fold to the control, the level of Gli1 increased significantly when treated with 10 or 20 nM Shh (Fig. 1I).

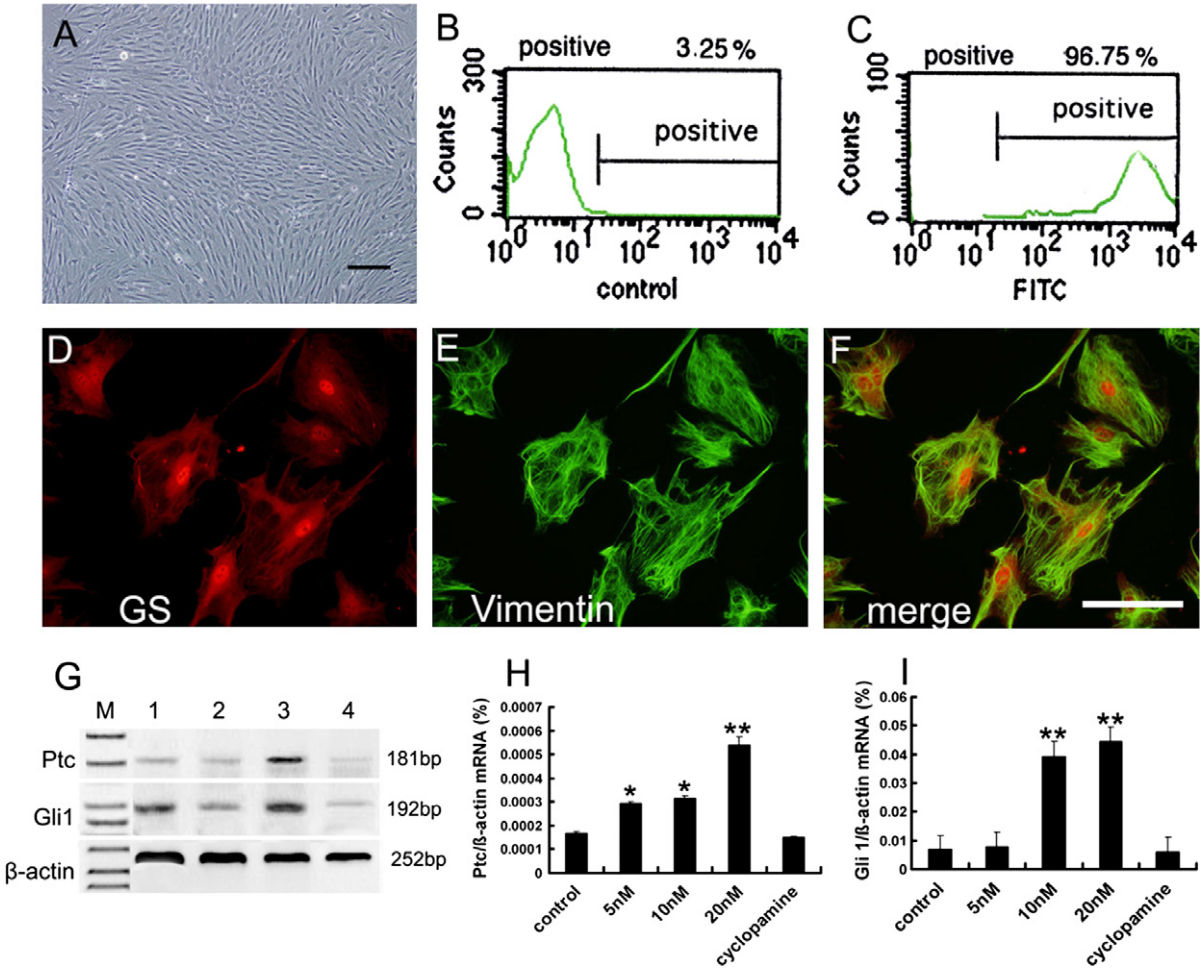

Fig. 1. Identification of cultured Müller glia and expression of Shh signaling components. (A) Rat Müller glia appeared flattened and spindle-like after 14 days in culture. (B,C) FACS analysis showed that 94.5% cells were GS positive. (D–F) Double immunocytochemistry showed that cells co-expressed specific markers of Müller glia, (D) GS and (E) Vimentin. (G) RT-PCR analysis showed Müller cell expressed *ptc* and *gli1*. (H,I) Real-time RT-PCR quantified Shh increased (H) *ptc* and (I) *gli1* mRNA. Lane M = DNA marker; lane 1 = embryonic retina; lane 2 = control Müller cells; lane 3 = Shh-treated Müller glia; lane 4 = cyclopamine-treated Müller glia. Scale bars: (A) 50 μm; (D–F) 50 μm.

These data indicated that Müller glia contained target genes of Shh pathway and served as Hh responding cells.

Shh promotes proliferation of Müller glia

Müller cells were grown for 2 days under control condition in the presence of either Shh or cyclopamine, we did immunocytochemistry for BrdU to assay the proportion of cells entering S-phase. Compared to the control (Fig. 2A), percentage of BrdU-positive Müller glia increased significantly after treated by Shh (Fig. 2B), cyclopamine led to fewer Müller cells incorporating BrdU (Fig. 2C). Mitogenic effect of Shh was relevant to its increasing concentrations (Fig. 2D). Twenty nanomolar Shh doubled proportion of BrdU-labeled cells however, when the concentration reached 100 nM, its mitogenic effects have no significant difference with that of 20 nM (data not shown). Therefore, we used the concentration of 20 nM in the following tests. FACS analysis also confirmed 20 nM Shh induced around triple fold cells entering S-phase to the control (Fig. 2E–G). These data showed Shh promoted proliferation of Müller glia in a dose-dependent manner.

Neuronal differentiation of Müller glia induced by Shh

We are interested in whether Shh would direct Müller glia toward a neuronal lineage since Shh can regulate adult neurogenesis in birds and mice [17,18]. Müller glia were cultured under condition of control, in the presence of 20 nM Shh for 7 days or grown with Shh for 2 days and then cyclopamine was added to medium and cultured for another 5 days. BrdU was added in the final 18 h to tag proliferating cells. We found Müller glia devoid of any mitogens did not expressed progenitor markers (data not shown). Shh induced 37.5 ± 2.2% cells expressed multiple retinal progenitor markers Pax6 (Fig. 3A) and 54.3 ± 3.0% cells expressed neurogenic genes Sox2 (Fig. 3B). Pax6 and Sox2 positive nuclei co-expressed with GS in cytoplasm. Shh-treated Müller glia maintained proliferation by incorporating BrdU, 61.2 ± 2.5% BrdU+ cells

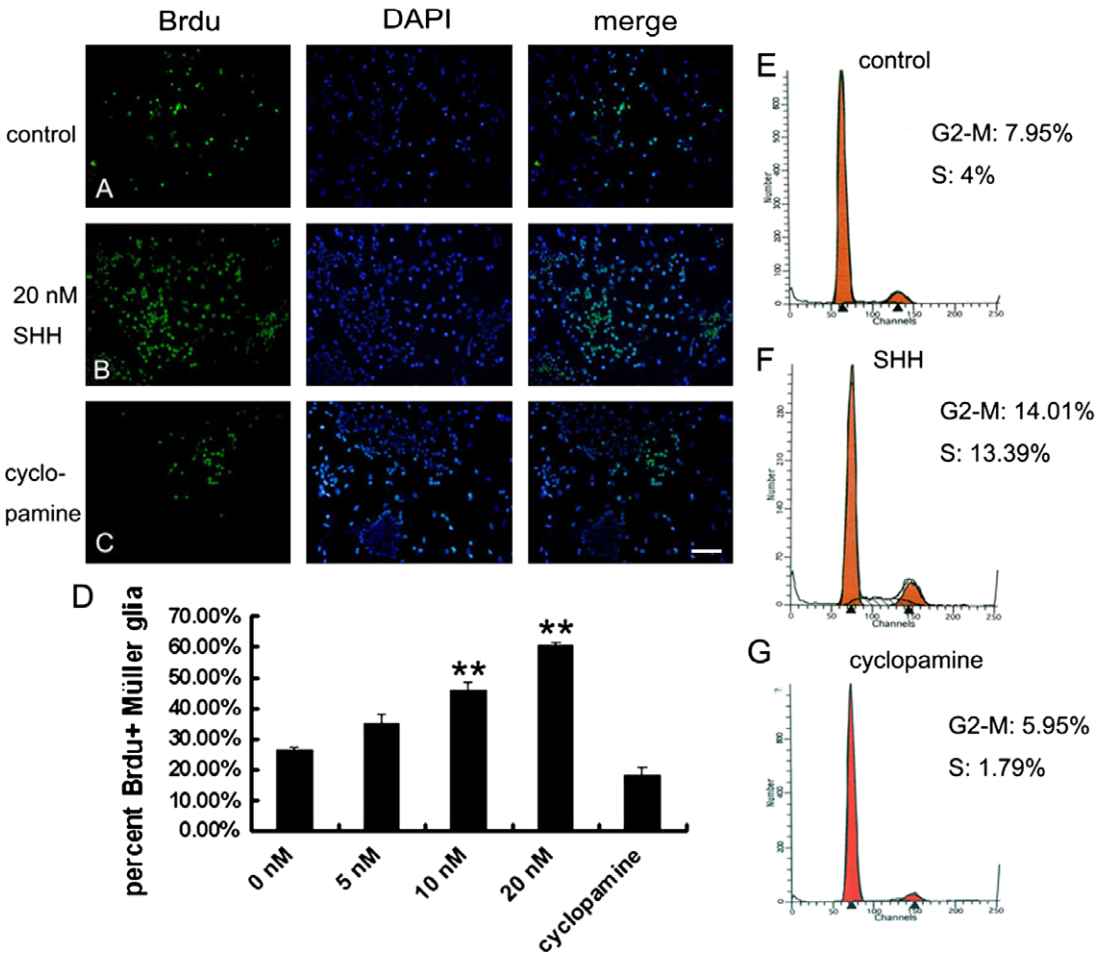

Fig. 2. Shh promoted proliferation of cultured Müller glia. (B) Shh increased BrdU positive cells compared to (A) control, (C) cyclopamine decreased BrdU-labeled cells. (D) At the concentration of 10 or 20 nM, proliferative effects were significant. (E,F) FACS analysis confirmed Shh promoted more Müller glia entering cell cycle.

expressed nestin (Fig. 3C). These progenitor markers were hardly detected if Müller glia were treated by cyclopamine (data not shown), which excluded the possibility that Shh activated other signaling pathways to stimulate Müller glia directly. We may conclude Shh induce Müller cells differentiate into progenitors.

We further test neurogenic potential of Müller glia. Cells were grown in Shh for 7 days, and cultured in fresh medium devoid of Shh for another 2 days, then cells were collected for immunofluorescent and RT-PCR analysis. Immunocytochemistry identified BrdU-tagged cells expressing neuronal marker β-tubulin ($18.6 \pm 2.1\%$) (Fig. 3D), confirming their shift along neuronal lineage. We examined their ability to generate retinal neurons by using specific retinal antibodies. Double staining revealed that $14.2 \pm 1.6\%$ BrdU+ cells expressed a rod photoreceptor-specific marker, rhodopsin (Fig. 3E). BrdU+/rhodopsin+ cells showed a characteristic bipolar shape. In addition, PKCα, a marker for bipolar cell was detected slightly in BrdU+ cells (data not shown), suggesting their differentiation along late-born retinal neuronal lineage. To verify whether Shh would determine Müller glia-derived progenitors to photoreceptor cell fate, we detected transcripts of photoreceptor development. RT-PCR results showed Shh preferentially induced expression of Nrl and Crx (Fig. 3F), which were reported to express in rod photoreceptors and regulate rhodopsin transcription [21,22]. Whereas, Müller glia grown in FBS were hardly detected to express any retinal neuron markers.

Shh promotes retinal regeneration after photoreceptor degeneration

As noted above that Shh stimulated proliferation and dedifferentiation of Müller glia in vitro. We are interested in whether activation of this pathway could control proliferative and regenerative competence of these cells in vivo. We made intraocular injection of Shh (or cyclopamine) and BrdU in MNU-exposed retina. In control retinas (untreated by Shh or cyclopamine), on average 355 ± 9.52 BrdU+ cells/section distributed in INL after retinal damage. To determine the cell type of retinal cells labeled with BrdU, we performed double immunostaining with antibodies to BrdU and cell-specific markers; we

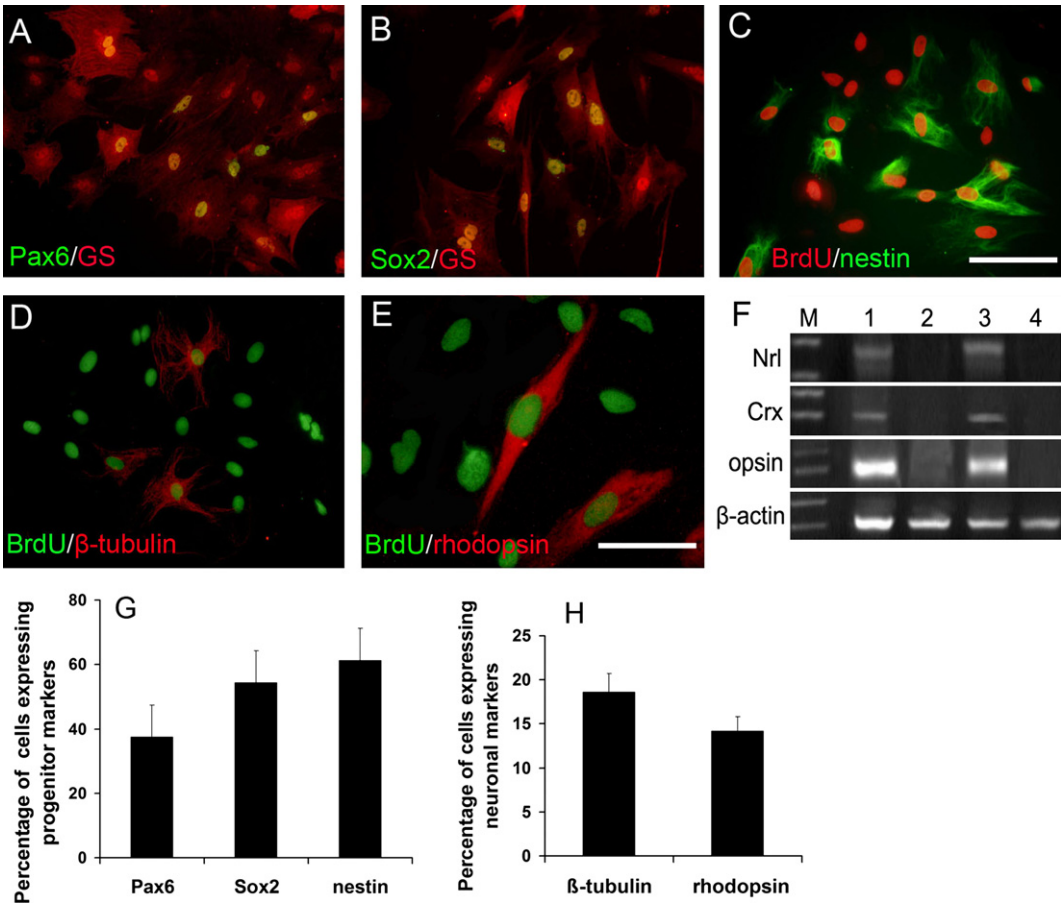

Fig. 3. Müller glia dedifferentiated into progenitors and generated rod photoreceptors. Double staining showed that Shh induced Müller glia expressed markers of progenitors: (A) Pax6 (green), (B) Sox2 (green), and (C) nestin (green). Then, Müller glia-derived progenitors differentiated into neuronal lineage by expressing (D) β-tubulin (red), and (E) produced rhodopsin-positive cells (red). (F) Transcripts of rod development, Nrl, Crx, and opsin were detected in Shh-treated Müller glia by RT-PCR. Lane M = DNA marker; lane 1 = PN 14 retina; lane 2 = control Müller cells; lane 3 = Shh-treated Müller glia; lane 4 = cyclopamine-treated Müller glia. (G,H) The percentage of cells expressing progenitor or neuronal markers is presented in the graph. Scale bars: (A–D) 50 μm; (E) 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

found BrdU-labeled cells co-expressed GS (Fig. 4A). Treating retinas with Shh for 3d markedly increased proliferating cells as 520 ± 12.36 cells/section were labeled with BrdU (Fig. 4B and L). To determine whether endogenous Shh functions in stimulating proliferation of Müller glia after retinal injury, we inhibited Shh signaling with cyclopamine, which binds to smoothened directly [23]. Proliferation was decreased as only 102 ± 6.12 BrdU+ cells/section detected (Fig. 4C and L). When Shh was injected into normal rat eyes, no BrdU-positive cells were found. In the adult rat retina, Pax6 is expressed in the nuclei of amacrine, ganglion, and horizontal cells. After retinal injury, 4.5 ± 1.4 cells/section were double positive for Pax6 and GS (Fig. 4D and M), indicating some Müller glia acquire the properties of retinal progenitors, this population increased after the application of Shh, with 14.3 ± 2.1 cells expressing Pax6 and GS (Fig. 4E and M). We may conclude that increasing levels of Shh stimulates proliferation of Müller glia-derived progenitors after retinal injury.

To study the effect of Shh signaling on the fate of Müller glia-derived progenitors, rats were injected daily for continuous 7 days immediately after MNU, and killed at day 21. In our previous study, we found a few Müller glia-derived cells expressed rhodopsin by day 15 after MNU (data unpublished). Animals were allowed to survive longer for us to investigate the fate of new-born cells. On day 21, $7.12 \pm 1.0\%$ BrdU-labeled cells expressed rhodopsin, and these cells sparsely distributed in INL (Fig. 4F–H and N). Shh application increased the number of rhodopsin+ cells, with $28.85 \pm 3.2\%$ BrdU+ cells expressing rhodopsin (Fig. 4I–K and N) (compared with untreated groups, $p < 0.01$, t-test). To examine whether BrdU-labeled cells differentiate into other cell types, we used specific antibodies for retinal neurons (bipolar cells, horizontal cells, amacrine cells, and ganglion cells), and we could not detect BrdU-labeled cells expressed these markers (data not shown).

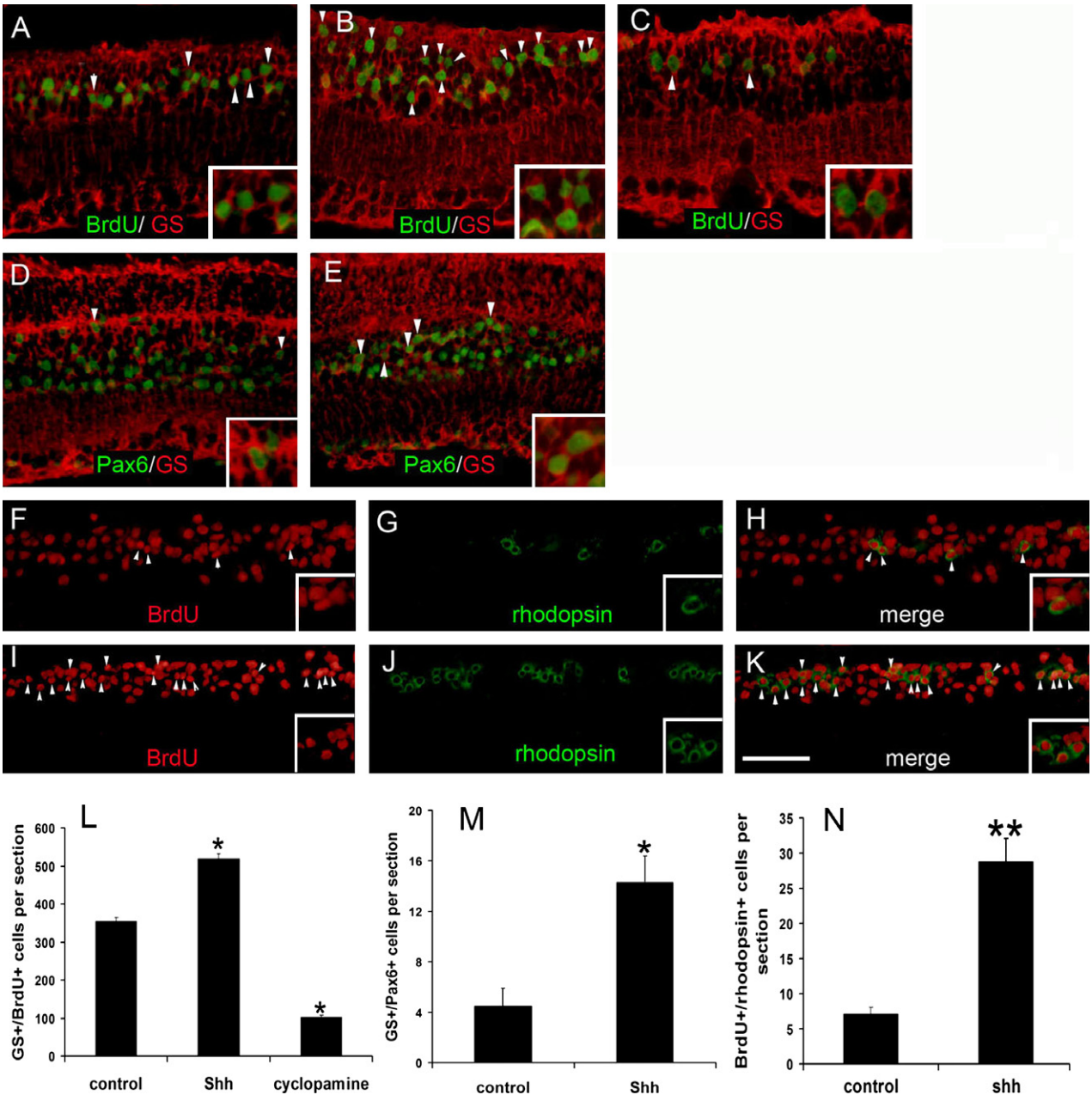

Fig. 4. Shh promoted retinal regeneration in photoreceptor damaged retina. Double staining of GS (red) and BrdU (green) showed that (B) intravitreal injection of Shh increased BrdU-labeled Müller glia *significantly* in INL compared to the control (A,L), (C,L) cyclopamine inhibited proliferation of Müller glia. (D) After retinal damage, Müller glia dedifferentiated into progenitors by expressing Pax6 (green). (E,M) Shh increased Müller glia-derived progenitors. (F–H) BrdU-tagged (red) cells generated rhodopsin-positive cells (green) in INL, (I–K) Shh promoted more rhodopsin-positive cells (green) produced from Müller glia, (N) the proportion of such cells was significantly higher as compared to the control. $^{*}p < 0.05$, $^{**}p < 0.01$. Double-labeling cells were indicated by arrow heads, and magnified in boxes. Scale bars: 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Discussion

In the present study, our data indicate that application of Shh promotes Müller glia proliferation, dedifferentiation into progenitors, and producing rod photoreceptors. Intraocular injection of Shh also accelerates retinal regeneration from Müller glia. Conversely, inhibition of Shh signaling with cylopamine inhibits stem-cell potential of Müller glia. We may conclude that endogenous neurogenic capacity of retinal Müller glia can be enhanced by Shh signaling.

Shh has been demonstrated to play important roles in the maintenance of stem cells and progenitors in CNS. Shh regulates proliferation of neuronal progenitors in the cerebral cortex [24] and granule neuron precursors in the cerebellum [10,11]. Studies in zebra fish also suggest Shh signaling is necessary for the proliferation and survival of retinal progenitors [15,25]. Shh stimulates proliferation of

rodent embryonic retinal progenitors in vitro [12,13]. Proliferative effects associated with Shh have also been described in adult CNS, including progenitors in hippocampus [16], the subventricular zone, and the olfactory bulb [26]. Retinal marginal progenitors in chicks and mice also depend on Shh as a mitogen [17,18]. We have obviously shown that Shh stimulates Müller glia to proliferate in vitro, and interestingly, we found exogenous Shh exert its proliferative effects on Müller glia in vivo only under the condition of retinal injury. Müller glia in intact retinas do not undergo activation even in the presence of Shh, which is consistent with previous observation that Müller glia do not show any signs of proliferation in Shh activating mice (*ptc*+/−) [17]. In summary, Shh maintains proliferation of progenitors derived from Müller glia.

We also found exogenously application of Shh induces Müller glia dedifferentiate to retinal progenitors by expressing Pax6, Sox2, and nestin. Further, Shh-treated cells shift to neural lineage by expressing β-tubulin and adopt cell fate of rod photoreceptor. Previous studies implicate Shh signaling is closely associated with photoreceptor development. Shh controls the timing and rate of photoreceptor differentiation in *Drosophila* eye [27,28]. *ptc*, a receptor of Shh, is expressed during retinal development in the neuroblast zone, which in later developmental stages contains primarily differentiating rod photoreceptors [12]. In addition, Shh promotes mammalian retinal progenitors proliferation and differentiation into more rod photoreceptors and Müller glial cells [13]. Both rod photoreceptor and Müller glia are late-born neurons, we hypothesize that Shh have a tendency to promote progenitors differentiation at late stage. It is also possible, that Shh act throughout photoreceptor differentiation since Müller glia treated by Shh express transcripts of rod development and mature marker. Though, Shh is secreted by ganglion cells and regulates their genesis, we could not detect regeneration of ganglion cells from Müller glia-derived progenitors.

Regenerative capacity of Müller glia can be regulated by exogenous factors, including insulin, fibroblast growth factor-2 [29], and Wnt [8]. Shh can direct progenitors in adult marginal zone to rod cell fate [17]. As the target cells of Shh in adult retina, Müller glia can also be modulated by Shh. Our results provide evidences that Shh enhances regenerative potential of Müller glia and produces encouraging number of rod photoreceptors, indicating that activating Shh pathway may provide an alternative strategy for cell replacement therapies by producing photoreceptors from endogenous progenitors. Furthermore, Müller glia may become a target for drug delivery and gene therapies for retinal degenerative diseases.

Acknowledgments

This study was supported by National Basic Research 973 Program (2005 CB 724306) and Innovation Foundation for Graduate Students of Fudan University (EYF101011).

References

[1] F.H. Gage, Mammalian neural stem cells, Science 289 (2000) 1433–1438.
[2] A. Alvarez-Buylla, D.A. Lim, For the long run: maintaining germinal niches in the adult brain, Neuron 41 (2004) 683–686.
[3] A.R. Kriegstein, M. Gotz, Radial glia diversity: a matter of cell fate, Glia 43 (2003) 37–43.
[4] A.J. Fischer, T.A. Reh, Müller glia are a potential source of neural regeneration in the postnatal chicken retina, Nat. Neurosci. 4 (2001) 247–252.
[5] S. Ooto, T. Akagi, R. Kageyama, J. Akita, M. Mandai, Y. Honda, M. Takahashi, Potential for neural regeneration after neurotoxic injury in the adult mammalian retina, Proc. Natl. Acad. Sci. USA 101 (2004) 13654–13659.
[6] A.V. Das, K.B. Mallya, X. Zhao, F. Ahmad, S. Bhattacharya, W.B. Thoreson, G.V. Hegde, I. Ahmad, Neural stem cell properties of Müller glia in the mammalian retina: regulation by Notch and Wnt signaling, Dev. Biol. 299 (2006) 283–302.
[7] A. Kubota, K. Nishida, K. Nakashima, Y. Tano, Conversion of mammalian Müller glial cells into a neuronal lineage by in vitro aggregate-culture, Biochem. Biophys. Res. Commun. 351 (2006) 514–520.
[8] F. Osakada, S. Ooto, T. Akagi, M. Mandai, A. Akaike, M. Takahashi, Wnt signaling promotes regeneration in the retina of adult mammals, J. Neurosci. 27 (2007) 4210–4219.
[9] L.V. Goodrich, M.P. Scott, Hedgehog and patched in neural development and disease, Neuron 21 (1998) 1243–1257.
[10] R. Wechsler-Reya, M. Scott, Control of neuronal precursor proliferation in the cerebellum by sonic hedgehog, Neuron 22 (1999) 103–114.
[11] N. Dahmane, A. Ruiz-i-Altaba, Sonic hedgehog regulates the growth and patterning of the cerebellum, Development 126 (1999) 3089–3100.
[12] E.M. Levine, H. Roelink, J. Turner, T.A. Reh, Sonic hedgehog promotes rod photoreceptor differentiation in mammalian retinal cells in vitro, J. Neurosci. 17 (1997) 6277–6288.
[13] A.M. Jensen, V.A. Wallace, Expression of sonic hedgehog and its putative role as a precursor cell mitogen in the developing mouse retina, Development 124 (1997) 363–371.
[14] X.M. Zhang, X.J. Yang, Regulation of retinal ganglion cell production by sonic hedgehog, Development 128 (2001) 943–957.
[15] D.L. Stenkamp, R.A. Frey, S.N. Prabhudesai, P.A. Raymond, Function for hedgehog genes in zebrafish retinal development, Dev. Biol. 220 (2000) 238–252.
[16] K. Lai, B.K. Kaspar, F.H. Gage, D.V. Schaffer, Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo, Nat. Neurosci. 6 (2003) 21–27.
[17] A. Moshiri, T.A. Reh, Persistent progenitors at the retinal margin of ptc−/− mice, J. Neurosci. 24 (2004) 229–237.
[18] A. Moshiri, C.R. McGuire, T.A. Reh, Sonic hedgehog regulates proliferation of the retinal ciliary marginal zone in posthatch chicks, Dev. Dyn. 233 (2005) 66–75.
[19] D. Hicks, Y. Courtois, The growth and behavior of rat retinal Müller cells in vitro. 1. An improved method for isolation and culture, Exp. Eye Res. 51 (1990) 119–129.
[20] J. Wan, H. Zheng, B.Y. Hu, H.L. Xiao, Z.J. She, Z.L. Chen, G.M. Zhou, Acute photoreceptor degeneration down-regulates melanopsin expression in adult rat retina, Neurosci. Lett. 400 (2006) 48–52.
[21] T. Furukawa, E.M. Morrow, C.L. Cepko, Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation, Cell 91 (1997) 531–541.
[22] A.J. Mears, M. Kondo, P.K. Swain, Y. Takada, R.A. Bush, T.L. Saunders, P.A. Sieving, A. Swaroop, Nrl is required for rod photoreceptor development, Nat. Genet. 29 (2001) 447–452.

[23] J.K. Chen, J. Taipale, M.K. Cooper, P.A. Beachy, Inhibition of hedgehog signaling by direct binding of cyclopamine to smoothened, Genes Dev. 16 (2002) 2743–2748.
[24] N. Dahmane, P. Sanchez, Y. Gitton, V. Palma, T. Sun, M. Beyna, H. Weiner, A. Ruiz iAltaba, The sonic hedgehog-gli pathway regulates dorsal brain growth and tumorigenesis, Development 128 (2001) 5201–5212.
[25] D.L. Stenkamp, R.A. Frey, D.E. Mallory, E.E. Shupe, Embryonic retinal gene expression in sonic-you mutant zebrafish, Dev. Dyn. 225 (2002) 344–350.
[26] R. Machold, S. Hayashi, M. Rutlin, M.D. Muzumdar, S. Nery, J.G. Corbin, A. Gritli-Linde, T. Dellovade, J.A. Porter, L.L. Rubin, H. Dudek, A.P. McMahon, G. Fishell, Sonic hedgehog is required for progenitor cell maintenance in telenc lic stem cell niches, Neuron 39 (2003) 937–950.
[27] C. Ma, Y. Zhou, P.A. Beachy, K. Moses, The segment polarity gene hedgehog is required for progression of the morphogenetic furrow in the developing *Drosophila* eye, Cell 75 (1993) 927–938.
[28] U. Heberlein, C.M. Singh, A.Y. Luk, T.J. Donohoe, Growth and differentiation in the *Drosophila* eye coordinated by hedgehog, Nature 373 (1995) 709–711.
[29] A.J. Fischer, C.R. McGuire, B.D. Dierks, T.A. Reh, Insulin and fibroblast growth factor 2 activate a neurogenic program in Muller glia of the chicken retina, J. Neurosci. 22 (2002) 9387–9398.