J Pharmacol Sci **109**, 168 – 173 (2009)

Journal of Pharmacological Sciences

Forum Minireview

Drug Development Targeting the Glycogen Synthase Kinase-3*β* (GSK-3*β*)-Mediated Signal Transduction Pathway: Targeting the Wnt Pathway and Transplantation Therapy as Strategies for Retinal Repair

Fumitaka Osakada[1,*] and Masayo Takahashi[1]

[1]*Laboratory for Retinal Regeneration, Center for Developmental Biology, RIKEN, 2-2-3 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan*

Received October 7, 2008; Accepted October 17, 2008

Abstract. Recent advances in stem cell biology have provided new insights that may lead to the development of regeneration therapy in the central nervous system to replenish lost neurons and to reconstitute neural circuits. The strategies for regeneration can be classified into two approaches: i) activation of endogenous neural stem cells and ii) transplantation of donor cells to replace lost cells. In the adult mammalian retina, Müller glia generate new retinal neurons in response to injury. The proliferation and differentiation of Müller glia–derived progenitors can be controlled by both intrinsic and extrinsic factors. Members of the Wnt/*β*-catenin signaling pathway, such as Wnt receptors and glycogen synthase kinase-3*β*, may be promising drug targets for neural regeneration. On the other hand, transplantation of photoreceptors or retinal pigment epithelia derived from human embryonic stem cells or induced pluripotent stem cells is also promising. Directed differentiation of pluripotent cells into retinal cells and purification to obtain retinal cells at a specific ontogenetic stage are required for donor cell preparation. Modulation of the host retinal environment to reduce the glial barrier is also critical for transplantation. To restore visual function, we need to understand the mechanisms underlying the integration of newly generated neurons or transplanted cells into the existing neural networks.

Keywords: neurogenesis, rod and cone photoreceptor, eye, cell replacement, induced pluripotent stem (iPS) cell, glycogen synthase kinase-3*β* (GSK-3*β*)

Introduction

For many decades, it was believed that neurons in the adult mammalian central nervous system (CNS) could not regenerate after injury, as postulated by Ramón y Cajal in 1913. However, recent evidence has overturned this long-held dogma. Neurogenesis does in fact take place in the adult mammalian CNS, with neural stem cells residing in at least two regions of the brain: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (1). Although newly generated neurons may be involved in processes such as learning, memory, and depression, their contribution to and significance for CNS pathophysiology remain poorly understood.

This finding opens up the possibility that the intrinsic ability of CNS cells to self-renew can be harnessed to develop treatments for injuries to the CNS or for diseases in which neurons are lost. Since the eye forms during embryonic development as an outpocketing of the neural tube, progress towards regenerating neurons in the CNS has important implications for retinal regeneration (2). The retina consists of three cell layers. The outer nuclear layer contains the cell bodies of rod and cone photoreceptors, while the inner nuclear layer (INL) contains the cell bodies of bipolar cells, horizontal cells, amacrine cells, and Müller glia. The ganglion cell layer contains the cell bodies of ganglion cells and displaced amacrine cells. Photoreceptor cells receive and transmit light signals to second order bipolar cells,

*Corresponding author. osakada@cdb.riken.jp
Published online in J-STAGE on January 29, 2009 (in advance)
doi: 10.1254/jphs.08R19FM

which relay the information to retinal ganglion cells. Retinal ganglion cells then send signals to lateral geniculate nuclei (LGN) neurons, which provide input to multiple areas of the visual cortex. Since damage to the retina, particularly the photoreceptors, causes irreversible visual impairment, the possibility that lost cells might be replenished and the neural circuitry reconstituted by neural regeneration has received much attention.

The strategies for regeneration can be classified into two approaches: i) activation of endogenous neural stem cells and ii) transplantation of donor cells to replace lost cells. As we learn more about the properties of endogenous neural stem cells, it may be possible to develop treatments in which stem cells in the retina are steered to differentiate into photoreceptors or other differentiated retinal cell types. On the other hand, transplantation of donor cells may be a fruitful approach. If embryonic stem (ES) cells can be differentiated into retinal neurons and transplanted into damaged or diseased retinas, it may be possible to recover some visual function. Moreover, transgenic expression of Oct3/4, Sox2, Klf4, and c-Myc in mouse (3) or human (4, 5) somatic cells has been shown to successfully reprogram cells so that they regain their pluripotential state. These induced pluripotent stem (iPS) cells may also be useful for transplantation therapies, particularly because they offer the potential of avoiding immune rejection of transplanted cells without ethical concerns. Here, we review recent findings regarding retinal repair in adult mammals.

Müller glia–derived regeneration in the adult retina

Neurogenic potential of Müller glia

Müller glia form processes that surround neuronal cell bodies in the nuclear layers and contact synapses in the plexiform layers. Their distal processes form the external limiting membrane of the retina, and their endfeet form the inner limiting membrane. Müller glia play important roles in regulating extracellular K^+ and pH and in the synthesis of the neurotransmitter glutamate.

The possibility that Müller glia might be an endogenous regenerative source was first raised by experiments in goldfish in which laser damage elicited the proliferation of Müller glia and the concomitant replacement of damaged cone photoreceptors (6). Müller glia in the avian retina have also been reported to possess the regenerative capacity (7). Moreover, several lines of evidence support a close relationship between Müller glia and retinal progenitors (8). In particular, recent gene expression profiling studies have demonstrated a large degree of overlap in genes expressed in both the Müller glia and late retinal progenitors.

We have demonstrated that in the adult rat retina, Müller glia act as neurogenic progenitors after retinal injury (9 – 11). Intravitreal injection of *N*-methyl-D-aspartate (NMDA), a glutamate-receptor agonist, causes neurotoxicity in amacrine cells and ganglion cells in the adult rat retina. After NMDA-induced retinal injury and subsequent labeling of dividing cells with BrdU, BrdU-labeled cells are mainly distributed in the INL. All the BrdU-labeled cells in the INL two days after injury are glutamine synthetase–positive Müller glia. These BrdU-labeled cells begin to express retinal progenitor markers such as Pax6 and Chx10. In addition, injury induces nestin expression in Müller glia. These observations indicate that Müller glia acquire progenitor-like properties after retinal injury. It should be noted that Müller glia are not neural stem cells under normal conditions; unlike neural stem cells in the SVZ of the lateral ventricle and the SGZ of the hippocampal dentate gyrus, de-differentiation and proliferation are required for Müller glia to become progenitors. Interestingly, we have observed heterogeneity of Müller glia (our unpublished observation). A subset of Müller glia might have the neurogenic potential, like astrocytes in the brain (11 – 13).

After proliferation in response to retinal damage, the BrdU-labeled cells migrate into the retinal neuron–specific layer and differentiate into cells positive for retinal neuron–specific markers such as rhodopsin (rod photoreceptors) and PKCα (rod bipolar cells). The Müller glia–derived progenitors appear to differentiate into the type of cell that was damaged, evidenced by the fact that Müller glia generate mainly photoreceptors in the photoreceptor-damaged retina. Moreover, experiments in which several types of neurotoxic injury were administered in the post-hatch chick retina also suggest that the type of neurons injured in the retina allows or promotes the regeneration of that neuronal type (14). Taken together, these results indicate that Müller glia proliferate and de-differentiate into retinal progenitors in response to retinal damage and then migrate and differentiate into retinal neurons.

The neural stem cell properties of Müller glia have been also verified in vitro (15). Dissociated Müller glia derived from injured retinas form neurospheres in vitro, as do neural stem/progenitor cells (16, 17). These neurospheres can differentiate into neurons and glia, demonstrating the multipotency of Müller glia. In addition, Müller glia–derived progenitors can be identified and purified as a distinct population by FACS analysis using Hoechst labeling, another characteristic of progenitor cells (15). After transplantation into the retina, these Müller glia–derived neurosphere cells can differentiate into retinal neurons (15).

Control of Müller glia–derived progenitors

In retinal regeneration, the differentiation of Müller glia–derived progenitors can be regulated by both intrinsic and extrinsic factors, similar to what has been observed with retinal progenitors during eye development (11). In the adult rat retina, retroviral gene transfer of *Crx* and *NeuroD* into Müller glia–derived progenitors induces their differentiation into rod photoreceptors (9). Misexpression of *NeuroD* and *Pax6* induces the differentiation of the progenitors into amacrine cells, and *Math3* and *Pax6* induce amacrine and horizontal cells after retinal injury. In addition, exogenous application of retinoic acid (10) and Sonic hedgehog (18) induce photoreceptor differentiation. Moreover, *NeuroD* expression is regulated by histone deacetylases, and the histone deacetylase inhibitor valproic acid induces *NeuroD* expression in neural stem cells. The application of valproic acid to the damaged retina promotes the differentiation of Müller glia–derived progenitors into photoreceptors (10).

In addition to cell fate determination, the proliferation of Müller glia–derived progenitors can be regulated by extrinsic factors. Wnt signaling, Notch signaling, and Sonic hedgehog signaling have all been shown to control proliferation (10, 15, 18). We have found that the Wnt signaling pathway is involved in retinal regeneration and that exogenous application of Wnt3a or Wnt2b increases the number of BrdU-labeled, proliferative cells in the injured retina, thereby increasing the number of newly generated photoreceptors (10). Interestingly, low molecular weight inhibitors of glycogen synthase kinase-3β (GSK-3β) mimic these effects and similarly promote the proliferation of Müller glia–derived progenitors (10). However, it should be noted that the degree of degeneration affects the effectiveness of this approach because retinas already exhibiting advanced retinal degeneration do not regenerate with this treatment.

In the canonical pathway, binding of Wnt ligand to its receptor Frizzled and co-receptor LRP5/6 inhibits the activity of the destruction complex that degrades β-catenin via an ubiquitin-proteasome, leading to the accumulation of β-catenin in the cytoplasm (Fig. 1). The accumulated β-catenin translocates into the nucleus, where it binds to lymphoid enhancer–binding factor /T-cell–specific transcription factor (LEF/TCF) transcription factors and then activates the transcription of target genes such as cyclin D1. Accumulating evidence points to a role for Wnt/β-catenin signaling in regulating various types of stem cells. In the adult hippocampus, Wnt3 derived from astrocytes promotes neuronal differentiation and enhances adult neurogenesis (19). Activation of β-catenin signaling by GSK-3β inhibitors increases the proliferation of neural stem cells in the SVZ, thereby promoting neurogenesis in the olfactory bulb (20). Thus, Wnt signaling may be a part of the natural regeneration mechanisms in the adult CNS.

Cell transplantation

ES cells are pluripotent cells derived from the inner cell mass of blastocyst stage embryos. They can maintain an undifferentiated state indefinitely in vitro and differentiate into derivatives of all three germ layers: the ectoderm, endoderm, and mesoderm. These characteristics make ES cells an attractive potential donor source for cell replacement therapies in tissues damaged by disease or injury. The treatment strategy of cell replacement is not new; dopaminergic neurons and retinal cells derived from fetal tissues have been transplanted into the adult striatum and eye, but because fetal tissues are limited in supply and ethically problematic, many efforts have been made to find alternative cell sources. Recent progress in the in vitro culture and differentiation of human ES cells has raised the possibility of using ES cell derivatives for cell transplantation therapy (2).

For photoreceptor transplantation, the stage and differentiation state of donor cells are important (21) (Fig. 2). P3-6 post-mitotic rod precursors are capable of integration when transplanted into the normal adult or degenerating retina, whereas proliferating progenitors or stem cells are not. The transplanted P3-6 photoreceptors are able to form functional synaptic connections with host bipolar cells and improve visual function. These results indicate that using cells already committed to photoreceptors rather than less committed retinal progenitors is crucial for successful integration. Thus, identification of appropriate surface antigens marking P3-6 photoreceptors should be crucial for future transplantation studies.

While somatic progenitors derived from the cilliary body (22) or iris (23) are limited in both differentiation potential and proliferation capacity, human ES cells can generate a large number of retinal cells. For this reason, retinal cells differentiated from ES cells are more suitable as donor cells for transplantation. In order to obtain transplantable retinal cells from ES cells, the developmental processes that regulate retinal differentiation must be at least in part recapitulated in vitro (2, 24, 25). We have been able to induce differentiation of ES cells in a stepwise fashion; ES cells are first differentiated into retinal progenitors (Rax+, Mitf+, Pax6+) and then to retinal pigment epithelia (RPE) and photoreceptors. We have also succeeded in generating photoreceptors and retinal pigment epithelia from human ES cells under defined conditions (25). It is important to

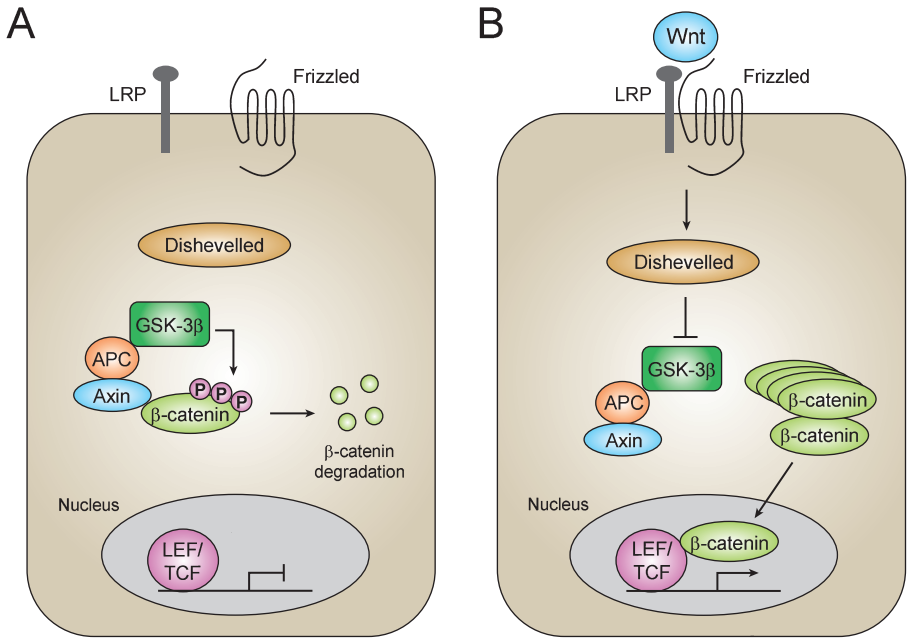

Fig. 1. Canonical Wnt signaling pathway. A: In the absence of Wnts, *β*-catenin forms a complex with Axin, APC, and GSK-3*β*; and it is degraded by the ubiquitin-proteasome system. B: When Wnts bind their receptors, such as Frizzled and LRP, GSK-3*β* is inactivated via Dishevelled. Cytoplasmic *β*-catenin is stabilized and the accumulated *β*-catenin is translocated into the nucleus, resulting in the activation of the transcription factor, LEF/TCF. LRP, low-density lipoprotein receptor–related protein; APC, adenomatous polyposis coli; GSK-3*β*, glycogen synthase kinase-3*β*; LEF/TCF, lymphoid enhancer–binding factor / T-cell factor.

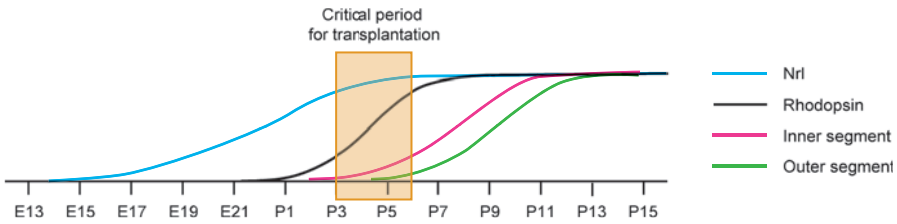

Fig. 2. Critical period for successful transplantation and photoreceptor development. During photoreceptor development, retinal progenitors begin to generate post-mitotic, Crx+ photoreceptor precursors at E12. Cones are born first, followed by rods. Expression of Nrl begins at E14, and rhodopsin expression begins at P0. The inner segment and outer segment begin to form at P2 and P5, respectively. The eye opens at P14, and mature rods are generated at P21. Rod photoreceptors at P3–6 stage are suitable as donor cells for integration in the host retina.

note that because xenogenic factors may cause rejection by the immune system following transplantation (26), the generation of differentiated cells from human ES cells without contamination from other animal-derived substances is essential for the clinical application of transplantation strategies.

The host environment is also critical for photoreceptor transplantation. The glial barrier in the host retina prevents integration of donor photoreceptors. However, robust integration of transplanted retinal cells into the retina of host mice deficient in both vimentin and glial fibrillary acidic protein has been reported (27). Moreover, chondroitinases and matrix metalloproteases-2 that degrade the extracellular matrix in the diseased retina aid in the integration of transplanted photoreceptors (28, 29). Disruption of the outer limiting membrane also increases photoreceptor integration following transplantation (30).

In addition to photoreceptors, the regeneration of RPE is important. RPE cells are essential for maintenance of retinal function, particularly for outer segment shedding, supplying nutrients to the retina, and maintaining the blood-retinal barrier. For example, in age-related macular degeneration, RPE cells are damaged, resulting in secondary loss of photoreceptors. We have demonstrated that transplantation of RPE cells derived from monkey ES cells can restore visual function in a retinal degeneration model, RCS rats (31). Human ES cell–derived RPE has also been shown to be effective for functional recovery in RCS rats (32).

In clinical trials, adverse effects after surgery, including tumors or vigorous immune reactions, must be avoided. To avoid tumor formation, we must establish methods to purify transplanted cells to exclude undifferentiated cells. In terms of the immune reaction, administration of immunosuppressant drugs is generally re-

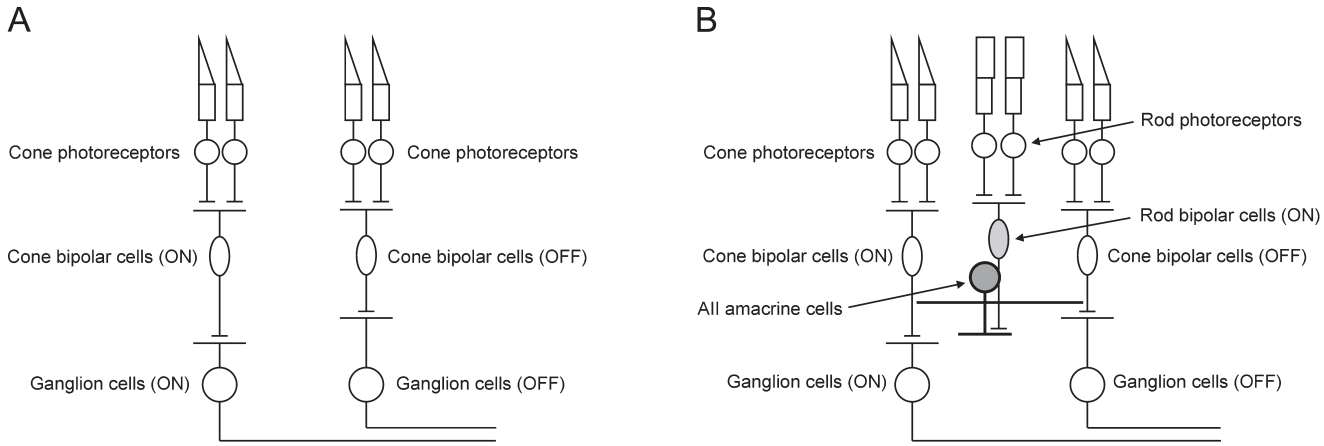

Fig. 3. Cone and rod pathway in visual transduction. A: Cone photoreceptors provide high acuity and color vision in daylight, while rod photoreceptors are specialized for high sensitivity at night. Phototransduction occurs when photons of light are absorbed by photopigments located in the outer segments of photoreceptors. Cone photoreceptors contact ON and OFF bipolar cells, which in turn contact ON and OFF ganglion cells, respectively. Ganglion cell axons fasciculate to form the optic nerve and carry visual information to higher visual centers. B: Rod photoreceptors send input to ON rod bipolar cells, which in turn synapse with AII amacrine cells. ON signals enter the cone pathways via gap junctions between AII amacrine cells and ON cone bipolar cells. OFF signals are produced by a glycinergic synapse between AII amacrine cells and OFF cone bipolar cells.

quired; however, iPS cells may circumvent this problem. Expressions of Oct3/4, Sox2, and Klf4 are able to reprogram patients' cells so that they may produce their own iPS cells (4, 5). Although it is unclear whether or not iPS cells are completely equivalent to ES cells, retinal cells differentiated from patient-specific iPS cells could enable non-immunogenic autograft.

Concluding remarks

Since Müller glia are a potential source of neural regeneration in the adult mammalian retina, developing drugs that target these cells is a promising approach that may lead to new retinal regeneration therapies. In particular, signaling component molecules of the Wnt/β-catenin pathway are potential drug targets. Low-molecular compounds that stimulate the Wnt/β-catenin signaling, such as Wnt receptor agonists and GSK-3β inhibitors, may have therapeutic potential (Fig. 1).

It should be noted that mammalian aging is associated with reduced regenerative capacity in tissues containing stem cells. Several lines of evidence indicate that this decrease in regeneration potential is partly due to the senescence of progenitors with age (33). It is also important to note that the progression of disease may restrict the potential degree of recovery. However, the generation of cloned animals (34) and iPS cells (3 – 5) indicate that cells can in fact be reprogrammed, if epigenetic regulation of the genome can be altered. Therefore, better control of the epigenetic state of progenitor cells may allow us to prevent their senescence and reduction in potential and to restore differentiation and proliferation potential.

Finally, despite the neurogenic properties of Müller glia, there is no direct evidence that Müller glia–derived neurons contribute to the functional regeneration of the retina in vivo. In retinal neural circuits, cone and rod photoreceptors have different pathways in visual transduction (35). Cone photoreceptors connect with ON or OFF cone bipolar cells, which make synaptic contacts with retinal ganglion cells (Fig. 3A). In contrast, rod photoreceptors connect to ON rod bipolar cells, which subsequently send the signal to AII amacrine cells. The AII amacrine cells send the information to ON or OFF cone bipolar cells, which make synapses to ON or OFF ganglion cells, respectively (Fig. 3B). Horizontal cells provide negative feedback to photoreceptors. For retinal regeneration therapy to become a reality, we need to elucidate the mechanisms underlying the integration of newly generated neurons or transplanted cells into the existing neural networks and functional recovery in animal models closely resembling human diseases.

Acknowledgments

We thank the members of the Takahashi laboratory (RIKEN), Sasai laboratory (RIKEN), and Akaike laboratory (Kyoto University) for stimulating discussions. This study was supported by Grants-in-Aid from MEXT and the Leading Project (to M.T.) and by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to F.O.).

References

1 Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell. 2008;132:645–660.
2 Osakada F, Sasai Y, Takahashi M. Control of neural differentiation from pluripotent stem cells. Inflamm Regen. 2008;28:166–173.
3 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–676.
4 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–872.
5 Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318:1917–1920.
6 Braisted JE, Essman TF, Raymond PA. Selective regeneration of photoreceptors in goldfish retina. Development. 1994;120:2409–2419.
7 Fischer AJ, Reh TA. Muller glia are a potential source of neural regeneration in the postnatal chicken retina. Nat Neurosci. 2001;4:247–252.
8 Blackshaw S, Harpavat S, Trimarchi J, Cai L, Huang H, Kuo WP, et al. Genomic analysis of mouse retinal development. PLoS Biol. 2004;2:E247.
9 Ooto S, Akagi T, Kageyama R, Akita J, Mandai M, Honda Y, et al. Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. Proc Natl Acad Sci U S A. 2004;101:13654–13659.
10 Osakada F, Ooto S, Akagi T, Mandai M, Akaike A, Takahashi M. Wnt signaling promotes regeneration in the retina of adult mammals. J Neurosci. 2007;27:4210–4219.
11 Osakada F, Takahashi M. Neurogenic potential of Müller glia in the adult mammalian retina. Inflamm Regen. 2007;27:499–505.
12 Bachoo RM, Kim RS, Ligon KL, Maher EA, Brennan C, Billings N, et al. Molecular diversity of astrocytes with implications for neurological disorders. Proc Natl Acad Sci U S A. 2004;101:8384–8389.
13 Alvarez-Buylla A, Lim DA. For the long run: maintaining germinal niches in the adult brain. Neuron. 2004;41:683–686.
14 Fischer AJ, Reh TA. Exogenous growth factors stimulate the regeneration of ganglion cells in the chicken retina. Dev Biol. 2002;251:367–379.
15 Das AV, Mallya KB, Zhao X, Ahmad F, Bhattacharya S, Thoreson WB, et al. Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. Dev Biol. 2006;299:283–302.
16 Reynolds BA, Tetzlaff W, Weiss S. A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. J Neurosci. 1992;12:4565–4574.
17 Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science. 1992;255:1707–1710.
18 Wan J, Zheng H, Xiao HL, She ZJ, Zhou GM. Sonic hedgehog promotes stem-cell potential of Muller glia in the mammalian retina. Biochem Biophys Res Commun. 2007;363:347–354.
19 Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, et al. Wnt signalling regulates adult hippocampal neurogenesis. Nature. 2005;437:1370–1375.
20 Adachi K, Mirzadeh Z, Sakaguchi M, Yamashita T, Nikolcheva T, Gotoh Y, et al. Beta-catenin signaling promotes proliferation of progenitor cells in the adult mouse subventricular zone. Stem Cells. 2007;25:2827–2836.
21 MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt TE, Akimoto M, et al. Retinal repair by transplantation of photoreceptor precursors. Nature. 2006;444:203–207.
22 Tropepe V, Coles BL, Chiasson BJ, Horsford DJ, Elia AJ, McInnes RR, et al. Retinal stem cells in the adult mammalian eye. Science. 2000;287:2032–2036.
23 Haruta M, Kosaka M, Kanegae Y, Saito I, Inoue T, Kageyama R, et al. Induction of photoreceptor-specific phenotypes in adult mammalian iris tissue. Nat Neurosci. 2001;4:1163–1164.
24 Ikeda H, Osakada F, Watanabe K, Mizuseki K, Haraguchi T, Miyoshi H, et al. Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. Proc Natl Acad Sci U S A. 2005;102:11331–11336.
25 Osakada F, Ikeda H, Mandai M, Wataya T, Watanabe K, Yoshimura N, et al. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. Nat Biotechnol. 2008;26:215–224.
26 Martin MJ, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat Med. 2005;11:228–232.
27 Kinouchi R, Takeda M, Yang L, Wilhelmsson U, Lundkvist A, Pekny M, et al. Robust neural integration from retinal transplants in mice deficient in GFAP and vimentin. Nat Neurosci. 2003;6:863–868.
28 Suzuki T, Mandai M, Akimoto M, Yoshimura N, Takahashi M. The simultaneous treatment of MMP-2 stimulants in retinal transplantation enhances grafted cell migration into the host retina. Stem Cells. 2006;24:2406–2411.
29 Suzuki T, Akimoto M, Imai H, Ueda Y, Mandai M, Yoshimura N, et al. Chondroitinase ABC treatment enhances synaptogenesis between transplant and host neurons in model of retinal degeneration. Cell Transplant. 2007;16:493–503.
30 West EL, Pearson RA, Tschernutter M, Sowden JC, Maclaren RE, Ali RR. Pharmacological disruption of the outer limiting membrane leads to increased retinal integration of transplanted photoreceptor precursors. Exp Eye Res. 2008;86:601–611.
31 Haruta M, Sasai Y, Kawasaki H, Amemiya K, Ooto S, Kitada M, et al. In vitro and in vivo characterization of pigment epithelial cells differentiated from primate embryonic stem cells. Invest Ophthalmol Vis Sci. 2004;45:1020–1025.
32 Lund RD, Wang S, Klimanskaya I, Holmes T, Ramos-Kelsey R, Lu B, et al. Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats. Cloning Stem Cells. 2006;8:189–199.
33 Molofsky AV, Slutsky SG, Joseph NM, He S, Pardal R, Krishnamurthy J, et al. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. Nature. 2006;443:448–452.
34 Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature. 1997;385:810–813.
35 Field GD, Sampath AP, Rieke F. Retinal processing near absolute threshold: from behavior to mechanism. Annu Rev Physiol. 2005;67:491–514.