·Review·

# The role of Toll-like receptors in retinal ischemic diseases

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## Abstract

**Toll-like receptors (TLRs) are commonly referred to a series of evolutionary conserved receptors which recognize and respond to various microbes and endogenous ligands. Growing evidence has demonstrated that the expression of TLRs in the retina is regulated during retinal ischemic diseases, including ischemia-reperfusion injury, glaucoma, diabetic retinopathy (DR) and retinopathy of prematurity (ROP). TLRs can be expressed in multiple cells in the retina, such as glial cells, retinal pigment epithelium (RPE), as well as photoreceptor cells and endothelium cells. Activation of TLRs in retina could initiate a complex signal transduction cascade, induce the production of inflammatory cytokines and regulate the level of co-stimulatory molecules, which play prominent roles in the pathogenesis of retinal ischemic diseases. In this review, we summarized current studies about the relationship between TLRs and ischemic retinopathy. A greater understanding of the effect of TLRs on ischemic injuries may contribute to the development of specific TLR targeted therapeutic strategies in these conditions.**

**KEYWORDS:** Toll-like receptors; retinopathy; retinal ischemic diseases; retinal regeneration

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## INTRODUCTION

Ischemic retinopathy presents a major common cause of vision loss and blindness in the industrialized world[1]. As retina being the most metabolically active tissue in the body, when retinal blood flow is insufficient to match the metabolic demands, a pathological process, incorporated with oxidative stress initiated by energy failure, activation of glial cells and retinal pigment epithelium (RPE) and the death of endothelium, photoreceptor cells, may be presented and in the late stage, pathological neovascularization occurs [2]. Extensive researches have confirmed the involvement of Toll-like receptors (TLRs) in retinal ischemic diseases. In the processes mentioned above, TLRs cause immunological damage to retinal cells by regulating a mass of cytokines and co-stimulatory molecules as well as inducing oxidative damage to DNA. Furthermore, TLRs also stimulate the secretion of related angiogenic growth factors, inducing the procedure of angiogenesis in retina[3-4]. Here we will discuss the role of TLRs in ischemic diseases and their underlying therapeutic effect.

**Toll-like Receptors** In 1980, Nüsslein-Volhard and Wieschaus [5] found a mutate gene which led to change of drosophila embryo development, they called it Toll gene. Subsequently, Toll was known as receptors recognizing microbe-specific molecular signatures such as pathogen-associated molecular patterns (PAMPs) and self-derived molecules derived from damaged cells and referred as damage associated molecules patterns (DAMPs) [6]. TLR family comprises 10 members (TLR1-TLR10) in human and 12 members (TLR1-TLR9, TLR11-TLR13) in mouse.

Cell surface TLRs include TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10, mainly recognizing microbial membrane components such as lipids, lipoproteins and proteins. While intracellular TLRs are located in the endosome and include TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13, mainly recognizing components of virus [7]. With the exception of TLR3, all TLRs initiate a myeloid differentiation factor 88 (MyD88) dependent signaling pathway. Upon TLR activation, MyD88 is recruited to the TIR domain of the activated TLR, leading to activation of NF-κB pathway and the mitogen-activated protein kinase (MAPK) pathway [8]. MyD88 independent TLR3 signaling requires the adaptor molecule TIR-domain-containing adapter-inducing interferon-β (TRIF) to activate downstream signaling pathways, including the activation of interferon regulatory factor 3 (IRF3) and the production of type 1 interferon [9]. TLR4 could also activate type 1 interferon response *via* TRIF signaling[10].

Activation of TLRs by pathogens would be expected to result in production of cytokines and chemokines which are important for stimulating the innate immune response and inflammatory cells infiltration into the lesion to alleviate the

microbial load and resolve infections. Moreover, TLRs activation may also help activating the acquired immune response *via* enhancing co-stimulatory molecules expression on antigen presenting cells. These two aspects of the immune response can help protecting the organism from microbial infections. In addition to pathogens, ligands derived from necrotic cells or extracellular matrix components are also important for TLRs activation [11]. As the endogenous ligands are mainly generated as consequences of tissue injury, TLRs could also take part in tissue repair and maintain tissue homeostasis as well as aggravate tissue damage.

**Expression and Function of Toll-like Receptors in Retina** In vertebrate embryonic development, the retina and the optic nerve are both originated as outgrowths of the developing brain, so the retina could be regarded as a part of the central nervous system (CNS) and is actually the brain tissue. Retina is a subtle tissue constituted by diverse cells and divided into ten distinct layers in the cross section [12]. In the next part we will discuss the expression of TLRs in different retinal cells.

**Toll-like receptors in retinal pigment epithelium** In addition to constituting an epithelial outer barrier towards the blood supply, RPE also constitutes the outer blood retinal barrier. RPE has a wide variety of functions, such as nutrient supply, oxidative stress protection, secretion of vascular endothelial growth factor (VEGF) and phagocytosis[13].

Kumar *et al* [14] first reported the presence of mRNA of TLR1 through 10, except TLR8 on human RPE cells in 2004, which was confirmed by Hooks *et al* [15]. Activation of TLRs in RPE has diverse effects from anti-pathogen to interaction with other cells. For instance, polyinosinic-polycytidylic acid [Poly (I:C)], the agonist of TLR3 could induce RPE cells to secrete monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8) and intercellular adhesion molecule-I (ICAM-I), which were critical mediators of viral clearance by RPE cells after infection; And CpG-DNA, an agonist of TLR9 greatly enhanced the ability of phagocytosis of RPE cells [16-17]. Meanwhile, RPE could alter the proinflammatory response of retinal microglia cells to TLR3 stimulation *via* some soluble factors, such as cytokines IL-6, IL-1β, tumor necrosis factor-α (TNF-α), and the effect enzymes cyclooxygenase-2 (Cox2) and inducible nitric oxide synthase (iNOS). RPE are in close contact with the blood and constitute a border, they contact with blood-borne danger signals easily, while microglia cells lie deep in the immune-privileged zone of the retina and are less likely to be the first to interact with blood-borne danger signals, there may have a crosstalk between RPE cells and microglia, and other cells[18].

**Toll-like receptors in glial cells** There are three types of glial cells found in the retina: Müller, astrocytes and microglia cells. Müller cells are the most abundant glial cell type in the retina. They span the entire thickness of the retina and have secondary processes that closely wrap around neuronal cell bodies and dendrites, forming the retinal margins at the inner limiting membrane (ILM) and external limiting membrane (ELM)[19].

The first report describing the expression and function of all known human TLRs in retinal Müller cells came in 2012 when Kumar and Shamsuddin [20] showed that the gene expression and protein levels of TLR1-10, and TLR4 co-receptors myeloid differemiation-2 (MD2) and cluster of differentiation-14 (CD14) were detected in the mouse retinal sections, human retinal Müller cell line and primary mouse retinal Müller cells. Among them, TLR2, TLR3, TLR4 and TLR5 were highly expressed in Müller cells [21]. Müller cells could contribute directly to retinal innate defense by directly recognizing microbial patterns under infectious conditions. TLR2 is a key sensor implicated in recognizing Gram-positive bacteria, the activation of TLR2 by Pam3Cys and S.aureus in Müller cells induced the expression of inflammatory cytokines and chemokines as well as antimicrobial peptide. Moreover, the conditioned media derived from Pam3Cys or S.aureus-treated Müller cells dramaticlly inhibited the growth of two S.aureus strains, which exhibited the strongest anti-staphylococcal activity of Müller cells. The finding suggested that Müller cells were actively participant in retinal defense against bacterial pathogens *via* the action of TLRs[22].

Microglia cells are inherent immunological cells in the retina, they stay in inner part of the retina normally and act as surveillance guard. When confronted with infection/ inflammation, they could proliferate and immigrate to outer part of the retina, recognize and phagocytose pathogens, induce inflammatory response [23]. The location of TLR4 on the retinal microglia cells was first detected by use of immunohistochemically method in 2011 [24]. A year later, TLR1, TLR2 and TLR6 mRNA were also found on retinal microglia cells [25]. Although studies have revealed that brain microglia cells could express all TLRs, so far, no other types of TLRs have been detected on retinal microglia cells. Microglia cells play a prominent role in immune surveillance in retina and recognize pathogen *via* their cell receptors, especially TLRs. The inflammation mediate by TLRs is a double edged sword which must be precisely regulated. For instance, in primary retinal microglia cells, most agonists of TLR2, like S.aureus, and its cell wall components, peptidoglycan (PGN) and lipoteichoic acid (LTA) mediated the secretion of abundant proinflammatory cytokines and chemokines, but excessive inflammation cause tissue damage and maybe lethal to the host [26]. However, when pretreated with a low dose (0.1 or 1 μg/mL) of Pam3Cys to retinal microglia cells, it dramatically reduced proinflammatory mediators, such as TNF-α and macrophage inflammatory

protein-2 (MIP-2), but strengthened phagocytic ability significantly [25]. *In vivo* study, intravitreal injection of Pam3Cys prior to S.aureus inoculation significantly decreased inflammatory infiltrates as well, moreover this method preserved retinal structural integrity and retinal function, reduced the bacterial load in the retina and induced the activation of retinal microglia cells [27]. Another study showed that TLR9 ligand CpG-ODN applied to the injured mouse cornea could elicit microglia cells activation and retinal inflammation mainly by the transduction of macrophages which were adjacent to retina [28]. It has been demonstrated that although microglia cells were situated in the immune privileged zone of the inner retina, they could have a response to infection indirectly *via* the induction of other immune cells.

In retinal astrocyte cultures *in vitro*, the mRNA and protein of TLR2, TLR3 and TLR4 have been found [29]. With the addition of the agonist of TLRs, retinal astrocytes produced different kinds of inflammatory factors as well. As a residential glial cell in the retina, astrocytes had the ability to express major histocompatibility complex II (MHCII) molecules, an essential molecular in antigen-presentation and adaptive immune system [30]. Activating astrocytes *via* TLR3 by Poly(I:C) had an intense effect on promoting the potential of astrocytes in stimulating T cells by up-regulating MHCII expression and regulating co-stimulatory molecules level, which enhanced adaptive immune response[29,31].

**Toll-like receptors in photoreceptor cells** The expression of TLR4 was also found in primary photoreceptor cells, as well as the photoreceptor cell line 661W. Photoreceptor cells could also produce IL-6 and chemokine (C-X-C motif) ligand-1 (CXCL1) after endotoxin (LPS) stimulation, suggesting that they could be another local source of inflammatory cytokines/chemokines during retinal infections [30]. Another study found that TLR4 mediated mitochondrial oxidative stress and the resultant mitochondrial DNA (mtDNA) damage due to the generation of TNF-α and iNOS were primarily localized to photoreceptor cells, leading to visual impairment[24].

**Toll-like receptors in retinal vascular endothelial cells** The high level of the TLR3 gene was expressed constitutively in human retinal vascular endothelial cells, while TLR2, TLR4, TLR7, TLR8 and TLR9 were low [32]. Recently, TLR6 and TLR10 mRNA were found to be expressed in human retinal endothelial cells besides TLR2 and TLR9[33].

**Toll-like Receptors and Ischemic Retinal Diseases** Ischemic diseases represent the leading causes of vision loss, including retinal vascular occlusion, acute glaucoma, as well as diabetic retinopathy (DR) and retinopathy of prematurity (ROP). Ischemia result in retinal circulatory disorders and cellular hypoxia, produce inflammatory cytokines and is associated with vascular growth factors, such as VEGF, insulin like growth factor-1 (IGF-1), platelet derived growth factor (PDGF), *etc.* which induce angiogenesis. Recent studies have revealed that TLRs were correlated with ischemic diseases of the retina. In the next part of this review, we will discuss the implications of TLRs in ischemic retinal diseases.

**Retinal ischemia/reperfusion injury** A number of studies have demonstrated the role of TLRs in ischemia/reperfusion (I/R) injury, including lung, renal, intestinal, hepatic, myocardial, spinal cord and so on [34]. However, there has no relevant literature summarized TLRs in retinal I/R injury.

There are many different retinal ischemic mouse models. The most frequently used model was to elevate the intraocular pressure to 120 mm Hg for 60min[35], while some other researchers used a new retinal ischemic mouse model in which the pterygopalatine artery (PPA) were ligated and ischemia was maintained for 3h, 5h, or 5d, then ligatures were removed[36]. Another experiment showed that retinal I/R can also be induced by clipping the retinal vessels for 30min[37]. These methods all could cause retinal inflammatory reaction and neuronal cell damage as well as a decrease in the thickness of the inner plexiform layer (IPL) and inner nuclear layer (INL) with a high number of necrotic cells at an early stage of pathology. Recently, results of some studies indicated that TLR4 signaling was involved in the pathogenesis of retinal I/R.

In 2010, Dvoriantchikova *et al* [38] found that TLR4 was involved in the retinal damage triggered by ischemic injury. They showed that TLR4 deficiency mice significantly promoted survival of retinal neurons in the ganglion cell layer, reduced inflammatory response, including lower secretion of inflammatory factors, chemokines and adhesion molecules as well inhibiting oxidative stress. At the early stage of I/R, a lot of factors could liberate from necrotic cells necrotic cells, and some of them were endogenous ligand of TLR4. First, heat shock proteins 70 (Hsp70), which increased in the vitreous humor of ischemic eyes after reperfusion and could promoted neuronal death by mediating production of cytotoxic levels of TNF-α [39]. *In vitro*, astrocytes and microglia cells treated with Hsp70 would cause proinflammatory response, either [29]. Another endogenous ligand, high mobility group protein -1 (HMGB1) could accumulate in the vitreous humor 24h after I/R, and cause a loss of retinal ganglion cells (RGCs) *in vivo* and *in vitro*[40]. However, there existed some controversies about the effect of HMGB1 in I/R injury. One study showed that in the experiment of retinal I/R, intraperitoneal injection of anti-HMGB1 monoclonal antibody played a substantial deleterious role in keeping retinal function by increasing the production of reactive oxygen species (ROS)[41]. However, in the rat model of brain I/R, in vein or intracerebroventricular

injection of anti-HMGB1 monoclonal antibody could reduce the infarct size as well as protect morphology and function[42]. This difference may caused by the fact that the effect of anti-HMGB-1 monoclonal antibody depend on the organ, for instance, anti-HMGB1 monoclonal antibody increased I/R injury in the rat heart, either [43]. It was not sure whether the method and dosage of administration could affect the result.

The existence of TLR4 downstream TRIF-dependent and MyD88-dependent signaling pathway in I/R injury were pivotal, which contributed significantly to retinal damage after I/R, especially the RGCs death and could active astrocytes and microglia cells in quantity, size and morphology as well as mediating a cytotoxic pro-inflammatory response. Moreover, a report showed MyD88 signaling mainly initiated inflammatory response in early stage and TRIF signaling had an effect on late phase[24,44]. The TLR4-nuclear factor-κ-gene binding (NF-κB) signaling may trigger inflammation and damage in I/R injury. First, the immunoreactivity of NF-κB was elevated following retinal I/R and activation of NF-κB was attenuated in the TLR4 knockout animals [35,45]. Second, NF-κB was involved in oxidative stress by regulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and inflammatory events associated with retinal ischemia [46]. Third, inhibition of astrocytes NF-κB reduced severity of I/R damage to the retina and the expression of pro-inflammatory genes following retinal I/R [47]. Studies above-mentioned showed that TLR4 and downstream molecules modulated retinal I/R injury, including inflammation reaction and RGCs death, besides, astrocytes and microglia cells played important role in terms of mechanism.

**Glaucoma** TLR2, TLR3 and TLR4 were upregulated in the glaucomatous human retina, and the expression of TLR2 and TLR4 was prominently detectable in microglia cells, while the expression of TLR3 was predominant in astrocytes[29]. *In vitro* studies of rat retinal microglia cells and astrocytes, it had been shown that components of the glaucomatous tissue stress, including up-regulated heat HSPs and oxidative stress [48-49] could similarly stimulate innate and adaptive immune responses through the glial TLR signaling as evident by increasing inflammatory and immunostimulatory cytokines production [50]. Results from human glaucoma and animal models, along with the findings of *in vitro* treatment experiments, supported that the glial TLR signaling initiated by glaucomatous stress related ligands include MyD88 dependent pathways and activated NF-κB[29,51].

**Diabetic retinopathy** DR is a kind of microvascular complications of diabetic which begins with biochemical and cellular alterations that are not clinically evident. During this period, attachment of leukocyte to the vessel wall and death of retinal pericytes can be seen. Then, the death of vascular endothelial cells lead to capillary closure and non-perfused vessels, causes focal hypoxia which lead to increased tissue production of angiogenic factors. Moreover, loosening of the endothelial cell-cell junctions result in subtle increase in vascular permeability, causing the release of growth factor for pathological neovascularization. And when the neovascular growth at the vitreo-retinal interface, it could be defined as proliferative retinopathy [52]. There is an increasingly accepted concept that tissue inflammation plays a significant role in the pathogenesis of DR, TLRs take part in it. First, activating TLRs could stimulate the expression of a spectrum proinflammatory cytokines, like TNF-α and IL-6, many of which had been implicated in insulin resistance[53-54]. And insulin resistance was considered to be one of the factors contribute to the development of microvascular complication of diabetes [54]. Second, the release of inflammatory cytokines and co-stimulatory molecules by activating TLRs in immune cells could bring oxidative stress damage and inflammatory injury to pericytes and endothelium cells[55-56], which causing the initial lesion in DR. Additionally, TLRs also promoted the secretion of angiogenic growth factors and led to neovascularization[57-58].

Numerous studies have shown that HMGB1, one ligand of TLR4 is involved in the progress of DR. In the clinical study, data showed the levels of HMGB1 in proliferative diabetic retinopathy (PDR) with active neovascularization were two fold and three fold higher than that in inactive PDR and nondiabetic patients, respectively. Mean HMGB1 level in PDR patients with hemorrhage were significantly higher than those in PDR patients without hemorrhage and nondiabetic patients. In the retinas of diabetic mice, similar results had been reported. This phenomenon brought a hint that HMGB1 might contribute to the progression of PDR [59]. *In vitro*, HMGB1 could induce cytotoxic activity of glial cells in a TLR4 dependent manner, which contributed to pericytes and endothelial death *via* cytokine or chemokines activity and reactive oxygen species production [60]. Increased protein expression of cytosolic HMGB1 had also been found in ARPE-19 cells treated with high glucose, and it regulated NF-κB activity and VEGF production [61]. In type 2 diabetic rat retina, HMGB1 and its receptors, incorporating receptor for advanced glycation end products (RAGE), TLR2, TLR4 were significantly upregulated as well. HMGB1 was mainly expressed in the ganglion cell layer (GCL), the INL and the RPE layer, while TLR4 was expressed mainly in the RPE layer [61]. It can be inferred that in the RPE cells of DR, HMGB1 may regulate VEGF production *via* TLR4. Not only HMGB-1, LPS, the exogenous ligand of TLR4 also activated the innate inflammatory of the retina of a transgenic mice model of type 1 diabetic, as well as causing the injury of capillary endothelium and *in vivo* thinning of the retina[62]. The above studies proved that TLR4 exogenous and endogenous ligand caused or sharpened the pathology in

the retina. There had another study found an interesting phenomenon, they grafted TLR4 wild type mice bone marrow-derived cells (BMC) into TLR4 mutant mice, verifying TLR4 positive BMCs contributed to the angiogenesis of small vessels in morphological and gene level in DR and increased the levels of TNF-α, IL-1β, and MIP-2 as well[63].

Besides TLR4, TLR2/MyD88 pathway could also be stimulated by Pam3Cys only in diabetic mice, but not in normal retinas, and deficiency of TLR2 in diabetes mice protected retinal vascular endothelial from death [64]. But to date no causal link has been reported whether the TLRs mediated angiogenesis in DR except some conjectures, this may partly due to the deficiency of an ideal animal model.

For the past few years, two polymorphisms in TLR4 gene have been widely studied, they are an aspartic acid for a glycine at a position 299 (Asp299Gly, rs4986790) and a threonine for an isoleucine at position 399 (Thr399Ile, rs4986791), which act as a role of amino acid exchanges and resulting in a change of the ligand-binding site of the TLR4, so the response of TLR may be attenuated after stimulus. Asp299Gly had been found to be present in co-segregation with Thr399Ile in different populations mainly because of evolutionary pressure during population migration events and selection due to infectious diseases [65]. Asp299Gly carriage was associated with a lower prevalence of diabetes mellitus (DM), besides, Thr399Ile polymorphism associated with the protection of type 2 DM [66]. In 2004, one research reported the function and expression of TLR4 in diabetic complications. They found there existed a strong association of the Asp299Gly/Thr399Ile genotypes of TLR4 with reduced prevalence of peripheral neuropathy in type 2 diabetic patients [67]. After 4y, in 2008, researchers investigated the potential association of the TLR4 Asp299Gly polymorphism with retinopathy in type 2 diabetes in Polish population. Their results showed that Asp299Gly carriage (GG and AG genotypes) was associated with a higher prevalence of retinopathy and the carriership of the G allele of this polymorphism was strongly associated with an early onset DR [68]. Another study also found that Asp299Gly genotype exclusively associated with DR in an Indian population. Moreover, the heterozygous genotype AG of TLR4_1859 and the combined risk genotype (AG+GG) as well as TLR4_2437 heterozygous genotype TC and combined risk genotype (TC+CC) to be significantly associated with the development of DR in an Indian population[65].

In conclusion, these results further back up the association of TLR4 with DR. However, much additional investigation should be conducted in this field, as the interactions between TLR4 and DR must be confirmed in different populations and across different ethnic groups. Moreover, the associations between genetic polymorphisms of other TLRs and DR remain poorly understood, and this may represent a significant field of research that can be explored to further define the etiology and predict the occurrence of DR.

**Retinopathy of prematurity** ROP is a devastating disease in premature infants and a major cause of childhood vision impairment. ROP is a complicated condition of the developing retina, in which various factors participate at different stages of the disease leading to neurovascular degeneration followed by neovascularization and photoreceptor cells injury[69].

The influence of inflammation in ROP had also came to people's sight in recent years. In clinical study, Dammann *et al* [70] reported clinical chorioamnionitis (CAM) and neonatal systemic inflammatory response syndrome (SIRS) were associated with ROP occurrence. Other studies incorporated neonatal bacteremia [71], systemic fungal infection as the possible influencing factors as well[72]. In lab, studies found that giving the mouse pups intraperitoneal injection of LPS could impair retinal vessel development, the characteristic of the lesion were similar to ROP, VEGF expression was elevated in the retina as well [73-74]. In the pathological process, there localized a large number of microglia cells and astrocytes in the lesion of abnormal vessels. And the increase in number of microglia cells was accompanied by a rise of TLR2 and TLR4. These activated glial cells are intimately associated with retinal vessels by disorganizing vascular growth.

A widely used animal model of ROP is the oxygen induced retinopathy (OIR) model, whereas the mouse model utilizes exposure to the constant hyperoxia for 5d before returning to the room air, the rat OIR model uses alternating hyperoxia-hypoxia cycles [75]. In OIR mice model, at P17, TLR3 and TLR4 were highly expressed [76-77]. Couples of studies found that glial cells have been activated as well, which may be the source of VEGF [78]. Intravitreous injection of HMGB1 to OIR mouse caused higher TLR4 expression, activating Müller cells and astrocytes, promoting the expression of VEGF, b-FGF, and IL-6[77]. These effects were alleviated in $TLR4^{-/-}$ OIR mice. Pulmonary surfactant protein A (SP-A), could be up-regulated in retina and Müller cells by activation of TLR2 and TLR4, and absence of SP-A attenuated retinal neovascularization in the OIR model [79]. These phenomena suggest that activation of TLR2, TLR3 and TLR4 in different glial cells contribute to pathology of OIR, partly by regulating VEGF expression and other angiogenetic factors. Müller glia were a major source of retinal VEGF and its expression level was reported to be increased during the hypoxic phase of OIR, by subretinal injection of lentivector-driven Short hairpin RNA to Müller cell $VEGF_{164}$ maintained long-term inhibition of intravitreal neovascularization and limited retinal cell death in the model

of OIR [80]. It was unknown how VEGF affect neovascularization in ROP. However, there only had definite conclusion said TLR3 is a key factor in CNV, for VEGF siRNA had an effect in inhibiting the expression of VEGF in endothelial cells *via* TLR3[81].

**Toll-like Receptors in Retinal Regeneration** Apart from the vascular pathology, neuronal dysfunction have also been proved in ischemic retinopathy, including dysfunction of photoreceptors and death of retinal ganglion cells [82-83]. Retinal regeneration may be an effective way in rescuing visual acuity. However, regeneration of the retina is still a difficult problem on the account of it is part of central nervous system. Until now, induced pluripotent stem cells (iPSC), retinal progenitor cells (RPCs) and Müller cells have been shown their potent role in retinal regeneration. Recently, a structured outer-segment disc formation and photo sensitivity *in vitro* had been successfully generated by the inducing of human iPSC (hiPSC), which brings one step closer to the anticipated use of hiPSC for disease modeling and open possibilities for future therapies[84].

A series of studies showed TLRs played an important role in the proliferation of progenitor cells. For instance, TLR4 and the signaling downstream, MyD88 or TRIF inhibited neural progenitor cell proliferation in the adult hippocampus, while TLR2 increased neural progenitor cells differentiation *in vitro*[85-86]. In addition, the negative role of TLR3 in regulation of neural progenitor cells (NPCs) had been certified, as in wild type NPCs, TLR3 ligand poly (I:C) could reduce proliferating cells and neurosphere formation of cultured embryonic cortical neurospheres, while NPCs from TLR3 deficient embryos formed greater numbers of neurospheres[87]. These results illustrated that TLRs played divergent roles, for TLR2 had a beneficial effect while TLR3 and TLR4 were detrimental, which allowed for regulating progenitor cells from different molecular levels. Müller cells were another potential source of neural regeneration, either. Recently, Gomez-Vicente *et al* [88] obtained a new murine retinal cell line (MU-PH1), which may have progenitor characteristics and potential interest in regeneration processes, the activation of TLR2 increased neurosphere formation in this cell line.

In addition to above, Hauk *et al* [89] also found repeated intravitreal Pam3Cys application could delay axotomized RGC cell death and stimulate the regeneration of axons *in vivo* following optic nerve injury via glial cells in retina. And TRIF$^{-/-}$ mice exhibited increased retinal axon regeneration and less RGC loss, as well as attenuating microglia cells activation and limiting the release of inflammatory cytokines following optic nerve injury[90].

All of the data mentioned above suggest that TLRs system exert complicated effects on retinal neurodegenerative and regeneration, on one hand, TLRs activation trigger the release of inflammatory reaction which lead to neuronal dysfunction or death. On the other hand, they also take part in the process of retinal and axon regeneration. TLR based treatments might become a promising research area, but further investigation should be performed to clarify the roles of TLRs in this aspect.

## DISCUSSION

As discussed here current evidence indicate that TLRs are expressed in retina to mediate inflammatory responses and that activation of TLRs are related to the retinal ischemic diseases. TLRs also take part in the regeneration of neuronal cells, the death of which is the mainly terminal pathology in ischemic retinal disease. In chronic hypoxic-ischemic retinal diseases, like DR and ROP, TLRs participate in new blood vessel growth, which may associate with the result of regulating pro-angiogenetic molecular level and activity. Furthermore, providing a better understanding of how TLRs regulate the processes of retinal hypoxic and ischemic diseases may be of great significance in identifying new markers and targets for the prediction and treatment of these diseases.

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