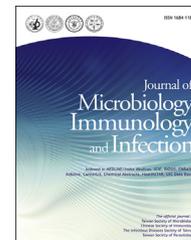




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Review Article

Roles of Toll-Like Receptor 4 for Cellular Pathogenesis in Primary Open-Angle Glaucoma: A potential therapeutic strategy



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Innate immunity

Abstract In recent years, glaucoma has been proposed as an autoimmune disease and an understanding immune-regulation concept has been applied for novel glaucoma therapy. Current evidence suggests an innate immunity is a keystone step for primary open angle glaucoma (POAG) pathogenesis resulting from trabecular meshwork (TM) cell fibrosis and retinal ganglion cell (RGC) death. Toll-like receptor 4 (TLR4) is a common player in the innate immunity, which appears on the TM and RGC of POAG. The activation of TLR4 regulates several molecules involving both fibrosis and cell death. Inhibition of TLR4 decreases TGF- β 2-induced fibrosis in TM cells and enhances cell survival of RGC in both optic nerve crush and ischemia models. In this review, we will summarize the molecular mechanisms of TLR4 related to POAG pathogenesis. An understanding of this mechanism may provide novel development of therapeutic strategies for POAG.

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Introduction

Glaucoma is a significant, disease-causing visual impairment of the eye that can cause permanent blindness. Importantly, even glaucoma patients who are not blind may have functional defects such as the decreased ability to read, which lasts for life. At present, the global population aged 40 to 80 living with glaucoma comprises approximately

3.5 percent of the total, while the number of people with glaucoma is expected to increase to 111 million by 2040.¹ Moreover, chronic glaucoma is asymptomatic, meaning an estimated 50 percent of the population of the industrialized world is obliviousness of their disease.² In addition, this disease not only causes health problems, but also affects economic burdens. Medication treatment cost is estimated at 2.5\$ billion per year in the United States. In

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Europe, the cost of treating glaucoma patients increased from €455 to €969 per person last year.^{3,4}

The most common type of glaucoma is Primary Open-Angle Glaucoma (POAG).⁵ POAG results from the loss of optic nerve functions via increased intraocular pressure (IOP). Under normal conditions, IOP is controlled by aqueous humor (AH) circulation regulated by a special cell called trabecular meshwork (TM). On the other hand, TM cell fibrosis results in the prevention of normal AH outflow, leading to high IOP in POAG, which causes retinal ganglion cell (RGC) death by IOP-related stress and high IOP-related ischemic.^{6–8} Furthermore, decreasing IOP by medication in pre-perimetric early stage of glaucoma patients enhances RGC functions as measured by an electroretinogram.⁹ For these reasons, TM cell fibrosis and RGC death play a pivotal role in the pathogenesis of POAG during elevation of IOP.

Innate immune activation causes the pathogenesis of several neurodegenerative diseases including glaucoma via expression of inflammatory cytokines.¹⁰ Among several molecules, Toll-like receptors (TLRs) are crucial to the production of inflammatory cytokines during a response to endogenous or exogenous antigens. In human glaucomatous donor eyes, up-regulated expression and stimulation of TLR2, TLR3 and TLR4 are expressions resulting from elevated IOP.^{10,11} Particularly, TLR4 plays an important role in liver, skin and lung tissue fibrosis.^{12,13} From this aspect, TLR4 should also play a role in TM cell fibrosis similar to other tissues. Generally, TLR4 signals through MyD88-dependent and TRIF-dependent pathways to enable activation of NF- κ B and IRF3 functions involving the inflammatory process and inflammasome in a brain injury and myocardial inflammation.^{14,15} Recently, several studies have suggested that TLR4 contributes to the pathogenesis of glaucoma.^{16–20} Therefore, this review will summarize the roles of TLR4 in the pathogenesis of POAG, including both TM fibrosis and RGC apoptosis. Finally, we also point out several research gaps for TLR4 which should be addressed to develop a novel strategy for overcoming glaucoma.

TLR4 gene polymorphisms associated with glaucoma

TLR4 gene polymorphisms have been involved with hypo-responsiveness to infection, auto-immunity and other diseases including glaucoma.^{21,22} A recent study showed that D299G (rs4986790) and T399I (rs4986791) of TLR4 increased the risk of POAG in a Mexican population.²³ This finding is contrary to a Saudi population study observed in a small sample size, which could not detect an association between T399I and POAG.²⁴

Although the roles of these mutations are controversially discussed for different diseases, there is evidence to suggest that these functional polymorphisms promote apoptosis in hepatic stellate cells via reducing Bcl-2.²⁵ Therefore, D299G and T399I were proposed to enhance RGC apoptosis.²³ Because a structural protein study via crystallography has shown that both D299G and T399I do not intrude lipopolysaccharide (LPS) binding.²⁶ One plausible hypothesis is that the mutations may alter the ability of TLR4 response to damage-associated molecular pattern

molecules (DAMPs). This may be due to the fact that 1) the D299G and T399I were shown to enhance the activation of TLR4-fibrinogen signaling and 2) several DAMPs such as high-mobility group protein-1 (HMGB-1), heat shock proteins 72 (HSP-72) and fibronectin increased in vitreous humane and AH in glaucoma and retinal ischemic disease.^{27–30} However, the exact mechanisms should be exported in the future.

Non-coding regions of TLR4 polymorphisms such as 5' untranslated region (rs10759930 and rs1927914), intron (rs1927911, rs12377632 and rs2149356) and 3' un-translated region (rs7037117) were associated with POAG in a Japanese population.^{31,32} Interestingly, the rs7037117 also strongly correlated with normal tension glaucoma.³³ However, these single nucleotide polymorphisms' (SNPs) function is under-investigated. Combined together, TLR4 might play a pivotal role in the molecular pathogenesis of glaucoma.

Activation of TLR4 causes fibrosis of TM cells

It is well established that the transforming growth factor- β (TGF- β) is a master regulator in various tissue fibrosis, which is characterized by the increasing of extracellular matrix (ECM) proteins expression and decreasing of ECM degradation, such as in eye, liver, kidney and skin fibrosis.^{34–36} Previous studies showed that the level of TGF- β 2 in AH was significantly increased in glaucoma patients compared to healthy controls.³⁷ Additionally, several studies in trabecular meshwork model have suggested that TGF- β 2 regulates ECM expression and induced AH outflow alteration.³⁸ TGF- β 2 induces both canonical (Smad-based) and non-canonical (non-Smad-based) signaling pathways to function as transcription factors for ECM genes expression, causing elevated IOP.^{39–41}

In recent decades, TLR4 signaling was proposed for fibroblasts, in which the TLR4/ligand interaction sensitizes the cells to sensitive TGF- β stimulation in the liver, skin, etc.⁴² In TM cells, cultures showed that TLR4 activation using fibronectin crosstalk to control TGF- β signaling resulted in ECM gene expression. A mutation of TLR4 gene blocks TGF- β 2 induced ocular hypertension in mouse model (C3H/HeJ).¹⁹ Moreover, the activation of TLR4 suppresses bone morphogenetic protein (BMP) and activin membrane-bound inhibitor (BAMBI) by NF- κ B.⁴³ Consequently, inhibition of BAMPI increases ECM expression and causes ocular hypertension.¹⁷

Activation of TLR4 disturbs the detoxification system of TM cells

The detoxification system is also important for resistance to several stresses such as hypoxia, xenobiotics, increased pressure and cytokine signaling.¹⁶ The ocular tissue of POAG, including TM cells, the optic nerves and retinas, express ATP binding cassette (ABC) transporters.⁴⁴ The most important ABC transporter is multidrug resistance protein 1, p-glycoprotein (ABCB1), which expresses on the surface of TM cells. Previous study has shown that the polymorphism of ABCB1 is associated with susceptibility to POAG.⁴⁵ The activation of TLR4 by hyaluronic acid (HA),

TLR4 ligand reduces ABCB1 activity. The effect of HA in ABCB1 activity can be impaired by using TLR4 inhibitors.¹⁶ Therefore, TLR4/DAMPs binding may be important for ABCB1 function in POAG. However, the molecular mechanisms of TLR4 regulating ABCB1 functions remains under-investigated.

The roles of TLR4 cause RGC death

Vision loss due to glaucoma results from RGC degeneration, in which IOP induces RGC apoptosis via stimulation of retinal ischemia/reperfusion injury.^{46,47} Moreover, acute elevated IOP can be used as an acute glaucoma model to induce RGC death.¹⁸ Therefore, inhibition of elevated IOP is able to reduce RGC death.

Previous evidence in animal and clinical studies showed the innate immunity played a pivotal role in neuro-inflammation.¹¹ RGC apoptosis is a consequential event of orchestral cells such as microglia and astrocytes.⁴⁸ However, RGC makes an effort to produce some inflammatory cytokines to induce apoptosis by itself. In this regard, the retinal tissue expresses TLR4, including RGCs, which responds to several endogenous ligands such as HMGB1, HSP-72, and fibronectin, occurring in AH and vitreous humor (VH) of retinal ischemic diseases and glaucoma patients.^{28–30} In the acute glaucoma model, the rapid increase of IOP induces retinal ischemia injury, while RGC apoptosis is activated by TLR4/HMGB1 interaction. The NF- κ B induces activation of the NLRP3-inducing caspase-1 pathway and non-caspase1 dependent caspase-8 pathway to process IL-1 β mediating RGC death.^{18,20} In order to

support the detrimental functions of TLR4 in RGC death, various TLR4 inhibition methods were applied in retinal injury models. In the IOP-induced ischemia model, TLR4 deficiency in mice reduced the inflammation of retinal neurons and significantly increased cell survival.⁴⁶ Inhibited TLR4 using inhibitors and knockouts enhance RGC survival in the optic nerve crush model.^{49–51} Moreover, the activation inhibitor NF- κ B, which is down-stream signaling TLR4 activation via MyD88 dependent pathway, is able to protect ganglion cell layer during HMGB1 treatments.⁵² In addition, knockdown TLR4 by using siRNA suppress amyloid- β induced pro-inflammatory response via NF- κ B activation in RGC.⁵³ Whereas, some evidences showed the Octreotide protects retinal ischemic by activation of NF- κ B.⁵⁴ Viral expression of active NF- κ B decreased RGC death.⁵⁵ Therefore, inhibiting TLR4/ligand binding may provide a new target that inhibits down-stream TLR4. The role of NF- κ B activation remains controversial.

Besides MyD88 activation of TLR4, the TRIF dependent pathway can play a contrary role in response to other ligands. Prothymosin- α /TLR4 interaction protects retinal ischemic via activating TRIF/IRF3 signaling.⁵⁶ However, the exact role of this pathway in RGC has yet to be fully understood.

TLR4 inhibitor in other diseases

Currently, TLR4 is known as an essential pathogenesis molecule in auto-immune and inflammatory diseases. For example, knock out TLR4 reduced vasculature inflammation in an atherosclerosis model and decreased autoantibody

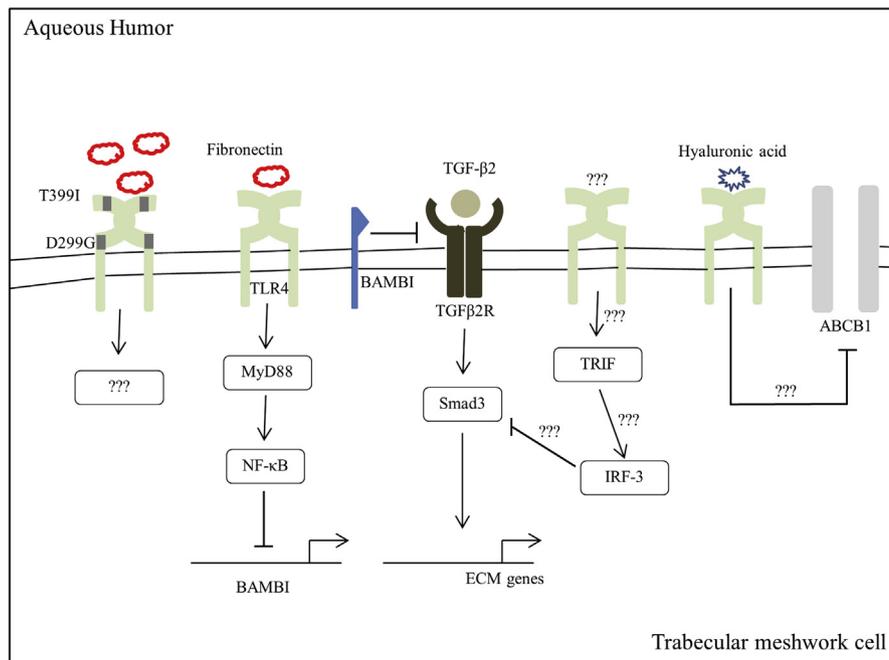


Figure 1. TLR4 activation plays a role in trabecular meshwork cell fibrosis and detoxification during POAG. The mutant TLR4 is able to interact with fibronectin. However, the mechanisms of TLR4 single nucleotide polymorphisms in fibrosis remain unknown. In addition, wild-type TLR4/fibronectin activates NF- κ B to inhibit BAMB1, TGFBR inhibitor. On the other hand, TLR4 activation can activate TRIF and IRF3 to inhibit TGF- β signaling pathways in other tissues. This evident is still under-investigated for TM cell fibrosis. Finally, the hyaluronic acid in TM cells activates TLR4 to inhibit ABCB1 via unknown mechanisms. ? indicates unknown mechanisms.

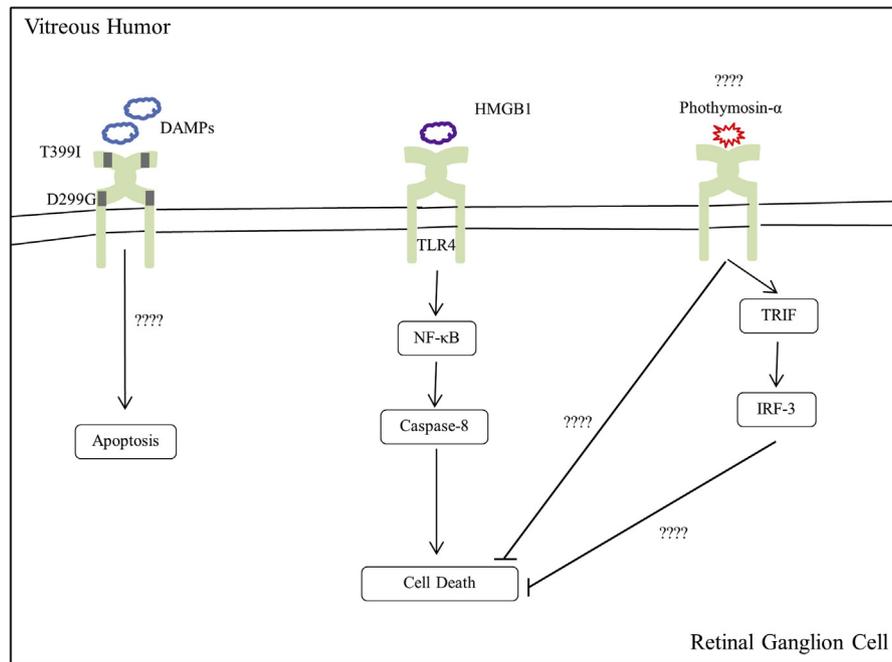


Figure 2. The role of TLR4 activation in retinal ganglion cell death. Unknown functions of mutant TLR4 may activate apoptosis. HMGB1, DAMPs in vitreous humor activates cell death by using NLRP3-inducing caspase-1 pathways and non-caspase1 dependent caspase-8 pathways. Surprisingly, phothymosin- α , the other DAMPs, also activates TLR4 to inhibit cell death via TRIF dependent pathways and other pathways under unknown mechanisms. However, the question of why TLR4 discriminates fibronectin and phothymosin- α is a gap in POAG research. ? indicates unknown mechanisms.

production in systemic lupus erythematosus (SLE).⁵⁷ Interestingly, several TLR4 antagonists are under clinical trials for various diseases. In phase II trials, there are JKB-121, TLR4 inhibitor for non-alcoholic steatohepatitis treatment (NCT02442687) and anti-TLR4 antibody treatment for rheumatoid arthritis (NCT03241108). Additionally, Eritoran is also in phase III trials, which complete patient recruitment for severe sepsis (NCT00334828). As previously mentioned, the inhibition of TLR4 signaling may provide a novel therapeutic strategy for POAG.

Conclusion and perspective

In this review, the functions of TLR4 in POAG were divided into two sections. First, TLR4 involves TM cells developing fibrosis. Fibronectin activates TLR-4 through MyD88 and NF- κ B signaling to inhibit BAMB1 expression. However, the roles of D299G and T399I for TLR4/fibronectin interaction during TGF- β stimulation remain undetermined. Moreover, the role of TRIF/IRF-3 should be accessed in TLR4 activation during fibrosis status. Previous evidence suggested that phosphor-IRF3 is able to inhibit Smad3 activation via competitive binding in hepatic cell fibrosis.⁵⁸ In addition, the detoxification system of TM cells is regulated by TLR4, though the mechanisms are still unknown (Fig. 1). Consequently, the abnormal function of TM cells causes fibrosis and increased IOP. Elevated IOP reduces RGC survival. At present, DAMPs (HMGB1) interacts with TLR4 to activate both NLRP3 inflammasomes and non-caspase-1 dependent caspase-8 pathways via MyD88 and NF- κ B. However, TRIF/IRF-3 signaling resulting from TLR4/Prothymosin interaction

reduces RGC death. Interestingly, TLR4 is one ligand binding site that takes responsibility for PAMPs and DAMPs. This topic requires further experimentation to answer why TLR4 and different DAMPs interactions cause various pathways. Finally, the function of polymorphisms for TLR4 in RGC death must be identified clearly (Fig. 2). In sum, understanding of the molecular mechanisms of TLR4 may shed new light to overcome POAG.

Conflicts of interest

The author declares no conflicts of interest with the subject matter and funding.

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