NLRP3 and autophagy in retinal ganglion cell inflammation in age-related macular degeneration: potential therapeutic implications

Xiao-Li Wang, Yun-Xia Gao, Qiong-Zhen Yuan, Ming Zhang

Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Correspondence to: Ming Zhang. Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China. mingzhangdoctor@163.com

Received: 2023-11-30 Accepted: 2024-04-22

Abstract
● Retinal degenerative diseases were a large group of diseases characterized by the primary death of retinal ganglion cells (RGCs). Recent studies had shown an interaction between autophagy and nucleotide-binding oligomerization domain-like receptor 3 (NLRP3) inflammasomes, which may affect RGCs in retinal degenerative diseases. The NLRP3 inflammasome was a protein complex that, upon activation, produces caspase-1, mediating the apoptosis of retinal cells and promoting the occurrence and development of retinal degenerative diseases. Upregulated autophagy could inhibit NLRP3 inflammasome activation, while inhibited autophagy can promote NLRP3 inflammasome activation, which leaded to the accelerated emergence of drusen and lipofuscin deposition under the neurosensory retina. The activated NLRP3 inflammasome could further inhibit autophagy, thus forming a vicious cycle that accelerated the damage and death of RGCs. This review discussed the relationship between NLRP3 inflammasome and autophagy and its effects on RGCs in age-related macular degeneration, providing a new perspective and direction for the treatment of retinal diseases.
● KEYWORDS: autophagy; age-related macular degeneration; NLRP3 inflammasome; retinal degeneration; retinal ganglion cells
DOI:10.18240/ijo.2024.08.20

INTRODUCTION

Inflammation is a defensive response initiated by the human body in response to external stimulation. Recent studies have demonstrated a close connection between inflammation and aging, suggesting that inflammation drives the development of many age-related diseases[1]. Neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, muscular dystrophy, lateral sclerosis, and multiple sclerosis, were primarily characterized by the damage and loss of ganglion cells, which were triggered by the increased involvement of damaged DNA. This damage stimulated cells to produce inflammatory cascade reactions, ultimately leading to the degeneration and death of ganglion cells[2]. The retinal nerve fibers were the only neurons in the body that directly connect the outside world with the brain, transmitting light sensing signals to the brain to form visual sensations[3]. Retinal degeneration was a group of chronic fundus diseases, included age-related macular degeneration (AMD), characterized by the progressive loss of cells, such as retinal ganglion cells (RGCs), retinal pigment epithelial (RPE) cells, and structural damage to the retinal layers, ultimately leading to the loss of visual function[3]. Research suggested that chronic inflammation is an important initiating mechanism for the degeneration and death of RGCs[4]. The nucleotide-binding oligomerization domain-like receptor 3 (NLRP3) inflammasome may be involved in multiple inflammatory responses throughout the body and has become a potential therapeutic target for many diseases. Relevant evidence had also been found in ocular inflammatory diseases[5]. NLRP3 played a pivotal role in immune regulation, specifically participating in the formation and signaling of the inflammasome. The abnormal triggering of NLRP3 had been linked to the development of several inflammatory condition. NLRP3 in the inflammatory process emerged as a potential new therapeutic target for treating inflammatory disorders[6]. In neurodegenerative diseases, autophagy had been observed to have a strong correlation with inflammatory reactions. Specifically, mitochondrial autophagy had been
Ganglion cell inflammation

shown to trigger the inhibition of the NLRP3 inflammasome, subsequently mitigating neuroinflammation[7]. Autophagy was a physiological process in which eukaryotes self-phagocytose and maintain homeostasis, and played a certain role in pyroptosis caused by inflammation of retinal cells[8]. Therefore, the purpose of this review was to explore the possible regulatory mechanisms in the inflammatory response of RGCs, including the NLRP3 inflammasome and autophagy pathway, and to identify possible therapeutic strategies and potential therapeutic targets.

Search Strategy The PubMed database, Google scholar and Ovid technologies (OVID) medicine was selected for literature search, and the following key words were used: ‘ganglion cells’ AND ‘inflammation’; ‘retinal degeneration’ AND ‘NLRP3’; ‘NLRP3’ AND ‘autophagy’; ‘retinal degeneration’ AND ‘autophagy’; ‘retinal ganglion cell’ AND ‘NLRP3’; ‘inflammation’ AND ‘autophagy’. After conducting preliminary retrieval, in case of an overwhelming number of documents, utilize a combination of over three keywords to refine the search scope. Conversely, if the retrieved documents are scarce, broaden the search by decreasing the number of keywords. We selected literatures published in English between January 2000 and November 2023, including clinical and basic studies.

Inflammatory Factors Related to Retinal Ganglion Cell Inflammation AMD are ocular neurodegenerative diseases. The irreversible degeneration of RGCs was the primary cause of vision loss[9]. There were currently no effective and reversible therapies to improve neuroretinal degeneration[10-11]. The retina and choroid were the most energy-consuming eye tissues, requiring a large amount of adenosine triphosphate (ATP) to maintain normal physiological functions[12]. Some external factors could induce the body to produce an oxidative stress response. When the excessive oxidative stress load was unable to be digested, oxidative stress damage to the RGCs and photoreceptors would occur[13]. Some intrinsic factors, such as drusen, were also involved in oxidative stress in AMD[14-15]. Oxidative stress lead to an increase in reactive oxygen species (ROS) in retinal cells, causing the death of photoreceptor cells, RGC and RPE cells by inducing changes in autophagy levels[16]. Under harmful stimulation, retinal tissue could develop a chronic inflammatory state, contributing to the degeneration and death of RGCs and other cells in AMD[17]. Autophagy also played a critical role in maintaining the homeostasis of the intracellular environment[18]. Typically, autophagy-related proteins expressed in RGCs and RPE cells maintain normal visual function. However, if the autophagy protein gene was mutated, autophagy would be activated, leading to the development of retinal diseases[19]. Autophagy could effectively reduce the toxic effects of cells[20].

Aging was closely associated with cell death, which in turn stimulated inflammation and triggered a series of cascade reactions[21]. RGCs were the only neurocytes could directly receive external light stimulation. When the body’s functions declined, such as chronic light stimulation, metabolic disorders, or other external factors, these could lead to the damage and degeneration of RGC. This in turn caused the release of cytokines, affecting the surrounding cellular environment[22]. The cytokines involved in the inflammation of RGCs and RPE cells are listed in Table 1[23-46].

Interleukins Interleukins (ILs) were classic cytokines which primary functions included transmitting signals, regulating the immune system, participating in inflammation and mediating the activation, proliferation[45]. Among them, IL-1β, IL-4, IL-6, IL-8, and IL-18 were particularly relevant to ophthalmology. IL-1β is involved in a variety of immune and inflammatory responses and cellular activities, including cell proliferation, differentiation, and apoptosis[23]. IL-1β, in conjunction with IL-18, collaborates to regulate immune responses through various downstream mechanisms, which were associated with pain, inflammation, neuroprotection[23]. IL-4 was an immunomodulatory cytokine that regulated the survival and differentiation of nerve cells, while stimulating the cholinergic differentiation of retinal cells[24,46]. The application of IL-6-like cytokines or the activation of their corresponding downstream signaling pathways could promote neuroprotection and optic nerve regeneration[25]. Signal transduction of IL-8 could be enhanced by inflammatory signals, ROS and death receptors, which may be related to primary or secondary ocular inflammation[26]. The inflammatory response of RGCs could be reduced by decreasing the expression of IL-8[26]. IL-10 was an anti-inflammatory cytokine that could downregulate inflammation and antagonize inflammatory mediators in neuroretina[47-48].

Interferons Interferons (IFNs) were immune system regulators, with IFN-γ being particularly relevant to the eye. It could induce microglia and astrocytes to produce nitric oxide synthase, which was related to the occurrence or protection of certain diseases in the central nervous system. If the expression of IFN-γ increases in response to stimuli in the retina, it may lead to the death of RGCs[48-50]. Du et al[51] found that in vitro, IFN-γ-stimulated retinal microglia increased the release of various pro-inflammatory factors, which then further affected RGCs and photoreceptors. Therefore, IFN-γ could produce a variety of cellular effects.

Tumor necrosis factor-alpha Tumor necrosis factor-alpha (TNF-α), a multifunctional molecule within cells, played roles in inflammation, apoptosis, and immune responses. TNF-α could promote local inflammatory reactions through cell death[51-52]. TNF-α exerted a harmful effect on RGCs. By
binding to the TNFR1 receptor, TNF-α significantly enhanced the function and expression of Nav1.6 channels on the surface of RGCs, leading to their hyper-excitability and, ultimately, apoptosis[53]. By utilizing antagonists to regulate TNF-α, the inflammatory cellular response could be effectively downregulated, ultimately mitigating local chronic inflammation within the body[54].

**Transforming growth factor-beta family** Transforming growth factor-beta (TGF-β) regulated the growth, differentiation, and apoptosis of retinal cells. Additionally, TGF-β also played an important role in some retinal diseases. TGF-β exhibited a protective effect on RGCs, as it could inhibit the apoptosis of RGCs through certain signaling pathways, thus playing a protective role[55]. Through the regulation of the TGF-α-related signaling pathway, the inflammatory response of RGCs can be mitigated, thereby minimizing local damage to the retina[56].

**Growth factors** Vascular endothelial growth factor (VEGF) promoted the survival and synaptic growth of RGCs, suggesting that VEGF may play a positive role in the growth and survival of RGCs[57]. Hepatocyte growth factor has a protective effect on RGCs, and it may exert its protective function by promoting the survival of RGCs and inhibiting cell apoptosis[58]. The main protective mechanism of insulin-like growth factor-1 on RGCs included activating certain intracellular signaling pathways to promote cell survival; promoting the growth of synapses in RGCs helping to maintain the normal structure and function of the retina[59].

**Chemokine family** Monocyte chemoattractant protein-1/chemokine (C-C motif) ligand 2; MMP: Matrix metalloproteinase; NLRP3: NOD-like receptor family pyrin domain containing 3; TGF: Transforming growth factor; TNF-α: Tumor necrosis factor-alpha; tPA: Tissue-type plasminogen activator; VEGF: Vascular endothelial growth factor; RPE: Retinal pigment epithelial; AMD: Age-related macular degeneration..

---

### Table 1 Inflammatory cytokines of retinal ganglion cells and RPE cells in AMD

<table>
<thead>
<tr>
<th>Categories</th>
<th>Cytokines</th>
<th>Functional mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin</td>
<td>IL-1β</td>
<td>Involved in proliferation, differentiation, and apoptosis, inflammatory factor</td>
<td>Natori et al[27], 2017</td>
</tr>
<tr>
<td></td>
<td>IL-4</td>
<td>Regulated humoral and adaptive immunization</td>
<td>Zhou et al[30], 2022</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>Involved in cell growth and differentiation</td>
<td>Coorey et al[31], 2015</td>
</tr>
<tr>
<td></td>
<td>IL-8</td>
<td>Stimulated angiogenesis and capillary leakage</td>
<td>Hautamäki et al[34], 2014</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>Increase retinal ganglion cell survival similar to IL-2</td>
<td>Colares et al[37], 2021</td>
</tr>
<tr>
<td></td>
<td>IL-18</td>
<td>Upregulated through NLRP3 pathway, inflammatory factor</td>
<td>Peng et al[39], 2020</td>
</tr>
<tr>
<td>Interferon</td>
<td>IFN-γ</td>
<td>Inflammatory factor, immune stimulation</td>
<td>Yu et al[40], 2016</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>TNF-α</td>
<td>Induces retinal ganglion cell degeneration, inflammatory factor</td>
<td>Ko et al[41], 2020</td>
</tr>
<tr>
<td>Transforming growth factor-β family</td>
<td>TGF-β1</td>
<td>Regulated cell proliferation, differentiation and activation</td>
<td>Tarallo et al[42], 2012</td>
</tr>
<tr>
<td></td>
<td>TGF-β2</td>
<td>Growth inhibiting factor, immunosuppressive effect</td>
<td>Llorián-Salvador et al[45], 2022</td>
</tr>
<tr>
<td>Growth factor</td>
<td>VEGF</td>
<td>Promoted angiogenesis and vascular permeability, and stimulated the proliferation and survival of endothelial cells</td>
<td>Liber et al[46], 2022</td>
</tr>
<tr>
<td></td>
<td>HGF</td>
<td>Promoted the proliferation of vascular endothelial cells and the formation of neovascularization</td>
<td>Chu et al[47], 2006</td>
</tr>
<tr>
<td></td>
<td>IGF-1</td>
<td>Stimulated cell growth and differentiation, reduce protein breakdown</td>
<td>Subramani et al[48], 2023</td>
</tr>
<tr>
<td>Chemokine family</td>
<td>MCP-1/CCL2</td>
<td>Involved in chronic inflammation, body defense, and anti-tumor</td>
<td>Lechner et al[49], 2017</td>
</tr>
<tr>
<td>Other factors</td>
<td>CFH</td>
<td>Regulated the complement-mediated immune system</td>
<td>Hayashi et al[50], 2010</td>
</tr>
<tr>
<td></td>
<td>CRP</td>
<td>Activated complement and enhances phagocytosis</td>
<td>Colak et al[51], 2012</td>
</tr>
<tr>
<td></td>
<td>ApoE</td>
<td>Involved in nerve regeneration, immune regulation, lipid metabolism, and enzyme activation</td>
<td>Vessey et al[52], 2022</td>
</tr>
<tr>
<td></td>
<td>tPA</td>
<td>Activated the conversion of endogenous plasminogen to plasmapause</td>
<td>Igllicki et al[53], 2024</td>
</tr>
<tr>
<td></td>
<td>Bcl-2</td>
<td>Decreased Bax activity and cell apoptosis</td>
<td>Nir et al[54], 2000</td>
</tr>
<tr>
<td></td>
<td>MMP-2</td>
<td>Zinc-dependent enzyme, cuts extracellular matrix components</td>
<td>De Groef et al[55], 2015</td>
</tr>
<tr>
<td></td>
<td>MMP-9</td>
<td>Degradation and remodeling of extracellular matrix homeostasis</td>
<td>Descamps et al[56], 2008</td>
</tr>
<tr>
<td></td>
<td>NLRP3</td>
<td>Lead to the occurrence of autoimmune diseases</td>
<td>Weinberger et al[57], 2023</td>
</tr>
</tbody>
</table>

ApoE: Apolipoprotein E; Bcl-2: B-cell lymphoma-2; CFH: Complement factor H; CRP: C-reactive protein; HGF: Hepatocyte growth factor; IFN-γ: Interferon-γ; IGF-1: Insulin-like growth factor-1; IL: Interleukin; MCP-1/CCL2: Monocyte chemoattractant protein-1/chemokine (C-C motif) ligand 2; MMP: Matrix metalloproteinase; NLRP3: NOD-like receptor family pyrin domain containing 3; TGF: Transforming growth factor; TNF-α: Tumor necrosis factor-alpha; tPA: Tissue-type plasminogen activator; VEGF: Vascular endothelial growth factor; RPE: Retinal pigment epithelial; AMD: Age-related macular degeneration.
Ganglion cell inflammation

Table 2 Timeline of the development of the NLRP3 inflammasome

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Findings</th>
<th>Significance</th>
<th>Team and contributions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>NLRP3 was discovered for the first time</td>
<td>The gene responsible for encoding the NLRP3 protein was identified as being a member of the NOD-like receptor family.</td>
<td>This initial understanding of NLRP3 serves as a foundation for further investigations and advances in the field.</td>
<td>Research team of the Immunity Center in Marseille, France.</td>
</tr>
<tr>
<td>2004</td>
<td>Structure analysis of NLRP3</td>
<td>The researchers studied the structural properties of NLRP3 and elucidated its connection to the disease.</td>
<td>A clear understanding of the structural and functional aspects of NLRP3 is essential for developing effective therapeutic strategies aimed at targeting this critical protein.</td>
<td>The three-dimensional structure of the NLRP3 protein has been resolved through X-ray crystallography. Researchers have genetically engineered mice to investigate the effects of either lacking or excessively expressing NLRP3.</td>
</tr>
<tr>
<td>2008</td>
<td>Relationship between NLRP3 and inflammation</td>
<td>NLRP3 was discovered to participate in inflammatory responses, and a connection to autoimmune diseases was confirmed.</td>
<td>The critical importance of NLRP3 in the inflammatory response has been well documented, paving the way for NLRP3 to serve as a promising new target for anti-inflammatory treatments.</td>
<td>There is an expert consensus on the critical role of NLRP3 in the inflammatory pathway.</td>
</tr>
<tr>
<td>2012</td>
<td>NLRP3 and crystal structure</td>
<td>The crystal structure of NLRP3 in complex with its ligand was published, providing further insight into its activation mechanism.</td>
<td>This understanding helps clarify how NLRP3 is activated, providing a critical foundation for the design of drugs targeting NLRP3.</td>
<td>Gene editing technology was used in the NLRP3 study.</td>
</tr>
<tr>
<td>2016</td>
<td>NLRP3 and the study of disease</td>
<td>NLRP3 had been associated with a wide spectrum of conditions, including gout, diabetes, and neurodegenerative disorders.</td>
<td>The pivotal role of NLRP3 in a diverse array of diseases has been firmly established, making it a crucial target for the treatment of various medical conditions.</td>
<td>Through large-scale genetic studies such as genome-wide association analysis (GWAS).</td>
</tr>
<tr>
<td>2020 to present</td>
<td>Study on NLRP3 inhibitors</td>
<td>Inhibitors targeting NLRP3 were currently undergoing clinical trials to evaluate their efficacy and safety.</td>
<td>The research on NLRP3 inhibitors has unveiled novel treatment strategies for inflammatory diseases in the future.</td>
<td>The Inflammation and Host Defense Research Center at Harvard Medical School conducts clinical research.</td>
</tr>
</tbody>
</table>

NLRP3: NOD-like receptor family pyrin domain containing 3 protein (CRP)\(^{[61-62]}\), apolipoprotein E (APOE), tissue-type plasminogen activator (tPA). The activation of the inflammasome was a crucial component of the innate immune response, among which the NLRP3 inflammasome was the well-studied (Table 2, Figure 1). NLRP3 was associated with various diseases throughout the body (Figure 2) and also closely linked to eye disease. It belonged to the NOD-like receptor family, whose members all possess nucleotide-binding and oligomerization domain (NACHT) with similar structures. In addition, NLRP3 included an N-terminal pyrin domain (PYD) and a C-terminal leucine-rich repeat (LRR). The NLRP3 inflammasome was primarily composed of NLRP3, apoptosis-associated spot-like protein (ASC) and caspase-1. The inflammasome activated caspase-1, which promoted the apoptosis-associated spot-like protein (ASC) and caspase-1.

The NLRP3 inflammasome was the well-studied component of the innate immune response, among which the NLRP3 inflammasome was the first time discovered. The three-dimensional structure of the NLRP3 protein has been resolved through X-ray crystallography. Researchers have genetically engineered mice to investigate the effects of either lacking or excessively expressing NLRP3.

The critical importance of NLRP3 in the inflammatory response has been well documented, paving the way for NLRP3 to serve as a promising new target for anti-inflammatory treatments. There is an expert consensus on the critical role of NLRP3 in the inflammatory pathway.

This understanding helps clarify how NLRP3 is activated, providing a critical foundation for the design of drugs targeting NLRP3. Through large-scale genetic studies such as genome-wide association analysis (GWAS).

The research on NLRP3 inhibitors has unveiled novel treatment strategies for inflammatory diseases in the future. The Inflammation and Host Defense Research Center at Harvard Medical School conducts clinical research. in the release of IL-1β in AMD\(^{[67]}\). When the function of retinal cells were impaired or their metabolites were not efficiently excreted, the cells experience swelling, vacuolar degeneration and death, which activated the NLRP3 and promoted cytokine secretion\(^{[68]}\), leading to the death of surrounding RGCs\(^{[69]}\) and creating an inflammatory cascade. Apolipoprotein C3 (ApoC3) had been discovered to trigger the activation of the NLRP3 inflammasome via particular mechanisms, ultimately resulting in immune inflammation\(^{[73]}\). C5 potentially enhanced the NLRP3-mediated inflammatory response\(^{[70]}\). The activation of the NLRP3 inflammasome was shown in Figure 3.

Relationship Between NLRP3 and Inflammatory Diseases Related to RGCs The NLRP3 inflammasome played a decisive role in various diseases, including Alzheimer’s disease, autoimmune inflammatory diseases and various neurodegenerative diseases\(^{[71]}\). Ocular neurodegeneration also encompasses AMD, retinitis pigmentosa, optic nerve atrophy, and retinitis neurtitis. In diseases like AMD, RGCs undergo degenerative changes, leading to retinal dysfunction and vision loss. NLRP3...
was an essential component of the inflammasome. Upon activation of NLRP3, it triggered a cascade of inflammatory responses. RGCs were primarily responsible for receiving and transmitting visual signals. When RGCs were stimulated by long-term chronic inflammation, inflammatory responses occur, causing cellular damage and dysfunction[72]. NLRP3 activation was closely associated with the onset and development of diseases related to RGCs inflammation[73]. In AMD, NLRP3 activation could lead to an increased inflammatory response in RGCs, further aggravating the symptoms and progression of the disease[74]. Therefore, targeting the regulation of NLRP3 may become a novel strategy for the treatment of inflammatory diseases related to RGCs. NLRP3 expression was significantly up-regulated in the microinflammatory environment of the retina, and activation of the NLRP3 could promote the maturation and secretion of IL-1β and IL-18, ultimately leading to a chain inflammatory response in peripheral RGCs and other cells[23]. Targeting NLRP3 or IL-18 could inhibit RPE degeneration, thereby reducing local inflammation and reducing RGCs damage[29].

The X-linked apoptosis suppressor protein could negatively regulate the secretion of IL-1β and IL-18. Located downstream of caspase-1, the X-linked apoptosis suppressor protein was reduced by the activation of NLRP3[30]. The secretion of IL-1β and IL-18 depended on the NLRP3[75]. When the NLRP3 was stimulated and activated, it promoted the conversion of pro-caspase-1 to active caspase-1, which then participated in mediating the programmed death of RPE cells and RGCs, ultimately promoting the development of AMD and leading to visual impairment due to the degeneration and death of RGCs and photoreceptors[24]. TRIM31 also interacted with NLRP3. TRIM31 inhibited the formation of NLRP3 by promoting NLRP3 ubiquitination, thus inhibiting the death of RPE cells[76]. Hwang and Chung[77] found that salazosulfapyridine could reduce the apoptosis of RPE cells induced by tamoxifen by inhibiting caspase-1-mediated inflammation, which proved that the NLRP3 was associated with the onset of AMD.

**Relationship Between Autophagy and Inflammatory Diseases Related to RGCs**

Autophagy was a cellular self-degradation process that helped remove damaged proteins, organelles, and more to maintain cellular homeostasis and balance. Autophagy also played an important protective role in RGCs. When RGCs were stimulated by inflammation, autophagy could be activated to help the cells clear damaged proteins and organelles, reduce the inflammatory response, and promote cell repair. However, in some cases, the activation of autophagy may not be sufficient to respond to a strong inflammatory response, resulting in increased damage to RGCs. At this point, regulating the autophagy process may help reduce the inflammatory response and protect RGCs.
from further damage. Pathological changes in AMD include RPE degeneration, drusen, lipofuscin deposition, and more. Autophagy had a certain regulatory effect on these pathological changes, which could reduce the damage of these pathological changes to RGCs[78-79]. The presence of ROS was also a critical factor. In RGCs, a delicate balance between ROS production and clearance was essential for maintaining the normal function of the cells. However, in certain circumstances, such as inflammation, oxidative stress, and other pathological conditions, ROS production may increase, resulting in elevated intracellular ROS levels. This state of oxidative stress could cause harmful effects on RGCs, leading to cell damage and dysfunction. Prolonged or excessive oxidative stress may accelerate the degeneration of RGCs, exacerbating vision loss. RGCs were particularly sensitive to ROS. The retina consumes vast amounts of oxygen during normal human eye activity and generates a significant amount of ROS. Under normal conditions, ROS was eliminated by intracellular antioxidant enzymes, maintaining an intracellular ROS balance. If ROS accumulation occurs in an emergency, it could cause cell damage. The chronic oxidative stress response can impair the autophagy ability of retinal cells, leading to retinal damage, including that of RGCs. Therefore, improving autophagy could prevent retinal damage caused by oxidative stress[80]. However, acute oxidative stress can stimulate autophagy activity, while chronic stress may reduce it, as confirmed in an experiment. It was found that the severity of oxidative stress had different effects on autophagy[31]. Oxidative stress could also stimulate the formation of lipofuscins, the main component of N-retinylidene-N-retinylidene ethanolamine (A2E). Autophagy could be induced in ARPE-19 cells when they are incubated with A2E. Autophagy inhibitors combined with A2E can promote cell death, while A2E alone cannot cause cell death[81], and this reduced the release of inflammatory factors that affect RGCs. Chronic inflammation was also another important cause of RGCs damage in AMD. In AMD, chronic inflammation could lead to pathological changes in RGCs and RPE cells under the continuous action of RGCs[82]. When drusen form and deposit between the RPE basement membrane and the Bruch membrane, they could lead to chronic damage and apoptosis of RPE cells, photoreceptors, and RGCs, as drusen contain various inflammatory components, including immunoglobulin, CRP, and complement components[83]. Complement was a protein which activation could attack RPE cells and participated in RGCs death, which were the main processes involved in the pathogenesis of dry AMD[84]. Autophagy could regulate inflammation, and inflammatory factors could also affect autophagy[85]. AMD was mainly characterized by the degeneration of RPE cells and the damage of photoreceptor cells in the macula. Autophagy played a crucial role in maintaining cellular homeostasis and removing damaged organelles. However, with aging, autophagy gradually weakens, resulting in a decrease in the efficiency of intracellular waste removal and damaged organelle degradation[86]. This decrease in autophagy function was closely related to AMD. In the retinal cells of patients with AMD, autophagy activity was reduced, leading to the accumulation of intracellular waste, further exacerbating cell damage and degradation[87]. Activating the autophagy pathway could promote waste removal and cells repairment, potentially slowing the progression and reducing symptoms of AMD[88]. In dry AMD, oxidative stress and inflammation play crucial roles in its development, and autophagy could influence the disease course by regulating these factors. Enhancing autophagy function therefore offered new possibilities for the treatment of AMD.

**Effect of NLRP3 Interaction with Autophagy on RGC Inflammation** When NLRP3 was activated, it stimulated inflammatory responses, leading to the infiltration of inflammatory cells and the release of inflammatory mediators, exacerbating the damage and dysfunction of RGCs. Autophagy, as a cytoprotective mechanism, could reduce inflammatory responses by removing damaged proteins and organelles. In RGCs, activation of autophagy could help remove intracellular waste and promote cell repair and regeneration. When NLRP3 interacted with autophagy, the balance between them was critical for the inflammatory response of RGCs. On one hand, if NLRP3 was overactivated and the inflammatory response was intensified, autophagy may not be able to effectively clear the damaged cell components, resulting in increased cellular inflammatory damage. On the other hand, by enhancing autophagy activity, it could promote the remission of inflammatory response and reduce the damage of RGCs. Therefore, regulating the interaction between NLRP3 and autophagy may protect RGCs from inflammatory damage by inhibiting NLRP3 overactivation or enhancing autophagy activity. The mechanisms of NLRP3 and autophagy inflammasome in different ocular diseases were shown in Table 3[89-95]. The schematic representation of the interaction between NLRP3 and autophagy inflammasome in RGCs is shown in Figure 4. The NLRP3 inflammasome primarily drove the production and secretion of inflammatory factors, while autophagy regulated and affected the inflammatory response. AMD was a degenerative disease of the retina, which primarily associated with the functional degeneration of RGCs, RPE cells, and choroidal blood vessels[96]. Autophagy could maintain the homeostasis of the internal environment of RGCs and RPE cells. If autophagy was disrupted, it would lead to the death of RGCs, which may accelerate the pathological process.
senescence and death of peripheral inflammatory ganglion cells, which then promote chronic inflammation of RPE cells and ultimately resulted in the activation of the NLRP3 inflammasome, which then promoted chronic inflammation of AMD and vision loss. Chronic inflammation in the retina may lead to aggregation of lipofuscin and activation of the NLRP3 inflammasome, which then promoted chronic inflammation of RPE cells and ultimately resulted in the activation of the NLRP3 inflammasome, which in turn could promote chronic inflammation of AMD and vision loss.

The interaction between the NLRP3 inflammasome and autophagy may be regulated by various intracellular and extracellular factors, such as the state of intracellular signaling pathways, cell type, and environmental conditions. The regulation of this balance was essential for maintaining the normal operation of the inflammatory response and preventing tissue damage caused by excessive inflammatory response. The interaction between NLRP3 and autophagy may be regulated by various intracellular and extracellular factors, such as the state of intracellular signaling pathways, cell type, and environmental conditions. The regulation of this balance was essential for maintaining the normal operation of the inflammatory response and preventing tissue damage caused by excessive inflammatory response.

The interaction between NLRP3 and autophagy was manifested in the following aspects: 1) Autophagy could reduce the production of NLRP3 by directly degrading damaged mitochondria, and could also reduce the production of ubiquitinated NLRP3 by recognizing it. This could target pro-IL-1β and pro-IL-18 to reduce their inflammatory products, IL-1β and IL-18, respectively, thereby reducing inflammation. The nucleus could be stimulated by ROS to activate the complement pathway. IL: Interleukin; NLRP3: NOD-like receptor family pyrin domain containing 3 protein; NF-κB: Nuclear factor kappa-B; ROS: Reactive oxygen species; ASC: Apoptosis-associated speck-like protein.

2) The inflammasome could reduce the activation of inflammasome by inducing autophagy, thereby reducing the production of NLRP3 by directly degrading damaged mitochondria, and could also reduce the production of ubiquitinated NLRP3 by recognizing it. This could target pro-IL-1β and pro-IL-18 to reduce their inflammatory products, IL-1β and IL-18, respectively, thereby reducing inflammation. The nucleus could be stimulated by ROS to activate the complement pathway. IL: Interleukin; NLRP3: NOD-like receptor family pyrin domain containing 3 protein; NF-κB: Nuclear factor kappa-B; ROS: Reactive oxygen species; ASC: Apoptosis-associated speck-like protein.

3) The inflammasome could inhibit autophagy. Lipopolysaccharide-induced inflammation led to an increase in NLRP3 inflammasome protein levels. Melatonin played an anti-inflammatory role by restoring the aforementioned blocked autophagy pathway, which was due to impaired autophagy flux. A2E had been shown to cause significant autophagic damage and upregulate the negative regulator of autophagy in RPE cells, suggesting that lipofuscin in the retina may inhibit autophagy. In vitro cultures of retinitis pigmentosa cells revealed that lipofuscin-like substances make them more sensitive to lysosomal phototoxicity, leading to the activation of NLRP3 inflammasome and the secretion of inflammatory factors. The autophagy agonist resveratrol possesses antioxidant and anti-inflammatory properties. It could effectively prevent the production and increase of ROS, IL-6, and IL-8 in retinal cells, and reduce the expression of lipofuscin, demonstrating that autophagy could inhibit the activation of NLRP3 inflammasome. Rapamycin could inhibit the activation of NLRP3 inflammasome by inducing autophagy, and nuclear factor erythroid 2-related factor 2 (Nrf2) could partially mediate the inhibitory effect of rapamycin on ROS, thus regulating the activation of NLRP3 inflammasome. Amyloid-β (Aβ) deposition could drive microglial activation and trigger the NLRP3 inflammasome, which in turn could promote chronic inflammation of AMD and vision loss.

Table 3 NLRP3 inflammasome and autophagy in different ocular neuropathic diseases

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Involved in mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal degeneration</td>
<td>Cyn protects against blue light-induced retinal degeneration by modulating autophagy and decreasing the NLRP3 inflammasome</td>
<td>Feng et al,[99], 2021</td>
</tr>
<tr>
<td>AMD</td>
<td>Chronic inflammation was regulated by autophagy degradation of NLRP3 inflammasomes in AMD</td>
<td>Phippo et al,[100], 2018</td>
</tr>
<tr>
<td>Dry eye</td>
<td>Melatonin inhibited the activated NLRP3 inflammation cascade and maintains normal levels of autophagy.</td>
<td>Wang et al,[101], 2021</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Autophagy cleared the extracellular Aβ fibrils by microglia and regulating the Aβ-induced NLRP3 inflammasome.</td>
<td>Cho et al,[102], 2014</td>
</tr>
<tr>
<td>Corneal transplantation rejection</td>
<td>Blocking NLRP3 inflammasome signaling had been shown to prolong the survival of corneal allografts.</td>
<td>Wei et al,[103], 2022</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>It could reduce the bacterial inflammatory response by inhibiting NLRP3 inflammasome and promote the degradation of ocular bacterial infections by autophagosomes</td>
<td>Singh et al,[104], 2023</td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>High glucose had been found to increase autophagy, while treatment with 3-MA led to the accumulation of ROS and the activation of NLRP3 inflammasome.</td>
<td>Shi et al,[105], 2015</td>
</tr>
</tbody>
</table>

AMD: Age-related macular degeneration; NLRP3: NOD-like receptor family pyrin domain containing 3; ROS: Reactive oxygen species; 3-MA: 3-Methyladenine.

Figure 4 Relationship between NLRP3 inflammasome and autophagy

As shown in the figure, when cells were subjected to long-term, chronic harmful stimuli from the outside world, mitochondria could be damaged, leading to the release of ROS. This ROS could then activate NLRP3 through the NF-κB and other pathways. Meanwhile, autophagy could reduce the production of NLRP3 by directly degrading damaged mitochondria, and could also reduce the production of ubiquitinated NLRP3 by recognizing it. This could target pro-IL-1β and pro-IL-18 to reduce their inflammatory products, IL-1β and IL-18, respectively, thereby reducing inflammation. The nucleus could be stimulated by ROS to activate the complement pathway. IL: Interleukin; NLRP3: NOD-like receptor family pyrin domain containing 3 protein; NF-κB: Nuclear factor kappa-B; ROS: Reactive oxygen species; ASC: Apoptosis-associated speck-like protein.
Table 4 Summary of small molecule drugs targeting autophagy in the treatment of inflammatory diseases

<table>
<thead>
<tr>
<th>Names</th>
<th>Targets</th>
<th>Functions</th>
<th>Cell lines/diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC950</td>
<td>P2X7R</td>
<td>Inhibit NLRP3</td>
<td>Retinal microglia</td>
<td>Zheng et al, 2019</td>
</tr>
<tr>
<td>CY-09</td>
<td>Directly bound to the ATP-binding motif of the NLRP3 NACHT domain and inhibited the activity of NLRP3 ATPase</td>
<td>Inhibit NLRP3</td>
<td>Macrophage</td>
<td>Shen et al, 2021</td>
</tr>
<tr>
<td>Orlistat</td>
<td>Blocked the assembly and activation of NLRP3 inflammasome</td>
<td>Inhibit NLRP3</td>
<td>Macrophage</td>
<td>Xu et al, 2022</td>
</tr>
<tr>
<td>NLRP3-IN-18</td>
<td>Pyrazine compounds</td>
<td>Inhibit NLRP3</td>
<td>Still on study</td>
<td></td>
</tr>
<tr>
<td>Pyridazine derivatives</td>
<td>Direct inhibitor</td>
<td>Inhibit NLRP3</td>
<td>Alzheimer’s disease</td>
<td>Sabin et al, 2023</td>
</tr>
<tr>
<td>JT002</td>
<td>Decrease NLRP3-dependent proinflammatory cytokine production</td>
<td>Inhibit NLRP3</td>
<td>Neuroinflammation</td>
<td>Ambrus-Akel et al, 2023</td>
</tr>
<tr>
<td>Oxidized ATP</td>
<td>P2X7R</td>
<td>Inhibit NLRP3</td>
<td>Endothelial cells</td>
<td>Fang et al, 2011</td>
</tr>
</tbody>
</table>

NLRP3: NOD-like receptor family pyrin domain containing 3; 3-MA: 3-Methyladenine; ATP: Adenosine Triphosphate; NACHT: Nucleotide-binding and oligomerization domain.

Table 5 Summary of small molecule drugs targeting autophagy in the treatment of inflammatory diseases

<table>
<thead>
<tr>
<th>Names</th>
<th>Targets</th>
<th>Functions</th>
<th>Cell lines/diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MA</td>
<td>PI3K</td>
<td>Inhibited the formation of autophagosomes</td>
<td>HeLa cell</td>
<td>Kosic et al, 2021</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Autophagy and toll-like receptors</td>
<td>Inhibited activity of autophagy and TLRs</td>
<td>Macrophage</td>
<td>Chen et al, 2018</td>
</tr>
<tr>
<td>Bafilomycin A1</td>
<td>Autophagosome and lysosome</td>
<td>Blocking autophagy flow and lysosomal degradation pathway</td>
<td>Neurodegenerative diseases</td>
<td>Lee et al, 2021</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>mTOR</td>
<td>Autophagy agonists</td>
<td>Neurodegenerative diseases</td>
<td>Querfurth et al, 2021</td>
</tr>
<tr>
<td>EBSS</td>
<td>Regulates various ions and osmotic pressure during cell growth and metabolism</td>
<td>Maintained intracellular and intracellular balance, positively regulates autophagy</td>
<td>Nerve cell</td>
<td>Mohamed et al, 2014</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>GSK3β, regulates neurotransmitters</td>
<td>Positively regulated autophagy</td>
<td>Nerve cell</td>
<td>Grandjean et al, 2009</td>
</tr>
</tbody>
</table>

NLRP3: NOD-like receptor family pyrin domain containing 3; 3-MA: 3-Methyladenine; PI3K: Phosphatidylinositol 3-kinase; mTOR: Mammalian target of rapamycin; GSK3β: Glycogen synthase kinase-3β; EBSS: Earle’s balanced salt solution.

Further exacerbate Aβ deposition, leading to damage to RGCs. Inhibition of microglial autophagy enhances NLRP3 inflammasome activation[105]. Conversely, promoting autophagy inhibited NLRP3 inflammasome activation, suggesting that autophagy may reduce Aβ deposition and vision loss caused by RGC death by inhibiting the NLRP3 inflammasome[105].

Potential Therapeutic Agents for NLRP3 and Autophagy

Currently, small molecule drugs targeting NLRP3 and autophagy in cells had been developed, mainly based on cell experiments, animal experiments, and some clinical trials. Therapeutic agents for NLRP3 in RGCs included MCC950, a small molecule inhibitor that specifically inhibits the activation of NLRP3 inflammasome and can protect RGCs from death[104]. By directly inhibiting NLRP3, CY-09 may help protect RGCs from inflammation-mediated damage and maintain the normal function of nerve cells[105]. NLRP3-in-18 was a novel and highly selective NLRP3 inhibitor, and its effect was still under further study. Pyrazine derivatives could be used as NLRP3 inhibitors for the treatment of inflammation-related and neurodegenerative diseases such as asthma, chronic obstructive pulmonary disease, Parkinson’s disease, and Alzheimer’s disease[106] inhibited the inflammatory response in macrophages by blocking the NLRP3/caspase-1 pathway. Oxidized ATP could also inhibit NLRP3 by targeting P2X7R, thereby reducing nervous system inflammation[107]. JT002 was a novel NLRP3 inhibitor that effectively reduced the production of NLRP3-dependent pro-inflammatory cytokines and prevents pyrosis[108]. The functional roles of NLRP3 and autophagy and their potential in the treatment of RGC inflammation were summarized in Table 4[109-114].

Autophagy had a variety of inhibitors and activators. Inhibitors included 3-Methyladenine (3-MA), chloroquine, and bafilomycin A1[115-117]. Autophagy agonists included rapamycin, Earle’s equilibrium salt solution, and lithium chloride, which could activate the autophagy process and promote the degradation and recycling of intracellular substances[118-119]. The functional effects of autophagy and its potential in the treatment of RGC inflammation were summarized in Table 5[120-125].

Future Direction

RGC inflammation was a pathological process in retinal diseases that could lead to vision loss and retinal dysfunction. During this process, the activation of the NLRP3 inflammasome played a key role, which promoted the occurrence and development of the inflammatory response[126]. The NLRP3 gene was first identified and characterized in 2002, and it was considered to be a member of the NOD-like receptor family. Subsequently, the structure and function of NLRP3 were studied[127], and its relationship with inflammation was initially explored[128]. Over the next few years, the association between NLRP3 and inflammation was confirmed, and NLRP3 was found to play a crucial role in the inflammatory response, particularly in autoimmune diseases[129]. This led to an expert consensus that NLRP3 was a key molecule in the inflammatory pathway[130]. Subsequently, the crystal structure of NLRP3 and its ligand was published[131], revealing its specific activation mechanism. One study utilized gene-editing technology to create a mouse model...
lacking NLRP3\textsuperscript{[122]}, suggesting the crucial role of NLRP3 in inflammation during animal research\textsuperscript{[111]}. Since 2016, large-scale genetic research methods such as GWAS have been used to further confirm the association of NLRP3 with a variety of diseases\textsuperscript{[130]}. Potential treatments targeting NLRP3 were entering clinical trials to assess their effectiveness and safety\textsuperscript{[123]}. However, an excessive inflammatory response could lead to further damage and degeneration of RGCs. Autophagy, as a cytoprotective mechanism, had potential therapeutic significance in RGCs inflammation. By enhancing autophagy activity, it could promote the removal of intracellular waste, reduce inflammatory responses, and promote cell repair and regeneration\textsuperscript{[121-122]}. Therefore, regulating the autophagy process had the potential to become a new therapeutic strategy to protect RGCs from inflammatory damage. The interaction between NLRP3 and autophagy also provided a new perspective for the treatment of RGC inflammation. By regulating the balance between NLRP3 and autophagy, it may be possible to control the extent and timing of the inflammatory response, thereby reducing the damage of inflammation to RGCs\textsuperscript{[120]}. AMD was characterized by the degeneration and death of RGCs and RPE cells and was the main cause of vision loss in these patients. Chronic inflammation played an important role in the pathogenesis of AMD, among which the NLRP3 inflammasome played a driving role in inflammation in various diseases, and autophagy was also involved in the process\textsuperscript{[130]}. The synergistic action of these two factors may lead to the degeneration and necrosis of RGCs. In the chronic inflammatory state of RGCs stimulated by aging, the NLRP3 inflammasome and its related inflammatory factors, as well as the participation of retinal cell autophagy, all produce a cascade reaction, leading to further damage of normal ganglion cells and RPE cells around the retina\textsuperscript{[124]}. Therefore, this paper reviews the two important molecular mechanisms of RGC inflammation, namely NLRP3 and autophagy, and the relationship between NLRP3 and autophagy as well as their potential significance in treatment. At the same time, it summarized the possible inflammatory mechanisms and other mechanisms of AMD, a representative irreversible disease of retinal degenerative diseases. This was a comprehensive analysis of basic research on diseases that severely impair vision. The schematic diagram of NLRP3 targeted therapy and autophagy was shown in Figure 5.

AMD remained a difficult-to-treat disease, with numerous factors involved in its entire progression, including external and internal factors, and the timing of interventions further complicated its study. NLRP3 and autophagy were merely fragments of the intricate internal milieu of the body, and their interventions and the effects on their upstream and downstream pathways cannot completely prevent complications from arising. In addition, compared with wet AMD, dry AMD was more related to the apoptosis of RGCs, photoreceptors, and RPE cells, which was an irreversible process, so there were still many things to explore.

CONCLUSION

In summary, the NLRP3 inflammasome was closely linked to autophagy, and their interaction affected the degree of pyroptosis in RGCs, which in turn impacts the occurrence and development of RGC inflammation. The primary challenges of conducting in vitro research on RGCs encompass the significantly reduced survival rate and proliferation capacity of these cells, as well as the potential loss of their inherent physiological traits and functions. Furthermore, accurately replicating the natural growth environment of the cells poses a significant hurdle in such studies. The NLRP3 inflammasome promoted the onset and development of inflammatory responses. Conversely, pathological changes such as drusen and lipofuscin deposition generated in related diseases could further activate the NLRP3 inflammasome, promoting the secretion of inflammatory factors. Autophagy could affect the course of disease by inhibiting or activating the NLRP3 inflammasome. The interaction between autophagy and NLRP3 and its relationship with AMD may provide new insights into exploring the treatment of AMD, opening up new hopes for...
Ganglion cell inflammation

clinical practice. In the future, further research could explore specific drugs or therapies targeting NLRP3 and autophagy to achieve effective treatment of RGC inflammation.

ACKNOWLEDGEMENTS

Authors’ contributions: All authors contributed to study conception and design and definition of intellectual content. Wang XL was responsible for data analysis and manuscript preparation. Gao YX, Yuan QZ, and Zhang M revised the article critically for important intellectual content. Zhang M is guarantor of this work, who had full access to all the data in this study and take responsibility for the integrity and accuracy of the data. All authors approved the final version of the manuscript.

Foundations: Supported by the Project of Sichuan Medical Association (No.S22058); National Key R&D Project (No.2018YFC1106103).

Conflicts of Interest: None.

REFERENCES

24. Zhou T, Yang ZQ, Ni BY, et al. IL-4 induces reparative phenotype of RPE cells and protects against retinal neurodegeneration via Nrf2 activation. *Cell Death Dis* 2022;13(12):1056.
27. Colares TG, de Figueiredo CS, de Oliveira Jesus Souza L, dos Santos
AA, Giestal-de-Araujo E. Increased retinal ganglion cell survival by exogenous IL-2 depends on IL-10, dopamine D1 receptors, and classical uIL-2/IL-2R signaling pathways. *Neurochem Res* 2021;46(7):1701-1716.


36 Lechner J, Chen M, Hogg RE, Toth L, Silvestri G, Chakravarthy U, Xu HP. Peripheral blood mononuclear cells from neovascular age-related macular degeneration patients produce higher levels of chemokines CCL2 (MCP-1) and CXCL8 (IL-8). *J Neuroinflammation* 2017;14(1):42.


40 Iglicki M, Khoury M, Donato L, Quispe DJ, Negri HP, Melamud JI. Comparison of subretinal aflibercept vs ranibizumab vs bevacizumab in the context of PPV, pneumatic displacement with subretinal air and subretinal tPA in naïve submacular haemorrhage secondary to nAMD. The Submarine Study. *Eye (Lond)* 2024;38(2):292-296.


Ganglion cell inflammation


78 D’Souza S, Lang RA. Retinal ganglion cell interactions shape the developing mammalian visual system. *Development* 2020;147(23):dev196535.


83 Voigt AP, Mulfaul K, Mullin NK, et al. Single-cell transcriptomics of the human retinal pigment epithelium and choroid in health and


107 Han JX, Xu TS, Fang QJ, Zhang HJ, Yue LJ, Hu G, Sun LY. Quercetin hinders microglial activation to alleviate neurotoxicity via the interplay between NLRP3 inflammasome and mitophagy. Redox Biol 2021;44:102010.


123 Querfurth H, Lee HK. Mammalian/mechanistic target of rapamycin (mTOR) complexes in neurodegeneration. Mol Neurodegener 2021;16(1):44.


