• Review Article •

Progress of autophagy in the pathogenesis of dry age-related macular degeneration

Wen Gao, Feng–Ming Liang

Department of Ophthalmology, the First Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300000, China

Correspondence to: Wen Gao. Department of Ophthalmology, the First Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300000, China. gaowenxiuwen@163.com

Received: 2020-03-09 Accepted: 2021-01-15

Abstract

● Age-related macular degeneration (AMD) is a major clinical blind-inducing eye disease, and its pathogenesis is closely related to the autophagy of RPE cells and the signaling pathway of nuclear factor erythroid-2 related factor 2 (Nrf2). Autophagy is one of the common and important physiological phenomena in human body, which is of vital significance for maintaining the stability and metabolism of cells. Nrf2 is a key transcription factor regulating cells to fight against foreign bodies and oxidative damage, and Nrf2 signaling pathway plays a wide range of cell protective functions in anti-tumor, anti-stress and other aspects. With the development of research, it is found that there are extensive interaction mechanisms between autophagy and Nrf2 signaling pathway. Inhibition of autophagy leads to accumulation of p62, which activates the Nrf2 signaling pathway by binding with Keap1 (kelch-like ech-associated protein1). At the same time, studies have also found that reactive oxygen species (ROS) and other factors also participate in the mutual regulation between autophagy and Nrf2.This paper will review the recent research progress on the interaction between Nrf2 signaling pathway and autophagy in the development of AMD. Hope to provide a new perspective for the treatment of AMD.

● **KEYWORDS:** autophagy; Nrf2; p62; reactive oxygen species

DOI:10.18240/ier.2021.04.10

This article is based on a study first reported in the *Guoji Yanke Zazhi (Int Eye Sci)* 2021;21(3):402-405.

Citation: Gao W and Liang FM. Progress of autophagy in the pathogenesis of dry age-related macular degeneration. *Int Eye Res* 2021;21(4):233-236

INTRODUCTION

Age-related macular degeneration (AMD) is a major clinical blinding eye disease, affecting 30 million to 50 million AMD patients worldwide, including over 5 million in China, and its prevalence rate is increasing year by year with an aging population[1-2]. AMD is divided into atrophic (dry) and exudative (wet) types clinically. The dry type of AMD is the most common type clinically, with a prevalence rate of 85%-90%, and its pathological signs include glass membrane warts (drusen) deposition, retinal pigment epithelium (retinal pigment epithelium, RPE) degeneration and geographic atrophy (geographic atro-phy, GA); Although dry AMD progresses slowly, it can still progress to wet AMD, causing irreversible visual impairment, and there is no recognized treatment drugs, so it is still a difficult problem in ophthalmology to explore the pathogenesis of dry AMD and find a therapeutic method that is reasonable and economic. In recent years, it has been found that the reduction of autophagy function of RPE cells plays a crucial role in the pathogenesis of dry AMD. Autophagy is closely related to Nrf2 signaling pathway, and its central molecule is ubiquitin binding protein P62, which is expected to become a new target for drug control of dry AMD[3-4].

Lack of Autophagy in RPE Cells is an Important Part of the Pathogenesis of Dry AMD The pathogenesis of dry AMD has been a focus of attention in the field of ophthalmology. Oxidative stress, hemodynamics, inflammatory immunology and genetic inheritance have been studied for many years, and each mechanism has been proved to be highly correlated with the occurrence and development of dry AMD, suggesting that dry AMD is a chronic degenerative disease mediated by multiple factors. In recent years, studies on the correlation between autophagy in RPE cells and the pathogenesis of dry AMD have drawn wide attention[5].

Autophagy is an important cell homeostasis process that ensures the physiological turnover of senile and damaged organelles. It plays an important role in maintaining the body's metabolism, which can update peroxides, longevity proteins

and mitochondria, remove damaged organelles and metabolites in the cytoplasm, reconstruct sub-cellular level, and protect damaged cells[6].

RPE cells have a typical autophagy ability, which can constantly devour the detached retinal photoreceptor outer ganglion membrane disc, playing a role of "scavenger". RPE cells absorb light and reduce light scattering, thus protecting tissues from oxidative damage, maintaining the visual cycle and providing nutrients. Decreased autophagy activity of RPE cells can lead to the accumulation of abnormal proteins and lipobrowcin in the eyes, triggering the production of reactive oxygen species (ROS) and protein aggregation, which can not only lead to the formation of dry AMD, but also activate inflammatory responses, accelerate cell aging, and further accelerate the process of dry AMD[7].

The specific process of autophagy is divided into four steps: autophagy induction/initiation; Nucleation of solitary membrane; Extended solitary membrane and closed (elongation and closure); Degradation. Each of these steps has its key regulatory proteins[8]. Among them, the initial stage requires the involvement of the unc51-like kinase (ULKl) complex, which is directly regulated by the autophagy central regulatory molecule mTOR. During the stress of hunger, mTOR activity is inhibited, thus initiating the first step of autophagy. When nutrients are sufficient, mTOR inhibits autophagy activity by inhibiting ULKl activity[9]. Solitary membrane nucleation stage refers to the aggregation and binding of the solitary membrane around the material to be degraded, at this stage the key regulatory proteins are spiral, membrane protrusions sympathetic BCL2 protein (coiled coil, moesin-like BCL2-Intel-acting protein, Beclin 1), Vsp34 and Atgl4 protein complexes. Beclin-1-vsp34-atgl4 complex can also bind to a variety of autophagy related proteins during autophagy, transmitting autophagy signals and promoting autophagy[10]. Atgl2-atg5-atgl6 binding system is required to promote the prolongation and closure of autophagosome membrane during the prolongation and closure of solitary membrane[11]. The degradation stage relies on autophagosome and lysosomaln association and fusion to form autophagy lysosomes. After the autophagy substrate is degraded, it receives new lysosomal hydrolases, and eventually forms mature lysosomes to complete lysosomal regeneration[12]. Cathepsins (CTS) are important proteases in lysosomes. CTSA, B, D, E and S have been found in RPE cells. Studies have shown that ctsd-deficient mice exhibit retinal degenerative changes[13].

Under oxidative stress, autophagosomes produced more markers and changed the expression of LC3 and P62 in RPE cells[14]. P62, as the downstream degradation substrate of autophagy pathway, is identified and encapsulated after the formation of vesicles to form autophagy vesicles, which are then degraded by lysosomes and encapsulated[15]. P62 mediates cell survival, apoptosis and autophagy through signal transduction, and can select autophagy receptors, which is very important for regulating autophagy effect[16-17]. In a word, reduced autophagy of RPE cells is not only a key link in the pathogenesis of dry AMD, but also a leading factor in accelerating the course of dry AMD.

Correlation Between Nrf2 Signaling Pathway and Dry AMD Oxidative stress plays an important role in the occurrence and development of dry AMD[10]. RPE has a set of endogenous antioxidant defense system, which is composed by a series of antioxidant genes, including SOD1, SOD2, Nrf2, catalase *etc.* Nrf2 belongs to the CNC alkaline leeucine zipper transcription activator family, and it is an important transcription factor regulating oxidative stress *in vivo* for self-protection. Nrf2 is expressed in almost all tissues, and Keapl protein is the main protein that affects the function of Nrf2. At rest, Nrf2 and its molecular partner (kelch-likeech-associated protein-1 (keap-1) were coupled to form a stable dimer that existed in the cytoplasm and was inactivated. Under the condition of oxidative stress, Nrf2 is activated after being separated from keap-1, dissociates from the dimer and undergoes nuclear metastasis, enters the nucleus and binds with the antioxidant response element (ARE), up-regulates hemo oxygenase 1 (ho-1) and P62 protein. Thus, ROS clearance can enhance the antioxidant capacity of cells, which can reduce the damage caused by oxidative stress to cells, tissues and organs[18]. In addition to the generally accepted keap1-mediated regulation, some studies have also found that Nrf2 can also spontaneously respond to external signals, and non-keap1 relies on the innervation of the nucleus to perform transcriptional functions[19].

Interaction Between Autophagy and Nrf2 Signaling Pathway

P62 protein is the bridge connecting autophagy and Nrf2 signaling pathway Studies have confirmed that Nrf2 signaling pathway can reduce inflammatory response, improve mitochondrial function, stimulate autophagy, and reduce the pathophysiological level of dozens of chronic inflammatory diseases, which has certain value for the prevention and treatment of many common chronic diseases. Nrf2 is an important transcription factor that has an anti-oxidative stress function in cells. SQSTMl (sequesto-some 1), also known as p62, is an intracellular protein induced by stress and acts as a multifunctional protein for selective autophagy. P62 protein is the bridge between autophagy and Nrf2, and its KIR domain is similar to Nrf2's ETGE or DLG domain, When autophagy is inhibited, so is the degradation of p62, and p62 is accumulated in large quantities. The accumulated p62 protein competitively binds to Nrf2 Keap1, causing Nrf2 to dissociate from Keap1,

while inhibiting keap1-cul3-e3 ubiquitin ligase complex to cause Nrf2 to undergo ubiquitination degradation and activate Nrf2. The activated Nrf2 will promote the expression of p62, thus forming a positive feedback loop of antioxidant reaction[10]. In recent years, experiments have found that isodeoxydistantin can activate autophagy through nrf2-p62-keap1 positive feedback loop, and Nrf2 signaling pathway can also regulate autophagy. Autophagy regulates Nrf2 signaling pathway through p62-dependent regulation, which can affect the occurrence and development of a variety of diseases. In mice knocked out by Atg7(autophagy related 7), p62 accumulation was positively correlated with Nrf2 activation. Multiple studies have found that Keap1 inactivation mediated by p62 activates Nrf2, which can cause liver injury, liver fibrosis, and liver cancer[20]. In addition to its close relationship with liver related diseases, p62 is also involved in other pathophysiological processes. For example, fenofibrate is a highly effective lipid-lowering drug. Studies have found that fenofibrate degrades Keap1 through p62-dependent autophagy, thus activating Nrf to remove reactive oxygen species[21]. P62 provides dual, mutually reinforcing protection for RPE cells from protein misfolding and aggregation caused by environmental stress by promoting autophagy and nrf2-mediated antioxidant response, which may be a potential therapeutic target for dry AMD[4].

The role of reactive oxygen species (ROS) in autophagy and Nrf2 signaling pathways ROS is a general term for a class of active oxygen-containing compounds produced by aerobic metabolism, including superoxide anions, free radicals and peroxides. Studies have found that during starvation, ROS activates autophagy through the type III pi3k-m TOR signaling pathway, which is conducive to cell survival. At the same time, ROS induced oxidative stress activates Nrf2 through the PI3K signaling pathway to promote the elimination of the products of oxidative stress[22]. The effect of ROS on autophagy and Nrf2 signaling pathway is related to the development of various diseases. According to a study in human lung adenoma cells A549, ROS induced by low doses of alpha particle radiation promotes autophagy and the activation of Nrf2 signaling pathways, Nrf2 signaling pathways promote the resistance of radiation, and the Nrf2 signaling pathways can be inhibited by removing ROS or inhibiting autophagy. It is speculated that ROS inducing autophagy and increasing Nrf2 signaling pathway is an influence factor of the resistance of radiation[23]. During ROS exposure, the Nrf2 signaling pathway and autophagy in pancreatic cancer cells are negatively regulated by each other, suggesting that the mechanism may be that autophagy inhibition not only activates the Nrf2 signaling pathway by increasing the accumulation of p62, but also activates the Nrf2 signaling pathway by excessive ROS[24].

Oxidative stress is involved in pigment disorders, intracellular lipofuscin accumulation, extracellular glass membrane wart formation and other processes[25], which plays an important role in the development of AMD. Oxidative stress includes electron leakage of mitochondrial electron transport chains and the formation of reactive oxygen species (ROS) such as hydroxyl radicals, superoxides and hydrogen peroxide[26]. The imbalance between the production and clearance of these ROS eventually leads to cell damage[27], and autophagy is a key factor to maintain this balance. Once the balance is broken, RPE cells will be damaged or even die. The autophagy ability decreases with the accumulation of lipofuscin, which can accelerate the production of ROS and protein aggregation. The accumulation of lipofuscin in lysosomes of RPE cells, the formation of extracellular glass vermicular and chronic inflammation can stimulate the active inflammation reaction, further damage RPE and accelerate the aging process[28]. Activated Nrf2 signaling pathway plays a therapeutic role in AMD by removing ROS[10]. In conclusion, it can be known that there is a complex regulatory mechanism between autophagy and Nrf2, and many molecular mechanisms are still unclear, so it is worth further exploring the relationship between them.

CONCLUSION

There is a wide connection between autophagy and Nrf2 signaling pathway, and in-depth study of the correlation plays an important role in the pathological mechanism and clinical treatment of dry macular degeneration. P62, ROS and other factors may be important conditions involved in the mutual regulation of autophagy and Nrf2 signaling pathway of RPE cells in dry macular degeneration. This target can provide a new idea for the prevention and treatment of dry macular degeneration, but the relationship between Nrf2 signaling pathway and autophagy is complex, how to influence each other remains to be explored through more experiments, providing theoretical guidance for clinical application[29].

ACKNOWLEDGEMENTS

Foundations: Supported by National Natural Science Foundation of China (No.81373694).

Conflicts of Interest: Gao M, None; **Liang FM,** None.

Peer Review File Available at: http://ier.ijo.cn/gjykier/ch/reader/download_attache_file.aspx?seq_id=20211227115242001&flag=1&journal_id=gjykier&year_id=2021&issue=4

REFERENCES

1 Leasher JL, Braithwaite T, Furtado JM, Flaxman SR, Lansingh VC, Silva JC, Resnikoff S, Taylor HR, Bourne RRA. Prevalence and causes of vision loss in Latin America and the Caribbean in 2015: magnitude, temporal trends and projections. *Br J Ophthalmol* 2019;103(7):885-893.

2 Zhang JJ, Xu F. Research progress in the clinical treatment of age-related macular degeneration. *Medical Recapitulate* 2016;22(12): 2352-2355.

3 Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. *Biochim Biophys Acta* 2014;1841(1):108-120.

4 Wang Y, Zhang J, Huang ZH, Huang XH, Zheng WB, Yin XF, Li YL, Li B, He QY. Isodeoxyelephantopin induces protective autophagy in lung cancer cells via Nrf2-p62-keap1 feedback loop. *Cell Death Dis* 2017;8(6):e2876.

5 Josifovska N. Albert R. Nagymihály R. Lytvynchuk L. Moe MC. Kaarniranta K. Veréb ZJ. Petrovski G. resveratrol as inducer of autophagy, pro-survival, and anti-inflammatory stimuli in cultured human RPE cells. *Int J Mol Sci* 2020,21(3):813.

6 Sun T. Long noncoding RNAs act as regulators of autophagy in cancer. *Pharmacol Res* 2018;129:151-155.

7 Kaarniranta K, Sinha D, Blasiak J, Kauppinen A, Veréb Z, Salminen A, Boulton ME, Petrovski G. Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. *Autophagy* 2013;9(7):973-984.

8 So KY, Oh SH. Cadmium-induced heme-oxygenase-1 expression plays dual roles in autophagy and apoptosis and is regulated by both PKC-δ and PKB/Akt activation in NRK52E kidney cells. *Toxicology* 2016;370:49-59.

9 Ndayisaba A, Wenning GK. Inhibition of the mammalian target or rapamycin (mTOR):a potential therapeutic strategy for multiple system atrophy. *Clin Auton Res* 2020;30(1):7-8.

10 Liu B, Deng X, Jiang Q, Li G, Zhang J, Zhang N, Xin S, Xu K. Scoparone improves hepatic inflammation and autophagy in mice with nonalcoholic steatohepatitis by regulating the ROS/P38/Nrf2 axis and PI3K/AKT/mTOR pathway in macrophages. *Biomed Pharmacother* 2020;125:109895.

11 Codogno P, Mehrpour M, Proikas-Cezanne T. Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat Rev Mol Cell Biol* 2012;13(1):7-12.

12 Nakatogawa H. Autophagic degradation of the endoplasmic *Reticulum*. *Proc Jpn Acad Ser B Phys Biol Sci* 2020;96(1):1-9.

13 Im E, Kazlauskas A. The role of cathepsins in ocular physiology and pathology. *Exp Eye Res* 2007;84(3):383-388.

14 Li C, Guan XM, Wang RY, Xie YS, Zhou H, Ni WJ, Tang LQ. Berberine mitigates high glucose-induced podocyte apoptosis by modulating autophagy via the mTOR/P70S6K/4EBP$_1$ pathway. *Life Sci* 2020;243:117277.

15 Barth S, Glick D, MacLeod KF. Autophagy: assays and artifacts. *J Pathol* 2010;221(2):117-124.

16 You ZY, Xu YF, Wan W, Zhou L, Li J, Zhou TH, Shi Y, Liu W. TP53INP$_2$ contributes to autophagosome formation by promoting LC3-ATG7 interaction. *Autophagy* 2019;15(8):1309-1321.

17 Chang HZ, Liu XD. Research Progress of Nrf2/Keap1/ARE Signaling Pathway and Autophagy in Nucleus Pulposus Cells. *Medical Recapitulate* 2019;18(25)3567-3572.

18 Oliveira AS, Sousa E, Vasconcelos MH, Pinto M. Curcumin: a natural lead for potential new drug candidates. *Curr Med Chem* 2015;22(36):4196-4232.

19 Jiang ZY, Chu HX, Xi MY, Yang TT, Jia JM, Huang JJ, Guo XK, Zhang XJ, You QD, Sun HP. Insight into the intermolecular recognition mechanism between Keap1 and IKKβ combining homology modelling, protein-protein docking, molecular dynamics simulations and virtual alanine mutation. *PLoS One* 2013;8(9):e75076.

20 Li DL, Gao J. Regulation of Nrf2/ARE pathway by autophagy and its role in liver disease. 2016.

21 Park JS, Kang DH, Lee DH, Bae SH. Fenofibrate activates Nrf2 through p62-dependent Keap1 degradation. *Biochem Biophys Res Commun* 2015;465(3):542-547.

22 Jiang XM, Hu JH, Wang LL, Ma C, Wang X, Liu XL. Ulinastatin alleviates neurological deficiencies evoked by transient cerebral ischemia via improving autophagy, Nrf-2-ARE and apoptosis signals in *Hippocampus*. *Physiol Res* 2018;67(4):637-646.

23 Chen N, Wu L, Yuan H, Wang J. ROS/autophagy/Nrf2 pathway mediated low-dose radiation induced radio-resistance in human lung adenocarcinoma A549 cell. *Int J Biol Sci* 2015;11(7):833-844.

24 Zhang L, Li J, Ma J, Chen X, Chen K, Jiang Z, Zong L, Yu S, Li X, Xu Q, Lei J, Duan W, Li W, Shan T, Ma Q, Shen X. The relevance of Nrf2 pathway and autophagy in pancreatic cancer cells upon stimulation of reactive oxygen species. *Oxid Med Cell Longev* 2016;2016:3897250.

25 Zhu DL, Xie MY, Gademann F, Cao JX, Wang PY, Guo YL, Zhang L, Su T, Zhang J, Chen JS. Protective effects of human iPS-derived retinal pigmented epithelial cells on retinal degenerative disease. *Stem Cell Res Ther* 2020;11(1):1-15.

26 Alexeyev MF. Is there more to aging than mitochondrial DNA and reactive oxygen species? *Febs J* 2009;276(20):5768-5787.

27 Biarnés M, Monés J, Alonso J, Arias L. Update on geographic atrophy in age-related macular degeneration. *Optom Vis Sci* 2011;88(7):881-889.

28 Kim KH, Lee MS. Autophagy-a key player in cellular and body metabolism. *Nat Rev Endocrinol* 2014;10(6):322-337.

29 Gao M, Liang FM. Progress of autophagy in the pathogenesis of dry age-related macular degeneration. *Guoji Yanke Zazhi (Int Eye Sci)* 2021;21(3):402-405.