

Autophagy: a potential target for the treatment of intraocular neovascularization

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Abstract

• **The formation of neovascularization is a common pathological feature of many ocular vascular diseases, and is an important cause of vision loss in patients. Neovascularization can cause retinal hemorrhage, vitreous hemorrhage, and other serious complications, leading to loss of vision. The treatment of intraocular neovascularization is the focus of ophthalmology research. In recent years, some studies have found that autophagy is closely related to vascular endothelial growth factor and the formation of neovascularization. Autophagy is expected to become a new target for the treatment of intraocular neovascularization. Therefore, this article reviews the research on autophagy and the formation of intraocular neovascularization.**

• **KEYWORDS:** autophagy; angiogenesis; vascular endothelial growth factor

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INTRODUCTION

The formation of neovascularization is a common pathological feature of many ocular vascular diseases, such as proliferative diabetic retinopathy, age-related macular degeneration, ischemic retinal vein occlusion, retinopathy of prematurity. In recent years, many studies have found that autophagy is closely related to the formation of neovascularization^[1-2]; autophagy is expected to become a new target for the treatment of intraocular neovascularization. Therefore, this article reviews the research on autophagy and the formation of intraocular neovascularization.

Autophagy is a key mechanism of self-protection to maintain normal cellular function and stable internal environment. And it is closely related to aging, oxidative damage protection and malignant tumor cells proliferation and neurodegenerative diseases. Autophagy can degrade the catabolism pathway of macromolecular substances in the cell and damaged organelles that maintaining metabolic balance and cell environment stabilization. This process can provide energy for poor nutrition status of cells, and it is mainly adjusted by autophagy related gene^[3]. Autophagy process are maintained at a basic level in all cells, but it can be activated when cells are lack of nutrition, oxidative stress, hypoxia, endoplasmic reticulum stress, and so on^[4-5].

Autophagosome is the cytoplasm of bubble that formed by degradation of the material wrapped by double membrane, and it will merge with lysosomes to form autophagy-lysosome, then the degradable material is digested and hydrolyzed by lysosomal acidic hydrolysis enzyme, and will be released back into the cytoplasm. This process can realize some organelles cyclic utilization and update. Autophagy has great significance to maintain cellular homeostasis.

It has been found more than 30 specific genes associated with autophagy [autophagy-related genes (Atg)]. Atg coding protein involved in the stages of formation of autophagosome in the form of collaborative^[6]. By detecting Atg and its related protein expression level can evaluate the level of autophagy^[7-9]. In organism, there exists a certain degree of autophagy in physiological and pathological conditions, and it plays a protection and restoration for cells in the normal range. However, if autophagy is over activated, it will cause the cells injury and death. Autophagy include three forms, macroautophagy, microautophagy and chaperone-mediate autophagy (CMA). For molecular partner mediated autophagy, it does not need vesicles in transportation, and no autophagosome is formed^[10].

STUDY ON THE RELATIONSHIP BETWEEN AUTOPHAGY AND VEGF

Vascular endothelial growth factor (VEGF) is the key cytokine to promote the formation of neovascularization. VEGF can promote vascular endothelial cell division, proliferation and increase vascular permeability. There are VEGF high affinity binding sites on endothelial cells, which can directly act on vascular endothelial cells and significantly promote the

mitosis of vascular endothelial cells^[11]. In clinical studies, it is also found that inhibiting VEGF can effectively inhibit the formation of neovascularization in the eye, and anti-VEGF therapy has become the primary means of treating neovascularization in the eyes. In recent years, several studies have confirmed that autophagy and VEGF are interrelated.

Chu *et al*^[12] have found that the activation of autophagy of the retinal pigment epithelial (RPE) cells in smokers promoted the expression of VEGF, which may protect RPE cells and reduce cell apoptosis. Cigarette smoking extract (CSE) affects cell vitality and induces expression of VEGF and its receptor. Exogenous VEGF plays a role in alleviating CSE induced RPE cell death by increasing autophagy fluxes. Ye *et al*^[13] have found that malondialdehyde (MDA) could increase the expression of VEGF in RPE-19 cells, while this process can be inhibited by autophagy-lysosome inhibitor. However, Miaomiao *et al*^[14] have found that in the renal podocyte the activation of autophagy reduced the expression of VEGF and inhibition of autophagy increased the expression of VEGF. Podocyte is the main cells producing VEGF in the kidney. Studies have found that high sugar can induce the VEGF expression and reduce the cell vitality in the podocytes. The expression of VEGF can be suppressed by rapamycin (an autophagy activator), while the expression of VEGF was increased after the treatment of 3-MA (an autophagy inhibitor) in the podocytes. Wang *et al*^[15] have found that VEGF could up-regulate the expression of autophagy associated protein Beclin-1 and LC3B, which suggest that VEGF could activate cell autophagy. In addition, the VEGF-C/Neuropilin-2 (NRP-2) signaling pathway is involved in the activation of autophagy, which helps the cancer cells self-protection, allowing cancer cells to survive after the drug treatment^[16]. Therefore, there may be a variety of interactions between autophagy and VEGF, and its specific function and mechanism should be further studied.

AUTOPHAGY AND NEOVASCULARIZATION IN THE EYE

In recent years, several studies have found that autophagy is closely associated with the formation of intraocular neovascularization. Inhibition of autophagy can inhibit cell migration and tube formation in aortic endothelial cells, and can also inhibit the formation of neovascularization induced by VEGF^[1]. Beclin-1 is a core protein in autophagy, which is homologous with Atg6, and plays a key role in autophagy regulation^[17]. Beclin-1 shRNA can block the cell migration and tube formation induced by VEGF^[11]. Cell autophagy can be activated in the condition of nutritional deficiency, oxidative stress, hypoxia, and endoplasmic reticulum stress^[4-5]. The level of cell autophagy can be activated under hypoxia conditions. Hypoxia inducing factor 1 (HIF-1) is one of the important genes that regulate cell oxygen concentration, and play an important role in the process. The main mechanism is

that HIF-1 activate BNIP3; BNIP3 and Beclin-1 competitively binding bcl-2 and release Beclin-1 to activate autophagy^[18-22]. Li *et al*^[23] have found that hypoxia can activate rhesus monkeys choroid retinal vascular endothelial cells (RF/6A) autophagy, promote vascular endothelial cell migration and tube formation. Inhibition autophagy can suppress RF/6A cell migration and tube formation *in vitro*. It is suggested that autophagy plays an important role in the formation of hypoxia induced angiogenesis.

The expression of chemerin, one of adipokines, was elevated in serum and vitreous humors of diabetic retinopathy, and was associated with proliferative diabetic retinopathy^[24-25]. Du *et al*^[26] have found that chemerin may activate autophagy of the RF/6A cells *in vitro*, promote the cells proliferation, migration and tube formation; the autophagy inhibitor 3-MA can inhibit cell proliferation, migration and tube formation. This indicated autophagy participate in retinal neovascularization formation that induced by chemerin. Du *et al*^[27] have found that high sugar can activate autophagy of the RF/6A cells by promoting the expression level of reactive oxygen species (ROS), promote the formation of neovascularization *in vitro*, and 3-MA can inhibit cell migration and tube formation. These studies suggest that autophagy may be involved in the formation of neovascularization in the retina of diabetic patients. Tumor necrosis factor- α (TNF- α) is a kind of inflammatory factor, has close relationship with the formation of neovascularization. Studies have found that TNF- α can promote cell proliferation, migration and tube formation by activating the RF/6A cell autophagy, inhibiting cell proliferation, migration and tube formation by autophagy. Autophagy activation may also involved in the formation of the inflammation induced retinal neovascularization^[28].

Fu *et al*^[29] have found that autophagy may plays a dual role in diabetic retinopathy. Their results showed that heavily-oxidised glycated low density lipoprotein (HOG-LDL) 50 mg/L treatment human retinal capillary pericytes induced autophagy, without changing cell vitality, and inhibiting autophagy would reduce cell survival. HOG-LDL 100-200 mg/L treatment causes obvious cell death. Inhibiting autophagy can promote apoptosis. In the retinal of diabetic rats, injection of 50 mg/L HOG-LDL in the vitreous caused autophagy and endoplasmic reticulum stress without causing apoptosis while the injection of 200 mg/L caused more obvious endoplasmic reticulum stress and apoptosis. Therefore, autophagy plays a dual role in diabetic retinopathy. It is protective under moderate stimulation (50 mg/L HOG-LDL), but it promotes cell death in more severe stimuli (200 mg/L HOG-LDL).

In diabetic retinopathy, Müller cells are the main cells that produce VEGF, and VEGF is an important promoting factor for diabetic retinopathy. Lopes de Faria *et al*^[30] have found that rat retinal Müller cells were exposed to high glycemic

environment could induce the increase of early and late autophagy markers. Under high sugar, inhibition of autophagy can promote Müller cell apoptosis. In high sugar, Müller cells can be protected from apoptosis when treated with rapamycin. Rapamycin activates autophagy and prevents VEGF release. In the animal model of diabetes, the expression of autophagy associated proteins in the retina increased compared with the control group. The results of this study confirmed that high sugar can increase autophagy, but due to lysosomal dysfunction, cause a large number of VEGF release and Müller cells death. Lysosome damage and autophagy dysfunction happened in early stage of diabetic retinopathy. Therefore, autophagy may be a new target for the research and treatment of diabetic retinopathy.

AUTOPHAGY AND VASCULAR INHIBITOR DRUG RESISTANCE

In recent years, several studies have shown that vascular inhibitor can lead to the activation of autophagy, including ranibizumab, bevacizumab and several other vascular inhibitors^[31-33]. Ranibizumab and bevacizumab are VEGF inhibitors, which can significantly inhibit the formation of blood vessels and play an important role in the treatment of neovascularization in the eye. Anti-angiotherapy is also widely used in tumor therapy. Guo *et al*^[34] have found that bevacizumab therapy can cause liver cancer SMMC-7721 cells autophagy related proteins Beclin-1 and LC3 increased. Inhibiting autophagy of hepatoma cells under low oxygen culture can reduce cell vitality and promote cell apoptosis. Combination of bevacizumab and autophagy inhibitor can significantly inhibit tumor growth and promote apoptosis of tumor cells. Hu *et al*^[35] have also found that bevacizumab can raise the level of malignant glioma cell autophagy, combined with bevacizumab and autophagy inhibitors can promote cell death. *In vivo* studies also confirmed that combination of bevacizumab and autophagy inhibitors can significantly inhibit the growth of malignant glioma. These results show that combining autophagy inhibitors may help to improve the resistance of the anti-angiogenesis therapy. Another study showed that angiogenesis inhibitors can induce endothelial cells autophagy, accelerate metabolism, and provide the cells necessary nutrition in hungry condition. This process will help the endothelial cells passed through the dangerous period, while lead to a relapse of neovascularization. Blocking autophagy by knockout Beclin-1 can significantly increase endothelial cell apoptosis. These results suggest that in the treatment of neovascular diseases, autophagy can serve as a new target to improve the therapeutic effect of angiogenesis inhibitors^[2]. In the treatment of intraocular neovascularization, whether bevacizumab and other vascular inhibitors increase the autophagy level of endothelial cells were still not clear. Whether the activation of autophagy is involved in the

protective mechanism of endothelial cells, and cause the recurrence of neovascularization were still unclear. It is still unclear whether the combination of autophagy inhibitors and vascular inhibitors can better inhibit neovascularization. These are worthy of our further study.

PROSPECT

In recent years, the research on autophagy and neovascularization has made some progress. However, the mechanism of autophagy in the neovascularization of the eye is still unclear. By regulating autophagy, we may be able to better control the development of intraocular neovascularization. In addition, autophagy may play an important role in the relapse and drug resistance mechanism after vascular inhibitor treatment. It is expected to improve the resistance of vascular inhibitors by targeting autophagy. However, most studies on autophagy and intravitreal neovascularization are still in the primary research stage, and further research will be needed by ophthalmologists.

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