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Chapter

Deficient Autophagy Contributes to the Development of Diabetic Retinopathy

Jacqueline M. Lopes de Faria and Marcella Neves Dátilo

Abstract

Autophagy is a self-degradation process essential to maintain intracellular homeostasis and cell survival, controlling elimination of pathogens, damage to organelles, and nutrient recycling to generate energy. Alterations in autophagic flux have been reported in the mechanisms of several diseases such as neurodegenerative diseases, cancer, diabetes mellitus, and its associated complications. Diabetic retinopathy (DR) is a microvascular complication of diabetes, affecting nearly 30% of diabetic patients. Several pathways are triggered and repressed in the development of DR, and autophagy showed to be relevant in the pathogenesis of this devastating complication. In this chapter, autophagy’s involvement in the development and progression of DR will be discussed, mainly in retinal pigmented epithelial cells and retinal microvascular endothelial cells, as well as in Müller cells—the more prominent retinal glial cell.

Keywords: retina, diabetic retinopathy, autophagy, ARPE-19, endothelial cell, Müller cell

1. Introduction

Autophagy (from Greek, meaning “self-eating”) refers to a highly conserved process in eukaryotic cells, which coordinates the degradation of intracellular components and nutrient recycling. This process is essential for cellular homeostasis, survival, and differentiation. In basal conditions, the autophagic process happens in low levels to maintain cellular homeostasis. However, in such conditions as low levels of adenosine triphosphate (ATP) or depletion of essential amino acids and glucose, autophagic flux can increase to generate energy and raise basal levels. More recently, the understanding of this process has gained attention due to its pivotal role in cellular physiology and a variety of diseases from cancer, chronic degenerative diseases, and immune diseases (Table 1).

Autophagy is a primary cell response to stress and can be induced by starvation, endoplasmic reticulum (ER) stress, hypoxia, cytotoxicity, and infection (Figure 1). Sensation, initiation, and regulation of the autophagy–lysosomal pathway is controlled by the heterotrimeric serine/threonine kinase AMP (AMPK) and rapamycin complex 1 (mTORC1), either triggering or repressing autophagy and mitophagy. Unc-51-like kinase 1 (ULK1) is a primary initiating protein, as is mTORC1-suppressed transcription factor EB (TFEB), which coordinates the synthesis of
Several cellular sensors regulate autophagic flux to maintain homeostasis.

- **Figure 1.**
  Several cellular sensors regulate autophagic flux to maintain homeostasis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>References</th>
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<tr>
<td>PS1[3], APP[4]</td>
<td>Alzheimer’s disease</td>
<td>Lee et al, 2010; Reddy et al, 2018</td>
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Table 1.
In this table, some examples of genetic diseases associated with autophagic impairment [1–8].

Autophagy can be constitutive or inducible, rapidly adjusting to alterations within the internal and external environment of the cells. Autophagy serves as a housekeeping system, demonstrated by animal models deficient in lysosomes and other essential proteins maintaining the autophagic flux [9–12]. In addition, sirtuin-1—a class III deacetylase dependent on nicotinamide adenine dinucleotide (NAD+)—becomes a positive autophagy regulator, since it may also be considered a cellular sensor [13].

This process is mainly regulated at a post-translational level, increasing mRNA expression of autophagy genes [14]. Under stress conditions, TFEB is translocated from cytosol to the nucleus, activating transcription of ATG genes and coordinating upregulation of the entire autophagy–lysosomal pathway [15].

In this table, some examples of genetic diseases associated with autophagic impairment [1–8].
autophagy-related genes (ATG). For example, deletion of specific neurons of ATG7 or 5 genes leads to postnatal neurodegeneration [16, 17].

Intrinsically, cellular sensors detect changes in levels of glucose, cytosolic Ca$^{2+}$, reactive oxygen species (ROS), and metabolic intermediates. Therefore, a decrease in glucose availability or impairment of mitochondrial respiration—compromising ATP production leads to an increase in the AMP/ADP ratio, activating the AMPK $\alpha$ subunit [10].

An example of extrinsic sensing occurs via drug-targetable mechanisms at the plasma membrane level. Tyrosine kinase receptors converge on mTOR, AMPK, or Beclin-1-Vps complex by modulating autophagy following growth factors [18, 19]. Even G-protein-coupled receptors (GPCRs) control autophagy via intracellular pathways that similarly modulate AMPK and mTOR [20–22].

This discussion includes a short overview of the more common types of autophagy and will highlight the role of autophagy in retinal diseases, with special attention to diabetic retinopathy.

2. Types of autophagy

There are three forms of autophagy previously described in the literature: macroautophagy, chaperone-mediated autophagy, and microautophagy (Figure 2).

2.1 Macroautophagy

Usually known as autophagy, this intracellular pathway includes cytosolic components such as proteins, lipids, organelles, and parts of the nucleus [23, 24]. Autophagy was first described by Christian du Duve 50 years ago and has been highly preserved across the species. From beginning to end, the whole process is controlled by the ATG protein family, and more than 35 genes have been identified to orchestrate the process [25].

Autophagosome formation is the hallmark of this process. The well-coordinated process begins with an initiation phase, when ULK1 kinase forms a complex with ATG13, ATG10, and FIP200 (known as RB1CC1) at a specific cell site located in the perivacuolar region known as the phagophore assembly site (PAS). ULK1 kinase activity triggers the formation of the phosphoinositide 3-kinase (PI3K) complex, which favors the formation of phosphatidylinositol 3-phosphate, initiating the nucleation phase [26]. Ubiquitin-like conjugation systems are then activated, catalyzed by ATG7. ATG12 is conjugated to ATG5, then phosphatidyethanolamine to microtubule-associated protein 1A/1B-light chain 3 (LC3) through ATG7 kinase, forming an autophagosome bound to LC3 (also called LC3-II) [27, 28]. The late stage of autophagy is controlled by molecules that regulate maturation of the autophagosome, fusion with lysosomes, acidification of the inside compartment of the autophagosome components, and recycling of metabolites from the lysosomal compartment. This coordinated process—including a sequence of protein–protein and protein–lipid interaction—is a dynamic process, where the autophagosome formation, fusion to the lysosome, and digestion of the inside components occur in less than 10 minutes. Therefore, any sort of autophagy dysfunction (such as blockage of lysosomal fusion or lysosomal function impairment) may lead to accumulation of harmful damaged organelles and protein aggregates inside the cell [29] (Figure 2).

2.2 Chaperone-mediated autophagy

In chaperone-mediated autophagy, there is no reorganization of the lysosomal membrane. This selective autophagy is only described in mammals [30], which
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mediates delivery of specific proteins to the lysosome. The distinction occurs because the cytosolic proteins need to be degraded by the presence of a pentapeptide amino acid sequence, KFERQ. This sequence permits recognition of the target protein by a family of chaperones and co-chaperones: the heat shock cognate, 70-kDa (Hsc70)—the most abundant in the family. After recognition of the KFERQ sequence, Hsc70 presents the unfolded proteins to the lysosome, one by one, where they are recognized by the transmembrane domain of lysosome-associated membrane protein type 2A (LAMP2-A). After this, the multimerization of LAMP2-A occurs, allowing transportation of the substrate into the lysosome for degradation. At the end of this process, the LAMP2-A complex is disassembled, and the chaperone Hsp70 is released to start a new cycle [31].

2.3 Microautophagy

Microautophagy is not well described in mammalian cells. However, recent evidence has shown that there is recognition and internalization of small cytoplasmic components in late endosomes. This type of autophagy requires the chaperone Hsc70. However, the microautophagy process is independent of the unfolding of KFERQ and the multimerization of LAMP2-A [32, 33].

2.4 Role of autophagy in disease development

Since the primary function of autophagy is to eliminate harmful components from cells (aggregated proteins, damaged organelles, and pathogens), malfunctioning of this mechanism implicates in diseases—such as Huntington's and Parkinson's diseases [34, 35]—results in protein accumulation.
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In physiological conditions, autophagy is involved in cellular homeostasis, as demonstrated in heart diseases, as seen in heart failure and ischemia–reperfusion injuries [36]. In the pancreas, autophagy is required to maintain function of β cells, revealing significance in the pathogenesis of diabetes. Alterations in autophagy have also been described in a more complex model in cancer research: it can suppress tumors but also helps the tumor adapt to metabolic stress in its late stages [37].

3. Diabetic retinopathy

Diabetes mellitus is a public health issue, estimated to affect about 500 million people by 2035 [38]. Nearly 30% of patients are likely to suffer from retinal microvascular complications and 10% may experience visual threatening due to macular edema or proliferative diabetic retinopathy [39, 40].

Multiple mechanisms are triggered under hyperglycemic conditions (hexosamine and polyol pathways [41], synthesis de novo of diacylglycerol-PKC [42, 43], low grade oxidative stress [44–46], inflammation [47–51], and advanced glycation end products [52, 53]). Although vascular changes are presumed to be the hallmarks of DR, abnormalities in retinal function are detected in patients with diabetes who have good visual acuity [54–59].

The characteristics of retinal neurodegeneration are apoptosis of neuro cells and dysfunction of glial cells—mainly Müller cells [29, 50, 60]. In microvascular disease of diabetic retinopathy, both inner and outer blood retinal barrier break down [61].

3.1 Autophagy in diabetic retinopathy

Since their pioneering studies, Remé et al. —describing the presence of active autophagy in photoreceptors during hibernation with a decreased number of mitochondria and organelles compared to animals in non-hibernating conditions— observed an increased number of autophagosomes [62]. These data show the pivotal role of autophagy in the retina, degrading cellular components (such as mitochondria) during hibernation.

Implications of autophagy in retinal ganglion cells (RGCs) attracted interest as a potential tool for neuroprotection in glaucoma. The first evidence of the cytoprotective role of autophagy in RGCs was shown by Rodríguez-Muela et al. using autophagy-deficient mice, which displayed increased axonal damage following optic nerve transection (ONT) models of optic neuropathy [63–65].

3.2 Autophagy in blood retinal barriers and implications on diabetic retinopathy

The main function of the blood-retina barrier (BRB) is maintenance of retinal homeostasis, regulating the transport of blood stream molecules to provide an appropriate supply for the neuroretina and to protect neural tissue against harmful agents present in the blood. The BRB is formed by two types of barriers: the inner blood-retina barrier (iBRB) and the outer blood-retina barrier (oBRB) [66].

Both outer and inner retinal barriers are affected by the toxic metabolic effects of hyperglycemia [67]. Alterations in the iBRB are more studied than the oBRB among the mechanisms of development and progression of DR [68–70]. The appropriated function of autophagy flux is important for maintenance of cellular viability and confers stress tolerance in retinal cells under adverse conditions such as DR [71].
Retinal endothelial cells of microcirculation of the retina form the iBRB. This barrier selectively allows passage of molecules from systemic circulation to retinal tissue. As a constituent of this barrier, there are tight junctions and adherens junctions such as zonula occludens-1 (ZO-1), occludin, VE-cadherin, and N-cadherin [72]. Endothelial cells are warped by pericytes, which are highly specialized. Pericytes play an essential role in the structure and stability of the iBRB, coordinating angiogenesis and vascular remodeling [73, 74].

Few articles have highlighted the autophagic process in retinal endothelial cells under diabetic conditions [75, 76]. Exposure to high glucose leads to an increase in retinal endothelial cell apoptosis, and this mechanism is mediated by the enhancement of ROS production. This phenomenon is correlated with a reduction in the AMPK pathway [76], which is well described as a direct activator of ULK-1 in the autophagy process [77]. Reestablishing the level of AMPK using specific activators—such as AICAR or antioxidant treatment—is effective in the protection of endothelial retinal cells from damage caused by diabetic conditions [75, 76]. A recent study from Niu et al. described the importance of the protective properties of metformin on retinal endothelial cells and human umbilical vascular endothelial cells (HUVECs) via autophagy in diabetic conditions. In this work, the authors showed that there was an increased LC3 puncta formation, which is an indicative of autophagy, in retinal vascular endothelium from db/db (diabetic) mice compared with control (non-diabetic) mice. This is indicative that metformin protects the retinal microvascular cells by diminishing LC3 formation. To further understand this mechanism, HUVECs were exposed to high levels of glucose and treated with metformin, resulting in a clear increase of LC3 formation. In HUVECs transfected with sh-PRKAA1/2 (AMP catalytic subunit), the protective effect of metformin was abrogated, indicating that metformin acts via AMPK activation [78] and improving autophagy in these cells.

The oBRB is a monolayer formed by retinal pigment epithelial cell layer that separates the neuro retina from choriocapillaris. Impairment of this barrier is implicated in diabetic retinopathy development [79–81]. The major functions of the oBRB are to provide glucose, fatty acids, and retinol to photoreceptors from choriocapillaris and reisomerise all-trans-retinal in 11-cis-retinal after photon absorption of the photoreceptor [66, 82, 83]. Therefore, any disturbance in this structure may have detrimental effects on the retina. A number of sight-threatening diseases display RPR dysfunction, such as age-related macular degeneration, proliferative vitreoretinopathy, and diabetic retinopathy [84].

It is well described in the literature that human retinal pigmented epithelial (RPE) immortalized cells (ARPE-19) exposed to high concentrations of glucose present molecular changes, including a decrease of proliferation, an increase in oxidative stress mediated by ROS production, and augmented lipid droplets and inflammation [85–88]. These alterations can activate or repress the autophagic flux in RPE cells. Studies have shown that, until 48 hours of exposure to high glucose levels, ARPE-19 cells present an increase in lipid droplets, which can contribute to ROS production [71, 85, 89]. This increase in ROS production can initiate autophagy, enhancing the numbers of autophagosomes, increasing conversion of LC3-I to LC3-II, and decreasing levels of p62/SQSTM1 as a defense mechanism against damage caused by high glucose. However, Chen et al. found that an increase in autophagic flux promoted by high glucose cannot be maintained long-term. After 7 days in high glucose, ARPE-19 presented impairment in the degradation of p62/SQSTM1 and an increase in apoptotic cells. These findings indicated that autophagy was the first defense against oxidative stress in high-glucose conditions. In the long-term, this protective pathway became saturated and inefficient, thus contributing to RPE degeneration in DR [87].
Zhang et al. have shown that high glucose concentrations can attenuate the PINK1 and parkin pathways involved in controlling cellular mitophagy. Downregulation of mitophagy can lead to an increase in cellular stress levels because the biogenesis of mitochondria becomes compromised [90].

The role of autophagy in retinal diabetic complications is not simply a matter of inhibiting its initiation or progression. Inhibition of autophagy in ARPE-19 during its initial phase with 3-methyladenine (3-MA) or during the fusion of autophagosome and lysosome using bafilomycin aggravates oxidative stress and exacerbates secretion of the pro-inflammatory interleukin-1β promoted by high glucose [88].

The appropriated autophagic process is important as a mechanism of cell homeostasis in diabetic conditions.

3.3 Autophagy in Müller glial cells and implications in diabetic retinopathy pathogenesis

Müller cells are the predominant glial cell in the retina. Its unique morphology allows the Müller cell to directly interact with neighboring neural and vascular cells, expanding through the entire retina from the inner limiting membrane to the photoreceptor layer. Müller cells are closely related with vitreous, blood vessels, and sub retinal space. Each Müller cell interacts with one cone and 10 rods [91]. This configuration of Müller cells inside the retina explains the diversity of its function, responsible for the metabolic, functional, and structural support of the retina [92].

There are several functions attributed to Müller cells, such as the release of trophic factors [93, 94], neurotransmitter recycling [95], and phagocytosis of external photoreceptor segments [96, 97]. Müller cells, depending upon the stimulus (trauma, vascular, or metabolic), may react with phenotype changes called gliosis, which consist in adaptive morphological, biochemical, and physiological alterations. Among the more interesting biochemical changes in Müller cells are increased vascular endothelial growth factor (VEGF) [98] and glial fibrillary acidic protein (GFAP) production, both with pro-angiogenic and pro-inflammatory effects. Massive VEGF release is present in the proliferative stages of DR and diabetic macular edema, representing a major therapeutic target for pharmacological treatment of these devastating complications.

There are few studies showing the effects of high glucose on autophagy in retinal Müller cells. Devi et al. described the implications of autophagy dysfunction in the mechanisms of DR [99]. In their study, Müller cells exposed to high glucose conditions for 5 days displayed an increase of autophagosome and mitophagosome in the cytosol, suggesting high glucose conditions activated the autophagy process. Despite activation of the protective process (autophagy), they observed an association with an increased proapoptotic caspase-3, leading to programmed cell death. This scenario elucidates that diabetic conditions induce activation of autophagy followed by dysfunction, leading to cellular death.

In the previously published work addressing the mechanism by which Müller cells exposed to high glucose release high amounts of VEGF feedback and trigger increased apoptosis, it was shown that the autophagic process was defective in Müller cells among diabetic conditions. In cells exposed to high glucose, autophagy markers—both early Beclin and late LC3-I and LC3-II—were increased, but p62/SQSTM1 accumulated in the cytosol compartment of Müller cells, accompanied by an increased apoptotic rate. To further understand how p62/SQSTM1 could modulate the autophagy and apoptosis in Müller cells exposed to high glucose, p62/SQSTM1 was suppressed. In this condition, there was less endoplasmic reticulum stress, lowering the interaction with caspase-8 and, by extension, less apoptosis. The presence of rapamycin, an mTOR blocker, triggered the formation of autophagosome
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and ameliorated the degradation of p62/SQSTM1. Rapamycin showed to improve proteolytic activity of the lysosome, reducing the release of VEGF. Corresponding findings were also demonstrated in models using diabetic animals. In the retinas of diabetic rats, there was a significant increase in p62/SQSTM1 accumulation, particularly in cells located in the inner nuclear layer [29]. Lysosomal impairment and autophagic flux dysfunction are early indicators of the pathogenesis of DR.

4. Conclusion

Diabetic retinopathy is a neurodegenerative disease presenting vascular changes in its late stages. Multiple factors are associated with the development and progression of DR. Recently, better understanding at cellular and molecular levels of its process has been identified through the pathways and intracellular signaling involved in cells exposed to diabetic conditions. This has allowed identification of new therapeutic approaches. Recent concepts of this disease have been analyzed here, with special focus on the process of autophagy using experimental models in different retinal cells targeted by hyperglycemia in the developmental stages of the disease.

Author details

Jacqueline M. Lopes de Faria* and Marcella Neves Dátilo
Faculty of Medical Sciences, State University of Campinas, São Paulo, Brazil

*Address all correspondence to: lopesdefariajm@gmail.com

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