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Review

Autophagy in the lens

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ABSTRACT

The lens of the eye is a transparent tissue composed of lens fiber cells that differentiate from lens epithelial cells and degrade all cytoplasmic organelles during terminal differentiation. Autophagy is a major intracellular degradation system in which cytoplasmic proteins and organelles are degraded in the lysosome. Although autophagy is constitutively activated in the lens and has been proposed to be involved in lens organelle degradation, its precise role is not well understood. Recent genetic studies in mice have demonstrated that autophagy is critically important for intracellular quality control in the lens but can be dispensable for lens organelle degradation. Here, we review recent findings on the roles of autophagy and lysosomes in organelle degradation and intracellular quality control in the lens, and discuss their possible involvement in the development of human cataract.

Contents

1. Introduction

Autophagy is an evolutionally conserved catabolic process that delivers cytosolic macromolecules and membrane-bound organelles to the lysosome for degradation. There are at least three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (Mizushima and Komatsu, 2011). Among them, macroautophagy (hereafter referred to as autophagy) has been the most widely investigated. Upon induction of autophagy, a portion of the cytoplasm is enclosed by an isolation membrane (or phagophore), which elongates and eventually seals to become the double-membrane structure of the autophagosome. The outer membrane of the autophagosome subsequently fuses with late endosomes and lysosomes to form the autolysosome, in which enclosed materials are degraded by lysosomal hydrolases. The resultant degradation products such as amino acids are recycled to the cytosol. Although autophagy degrades substrates mostly in a non-selective manner, recent studies have demonstrated that autophagy can selectively recognize particular substrates such as certain proteins (e.g., p62), ribosomes, mitochondria, and peroxisomes (Mizushima and Komatsu, 2011).

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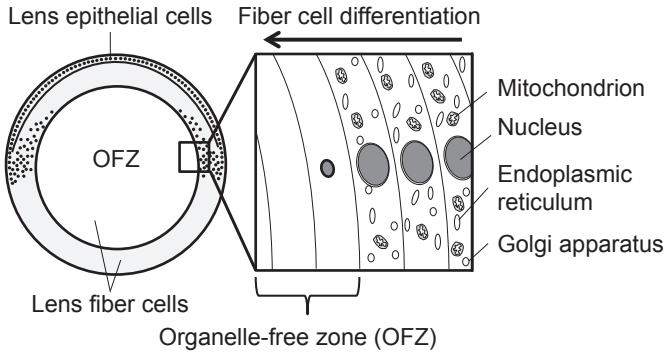

Fig. 1. The lens and programmed organelle degradation. A model of lens epithelial-to-fiber cell differentiation and organelle degradation. Differentiating secondary fiber cells around the organelle-free zone (OFZ) are shown (right panel). The nucleus, mitochondrion, endoplasmic reticulum, and Golgi apparatus are degraded almost simultaneously.

Genetic screens in yeast have identified a group of around 40 autophagy-related (*ATG*) genes essential for autophagy and its related processes (Mizushima et al., 2011). Most of these, including *Atg5* and *Pik3c3/Vps34*, are conserved among eukaryotes, and disruption of these genes in lower model organisms has revealed the importance of autophagy in the adaptive responses to nutrient starvation, cellular differentiation, and development (Mizushima and Levine, 2010). Analyses using systemic and tissue-specific *Atg* knock-out mice have demonstrated that autophagy is also critical in the prevention of various disorders including cancer, neurodegenerative diseases, infections, and metabolic diseases (Mizushima and Komatsu, 2011).

The lens of the eye is an avascular and transparent tissue that focuses light on the retina (Piatigorsky, 1981; McAvoy et al., 1999). The lens is composed of epithelial and fiber cells (Fig. 1). During early lens development, the anterior cells of the lens vesicle differentiate into lens epithelial cells, while the posterior cells differentiate into primary lens fiber cells (Cvekl and Ashery-Padan, 2014). As development proceeds, the lens epithelial cells proliferate and differentiate into secondary fiber cells that lay atop the pre-existing fiber cells. This differentiation of fiber cells is accompanied by cellular elongation, synthesis of crystallin proteins, and programmed degradation of all intracellular membrane-bound organelles. Impairment of these processes or cellular damage could result in cataract formation, a leading cause of blindness (Michael and Bron, 2011). Cytoprotective systems should therefore exist to keep the lens transparent. In this review, we describe recent findings on the role of autophagy in lens organelle degradation and intracellular quality control, and discuss unresolved issues awaiting further study.

2. Constitutive autophagy in the lens

The presence of autophagic vacuoles (autophagosomes or autolysosomes) in lens cells was originally suggested in 1984 from electron microscopy findings of an *in vitro* differentiation system of primary lens cells from rat (Walton and McAvoy, 1984) and chicken (Menko et al., 1984). In 2004, the autophagosomes in the lens *in vivo* were visualized using autophagy-indicator mice carrying a *GFP-LC3* transgene, coding a specific autophagosome marker (Mizushima et al., 2004). In the lens epithelial cells of *GFP-LC3* transgenic mice, a number of GFP-LC3 puncta can be detected by fluorescence microscopy. Of note, autophagy induction in lens epithelial cells is not affected by nutrient status, as evidenced by the fact that autophagy does not change even after mice were deprived of food for 1 or 2 days. Numerous autophagosomes are also detected in differentiating primary fiber cells (Matsui et al., 2006) and differentiating secondary fiber cells, but not in terminally differentiated fiber cells lacking organelles (Morishita et al., 2013). Thus, autophagy is programmed to be constitutively activated in lens epithelial cells and during the period of fiber cell differentiation.

In a 2004 study also using *GFP-LC3* transgenic mice, the ultrastructure of the double-membrane autophagosomes in *in vivo* lens epithelial cells was elucidated from immunoelectron microscopy findings (Mizushima et al., 2004). Later studies using transmission electron microscopy revealed the presence of autophagosomes containing cytosol and mitochondria in both lens epithelial and fiber cells in mice (Wignes et al., 2013), chicken (Costello et al., 2013a; Basu et al., 2014), and humans (Costello et al., 2013a). Although transmission electron microscopy is one of the principal methods for investigating the ultrastructure of autophagosomes (Mizushima et al., 2010), it is sometimes difficult to distinguish autophagosomes from other similar structures in the lens. During fiber cell differentiation, the plasma membrane is extensively remodeled to form interlocking membrane domains between neighboring cells such as ball-and-sockets and protrusions (Bassnett et al., 2011). These structures can appear as autophagosome-like double-membrane structures if they are cut perpendicular to the protrusion axis (Costello et al., 2013b). Thus, it is recommended that detection of autophagy in the lens be performed in combination with other methods such as immunohistochemistry of autophagosome markers (e.g., LC3) using antibodies, the specificity of which should be confirmed in the lens using autophagy-deficient animals (Morishita et al., 2013).

3. Involvement of autophagy in lens organelle degradation?

A long-standing question in developmental biology has been how lens fiber cells, keratinocytes, and erythrocytes degrade their cytoplasmic organelles during terminal differentiation (Bassnett, 2009; Wride, 2011). Both the primary and secondary fiber cells degrade all membrane-bound organelles, including the nuclei (Kuwabara and Imaizumi, 1974; Vrensen et al., 1991; Bassnett and Mataic, 1997), endoplasmic reticulum, Golgi apparatus (Bassnett, 1995), and mitochondria (Bassnett and Beebe, 1992) almost at the same time, forming the central organelle-free zone in the lens (Fig. 1). The molecular mechanisms of lens organelle degradation are as yet poorly understood.

It has been clearly shown that the nuclear DNA in fiber cells is degraded by DNase II-like acid DNase (DLAD/DNase2b) in mice (Nishimoto et al., 2003). DLAD is expressed in mouse and human fiber cells and *DLAD* knock-out mice develop nuclear cataract with defective degradation of DNA. On the other hand, in zebrafish, DNase1-like 3-like (Dnase1l3l), but not DLAD, is expressed in the lens and is critical for the degradation of DNA (Iida et al., 2014). Given the fact that DLAD is a lysosomal enzyme (De Maria and Bassnett, 2007; Nakahara et al., 2007) and autophagy is constitutively activated in the lens, it has been hypothesized that autophagy is involved in this process.

However, the results of several *in vivo* studies using autophagy-deficient mice suggest that autophagy is not required for lens organelle degradation. This finding first emerged from studies using systemic *Atg5* knock-out mice (Kuma et al., 2004). Atg5 is an evolutionally conserved factor essential for autophagosome maturation and completion (Mizushima et al., 2001; Kishi-Itakura et al., 2014). In $Atg5^{-/-}$ primary fiber cells, the nuclei and endoplasmic reticulum are normally degraded (Matsui et al., 2006). Because *Atg5* knock-out mice die soon after birth (Kuma et al., 2004), the