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The autophagic lysosomal system in outflow pathway physiology and pathophysiology

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ABSTRACT

Malfunction of the trabecular meshwork (TM)/schlemm's canal (SC) conventional outflow pathway is associated with elevated intraocular pressure (IOP) and, therefore, increased risk of developing glaucoma, a potentially blinding disease affecting more than 70 million people worldwide. This TM/SC tissue is subjected to different types of stress, including mechanical, oxidative, and phagocytic stress. Long-term exposure to these stresses is believed to lead to a progressive accumulation of damaged cellular and tissue structures causing permanent alterations in the tissue physiology, and contribute to the pathologic increase in aqueous humor (AH) outflow resistance. Autophagy is emerging as an essential cellular survival mechanism against a variety of stressors. In addition to performing basal functions, autophagy acts as a cellular survival pathway and represents an essential mechanism by which organisms can adapt to acute stress conditions and repair stress-induced damage. A decline in autophagy has been observed in most tissues with aging and has been considered responsible, at least in part, for the accumulation of damaged cellular components in almost all tissues of aging organisms. Dysfunction in the autophagy pathway is associated with several human diseases, from infectious diseases to cancer and neurodegeneration. In this review, we will summarize our current knowledge of the emerging roles of autophagy in outflow tissue physiology and pathophysiology, including novel evidence suggesting compromised autophagy in the glaucomatous outflow pathway.

1. Introduction

Autophagy is a dynamic catabolic process by which cytosolic material, including organelles, proteins and pathogens, are delivered to the lysosome for degradation. Although autophagy was initially thought to be a bulk cytoplasmic degradation mechanism in response to starvation, numerous studies now support a key role of autophagy in maintaining cellular and tissue homeostasis, as well as an adaptive cellular response to stress, providing protective functions during tissue injury. The trabecular meshwork (TM)/schlemm's canal (SC) outflow pathway is known to be subjected to different types of stress such as mechanical, oxidative, and phagocytic stress. Short-term exposure to these stresses is expected to elicit adaptive responses, however, long-term exposure may lead to permanent alterations in the tissue physiology and contribute to the pathologic increase in aqueous humor (AH) outflow resistance frequently associated with glaucoma. In this review, we will summarize our current knowledge of the emerging roles of autophagy in outflow tissue physiology and pathophysiology, including novel evidence supporting potential alterations of autophagy in the glaucomatous outflow pathway.

2. Autophagy, a cellular survival pathway against stress and adaptation

Autophagy, which means self-eating, is an evolutionarily conserved mechanism that allows for the degradation of cytosolic components, such as proteins and organelles, within lysosomes by lysosomal hydrolases. Three main autophagic pathways have been described in mammalian cells based on the delivery route of the cargo material to the lysosomal lumen: macroautophagy, microautophagy and chaperon-mediated-autophagy (CMA) (Fig. 1). Macroautophagy, commonly known as simply “autophagy”, involves the formation of a new organelle, the autophagosome, a double-membrane structure that encloses portions of the cytosol, including whole organelles. Autophagosomes then fuse with

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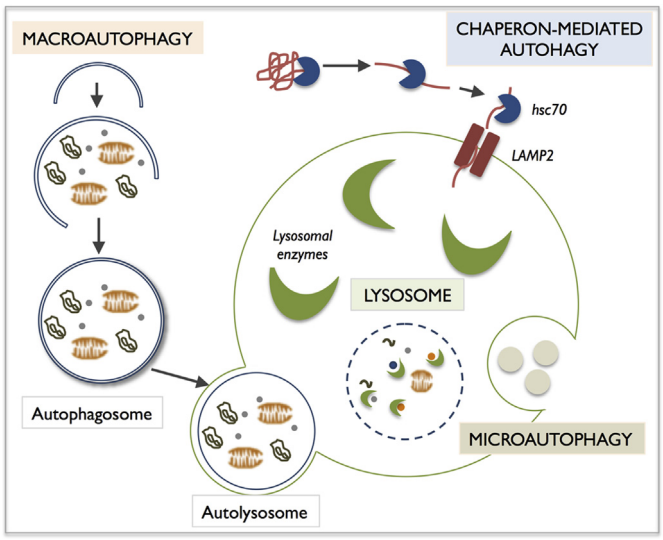

Fig. 1. Types of autophagy.

lysosomes, forming the autolysosomes, in which the luminal material is degraded by resident hydrolases (Mizushima et al., 2008). In microautophagy, small pieces of the cytoplasm are directly engulfed by inward invagination of the lysosomal or late endosomal membrane. In CMA, specific cytosolic soluble proteins, containing a KFERQ-like pentapeptide sequence, are recognized by the chaperone Hsc70 that delivers them to the lysosomal receptor, the lysosome-associated membrane protein type 2A (LAMP-2A), for translocation into the lysosomal lumen (Kon and Cuervo, 2010; Mizushima et al., 2008). After all three types of autophagy, the resultant degradation products are then transported back into the cytosol through the activity of membrane permeases and can be used for different purposes, such as new protein synthesis, energy production, and gluconeogenesis. Among all these types of autophagy, macroautophagy (referred to here as autophagy) is the most extensively studied.

Despite the earliest beliefs, autophagy does not just occur during starvation; autophagy occurs constitutively at basal levels to perform homeostatic functions such as protein and organelle turnover. In fact, the autophagy pathway has emerged as an essential component during development and in maintaining cellular and tissue homeostasis (Mizushima and Komatsu, 2011). A proof of that is that whole-body knockout of essential autophagy genes is incompatible with life; autophagy-incompetent mice die at early embryonic stages because of severe developmental defects, or within the first three days postpartum. In addition to performing basal functions, autophagy acts as a cellular survival pathway. Autophagic activity can be enhanced in response to a wide variety of intracellular and extracellular stimuli and represents an essential mechanism by which organisms can adapt to acute stress conditions and repair stress-induced damage. The importance of autophagy is highlighted by an increasing number of studies linking dysfunction in the autophagy pathway with several human diseases, from infectious diseases to cancer and neurodegeneration (Levine and Kroemer, 2008). Defects in all the steps of the autophagy pathway have been associated to disease; from autophagosome formation, as seen in several cancers, to defects in autophagosome maturation, like in Alzheimer's disease, or impaired autophagic clearance (i. e. lysosomal storage disorders). Moreover, a decline in autophagy has been observed in most tissues with aging and has been considered responsible, at least in part, for the accumulation of damaged cellular components in almost all tissues of aging organisms (Rubinsztein et al., 2011). This is particularly detriment in patients with proteinopathies, like Huntington's disease, as autophagy may reach a saturation point in which its capacity to degrade the mutant aggregate-prone proteins is exceeded.

Activation of autophagy is a highly regulated event controlled by a number of evolutionary conserved autophagy related genes (ATG genes) (Mizushima, 2007). The best well-known pathway controlling autophagy is the mTOR pathway, although an mTOR-independent activation of autophagy has been long suspected and is started to be characterized (Sarkar, 2013). In the case of the mTOR-dependent pathway, inactivation of mTOR triggers the recruitment of ATG proteins to the initiation site and activation of two ubiquitin-like conjugation systems (Atg12-Atg5-Atg16 and Atg8/LC3 conjugation systems) that culminate with the formation of the autophagosome. A key event required for autophagosome formation is the lipidation of the autophagosome marker LC3-I to LC3-II (Kimura et al., 2009; Mizushima and Yoshimori, 2007). LC3 is synthesized as a precursor form that is cleaved by the protease ATG4B, resulting in the cytosolic isoform LC3-I. Upon induction of autophagy, LC3-I is conjugated to phosphatidylethanolamine to form LC3-II. LC3-II is incorporated to the nascent and elongating autophagosome membrane and remains on the autophagosome until fusion with the lysosomes. In the autolysosomes LC3-II is then either degraded or delipidated by ATG4 and recycled. A number of different intracellular and extracellular signals and factors have been shown to regulate autophagy, i.e. lack of nutrients, reactive oxygen species or, as we will review here, mechanical strain or ascorbic acid (Kroemer et al., 2010).

3. The outflow pathway, a tissue controlling intraocular pressure

The TM/SC outflow pathway is a highly specialized tissue located at the angle formed by the cornea and the iris. This tissue is involved in intraocular pressure (IOP) homeostasis by modulating the outflow of AH from the anterior chamber to the venous system. Increased IOP resulting from abnormally high outflow resistance is commonly associated with primary open angle glaucoma (POAG), an age-related disease second leading form of permanent blindness worldwide (Bill and Phillips, 1971; Quigley, 2011; Stamer and Acott, 2012).

In addition to modulating AH outflow resistance, the conventional outflow pathway is believed to be involved in detoxification of the AH, phagocytosis of cellular debris, and the maintenance of immune privilege in the eye. To accomplish all these functions, the conventional outflow pathway is organized, despite its small size (100–150 μg, containing approximately 200,000–300,000 cells), as a complex structure composed of morphologically and functionally different cell types (Lütjen-Drecoll, 1999; Tripathi, 1977). The TM/SC tissue is structured into four differentiated layers through which the AH must pass before leaving the eye: the inner uveal meshwork, the corneoscleral meshwork, the juxtacanalinular tissue (JCT), and the inner wall of the SC. The uveal and the corneoscleral meshworks are composed of sheets of connective tissue beams lined by TM endothelial cells. The beams attach to each other in several layers forming a porous filter-like structure. TM cells covering the beams are involved in phagocytosis and tissue remodeling. The JCT is composed of loosely arranged extracellular matrix (ECM) in which JCT cells are randomly distributed. JCT cells are characterized by the presence of processes or extensions connecting the TM cells from the corneoscleral meshwork with cells from the inner wall of SC. The cells of the inner wall endothelium of SC constitute the only continuous cell layer in the outflow pathway. The JCT/SC region contains the locus of both normal outflow