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Review

Lacritin and other autophagy associated proteins in ocular surface health

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ABSTRACT

Advantage may be taken of macroautophagy ('autophagy') to promote ocular health. Autophagy continually captures aged or damaged cellular material for lysosomal degradation and recyling. When autophagic flux is chronically elevated, or alternatively deficient, health suffers. Chronic elevation of flux and stress are the consequence of inflammatory cytokines or of dry eye tears but not normal tears in vitro. Exogenous tear protein lacritin transiently accelerates flux to restore homeostasis in vitro and corneal health in vivo, and yet the monomeric active form of lacritin appears to be selectively deficient in dry eye. Tissue transglutaminase-dependent cross-linking of monomer decreases monomer quantity and monomer affinity for coreceptor syndecan-1 thereby abrogating activity. Tissue transglutaminase is elevated in dry eye. Mutation of arylsulfatase A, arylsulfatase B, ceroid-lipofuscinosis neuronal 3, mucolipin, or Niemann-Pick disease type C1 respectively underlie several diseases of apparently insufficient autophagic flux that affect the eye, including: metachromatic leukodystrophy, mucopolysaccharidosis type VI, juvenile-onset Batten disease, mucolipidosis IV, and Niemann-Pick type C associated with myelin sheath destruction of corneal sensory and ciliary nerves and of the optic nerve; corneal clouding, ocular hypertension, glaucoma and optic nerve atrophy; accumulation of 'ceroid-lipofuscin' in surface conjunctival cells, and in ganglion and neuronal cells; decreased visual acuity and retinal dystrophy; and neurodegeneration. For some, enzyme or gene replacement, or substrate reduction, therapy is proving to be successful. Here we discuss examples of restoring ocular surface homeostasis through alteration of autophagy, with particular attention to lacritin.

Contents

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1. Introduction

Macroautophagy ('autophagy') is a stimulatable self-catabolic process that constitutively clears damaged proteins and organelles to an autolysosomal compartment for degradation (Fig. 1), thus serving as a key regulator of homeostasis (Galluzzi et al., 2014). When insufficient, damaged proteins and organelles accumulate thereby promoting cellular toxicity and inflammation. Insufficient autophagic flux underlies many eye diseases, including stromal corneal dystrophy type 2 (see contribution by Kim in this issue; Choi et al., 2012) and corneal pathogenesis of herpes simplex virus Type 1 via viral sequestration of autophagy protein beclin 1 (Leib et al., 2009). Other examples include: cataract formation in the lens (see contribution by Mizushima and Morishita (Morishita et al., 2013)), glaucoma (see contributions by Liton (Porter et al., 2013) and Dickey (Suntharalingam et al., 2012)), retinal blindness (see contributions by Sinha (Valapala et al., 2014), Swarup (Sirohi et al., 2013), Maeda (Chen et al., 2013) and Yue (Shen et al., 2011)) and axonal degeneration of the optic nerve by Lingor (Knoferle et al., 2010). Accordingly, restoration or transient stimulation of autophagic flux is a potential treatment approach. One example is the tear protein 'lacritin' that rapidly stimulates autophagy in stressed human corneal epithelial cells (Wang et al., 2013) and when applied topically largely eliminates corneal lissamine green staining in dry eye mice (Vijmasi et al., 2014).

Gene 'autophagy' keyword search cross-referenced to expression sequence tag ('EST') libraries suggest that at least 460 different autophagy-associated genes are expressed in the eye (Supplemental Table 1). Some are well known autophagy mediators of the AuTophaGy related family 'ATG' series (Klionsky et al., 2003), most originally discovered in yeast – including ATG12 (Mizushima et al., 1998) and ATG16L1 (Mizushima et al., 1999) by issue contributor Noboru Mizushima who also discovered ATG101 (Hosokawa et al., 2009) out of HEK293 cells (see each in Fig. 1). Others include members of the upstream AKT serine threonine kinase (AKT1 – 3) family, BCL2 and the BCL2-associated family (BAD, BAG3, BAG5, BAX), BAK1, beclin 1 (BECN1), FOXO1 and FOXO3, the MAP1LC3 family (A, B, B2), MTOR, PIK3C3, RB1CC1, RIPK1 and the ULK1 – 3 family (see several in Fig. 1). Forty are NEIBank 'eye disease genes' (Fig. 2; Supplemental Table 1). Here we focus on all known ocular surface disease genes associated with autophagy, beginning with LACRT and its protein product lacritin (Sanghi et al., 2001).

2. Lacritin (LACRT)

Lacritin is a multifunctional tear glycoprotein (Fig. 3) (Sanghi et al., 2001) that transiently and rapidly triggers autophagy in cultured corneal epithelial cells under conditions of inflammatory cytokine stress to restore homeostasis (Wang et al., 2013). Lacritin is also a tear secretagogue – although a tear protein itself. It promotes corneal wound healing (Wang et al., 2014), exhibits latent bactericidal activity (McKown et al., 2014) and exists in active monomeric and inactive polymeric forms in human tears (Velez et al., 2013). Several proteomic studies suggest that lacritin monomer is selectively deficient in human dry eye (Aluru et al., 2012; Koo et al., 2005; Nichols and Green-Church, 2009; Srinivasan et al., 2012).

2.1. Discovery as a stimulator of basal tearing

An unbiased screen for factors triggering unstimulated tear protein secretion in rat lacrimal acinar cell culture led indirectly to the discovery of lacritin (Sanghi et al., 2001), an extracellular glycoprotein with SDS-PAGE mobility in tears of ~23–25 kDa vs ~18 kDa for lacritin generated recombinantly in *Escherichia coli*, and 12.3 kDa predicted from primary sequence. Aberrant mobility is in part thought to be a consequence of its C-terminal amphipathic α-helical structure (Fig. 3); (Karnati et al., 2013). Topical recombinant lacritin stimulates tear protein release both in dry eye mice (Vijmasi et al., 2014; Wang et al., 2015) and normal rabbits (Samudre et al., 2011). Similarly, lacritin monomer semi-purified from monkey tears triggers tear lipocalin secretion from monkey lacrimal acinar cells cultured in the presence of dry eye inflammatory cytokines, that under the same conditions are unresponsive to the acetylcholine receptor agonist carbachol (Fujii et al., 2013). Lacritin is itself a tear protein derived largely from the same lacrimal acinar cells that it stimulates (Fujii et al., 2013; Sanghi et al., 2001), and is expressed in human lacrimal gland as the sixth most common mRNA (Ozyildirim et al., 2005). Other human sources include accessory lacrimal glands of Wolfring (Ubels et al., 2012) and meibomian glands (Tsai et al., 2006). RT-PCR of ocular tissues in monkey validate these observations and point also to progressively lesser expression by conjunctiva, corneal, retinal and lens epithelia, as well as by the iris and ciliary body (Nakajima et al., 2007). The lacritin LACRT gene is one of the most eye-specific genes (Karnati et al., 2013).

2.2. Lacritin prosurvival activity

The avascular corneal epithelium is thought to be dependent on tears for nutrition and health. When basal tearing is insufficient and disruptions develop in the tear film, the epithelium becomes stressed and releases inflammatory cytokines such as tumor necrosis factor (Luo et al., 2004) and interferon gamma (Fig. 4) that