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Working your SOCS off: the role of ASB10 and protein degradation pathways in glaucoma

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Abstract

Evidence is accumulating to suggest that mutations in the Ankyrin and SOCS Box-containing protein-10 (*ASB10*) gene are associated with glaucoma. Since its identification in a large Oregon family with primary open-angle glaucoma (POAG), ASB10 variants have been associated with disease in US, German and Pakistani cohorts. ASB10 is a member of the ASB family of proteins, which have a common structure including a unique N-terminus, a variable number of central ankyrin (ANK) repeat domains and a suppressor of cytokine signaling (SOCS) box at the C-terminus. Mutations in *ASB10* are distributed throughout the entire length of the gene including the two alternatively spliced variants of exon 1. A homozygous mutation in a Pakistani individual with POAG, which lies in the center of the SOCS box, is associated with a particularly severe form of the disease. Like other SOCS box-containing proteins, ASB10 functions in ubiquitin-mediated degradation pathways. The ANK repeats bind to proteins destined for degradation. The SOCS box recruits ubiquitin ligase proteins to form a complex to transfer ubiquitin to a substrate bound to the ANK repeats. The ubiquitin-tagged protein then enters either the proteasomal degradation pathway or the autophagic-lysosomal pathway. The choice of pathway appears to be dependent on which lysine residues are used to build polyubiquitin chains. However, these reciprocal pathways work in tandem to degrade proteins because inhibition of one pathway increases degradation via the other pathway. In this publication, we will review the literature that supports identification of *ASB10* as a glaucoma-associated gene and the current knowledge of the function of the ASB10 protein. In addition, we present new data that indicates ASB10 expression is up-regulated by the inflammatory cytokines tumor necrosis factor- α and interleukin-1 α . Finally, we will describe the emerging role of other SOCS box-containing proteins in protein degradation pathways in ocular cells.

Keywords

ASB10; glaucoma; protein degradation; SOCS proteins; autophagy; ubiquitin; retinal ganglion cells

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1. The *GLC1F* gene

Primary open angle glaucoma (POAG) remains a leading cause of irreversible blindness (Quigley, 2011). Identification of genes impacting glaucoma has been a long and torturous path due to the heterogeneity and complexity of the disease. Family studies utilizing pedigrees, where a large majority of relatives are afflicted, have been the standard for identifying novel genes. Using this approach, we identified a glaucoma locus, *GLC1F*, on chromosome 7q35-q36 in an Oregon family with nine individuals with glaucoma encompassing 4 generations (Wirtz et al., 1999). All affected individuals had elevated (>22 mmHg) intraocular pressure (IOP). Sequencing all of the genes in the region, a synonymous mutation in ankyrin repeat and SOCS box containing-10 (*ASB10*), T255T, was identified in all nine affected individuals (Pasutto et al., 2012). This mutation, rs104886478, has a frequency of 0.04% in the ExAc Aggregated Population. The finding that this variant is extremely rare and segregates with POAG in this family supports our hypothesis that *ASB10* is a POAG susceptibility gene. Analysis of *ASB10* variants in POAG cohorts from the USA and Germany identified 26 amino acid changes in 70 patients accounting for 6% (70 of 1172) of the glaucoma population compared to 2.8% in the control group (Pasutto et al., 2012). Thus, a significant difference was found between patients and controls [$P=0.008$, two-tailed Fisher's exact test; odds ratio (OR) = 2.2, 95% confidence interval (CI) = 1.14-3.0]. The non-synonymous amino acid changes are distributed over the entire protein sequence impacting the N-terminus, the ankyrin repeats and the SOCS box.

Since our original identification, two subsequent studies have been published. In the first, eleven non-synonymous *ASB10* variants were identified in a small cohort of 158 POAG patients and 82 control subjects from Iowa (Fingert et al., 2012). However, comparison of the allele frequency of each of the non-synonymous *ASB10* variants between the patients and controls showed that none was statistically more common in patients ($P>0.05$). This paper has led to controversy over whether *ASB10* is a glaucoma-associated gene. A more recent report using a Pakistani cohort identified ten non-synonymous variants in 208 sporadic POAG patients and 151 healthy controls (Micheal et al., 2015). A burden test showed that there was a significant difference between the two groups ($p=0.0005$). Furthermore, screening of 30 POAG families identified three rare non-synonymous variants. While these variants did not segregate with disease in the three families, one family member was homozygous for the p.Arg453Cys mutation and had a much more severe phenotype compared to his sibs. Diagnosed with POAG at the age of 15, he subsequently lost vision in both eyes at age 35 in spite of having two trabeculectomies to control the IOP of both eyes (Micheal et al., 2015). This homozygous mutation lies in the functional site of the SOCS box of *ASB10* (see later).

Collectively, the three studies show that non-synonymous variants were detected in 106/1468 (7.2%) patients and in 18/694 (2.6%) controls subjects, excluding the common variants (rs62489646, rs919533 and rs201566253) (Micheal et al., 2015). Combining these studies results in a significant difference ($P=0.000006$) in the association of rare *ASB10* variants in POAG patients compared to controls (Micheal et al., 2015). Additional population studies, as well as molecular research, will be required to determine *ASB10*'s role in glaucoma.

2. The ASB family of SOCS-box containing proteins

The family of suppressor of cytokine signaling (SOCS)-box containing proteins has over 70 members (Piessevaux et al., 2008). All SOCS-box containing proteins are involved in ubiquitination of target proteins for proteasomal-mediated protein degradation. This family is categorized into the several sub-groups depending on what protein domain the SOCS box is linked to: the SOCS family, which are linked to Src homology 2 (SH2) domains; the WSB family, proteins that contain WD-40 repeats; SSB proteins, which have Spry domains; the RAR group, which have GTPase domains; and the ASB family, which contain ankyrin repeats (Hilton et al., 1998; Nicholson and Hilton, 1998). *ASB10* is one of 18 members of this sub-group (Kile et al., 2000). Members have common structural motifs including a unique N-terminal domain, a varying number of central ankyrin repeat domains and a SOCS box domain at the C-terminus. Each of these domains has distinctive functions, which we will now describe in more detail.

2.1 The N-terminus

The N-terminal region is the most divergent at the amino acid level and there is no consensus structural motif between family members. In *ASB10*, alternative mRNA splicing of exon 1 results in two protein isoforms called variant 1 (v1) and variant 3 (v3) (Pasutto et al., 2012). The function of the unique N-terminal regions is unknown, but POAG-associated mutations are detected in exon 1 of both of these alternatively spliced variants suggesting that they serve important biological functions (Marrs et al., 2013; Micheal et al., 2015; Pasutto et al., 2012). We performed bioinformatics analyses of the primary amino acid sequence of V1 and V3 (Fig. 1). These analyses predicted that V1 has a signal peptide and its N-terminus is likely located outside the cell. Conversely, the V3 isoform was not predicted to have a signal peptide and its N-terminus is likely located intracellularly. From these predictions, it appears that these two isoforms may perform different biological functions.

2.2 The ANK repeats

Ankyrin (ANK) repeats are one of the most common structural motifs in proteins (Mosavi et al., 2004; Mosavi et al., 2002). Each repeat, of approximately 33 residues, folds into a helix-loop-helix structure with the loop region projecting outward from the helices at a 90° angle. When multiple ANK repeats are stacked together, the loops form a concave L-shaped recognition face that binds specific proteins. The number and structure of ANK repeats contribute to the interaction with their substrates (Mosavi et al., 2002). For instance, the ANK repeats of *ASB3* interact with tumor necrosis factor- α receptor-2 (TNF α RII), while *ASB9* ANK repeats bind creatine kinase B (Chung et al., 2005; Debrincat et al., 2007). The interaction between *ASB11* and ribophorin 1 appears to be dependent on the fourth of six ANK repeats (Andresen et al., 2014). Yet, for the majority of the ASB proteins including *ASB10*, the protein bound by the ANK repeats has not been identified.

The T255T mutation identified in the Oregon family is predicted to impact the number of ANK repeats in the resultant protein. The mutation is at the center of an exon splice enhancer site (Pasutto et al., 2012). Sequencing of *ASB10* mRNA from four of the affected

family members revealed that exon 3 was skipped, which results in an altered reading frame and introduction of a stop codon in exon 4. The predicted mutant protein would have only two of the seven ankyrin repeats and the SOCS box would be completely absent. Analysis of the ASB10 protein produced from lymphoblasts from one of the affected family members showed the full-length product as well as smaller molecular weight bands (Pasutto et al., 2012). Since each of the affected individuals is heterozygous for the mutation, they have both normal and mutant ASB10 protein.

2.3 The SOCS box

The C-terminal SOCS box of approximately 40 amino acids is the most conserved motif in ASB proteins (Kile et al., 2002). The function of the SOCS box is to recruit components of the ubiquitin (Ub) ligase complex to ubiquitinate the substrate bound to the ANK repeats for degradation (Kile et al., 2002; Piesseaux et al., 2008). ASB proteins use ubiquitination to promote the turnover of their bound substrate. Thus, SOCS box-containing proteins have been implicated as negative regulators of cell signaling.

The multi-subunit Ub ligase complex include a platform protein (Cullin), a RING family protein (Rbx) and an adapter protein (elongin BC) (Kohroki et al., 2005). ASBs 1, 2, 6, 7 and 12 bind to cullin 5 (CUL5) and RING box protein-2 (RBX2) (Kohroki et al., 2005). Furthermore, a recent study used proteomics to confirm that CUL5 and RBX2 bind to ASB10 (Andresen et al., 2014). The CUL5-RBX2 ligase complex is categorized as an E3 ubiquitin ligase (Lamsoul et al., 2016). Because ubiquitination by E3 ligases control entry into the ubiquitination-mediated protein degradation pathways, these are promising targets for the development of therapeutic interventions. Several drugs targeting CUL5-RBX E3 ligases are currently in clinical trials for the treatment of some solid tumor and hematologic malignancies (Lamsoul et al., 2016; Zhao and Sun, 2013).

Primary amino acid sequence analysis of the ASB10 SOCS box shows the presence of CUL5 and Elongin BC consensus binding sequences (Fig. 2) (Kohroki et al., 2005). Interestingly, a putative POAG mutation (R438C) resides at the center of the CUL5 binding motif (Pasutto et al., 2012). Furthermore, a Pakistani individual with a particularly severe form of glaucoma was homozygous for this mutation (named R453C in their study) (Micheal et al., 2015). Because the R438C mutation sits at the center of the CUL5 binding site, it could disrupt recruitment of CUL5 to the SOCS box. It is conceivable that the substrate bound to the ANK repeats would not be ubiquitinated properly and entry into the degradation pathways would be limited. Alterations in proteolytic degradation activity might therefore predispose POAG patients to an accumulation of cellular ASB10 and/or its bound substrate(s).

3. ASB10 expression and function

3.1 ASB10 expression

Little research has been performed on ASB10. One study showed high mRNA expression in skeletal muscle and heart (Kile et al., 2001). Another study showed that ASB10 protein was present in caveolin-1-enriched fractions of keratinocytes (Lemaitre et al., 2005). Screening

of ocular tissues revealed that ASB10 mRNA was expressed in several ocular tissues including trabecular meshwork (TM), iris, ciliary body, retina, choroid, lamina cribrosa, and the optic nerve, but not the cornea or the lens (Pasutto et al., 2012). Of these, the TM and retina are particularly relevant to glaucoma since the TM regulates intraocular pressure (IOP) and axonal degeneration of RGCs is a characteristic phenotype of glaucoma. Indeed, RNAi gene silencing of ASB10 decreases outflow in anterior segment perfusion culture (Keller et al., 2013; Pasutto et al., 2012). These studies used two different shRNAs, which targeted exon 2 or exons 5-6 that are common to all ASB10 alternative transcripts. Since ASB10 was originally mapped in a POAG family with elevated IOP, our studies suggest that ASB10 functions in the conventional aqueous humor outflow pathway and support a role for ASB10 in IOP homeostasis.

3.2 ASB10 and ubiquitin-mediated protein degradation pathways

Proteins targeted for degradation are tagged with ubiquitin. Polyubiquitin chains are built by transferring ubiquitin molecules onto specific lysine (K) residues (Pickart, 2000). Two such linkages are at K48 and K63. K48-linked polyubiquitin chains are sorted into the ubiquitin-proteasomal (UPS) degradation pathway, while those tagged with K63-linked ubiquitin are degraded by the autophagic-lysosomal (AL) pathway (Fig. 3) (Tan et al., 2008). We used K-linkage-specific antibodies (Newton et al., 2008) to show that ASB10 is associated with biomarkers of both the UPS and AL degradation pathways in cells (Keller et al., 2013). These two pathways work reciprocally to clear proteins destined for degradation: Inhibition of one pathway activates the other pathway (Pandey et al., 2007). Our recent results show that if you inhibit the proteasomal pathway using MG132, outflow resistance is increased in human anterior segment perfusion culture (Keller et al., 2013). Thus, these degradation pathways appears to play a role in the IOP regulation.

The involvement of ASB10 in protein degradation pathways was further strengthened by a recent study identifying proteins that interact with ASB10 (Andresen et al., 2014). The open-reading frame was cloned into an expression vector and transfected into 4 different cell lines that had been labeled with SILAC (stable isotope labeling by amino acids in cell culture). After 48 hours, 22 interacting proteins were identified from immunoprecipitates using LC-MS/MS (Andresen et al., 2014). Several of these proteins are related to ubiquitin-associated degradation pathways. For instance, CUL5, RBX2, Elongin B (TCEB2) and Elongin C (TCEB1) are part of the Cullin-ring complex that interacts with the SOCS box (Fig. 2), while polyubiquitin C (UBC) is a source of ubiquitin (Thomas et al., 2013). Another ASB10-interacting protein was identified as HSPA1A, which is also known as HSP72 or HSP70. Our recent paper shows strong colocalization of HSP72 and ASB10 in intracellular vesicles (Keller et al., 2013). HSP72 is a biomarker of aggresomes, a pre-autophagic structure. Thus, both mass spectrometry and immunofluorescence studies show ASB10 is associated with molecules of ubiquitin-mediated protein degradation pathways.

The UPS and AL protein degradation pathways are functional in healthy cells of both the TM and the retina (Esteve-Rudd et al., 2010; Liton, 2016; Liton et al., 2009a; Munemasa and Kitaoka, 2015). Evidence is accumulating that dysfunction of these pathways in aged and glaucomatous tissues may lead to accumulation of toxic material and a progressive

cellular decline of cellular function (Caballero et al., 2004; Liton et al., 2009c). This may be particularly detrimental for non-dividing cells such as those in the TM and the retina since the accumulated material would not be diluted by distribution to daughter cells. A recent proteomics study showed many proteins linked to the ubiquitination pathway are up-regulated in hypertensive human retinas (Yang et al., 2015b). Interestingly, two up-regulated proteins identified in this hypertensive study, HSPB1 and UBC, were identified to bind to ASB10 (Andresen et al., 2014). Because of its expression in the TM and retina and its role in proteolytic degradation pathways, it is therefore possible that mutant ASB10 may contribute to cellular dysfunction. Mechanical stresses induced by elevated IOP and/or environmental factors such as chronic oxidative stress and aberrant expression of inflammatory cytokines may also predispose these tissues to progressive functional decline (Liton et al., 2009b; Luna et al., 2009).

4. Protein degradation pathways and other glaucoma-associated proteins

Several recent studies have linked other glaucoma-associated molecules with the UPS and AL degradation pathways. These have been extensively reviewed elsewhere so we shall only provide a brief overview of their findings (Liton, 2016; Liton et al., 2009a; Qiu et al., 2014; Sirohi and Swarup, 2016; Ying and Yue, 2016). All of these studies suggest that the pathways work in tandem to clear proteins from cells. For instance, optineurin, a gene associated with normal tension glaucoma (Ying and Yue, 2012, 2016), is usually degraded by the proteasome pathway in retinal ganglion cells (RGCs) (Ying et al., 2010). However, mutated or over-expressed optineurin is degraded via the autophagic pathway (Shen et al., 2011). Myocilin is the archetypal glaucoma-associated gene and mutant myocilin is known to accumulate in the endoplasmic reticulum, ultimately inducing cell death (Stothert et al., 2016). In normal cells, myocilin was found to be cleared by both the UPS and AL pathways (Qiu et al., 2014). However, mutant myocilin was preferably cleared via autophagic degradation. Thus, both the UPS and AL pathways are involved in clearing glaucoma-associated proteins and there appears to be a fine balance between which of these pathways is preferentially utilized.

5. ASB10 and other diseases

Recently, a whole exome sequencing study identified *ASB10* as a gene associated with a significant increased risk of systemic sclerosis (Gao et al., 2016). This autoimmune disease is characterized by excessive collagen production in the skin and other internal organs (Murdaca et al., 2016). Several studies have correlated an increased prevalence of glaucomatous neuropathy in patients with systemic sclerosis, although there was no difference in IOP between patients and controls, which may suggest a link to normal tension glaucoma (Allanore et al., 2004; Gomes Bde et al., 2011; Yamamoto et al., 1999). Systemic sclerosis is characterized by an accumulation of connective tissue components that leads to progressive hardening and scarring of the tissue (Murdaca et al., 2016). In the glaucoma field, much recent discussion has centered on the potential of fibrosis in the TM and optic nerve head leading to decreased compliance and increased stiffness of these tissues (Last et al., 2011; McDonnell et al., 2014; Overby et al., 2014; Yang et al., 2015a). Identification of

ASB10 mutations in glaucoma and systemic sclerosis, two diseases that are characterized by altered deposition of ECM components and increased tissue stiffness, is therefore intriguing.

6. ASB10 and inflammatory cytokines

While we and others have identified *ASB10* as a glaucoma-associated gene (Micheal et al., 2015; Pasutto et al., 2012), it is highly likely that there are additional modifiers that may impact the development and/or severity of the disease. Tumor necrosis factor- α (TNF α) has the potential for being a modifier based on its being a contributing factor in glaucoma neurodegeneration (Nakazawa et al., 2006; Tezel et al., 2001; Tezel et al., 2004; Yan et al., 2000; Yang et al., 2011). Several genetic studies have suggested an association between *TNF α* promoter polymorphisms and glaucoma risk (Fan et al., 2010; Lin et al., 2003; Razeghinejad et al., 2009; Wang et al., 2012). Interestingly, the *TNF α* (-857) variant appears to have an impact on severity of glaucoma in patients with the synonymous polymorphism Thr34Thr in optineurin (Funayama et al., 2004). Polymorphisms in the interleukin-1 (IL-1) gene cluster also appear to increase the susceptibility for developing POAG (Markiewicz et al., 2013; Mookherjee et al., 2010; Wang et al., 2006).

TNF α and IL-1 α are inflammatory cytokines that are released following laser trabeculoplasty, a common surgical intervention for glaucoma patients (Bradley et al., 2000). Both of these cytokines increase outflow in human anterior segment perfusion culture (Bradley et al., 2006). We therefore investigated whether *ASB10* expression was affected by TNF α or IL-1 α treatment of human TM cells. All methods are included in the supplement. Quantitative RT-PCR showed a ~3.8- and 2-fold up-regulation of *ASB10* mRNA after 24 and 48 hours of TNF α treatment, respectively (n=3; Fig. 4). For IL-1 α , *ASB10* mRNA was significantly up-regulated ~2.5-, 3.5- and 2.7-fold at 12, 24 and 48 hours after treatment, respectively. Western blotting of TM cell RIPA lysates confirmed the up-regulation of *ASB10* protein with TNF α or IL-1 α treatment at 48 hours. We also investigated the distribution pattern in cells by immunofluorescence. Using a monoclonal antibody that recognizes the V1 variant, confocal images clearly showed an increase in endogenous v1 immunostaining after 72 hours (Fig. 4C-E). The localization of *ASB10* V1 antibody appears to be on the cell surface, consistent with the prediction that the N-terminus of the V1 variant is outside the cell. Collectively, this data suggests that *ASB10* expression is increased by inflammatory cytokines. We hypothesize that this increase in *ASB10* expression is required to bind a specific target and send it for degradation, similar to the *ASB3*-TNF α RII interaction (Chung et al., 2005). It is conceivable that mutant *ASB10* is unlikely to respond appropriately to inflammatory signals in the eye, which could precipitate the deleterious changes that lead to glaucomatous vision loss.

6.1 The function of other SOCS box-containing proteins in the eye

Other SOCS box-containing proteins also appear to play a role in inflammatory events in the eye. The SOCS family of proteins has 8 members (Piessevaux et al., 2008). SOCS1 may have a neuroprotective role in the retina as it inhibits lymphocyte recruitment during inflammatory events (Yu et al., 2011). SOCS3 is a negative regulator of the janus kinase / signal inducer and activator of transcription (JAK/STAT) pathway. SOCS3 activity has been

linked to ubiquitin-mediated degradation of the photoreceptor rhodopsin (Ozawa et al., 2008). During retinal inflammation in SOCS3 knockout mice, the levels of rhodopsin were markedly reduced and the mice had worse visual disturbance than their wild-type counterparts. However, persistent expression of SOCS3 caused by chronic inflammation may induce insulin resistance and inhibit neurotrophic factors that are essential for retinal cell survival (Liu et al., 2008).

SOCS3 also appears to negatively regulate axon regeneration because RGCs in conditional SOCS3 knockout mice had a significant increase in regeneration up to two weeks post-injury (Smith et al., 2009). Double knockouts showed that axon regeneration induced by SOCS3 deletion is largely dependent upon gp130-mediated signaling, which triggers the JAK-STAT pathway. Moreover, SOCS3 may work in synergy with phosphatase and tensin homolog (PTEN), a negative regulator of the mammalian target of rapamycin (mTOR) pathway (Sun et al., 2011). Concurrent activation of both the STAT3 and mTOR pathways appears to promote long-distance axon regeneration (Sun et al., 2011). Collectively, these studies suggest that SOCS3 expression suppress axon regeneration in the mouse eye. Interestingly, both the mTOR and STAT3 pathways suppress autophagy (Jung et al., 2010; Shen et al., 2012). Thus, it appears that autophagy may negatively regulate axon growth in the eye. However, this may be over-simplistic since several other studies have reported contradictory findings (Koch and Lingor, 2016). The role of autophagy in glaucomatous optic neuropathy and the possible role in axonal protection has recently been discussed in detail elsewhere (Munemasa and Kitaoka, 2015).

Conclusions and Future studies

While *ASB10* mutations appear to contribute a rare familial form of POAG, they have the potential to identify important pathways in glaucoma. Rare familial forms of disease have been the basis for identifying important pathways in many diseases including neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Although *ASB10* mutations in POAG may be rare, identifying pathway(s) involving *ASB10* should lead us to insights about glaucoma pathogenesis not only in rare Mendelian families, but also in sporadic cases of POAG. Recent functional studies on *ASB10*, together with studies on the glaucoma-associated genes myocilin and optineurin, suggest dysfunction of the proteasomal degradation pathways may be a common feature that leads to the detrimental changes associated with glaucoma. Moreover, studies on SOCS box-containing proteins and their recruitment of the Cullin-RING E3 ligase complexes may lead to the development of novel clinical strategies for the treatment of glaucoma. Further studies on the normal function of *ASB10*, particularly deciphering the function of the N-terminal variants and identification of the substrate to which *ASB10* binds, should provide novel data of how proteolytic degradation pathways function in the eye. Moreover, development of an *ASB10* null mouse, or a R438C knock-in mouse, would allow us to study the effects of *ASB10* on glaucoma-associated phenotypes such as IOP, RGCs, and optic disc cupping and may also serve as animal model to test glaucoma therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

ASB10 variants are associated with glaucoma in USA, German and Pakistani cohorts

A homozygous mutation in the SOCS box may disrupt binding to E3 ubiquitin ligase

ASB10 functions in ubiquitin-mediated protein degradation pathways

ASB10 is up-regulated by TNF α and IL-1 α in trabecular meshwork cells

Figure 1. Schematic of ASB10

The modular structure of ASB10 is shown: the N-terminal region (green), the 6 ANK repeats (blue) and the C-terminal SOCS box (pink). Alternative mRNA splicing of exon 1 results in two protein isoforms called V1 and V3. Bioinformatics analyses of the primary amino acid sequence shows that variant 1 has a predicted signal peptide and its N-terminus is outside the cell, whereas V3 may not to have a signal peptide and its N-terminus is predicted to be inside the cell. Signal peptide prediction analyses were performed at www.cbs.dtu.dk/services/SignalP/ and transmembrane predictions were performed at www.ch.embnet.org/software/TMPRED_form.

Figure 2. The SOCS box of ASB10

Schematic showing the mechanisms used by ASB proteins to ubiquitinate and degrade target substrates. ANK repeats bind to the target substrate (purple). Components of the ubiquitin ligase complex (Cullin5 (blue), Elongin BC (red), Rbx2 (yellow), E3 ubiquitin ligase (green)) are recruited by the SOCS box and these transfer ubiquitin (Ub; black) to the bound substrate. The primary sequence of the SOCS box of ASB10 is shown including the positions of three glaucoma-associated mutations. Two of these are located in the Cullin-5 binding motif.

Figure 3. Ubiquitin-mediated protein degradation pathways

Schematic showing the reciprocal ubiquitin-proteasomal (UPS; top) and autophagy-lysosomal (AL) protein degradation pathways (bottom). ASB10 has been found to colocalize with biomarkers of both the UPS (K48-linked ubiquitin, 20S) and the AL (K63-linked ubiquitin, HSP70, P62, HDAC6, LC3, LAMP1) pathways.

Figure 4. Effects of TNF α and IL-1 α on ASB10 expression

(A) ASB10 mRNA levels were measured using quantitative RT-PCR from human TM cells treated for 12, 24 and 48 hours with 10 ng/ml TNF α or IL-1 α . Data shown are the average fold change compared to untreated control $\pm$ standard error of the mean. N=3; * $p < 0.05$. (B) Western immunoblotting of human TM cells treated for 48 hours with 10 ng/ml TNF α or IL-1 α or untreated control with an antibody recognizing the V1 variant. (C) Distribution of the V1 variant of ASB10 in human TM cells treated with 10 ng/ml TNF α or IL-1 α , or untreated control for 72 hours by confocal microscopy. Confocal acquisition settings were identical for all images. Scale bar = 20 μ m.