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Novel roles for α -crystallins in retinal function and disease

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Abstract

α -Crystallins are key members of the superfamily of small heat shock proteins that have been studied in detail in the ocular lens. Recently, novel functions for α -crystallins have been identified in the retina and in the retinal pigmented epithelium (RPE). α B-Crystallin has been localized to multiple compartments and organelles including mitochondria, golgi apparatus, endoplasmic reticulum and nucleus. α -Crystallins are regulated by oxidative and endoplasmic reticulum stress, and inhibit apoptosis-induced cell death. α -Crystallins interact with a large number of proteins that include other crystallins, and apoptotic, cytoskeletal, inflammatory, signaling, angiogenic, and growth factor molecules. Studies with RPE from α B-crystallin deficient mice have shown that α B-crystallin supports retinal and choroidal angiogenesis through its interaction with vascular endothelial growth factor. α B-Crystallin has also been shown to have novel functions in the extracellular space. In RPE, α B-crystallin is released from the apical surface in exosomes where it accumulates in the interphotoreceptor matrix and may function to protect neighboring cells. In other systems administration of exogenous recombinant α B-crystallin has been shown to be anti-inflammatory. Another newly described function of α B-crystallin is its ability to inhibit β -amyloid fibril formation. α -Crystallin mini-chaperone peptides have been identified that elicit anti-apoptotic function in addition to being efficient chaperones. Generation of liposomal particles and other modes of nanoencapsulation of these minipeptides could offer great therapeutic advantage in ocular delivery for a wide variety of retinal degenerative, inflammatory and vascular diseases including age related macular degeneration and diabetic retinopathy.

Keywords

α B-Crystallin; Angiogenesis; Apoptosis; Chaperone; Exosomes; Retinal pigment epithelium

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1. General background

The α -crystallins are molecular chaperones belonging to the small heat shock protein (sHSP) superfamily. The members of this family have been found throughout all animal kingdoms (Kappe et al., 2002). In spite of the differences in sequence and size, sHSPs share some features in common such as a highly conserved α -crystallin domain, a comparatively small molecular mass (12–43kDa), a dynamic quaternary structure, formation of large oligomers, induction by different stressors, and chaperone activity. The most widely studied sHSP α -crystallin, has two subunits, namely α A and α B-crystallin. Though once considered lens specific, α -crystallin has widespread non-lenticular distribution (Horwitz, 2003). It was once thought that sHSPs were exclusively intracellular molecules which functioned as intracellular molecular chaperones. Recent data reveal that certain HSPs are released from cells which are induced by endogenous and exogenous factors and exhibit extracellular functions. We have described the pathways of exosomal secretion of α B-crystallin from primary human RPE cells (Sreekumar et al., 2010) which have also been described in the ARPE-19 cell line (Gangalum et al., 2011). When released, HSPs have the potential to act on cell surface receptors; elicit autocrine signals; paracrine signals; or endocrine signals. In this respect, the sHSPs are considered akin to cytokines.

α -Crystallins are characterized by the presence of a conserved crystallin domain flanked by a variable N-terminal domain and C-terminal extension (Fig. 1). Each of the domains has chaperone function and this function can vary depending on post-translational modifications of the N-terminus such as oxidation, phosphorylation, deamidation, acetylation and truncation (Kapphahn et al., 2003; Asomugha et al., 2011). The N- and C-termini, together with part of the crystallin domain, are also involved in substrate binding. The α -crystallin domain consists of 6–8 β -strands organized in two β -sheets flanked by the NH_2 - and COOH -terminal regions, and this domain is considered an important hallmark of sHSPs, independent of their origin and nature (Kampinga et al., 2009). Very often the NH_2 -terminal region contains a number of sites phosphorylated by different protein kinases (Haslbeck et al., 2005; Vos et al., 2008). α -Crystallins have stable structures and are water soluble. In the lens, native α -crystallin is composed of subunits of α A and α B-crystallin (Horwitz, 2003). α A- and α B-crystallin occur as a heteroaggregate of variable size, ranging from 300,000 to over 1,000,000 daltons and each monomer is approximately 20 KDa in size. The proportion of α A-crystallin to α B-crystallin varies in each aggregate, but generally in a 3:1 ratio of α A- to α B-crystallin in the mammalian lens (Posner, 2003). Attempts to crystallize full-length human crystallins were unsuccessful until recently. In 2010, the structure of full length α B-crystallin was determined by means of solid-state NMR and small-angle X-ray scattering (Jehle et al., 2010). The NMR ribbon structure of α B-crystallin core domain dimer is shown in Figure 2.

In addition to their chaperone functions, α -crystallins and other sHSPs perform multiple additional tasks; they may assist in the efficient folding of newly translated proteins (Frydman, 2001), inhibit subcellular transport of some proteins (Liu et al. 2007; Mao et al. 2004), prevent cell death (Acunza et al. 2012; Adhikari et al., 2011; Andley 2000, 2007; Arrigo et al., 2007; Mao et al., 2001, 2004; Parcellier et al., 2005; Sreekumar et al., 2010, 2012, 2012b; Yaung et al., 2008, 2007; Whiston et al. 2008), inhibit inflammation (Arac et al., 2011; Arrigo et al., 2007; Masilamoni et al., 2005, 2006; Ousman et al., 2007; Rothbard et al., 2012; van Noort et al., 2012; Whiston et al., 2008), provide neuroprotection (Ousman et al., 2007; Sreekumar et al., 2010) and regulate proteosomal degradation of proteins (Adhikari et al., 2011; Boelens et al., 2001; den Engelsman et al., 2003; Kase et al., 2010; Singh et al., 2010). Further, sHSPs participate in angiogenesis (Dimberg et al., 2008; Kase et al., 2010), and interact with several proteins or cytokines and influence cell signaling (Andley, 2007; Ecroyd and Carver, 2009; Ghosh et al. 2007a, 2007b; Rekas et al., 2004;

Kase et al., 2010; Sreekumar et al., 2011). Thus, these chaperones broadly shape protein homeostasis by controlling protein quality control and turnover during both normal and stress conditions. Consistent with these diverse activities, genetic and biochemical studies have implicated their role in a wide range of pathological conditions, including neurodegeneration, cancer, and inflammation. Thus new roles of sHSPs important for physiology and disease are emerging in this rapidly expanding field. This review summarizes our knowledge on α -crystallins in the retina with special emphasis on their function under normal and pathological conditions and their potential as therapeutic agents.

2. Evolutionary aspects

In humans, the α A-crystallin gene encodes a 173 amino acid residue protein, while the α B-crystallin gene encodes a 175 amino-acid residue protein. The amino-acid sequence homology between α A and α B is about 57% (de Jong et al., 1998). Both α A- and α B-crystallin are encoded by single copy genes located on chromosomes 21 and 11, respectively, in humans (Quax-Jeuken et al., 1985; Ngo et al., 1989) and contain two introns at homologous positions. However, in some species such as rat, rabbit and mouse an additional 63 bp are added from intron 1 resulting in a protein 23 amino acids longer than the regular α A crystallin, named α A insert (Derham and Harding, 1999; Graw, 2009). A variant without the insert is also present in these species. Although some evidence suggests decreased chaperone function for the insert (Derham et al., 2001), this phenomenon remains to be further explored. The genes for α A- and α B-crystallin originated by duplication of an ancestral α -crystallin gene, which occurred well before the divergence of the cartilaginous fishes, and the higher vertebrates (de Jong et al., 1993).

3. Tissue and cellular distribution of α -crystallin and factors affecting its expression

α A and α B-crystallin have a differential tissue distribution. α A-crystallin is prominently localized to the eye and is the most abundant in the lens. However, its non-lenticular expression is well described (Kato et al., 1991; Srinivasan et al., 1992). It was reported that a significant amount of α A-crystallin was present in rat spleen and thymus and low levels were detected in the rectum, cecum, liver, kidney, adrenal, cerebellum and brain stem (Kato et al., 1991; Srinivasan et al., 1992). In the retina, α A-crystallin is expressed mostly in the ganglion cell and inner nuclear layers and in photoreceptors (Rao et al., 2008; Xi et al. 2003; Yaung et al., 2008). α A-crystallin is significantly upregulated in the early phase of experimental autoimmune uveitis (EAU), especially in the photoreceptor inner segments (Rao et al., 2008). It is also one of the components of drusen and was found to increase in retinal degenerative diseases such as age-related macular degeneration (AMD) (Nakata et al., 2005). α B-crystallin is ubiquitously expressed and its expression has been well documented in lens, neural retina, retinal pigment epithelium (RPE), heart, skeletal muscle, kidney, placenta, skin, brain, peripheral nerves, spinal cord, and liver; however, the highest level of α B-crystallin is found in the lens (Bhat and Nagineni, 1989; de Jong et al., 1993; Horwitz et al., 1999; Inaguma et al., 1995; Iwaki et al., 1990, 1989; Lowe et al., 1992). Expression of α B-crystallin is highly regulated during development (Robinson and Overbeek, 1996), under some pathological conditions such as AMD (De et al., 2007; Nakata et al., 2005), ischemia (Pangratz-Fuehrer et al., 2011), diabetes (Kandpal et al., 2012; Kumar et al., 2005), retinal and choroidal neovascularization (Kase et al., 2010), multiple sclerosis (Ousman et al., 2007; Rothbard et al., 2012), inflammatory disorders (Hegen et al., 2010; Wanschitz et al., 2008) and myofibrillar myopathies (Selcen, 2011). An especially large increase in α B-crystallin expression was detected in cancer cells from patients with retinoblastoma (Pineda et al., 1993), breast cancer (Chan et al., 2011; Launay et al., 2010), and glioblastoma (Goplen et al., 2010). In the retina, both α A-and α B-crystallins were

found to prominently expressed in the inner retina (ganglion cell layer and inner synaptic layer) and in photoreceptors and RPE (Piri et al., 2007; Xi et al., 2003; Yaung et al., 2008). Fig. 3 shows the distribution of α B-crystallin in the retina of a 10 week old 129 SvJ mouse. Expression is seen in the inner retina, the inner and outer segments of photoreceptors and in the RPE. The expression of α -crystallin is regulated by oxidative stress showing either an increase or a decrease depending on the severity of dose and duration. In general, α B-crystallin expression is increased with mild oxidative stress (Shin et al., 2009; Yaung et al., 2008, 2007); however, chronic oxidative stress with H_2O_2 causes a decrease in expression (Yaung et al., 2007). For instance, in mice treated with 33 nmol $CoCl_2$ for a week, increased expression of α B-crystallin was seen and expression was more diffusely distributed across the retina, whereas increased retinal degeneration and decreased levels of α B-crystallin were observed with a higher dose (Yaung et al., 2008). Further, in an intraocular hypertension induced ocular ganglion cell degeneration rat model, the mRNA and protein levels of α -crystallins showed an initial decrease at 2 weeks followed by recovery to levels found in control retina at 5 weeks post-IOP (Piri et al., 2007). Temperature also upregulated α B-crystallin expression and the temperature-dependent increase of α B-crystallin seems to be dependent on the activity of heat shock factors which interact with two regulatory heat shock elements (Kato et al., 1999). Intense green light exposure significantly upregulated α B-crystallin expression in rod outer segments of photoreceptor and RPE cells, and this could be a mechanism to protect photoreceptors from light damage (Sakaguchi et al., 2003). Recent evidence supports an important role for α B-crystallin in the pathogenesis of AMD. In pathologic specimens from patients with both late “dry” and “wet” AMD, α B-crystallin was heterogeneously expressed by RPE cells and the expression level was significantly increased in the RPE when compared with control samples (De et al., 2007). As shown in Fig. 4, we have confirmed that there is prominent and increased expression of α B-crystallin in RPE associated with drusen. In addition, α B-crystallin has been identified as a component of drusen (Crabb et al., 2002; De et al., 2007; Nakata et al., 2005).

Both α A- and α B-crystallin can be considered as soluble cytosolic proteins but they are also localized within subcellular organelles of unstressed cells including mitochondria and endoplasmic reticulum (Yaung et al., 2007). The cellular localization and distribution seems to be dependent on the physiological status of the cells. In the developing lens, there is a transient association of α B-crystallin with golgi-associated membrane (Gangalum and Bhat, 2009); an association between α B-crystallin and golgi in RPE cells and photoreceptors has also been shown (Deretic et al., 1994). In stressed cells, α B-crystallin also translocates to the nucleus (Adhikari et al., 2004; den Engelsman et al., 2005; Sreekumar et al., 2010; Verschuure et al., 2002) or to mitochondria (Adhikari et al., 2004; Jin et al., 2008; Whittaker et al., 2009; Yaung et al., 2007) or endoplasmic reticulum (Dou et al., 2010; Kase et al., 2010). Both HSP-27 and α B-crystallin localized to nuclear speckles in unstressed cardiac myocytes (den Engelsman 2004). Further it was shown that silencing of α B-crystallin sensitized ARPE-19 cells to a histone deacetylase inhibitor (HDAC1) SAHA by abolishing the association of HDAC1 with nuclear SC35 speckles (Noh et al. 2008). An upregulation of α B-crystallin in mitochondria with oxidative stress from H_2O_2 treatment is illustrated in Fig. 5. Some studies claimed that phosphorylation is required for the translocation (Golenhofen, 1998) while others showed translocation is independent of phosphorylation (Verschuure et al., 2002). However, a recent study demonstrated that during murine cardiac myocyte ischemia α B-crystallin translocates to the mitochondria and contributes to modulating mitochondrial damage upon reperfusion. In this model system α B-crystallin is, at least in part, phosphorylated in association with mitochondria (Whittaker et al., 2009). We studied the translocation of α B-crystallin using fluorescein labeled human recombinant α B-crystallin in the presence or absence of oxidative stress in RPE. Human RPE cells incubated with 500 μ M H_2O_2 for 1 h showed a marked uptake of α B-crystallin into the nucleus when compared with unstressed cells (Sreekumar et al., 2010). Chemically induced hypoxia

caused translocation of α B-crystallin to the ER where it colocalized with growth factors such as vascular endothelial growth factor (VEGF) (Kase et al., 2010). Hypoxia also caused translocation of α B-crystallin to the mitochondria (Yaung et al., 2008). Further work is needed to understand other mechanism(s) that may be involved in the subcellular translocation of α -crystallins such as role of putative transporters or phosphorylation.

4. Knockout models

To facilitate understanding the *in vivo* functions of α -crystallins, animal models have been developed where one or both of the α -crystallin gene products have been knocked out. With an idea of studying the significance of α A-crystallin in lens function, Brady et al. (1997) generated α A-crystallin knockout (KO) mice. Targeted elimination of the α A-crystallin gene induced cataract and accumulation of dense cytoplasmic bodies including α B-crystallin, HSP25 and α -crystallin in lens fiber cells (Brady et al., 1997) without any apparent changes in the retinal layers. α A-crystallin KO lenses were 40% smaller in size than age matched wild type mice; a mild cataract begins to develop as early as 7 weeks and a fully opaque mature cataract ensues by 18 weeks (Brady et al., 1997; Kannan et al., 2001). Targeted disruption of α B-crystallin did not produce cataracts (Brady et al., 2001) as observed in α A-crystallin KO mice. α B-crystallin KO mice are viable and fertile, with no obvious prenatal defects and normal lens transparency (Brady et al., 2001). Until recently, a retinal phenotype had not been described in the α B-crystallin KO mice. The major phenotype in these mice, which lack both α B-crystallin and HspB2 (an adjacent gene located 1kb upstream of α B-crystallin) was that as the mice become aged, homozygous KO mice developed degeneration of the musculature with severe spine curvature (kyphosis) (Brady et al., 2001). Histology of the eyes, cardiac muscles and skeletal muscle from α B-crystallin KO revealed no apparent structural abnormalities. A study from our laboratory tested the hypothesis that deficiency of α B-crystallin during development could result in changes in retinal vasculature (Kase et al., 2010). Retinal vessel density was lower at the junction between the inner plexiform layer and the outer nuclear layer in adult α B-crystallin KO mice (Kase et al., 2010) leading to the suggestion that localized changes in retinal vessel formation could result from decreased expression of vascular endothelial growth factor (VEGF) during development. The observed phenomenon was due to α B-crystallin deletion rather than HspB2 since HspB2 was not expressed retina or RPE (Kase et al., 2010). Further, HspB2 is not heat-shock-inducible, and is thus unlikely to act as a general chaperone (Doerwald et al., 2004).

α B-Crystallin deficient mice or α B-crystallin mutants (Sacconi et al., 2012) are highly susceptible to cell death when subjected to oxidative stress (Yaung et al., 2008, 2007) or in inflammatory disease models (Arac et al., 2011; Ousman et al., 2007). As in the case of α B-crystallin KOs, RPE cells isolated from α A-crystallin KO mice are highly susceptible to inflammation or oxidative stress-induced apoptosis (Rao et al., 2008; Yaung et al., 2007). In α A-crystallin KO mice, α B-crystallin could compensate for some of the functions of the missing protein, particularly in the lens, where both α A and α B are abundantly expressed. Therefore, to study the combined function(s) of α -crystallins, double deficient mice were generated which had gross abnormalities in the fiber cell structure that was not evident in single crystallin KO mice (Boyle et al., 2003). The lack of cellular organization and uniform cell shape at the equatorial region observed in α -crystallin double KO lenses suggests a role for α -crystallins in cytoskeletal organization.

5. Function and properties of α -crystallin

Mammalian sHSPs, including α A-crystallin and α B-crystallin, are constitutively expressed during development. Though chaperone activity is the first described function of α -

crystallins in the eye, the importance of α -crystallins in cellular function is further highlighted by their participation in a growing number of fundamental cellular processes such as prevention of apoptosis, protection against oxidant insults, and neuroprotection (Andley, 2007; Arrigo et al., 2007). These are discussed in detail below.

5.1 Chaperone function

Molecular chaperones are a class of unrelated families of proteins that assist the proper assembly of other polypeptide-containing structures *in vivo*, but are not components of these assembled structures when they are performing their normal biological function (Ellis, 1993). Molecular chaperones consist of different classes of proteins that can assist in stabilization of native protein conformations, protein folding, mediation and stabilization of correct oligomeric assembly of proteins, translocation of proteins, and protection of proteins from multiple stresses (Derham and Harding, 1999). The chaperone activity of α -crystallins was first characterized under *in vitro* experimental conditions using purified bovine α -crystallin where they inhibited aggregation of several enzymes, prevented heat-induced aggregation of β and α -crystallins and refolding of α -crystallins in the lens (Horwitz, 1992). The fundamental cellular processes, like protein synthesis, transport and turnover involve unfolded or partially denatured proteins, exposed hydrophobic regions which may interact incorrectly, even at low concentration (Jaenicke, 1987). The chaperone activity is generally studied by measurement of light scattering of proteins incubated at high temperature or to reduction induced denaturation. The protective properties of α -crystallin have been demonstrated with other *in vitro* assays including prevention of aggregation of insulin B by reduction of disulfide bonds (Das and Surewicz, 1995; Farahbakhsh et al., 1995), by refolding of guanidine hydrochloride (or urea)-denatured proteins (Horwitz, 1992; Raman et al., 1995), by prevention of UV-induced aggregation of proteins (Raman and Rao, 1994) and prevention of inactivation of enzymes by small molecules (Ganea and Harding, 1995, 1996; Heath et al., 1996; Hook and Harding, 1996). The ability of α -crystallin to confer thermotolerance *in vivo* which supported the *in vitro* findings has also been described (Plater et al., 1996; van den Ijssel et al., 1994).

The mechanism by which α -crystallin prevents protein aggregation or its chaperone activity has been studied by several investigators. These studies suggest that there are multiple substrate binding sites in each α -crystallin oligomer and the substrates that bind with are in a molten globular state (Carver and Lindner, 1998; Carver et al., 2002). The substrate- α -crystallin complex is very stable. α -Crystallins do not interfere with intermediates of protein folding pathway but are specific for the destabilized, precipitation-bound, relatively unstructured intermediates that have the potential to aggregate (Carver et al., 2002). Thus, kinetic factors could be one of the major determinants in regulating the interaction of a protein with molecular chaperones; transient intermediates associate with protein folding pathway chaperones, e.g. GroEL and DnaK, whereas long-lived intermediates that are potentially aggregating, associate with off-folding pathway chaperones, e.g. sHSPs (Carver et al., 2002). Further, the association and dissociation of oligomer subunits play an essential role in the chaperone functions (Laskowska et al., 2010). A number of parameters such as temperature, phosphorylation, ionic strength, pH, Ca^{2+} concentration, tissue age, and protein concentration lead to the subunit exchange and dissociation of oligomers (Bova et al., 2000, 1997; Regini et al., 2004). Several *in vitro* studies suggest that α -crystallin activity does not require ATP hydrolysis and therefore, α -crystallin becomes particularly significant under stress conditions when the level of ATP is very low (Horwitz, 1992). However, ATP effects have been reported by others, primarily with α -crystallin (Barton et al., 2009; Biswas and Das, 2004; Muchowski and Clark 1998; Wang and Spector, 2000). The identification of $\beta 4$ - $\beta 8$ groove as the ATP interactive region in α B-crystallin, is in contrast to the existing paradigm that classifies sHSPs as ATP-independent chaperones (Dutta et al., 2010; Ghosh et

al., 2006). However, it should be noted that many sHSPs are not constitutively active; they are specifically activated in the presence of stress. Further studies are needed to clarify the importance of ATP in the chaperone activity of α -crystallins.

Different methods are utilized by investigators to identify potential sequences of α -crystallins showing chaperone activity. Biophysical studies (Berengian et al., 1999; Bova et al., 2000) and subunit interaction studies using peptide array (Sreelakshmi et al., 2004) and pin array (Ghosh et al., 2005) showed extensive contact and interaction occurs between the subunits. Regions 42–57 and 60–71 in α B-crystallin function as subunit interaction sites (Sreelakshmi et al., 2005; 2004). The residues 73–92 in α B-crystallin, called mini- α B-crystallin, can independently prevent the aggregation of denatured substrate proteins similar to the action of native α B-crystallin (Bhattacharyya et al., 2006). Using protein pin arrays Ghosh et al. (2005) identified seven interactive sequences (9–20, 43–58, 75–82, 113–120, 131–138, 141–148, and 157–164) in the human α B-crystallin using endogenous target proteins β H/ γ D crystallins and non-physiological targets alcohol dehydrogenase and citrate synthase for the chaperone activity. Two of the seven chaperone sequences were in the N-terminus, four in the conserved α -crystallin core domain, and one was in the C-terminal extension containing the highly conserved I-X-I/V motif. The authors also suggested that point mutations within the interactive domains could modify the chaperone activity of α B-crystallin and those outside the interactive domains could have little or no effect on chaperone activity. Oligomerization of sHsp subunits is essential in order for α -crystallins to function as molecular chaperones. Two interactive sequences, ${}_{73}\text{DRFSVNLDVKHFS}_{85}$ and ${}_{131}\text{LTITSSLSGDV}_{141}$, belonging to the α -crystallin core domain were synthesized as peptides and assayed for chaperone activity *in vitro* (Ghosh et al., 2005). Both synthesized peptides inhibited the thermal aggregation of β H crystallin, alcohol dehydrogenase, and citrate synthase *in vitro*. Moreover, it was found that specific α B-crystallin interactive peptides were capable of protecting key regulatory proteins from unfolding and aggregation. Several studies implicate that structure, hydrophobicity, and ionic charge are the major factors determining the chaperone activity of α -crystallins (Carver et al., 1995; Sharma et al., 2000, 1997; Santhoshkumar and Sharma, 2001). The chaperone efficiency of α -crystallin depends on its ability to recognize substrates undergoing denaturation, interact with the substrate, and keep the complex in soluble form and any alterations of these factors would result in a protein with reduced or altered chaperone function. For α A-crystallin Sharma et al. (2000) identified a 19-mer sequence which has similar chaperone activity to that of α A-crystallin by *in vitro* assays. The peptide sequence corresponded to the β 3 and β 4 region present in the α -crystallin domain (Sharma et al., 2000). Further studies revealed that the 19-mer peptide sequence of α A-crystallin as well as the region 42–57 derived from α B-crystallin elicited antiapoptotic properties in RPE cells challenged with oxidative stress (Kannan et al., 2010; Santoshkumar and Sharma, 2004). On the other hand, Santhoshkumar et al. (2011) found that a different α A-crystallin-derived peptide, $({}_{66}\text{SDRDKFVIFLDVKHF})_{80}$, which accumulates in the aging lens, could potentially inhibit the chaperone activity of α -crystallin and cause aggregation and precipitation of lens crystallins. They proposed that the α A-(66–80) peptide generated in human lenses interacts with α -crystallin and causes its aggregation and precipitation and suppresses the chaperone activity of α -crystallins. The direct functional demonstration of α -crystallin sequences provides support for the hypothesis that the chaperone function of α -crystallin is not limited to stress conditions and has an important function in regulating normal cellular growth and development. The physiologic significance of the chaperone function of α -crystallin is further substantiated by the findings that mutations in α -crystallin have a dramatic effect on its chaperone activity (Sun and MacRae, 2005). Although most of the chaperone activity studies are based on *in vitro* findings, *in vivo* studies using double heterozygous α -crystallin knock-out [α A (+/–) α B (+/–)] mice suggests that the total chaperone activity of α -crystallins is critical for maintaining lens transparency (Cheng et al., 2010). However, we

can't rule out that loss of lens transparency in these mice is a non-chaperone effect resulting from reduced expression of these abundant structural lens proteins. The role of overall chaperone activity of α -crystallin in retinal function and disease is yet to be delineated.

5.2. Angiogenesis & vascular remodeling

Angiogenesis and vascular remodeling are normal physiologic processes occurring during embryonic development, wound healing, and the development of compensatory collateral blood vessels in response to different diseases. Pathological angiogenesis or pathological neovascularization is the development of abnormal blood vessels or blood vessel formation in response to inflammation, ischemia and other pathologies. The role of α B-crystallin in regulating angiogenesis has attracted a lot of attention. α B-crystallin and HSP27 have been shown to participate in the regulation of angiogenesis (Dimberg et al., 2008; Kase et al., 2010; Keezer et al., 2003). We found that α B-crystallin KO resulted in attenuation of neovascularization in oxygen induced retinopathy while prominent neovascularization was observed in the wild type mouse (Fig. 6). α B-Crystallin regulates angiogenesis and vasculogenesis through multiple mechanisms, depending on the cell and tissue type. α B-Crystallin could act either directly on endothelial cells and/or indirectly on other cell types such as RPE (Kase et al., 2010). It has been shown that α B-crystallin directly acts as a chaperone for VEGF and other growth factors such as fibroblast growth factor-2 (FGF-2) (Ghosh et al., 2007a). Our laboratory demonstrated coimmunoprecipitation of α B-crystallin with VEGF in cultured RPE cells and extended the significance of this finding to α B-crystallin KO mice exposed to oxygen-induced retinopathy or laser induced choroidal neovascularization (CNV) (Kase et al., 2010). The stability of VEGF in these models depended on α B-crystallin and showed a decrease in the absence of α B-crystallin (Kase et al., 2010). Inner retinal vascular density was slightly lower in α B-crystallin KO, suggesting that α B-crystallin also plays a role in the development of the retinal vasculature (Kase et al., 2010). Recent *in vitro* studies show the importance of α B-crystallin in maintaining the intracrine stimulation of VEGF by endothelial cells (Ruan et al., 2011), while, interestingly, *in vivo* studies using α B-crystallin KO mice did not produce any major vascular defects, suggesting that α B-crystallin may not be strictly essential for intracrine VEGF signaling. However, it is not clear whether α B-crystallin phosphorylation or extracellular α B-crystallin could play a role in angiogenesis. In the mouse model of oxygen-induced retinal angiogenesis (Smith et al., 1994), phosphorylated serine 59 increased in WT mice which showed increased VEGF and retinal angiogenesis, suggesting a possible role for α B-crystallin phosphorylation in angiogenesis (Kase et al., 2010). This was further supported by additional evidence that under the same experimental conditions α B-crystallin KO mice showed decreased cellular and plasma VEGF levels and significant reduction in retinal angiogenesis. Similar findings were observed for the laser-induced choroidal angiogenesis model (Kase et al., 2010). Therefore, it can be argued that phosphorylation at serine 59 is essential for maintaining the chaperone function and retaining the stability of VEGF given that α B-crystallin binds to VEGF (Kase et al., 2010). Extracellular α B-crystallin could have a role in angiogenesis since α B-crystallin in normal blood vessel walls immediately responds to mechanical stress and is released from injured arterial wall into circulation (Kozawa et al., 2001). In the plasma, α B-crystallin inhibits aggregation of platelets which has crystallin binding sites on its surface (Enomoto et al., 2009). Extracellular α B-crystallin may inhibit release of granules from platelets and it has been reported that platelets contain both pro- and anti-angiogenic molecules (Enomoto et al., 2009; Sabrkhan et al., 2011). Therefore, it can be hypothesized that extracellular α B-crystallin could play a role in angiogenesis either by modulating platelet aggregation or by inhibiting granule release occurring during injury or inflammation. Further studies are needed to define the role of α B-crystallin phosphorylation (for eg. role of other phosphorylation sites, see below) on angiogenesis. Further the effect of role of extracellular α B-crystallin on retinal angiogenesis

either under physiological or pathological conditions is a useful area for further research. In this context, a recent study on the role of α A-crystallin in suture or chemical burn induced corneal neovascularization in mice is of interest (Zhu et al., 2012). The authors found that subconjunctival injection of α A-crystallin significantly attenuated suture-induced corneal neovascularization and this inhibition was shown to be mediated by the expression of soluble VEGFR1 (Zhu et al., 2012). Thus, it appears that the effect and mechanism of α -crystallins on neovascularization may vary depending on the crystallin isoform studied, the tissue type and the model of neovascularization.

Taking into consideration that α A-crystallin plays a significant role in ocular inflammation (Rao et al., 2008) and the fact that there is molecular crosstalk between inflammation and angiogenesis (Arroyo and Iruela-Arispe, 2010; Costa et al., 2007), it can be postulated that proteins that alter inflammation could also play a role in angiogenesis. Future studies will unravel the mechanisms of α A-crystallin participation in angiogenesis.

5.3. Phosphorylation and cell survival

Phosphorylation is one of the most important post-translational modifications controlled either enzymatically (e.g. phosphorylation and glycosylation) or occurring non-enzymatically (eg. oxidation). Post-translational modifications from phosphorylation have been studied extensively for α B-crystallin. Protein phosphorylation in eukaryotic cells takes place on serine, threonine, and tyrosine residues, with serine phosphorylation being the most common (McLachlin and Chait, 2001). α B-Crystallin is characterized by its capacity to be phosphorylated in response to a variety of stimuli originating from physiological changes or environmental insults (Hoover et al., 2000). For example, α B-crystallin undergoes multisite phosphorylation catalyzed by different protein kinases, and serine residues 19, 45, and 59 are the major sites that are phosphorylated *in vivo* (Smith et al., 1992). As illustrated in Fig. 1, the three serine residues in α B-crystallin are phosphorylated by different protein kinases, p44/42 MAP kinase (ERK), and MAPKAP kinase-2 (p38) targeting Ser-45 and Ser-59, respectively (Kato et al., 1998; Li and Reiser, 2011; Shu et al., 2005). Upstream of p38, RhoK, PKC and PKA are selectively involved in the activation of p38 and phosphorylation of α B-crystallin at serine 59 (Launay et al., 2006). The signaling pathway that results in phosphorylation of serine 19 is presently unknown. Under conditions of different stresses such as heat (Djabali et al., 1997), ischemia (Golenhofen et al., 1998), and hypoxia (Kase et al., 2010) the level of phosphorylated α B-crystallin increases. Phosphorylation of α B-crystallin at Ser-45 is enhanced in dividing cells (Kato et al., 1998), whereas phosphorylation at Ser-59 is high in centrosomes and midbodies (Inaguma et al., 2001). Human glioma cells treated with proteasome inhibitors, MG-132 or lactacystin, show enhanced phosphorylation of α B-crystallin suggesting that α B-crystallin (phosphorylated and total crystallin) could participate in the degradation of proteins via the ubiquitin-proteasome system (Ito et al., 2002). Studies using phosphorylation mimics demonstrated that an increase in phosphorylation leads to a decrease in the oligomerization of the protein (Ito et al., 2001) and a disruption in dimeric substructure within the oligomer (Aquilina et al., 2004). A slight decrease in the ability of a triple phosphorylation mutant (Ser-19D/Ser-45D/Ser-59D) to prevent the thermal aggregation of lactate dehydrogenase or to refold denatured firefly luciferase, compared with the wild-type recombinant protein was reported (Ito et al., 2001). However, individual mutations to mimic either phosphorylation or dephosphorylation of α B-crystallin showed that pseudophosphorylation of α B-crystallin at Ser-45 or Ser-59 provided powerful protection of astrocytes against oxidative stress (Li and Reiser, 2011). In addition, unphosphorylation of α B-crystallin at Ser-45 or Ser-59 resulted in the loss of protective function supporting a significant role for serine phosphorylation at position 45 and 59 in cell protection (Li and Reiser, 2011). It has to be noted that the

phosphorylation/unphosphorylation at Ser-19 does not affect the protection status, demonstrating that only Ser-45 and Ser-59 participate in cytoprotection.

As further evidence on the regulation of the chaperone activity of α B-crystallin by phosphorylation (Ecroyd et al., 2007), our laboratory has recently found that in mouse models of retinal and choroidal angiogenesis, phosphorylation of α B-crystallin at Ser-59 increased significantly suggesting increased and sustained chaperone activity throughout angiogenesis (Kase et al., 2010). These results suggest that the highly prevalent post-translational modification of α -crystallins may play an important role in alleviating the pathogenic effects associated with protein conformational diseases. Whether a direct link between phosphorylation and chaperone activity exists is unresolved because of the contradictory reports in the literature using phosphorylation-mimicking mutants of α B-crystallin (Ahmad et al., 2008; Ecroyd et al., 2007; Ito et al., 2001; Koteiche and McHaourab, 2003). For instance, it has been reported that the phosphorylation-mimicking mutant has decreased chaperone activity towards heat-induced aggregation and refolding of luciferase (Ito et al., 2001). On the other hand, an increase in the chaperone activity of α B-crystallin in binding to the destabilized T4 lysozyme mutants was found in another study (Koteiche and Mchaourab, 2003). In support, Ecroyd et al. (2007) reported that the phosphorylation-mimicking mutation leads to increased chaperone activity of α B-crystallin towards amorphous reduction-induced aggregation or heat stress-induced aggregation. Other studies demonstrated similar chaperone activity irrespective of the phosphorylation status of α B-crystallin (Wang et al., 1995). The discrepancies in results could arise partly from differences in the substrate-dependent behavior of α B-crystallin, the phosphorylation-mimicking mutants, experimental conditions and or changes the structure and stability of α B-crystallin. Therefore, to understand the role of phosphorylation in the structure and function of α B-crystallin one has to consider the chaperone activity of the phosphorylated α B-crystallin, differences in the structure and stability of phosphorylated α B-crystallin and/or the possibility of phosphorylated α B-crystallin forming mixed oligomers with α B-crystallin. At the functional level, ectopic expression of a pseudophosphorylated α B-crystallin mutant with three target serine residues (Ser-19E, Ser-45E, Ser-59E) substituted for aspartate did not have any anti-apoptotic activity (Kamradt et al., 2002). Further studies showed that phosphorylation of α B-crystallin at Ser-59 provided cytoprotection in stressed cardiac myocytes (Morrison et al., 2003). p38-MAPK and its downstream substrate MSK1 have shown to be involved in H₂O₂-induced α B-crystallin (Ser-59) phosphorylation and in cytoprotection (Aggeli et al., 2008). As mentioned earlier, our work has shown significant phosphorylation of Ser-59 in experimental models of retinal angiogenesis (Kase et al., 2010). It is of interest that a recent study demonstrated that Ser-59 phosphorylation of α B-crystallin down regulates its anti-apoptotic function by binding and sequestering Bcl-2 in breast cancer cells (Launay et al., 2010). In summary, while phosphorylation contributes to apoptosis prevention, the exact nature and phosphorylation sites involved in other functions of crystallins such as inflammation and angiogenesis remain to be determined.

Unlike α B-crystallin, detailed functional studies for α A-crystallin phosphorylation are scarce. Earlier studies had shown *in vivo* phosphorylation sites of α A-crystallin in the lens at Ser-122 and Ser-148 (Chiesa et al., 1987; Voorter et al., 1986). The major phosphorylation of α A-crystallin at Ser-122 appears to be an age regulated event in eye lenses (Takemoto, 1996). Later studies identified eight (Thr-4, Ser-20, Ser-45, Ser-59, Ser-122, Ser-148, Ser-155 and Ser-172 or 173) different phosphorylation sites of α A-crystallin in *in vivo* mouse lenticular samples (Schaefer et al., 2006). Nine phosphorylated sites (Ser-20, Ser-59, Ser-62, Ser-66, Ser-81, Ser-86, Ser-148, Ser-155, Ser-173) of α A-crystallin were identified *in vivo* from porcine lens by phosphoproteomic analysis (Chiou et al., 2010). It is noteworthy that Chiou et al. (2010) identified some reported phosphorylation sites but could not find some other sites from previous studies. Thus, there are discrepancies

in the literature regarding sites of α A-crystallin phosphorylation. Further, very little is known about the functional significance of these phosphorylation sites. Therefore, site-directed mutagenic studies by replacing phosphorylated sites of Ser- or Thr- residue to mimic the phosphorylation status of chaperoning α A-crystallin are needed to clearly understand the role of phosphorylation in relation to chaperone activity and biological significance. The precise mechanism of phosphorylation is also unclear. Serine-specific phosphorylation of α -crystallins is believed to occur via cAMP-dependent protein kinase pathways (Spector et al., 1985). An alternative pathway of phosphorylation is provided by the finding that α A-crystallin possesses autokinase activity (Kantorow and Piatigorsky, 1994); conversion from oligomers to tetramers enhances this activity (Kantorow et al., 1995). It is unknown which one of the phosphorylation pathways for α A-crystallin operates in RPE cells or in retina.

5.4. Cytoprotection

Although chaperone function was thought to be the only major function of α -crystallins, the ubiquitous tissue distribution (especially that of α B-crystallin) and studies in multiple tissues discovered several other functions which are of equal importance. One such function is its cytoprotective property (Andley, 2007, 2000; Arrigo et al., 2002; Kamradt et al., 2005, 2002, 2001, Liu et al., 2004a, 2004b; Ousman et al., 2007; Pasupuleti et al., 2010; Rao et al., 2008; Sreekumar et al., 2012, 2010; Yaung et al., 2007). The finding of increased expression of α -crystallin in different retinal disorders may be for neuroprotective purposes. Apoptosis is mediated by multiple signaling pathways and regulators such as the mitogen activated protein kinases (MAPKs) and or RAF/MEK/ERK or AKT Kinases (Liu et al., 2004a). α A-crystallin was reported to provide higher level of protection against cell death than α B-crystallin in cultured lens epithelial cells (Andley et al., 2000). However, RPE isolated from α A-crystallin KO mice were just as susceptible as α B-crystallin KO RPE to oxidative stress, despite the relatively low abundance of α A-crystallin in RPE (Yaung et al., 2007). Further, we found RPE cells overexpressing either α A- or α B-crystallin provided similar protection against H_2O_2 -induced cell death (Sreekumar et al., 2012). This phenomenon of augmentation of apoptosis in α A- or α B-crystallin deficiency and suppression of apoptosis in α A- or α B-crystallin overexpressing ARPE-19 cells is shown in Figure 7. Similar supportive data were also reported in lens epithelial cells irradiated with UVA light (Liu et al., 2004a) or staurosporine, etoposide and sorbitol (Mao et al., 2004). *In vivo*, in $CoCl_2$ induced hypoxia, retinas from α A- or α B-crystallin KO mice exhibited similar, rapid and more severe degeneration as compared to WT retinas, supporting *in vitro* findings (Yaung et al., 2008). The retinal layers, especially inner and outer nuclear layers from α -crystallin KO retina under hypoxia suffered severe degeneration while wild type retinas were not severely affected (Fig. 8). Based on these studies, one can conclude that the two α -crystallins display similar anti-apoptotic properties in the retina, irrespective of their relative abundance. However, the mechanisms associated with the protection may be different and may vary depending upon the stress stimulus (Mao et al., 2004). Accumulating evidence in several cell types including RPE suggests that α B-crystallin can provide potent anti-apoptotic protection against multiple stress stimuli such as staurosporine, tumor necrosis factor, ultraviolet A irradiation, okadaic acid, H_2O_2 , hypoxia and ceramide (Andley et al., 1998; Bluhm et al., 1998; Ito et al., 1997; Li and Reiser, 2011; Martin et al. 1997; Ousman et al., 2007; Sreekumar et al., 2012, 2010; Yaung et al., 2007). Accordingly, overexpression of α B-crystallin offers protection against a wide array of apoptotic stimuli while silencing renders cells susceptible to apoptosis (Bousette et al. 2010; Kamradt et al., 2005, 2002, 2001; Kase et al., 2010; Liu et al., 2004b; Mehlen et al., 1996; Nagaraj et al., 2005; Sreekumar et al., 2012). Deficiency of α B-crystallin increased levels of reactive oxygen species (ROS) and consequently, cells exposed to oxidative stress exhibited significant changes in mitochondrial permeability transition, and subsequent downstream activation of

caspase 3 (Noh et al., 2008; Yaung et al., 2007). Oxidative stress also caused mitochondrial translocation of α B-crystallin (Jin et al., 2008; Saraswathy and Rao, 2009; Shin et al., 2009; Whittaker et al., 2009; Yaung et al., 2008, 2007). Further, α B-crystallin interacts directly with the pro-apoptotic members Bax and Bcl-X_S and P53 polypeptides *in vitro* and *in vivo*, with sequestration of these proteins preventing translocation to mitochondria and hence suppressing apoptosis (Liu et al., 2007; Mao et al., 2004). Interestingly, studies in cardiomyocytes and cancer cells found that phosphorylation of α B-crystallin at the level of Ser-59 may be sufficient to provide maximal protection against apoptosis (Launay et al., 2010; Morrison et al., 2003). In association to its anti-apoptotic effects, α B-crystallin protects multiple cell types by augmenting expression of antioxidants, such as glutathione (Kannan et al., 2001; Mehlen et al., 1996; Sreekumar et al., 2012). In α A- and α B-crystallin overexpressing ARPE-19 cells, levels of cellular GSH were >2 fold higher than corresponding WT cells which was accompanied by increased resistance to oxidative stress-induced cell death (Sreekumar et al., 2012). Our work also demonstrated that exogenously added recombinant human α B-crystallin was taken up by stressed cells and protected these cells from apoptosis by inhibiting caspase 3 and by Poly (ADP-ribose) polymerase activation (Sreekumar et al., 2010). As shown in Fig. 9, while H₂O₂ alone caused significant apoptosis as evidenced by increase in TUNEL positive cells and cleaved caspase 3, co-treatment with recombinant α B-crystallin nearly abolished this effect. The role of α B-crystallin in mitochondria, given its antiapoptotic function, could be to uphold or maintain mitochondrial function by protecting protein folding and to restore and prevent subsequent downstream activation of apoptotic events (McGreal et al., 2012). Inhibition of apoptosis could occur through an increase in mitochondrial GSH or activation of transcription factors such as NF- κ B (Adhikari et al., 2011; Sreekumar et al., 2012). Munemasa et al. (2009) found that overexpression of α B-crystallin increased the number of retinal ganglion cells (RGCs) by 40% and 75% 1 week and 2 weeks after optic nerve axotomy-induced RGC degeneration, respectively. In experimental ischemic optic neuropathy, intravenous treatment with α B-crystallin enhanced the endogenous, post-ischemic response by decreasing microglial activation and promoting optic nerve oligodendrocyte survival (Pangratz-Fuehre et al., 2011). Studies in a cardiac ischemia-reperfusion model demonstrated that mitochondria may be among the key components in stressed cells with which S59 phospho- α B-crystallin interacts (Jin et al., 2008). Mitochondrial localization of α B-crystallin may protect the myocardium, in part, by modulating mitochondrial permeability transition pore opening and, thus, reducing ischemia/reperfusion injury (Jin et al., 2008). Other pathways of α B-crystallin's antiapoptotic function have also been described. For instance, genotoxic stress induced α B-crystallin by a p53-dependent mechanism whereby the p53 family inhibitor Δ Np73, activated α B-crystallin gene expression (Evans et al., 2010). In another study, exogenous VEGF or VEGF overexpressing myoblasts were resistant to cell death from oxidative or hypoxic-like stress and this protection was linked to the phosphorylation of VEGF receptor (Flk-1), activation of NF- κ B and/or the overexpression of α B-crystallin (Mercatelli et al., 2010). The involvement of NF- κ B pathway was further supported by the recent findings that α B-crystallin-dependent NF- κ B activation protected myoblasts from TNF- α induced cytotoxicity by enhancing the expression of the anti-apoptotic protein, Bcl2 (Adhikari et al., 2011).

The antioxidant function of α -crystallin is linked to the cellular GSH level (Mehlen et al., 1996; Pal et al., 1998; Sreekumar et al., 2012a, 2012b). A direct correlation between α A-crystallin and GSH was found in lens epithelial cells; overexpression of α A-crystallin increased GSH, whereas lack of α A-crystallin caused a decrease in GSH expression (Kannan et al., 2001). Our recent study revealed that in α -crystallin (α A- or α B-crystallin) overexpressing cells GSH levels increased significantly particularly in RPE mitochondria, while in murine retina lacking α -crystallin (α A-crystallin KO or α B-crystallin KO) GSH

decreased by 50% (Sreekumar et al., 2012). A corresponding increase in antiapoptotic function in α -crystallin overexpressing cells and an exacerbation of cell death in α -crystallin deficient cells was observed. In another study, the antiapoptotic function of α A-crystallin was shown to be directly interlinked with the chaperone function by enhancing P13K activity and reducing PTEN activity in HeLa and Chinese hamster ovary cells (Pasupuleti et al., 2010).

As has been mentioned earlier, distinct regions of α A- or α B-crystallin have been identified that exhibit chaperone functions. In experiments to test the hypothesis that some of these minipeptides are antiapoptotic, rat pheochromocytoma cells co-treated with β -amyloid peptide and α A-crystallin minipeptides were resistant to cell death (Santoshkumar and Sharma, 2004). However, the precise mechanism of this protection remains to be explored. Our preliminary observations in oxidatively stressed RPE cells suggest that peptides derived from both α A-crystallin and α B-crystallin have similar antiapoptotic properties to that of parent crystallins and this protection was through the inhibition of caspase 3 activation (Kannan et al., 2010). In additional studies, we found that poly(lactic-co-glycolic acid (PLGA) encapsulated α A- or α B-crystallin peptides were significantly more efficient in protection of RPE cells against oxidative stress than the free peptide in a dose and time dependent fashion. This suggests that nanocoated particle delivery of α -crystallin peptides to RPE could be an efficient approach in preventing RPE cell death and preserving cell function. We believe that conducting detailed investigation using *in vitro* and *in vivo* models with α -crystallin-derived chaperone peptides may prove to be valuable in developing therapeutic agents for retinal degenerative diseases.

5.5. Autophagy

Autophagy is an intracellular catabolic process which involves degradation of cell organelles and proteins through lysosomal pathways (Kaarniranta et al., 2009; Ryhanen et al., 2009; Wang et al., 2009a) and can also lead to non-apoptotic cell death (type 2 programmed cell death). Thus autophagy can either protect cells or promote cell death depending on the micro-environment of the cell. Among the three known autophagic mechanisms (Mizushima et al., 2008), we focus our comments mainly on chaperone-mediated autophagy (CMA) although the autophagic systems are not completely separated from each other. CMA is different from macroautophagy in that no vesicular trafficking is involved; instead a pentapeptide motif in substrate proteins allows their specific translocation to the lysosomal membrane (Cuervo, 2010; Dice, 2007).

Chaperone-mediated autophagy is involved in cellular stress response through selective targeting of cytosolic proteins for lysosomal degradation (Cuervo, 2010; Dice, 2007). Misfolded proteins and protein aggregates are linked to several pathological conditions associated with cardiovascular, oncological, and neurodegenerative disorders (Balch et al., 2008). The ability of α B-crystallin to promote either the destruction or renaturation of damaged proteins or unfolded proteins under diverse stress conditions potentially confers on them a major role in protein quality control and in the regulation of protein triage. Interestingly, α -crystallin expression is increased in neurodegenerative diseases such as AMD where it is a component of drusen (Crabb et al., 2002; De et al., 2007; Nakata et al., 2005). It has been hypothesized that increased autophagy, and exocytic activities in aged RPE could provide extracellular materials for the formation of drusen (Wang et al., 2009a). Consistent with this postulate, the presence of autophagic and exosomal markers in drusen from AMD patients has been reported (Wang et al., 2009a). We have presented experimental evidence that α B-crystallin is released from RPE cells through the exosomal route and co-localized with exosomal markers in RPE monolayers (Sreekumar et al., 2010). In polarized RPE monolayers, we evaluated secretion of α B-crystallin in exosomes by immunogold labeling and by immunofluorescence with CD63, an exosomal marker and

found that the secretion was predominantly apical (Fig. 10). In severe oxidative stress causing RPE barrier breakdown, α B-crystallin containing exosomes accumulated in the basolateral side of RPE, indicating that exosomes may be a possible source for α B-crystallin accumulation in the drusen (Sreekumar et al., 2010). Drusen also contain potentially toxic components such as β -amyloid (Isas et al. 2010; Luibl et al., 2006; Wang et al., 2009b). Thus we speculate that the presence of α B-crystallin in drusen could be to decrease the toxic protein aggregation and to increase autophagy-mediated clearance. This notion is supported by the finding that a missense mutation (CryAB^{R120G}) in the α B-crystallin gene results in protein aggregation and aggresome formation (Sanbe et al., 2004), mitochondrial toxicity (Maloyan et al., 2005), disruption of proteasome function (Chen et al., 2005), and induces a state of reductive stress (Rajasekaran et al., 2007). The mutation of α B-crystallin decreased the expression of Atg7, a mediator of autophagosomal biogenesis and reduced the autophagic function of rat cardiomyocytes (Pattison et al., 2011). These authors further observed that induction of autophagy using Atg7 overexpression can rescue the accumulation of misfolded mutant α B-crystallin and aggregates (Pattison and Robbins, 2011; Pattison et al., 2011).

Few studies have addressed the role of autophagy in the retina *in vivo*, reporting contrasting results on both cytoprotective and pro-survival effects in photoreceptors in *Drosophila* (Wang et al., 2009c), in mouse models of axonal degeneration (Knoferle et al., 2010) and optic nerve axotomy (Rodríguez-Muela et al., 2012). Detrimental effects of autophagy have also been described in a mouse model of retinitis pigmentosa (Punzo et al., 2009) and in a rat model of ischemia (Piras et al., 2011). The importance of autophagy during early stages of retinal development has been documented; inhibition of autophagy resulted in accumulation of apoptotic cells during neurogenesis and the autophagic machinery provides the retina with the energy required for proper removal of cell remains and further degradation of apoptotic cells (Mellén et al., 2008). Thus, while the role of autophagy in retinal development and degeneration is clear, the link between α B-crystallin and autophagy remains speculative but should be an important area of future investigation. Support for this notion is provided by the recent demonstration that mutations in β A3/A1-crystallin impairs phagosome degradation and results in a defect in autophagy in the RPE (Zigler et al., 2011).

5.6. Interaction with other proteins

Knowledge of protein-protein interactions is necessary to understand the functions of proteins and how these functions are regulated. Protein-protein interactions are decided by different factors, including hydrophobicity, solvent accessibility, and residue pairing preferences, which in turn are determined by the amino acid sequence and aspects of protein structure. The multiple β -strands of the β -sheet domain of α crystallin have been suggested as one of the sites of protein-protein interactions (Hoskins et al., 2006). In addition to stabilizing different structurally compromised proteins during multiple pathological conditions, α -crystallins have been found to interact with several other proteins such as β -crystallins, α -crystallin, actin filaments, vimentin, CP49, filensin, intermediate filaments, VEGF, HSP27, galectin-related interfiber protein, apoptotic mediators, mitochondrial proteins and nuclear proteins (Barton et al., 2009; FitzGerald et al. 1991; Ghosh et al., 2007a; Nicholl and Quinlan 1994; Singh et al. 2007). A detailed list of α -crystallin interacting partners based on functional categories is presented in Table 1. Two examples of α -crystallin interaction, one with an antioxidant enzyme and the other with an angiogenic factor are shown in Figure 11. The interaction of α A- and α B-crystallin with methionine sulfoxide reductase A (MsrA) in the retina of a patient with age-related macular degeneration is shown in Fig. 11A. The binding of α B-crystallin to VEGF in RPE cells from two hypoxia animal models (OIR and CoCl₂-induced hypoxia) is shown in Figure 11B. It

is believed that sHSPs participate in the dynamic assembly and stabilization of several filamentous cytoskeletal elements and some of these associations protect against multiple stress conditions (Singh et al., 2007). α B-crystallin interacts directly with an array of filament proteins such as phakinin (CP49), filensin (CP119), desmin, glial fibrillary acidic protein (GFAP), vimentin, and actin where it functions in the organization and stabilization of the filament networks formed by these proteins (Djabali, et al., 1999; Head et al., 2000; Launay et al., 2006; Muchowski et al., 1999; Prescott et al., 1996; Tomokane et al., 1991). Pin array analysis determined that the sequence, $_{57}$ APSWFDTG $_{64}$ of α B-crystallin had strong interactions with partially unfolded actin and this interactive sequence contains Ser-59, a key residue that is critical during several stress conditions (Ghosh et al., 2007b; Morrison et al., 2003). Phosphorylation of Ser-59 results in increased interactions between α B-crystallin and actin that is believed to protect actin filaments from unfolding and aggregation (Launay et al., 2006). α B-crystallin regulates actin filament dynamics *in vivo* and its association with actin helps maintenance of pinocytosis, a physiological function essential for survival of cells (Singh et al., 2007).

In addition, α B-crystallin interacts with several growth factors that are important for cell proliferation, differentiation and development such as fibroblast growth factor-2, nerve growth factor- β , VEGF, insulin, and β -catenin further suggesting its significance during normal development (Andley, 2007; Djabali et al., 1997; Gusev et al., 2002; Sun and Macrae, 2005). Although α B-crystallin is a cytosolic protein, under pathological situations or oxidative stress, α B-crystallin migrates to the nucleus, mitochondria or endoplasmic reticulum (Dou et al., 2010; Kase et al., 2010; Sreekumar et al., 2010; Yaung et al., 2008, 2007) and interacts with other structural or regulatory proteins (Ghosh et al., 2007a, 2007b). Cell migration is a physiological or pathological process and conformational changes in proteins have been associated with migration and the migrated proteins require special protective assistance to prevent protein aggregation or protein misfolding. For instance, α B-crystallin colocalizes with β -catenin during cell migration and is likely the chaperone for β -catenin (Maddala and Rao, 2005). The identification of chaperone sequence in α B-crystallin that mediates interactions with β -catenin during oxidative stress may protect and inhibit β -catenin degradation thereby enabling cell migration. A study by Ghosh et al. (2007a) provides *in vitro* evidence that fibroblast growth factor-2, nerve growth factor- β , VEGF, insulin, and β -catenin interact with specific sequences of α B-crystallin, previously reported to have chaperone activity (Ghosh et al., 2005) and further supports the known role of α B-crystallin in the pathways associated with these growth factors (Andley et al., 2000; Civil et al., 2000; Edwards et al., 2001; Maddala and Rao, 2005). In lens epithelial cells, FGF-2 treatment significantly induced the expression of all major crystallins, particularly α -crystallins (Mailankot et al., 2010) supporting the finding that α B-crystallin has specific interacting sequences for FGF-2 (Ghosh et al., 2007a). The overlap between the interactive sequences in α B-crystallin for chaperone activity and binding with VEGF is of high biological significance given the known role of α B-crystallin in VEGF related functions such as angiogenesis (Kase et al., 2010), vascular tube formation (Dimberg et al., 2008) and protecting VEGF from intracellular degradation (Ruan et al., 2011). The latter finding was reported in a recent study in which knockdown of α B-crystallin significantly increased the proteolytic degradation of VEGF in endothelial cells (Ruan et al., 2011). Further, VEGF levels were significantly lower in α B-crystallin KO mice exposed to hypoxia followed by minimal retinal angiogenesis probably due to VEGF proteolysis (Kase et al., 2010). However, it can be argued that since α B-crystallin is intracellular, the possibility of the interaction with growth factors will be minimal. However, recent evidence shows extracellular release of α B-crystallin (Gangalum et al., 2011; Sreekumar et al., 2010). Since α B-crystallin protects VEGF against unfolding, aggregation and proteolysis, it has therapeutic potential as a VEGF stabilizing molecule.

In addition, there are other α -crystallin binding partners and their number will increase as the research on finding new functions of crystallins intensifies. Methionines of α A- and α B-crystallins are oxidized in aged human lenses (Lund et al., 1996). Methionine oxidation damages α -crystallin chaperone activity, but methionine sulfoxide reductase A (MsrA) can repair oxidized methionines in α -crystallin and restore the chaperone function (Brennan et al., 2009). We have previously reported strong immunoreactivity for MsrA in sub-RPE macular drusen from patients with age-related macular degeneration (Sreekumar et al., 2011, 2005). Both α A- and α B-crystallins co-localize with MsrA in AMD retinas, particularly in the drusen, suggesting potential interaction between the two types of proteins (Fig. 11A). A recent study identified a direct interaction between α B-crystallin and Bcl-2-associated athanogene 3 (BAG3) in myoblast cells and the interaction could inhibit the aggregation and toxicity (Hishiya et al., 2011). Studies on peptides that inhibit this interaction in an *in vitro* binding assay indicate that two conserved Ile-Pro-Val regions of BAG3 are involved in the interaction with α B-crystallin, which is similar to results showing BAG3 binding to HspB8 and HspB6. Further, BAG3 overexpression increased α B-crystallin R120G solubility and inhibited its intracellular aggregation (Hishiya et al., 2011). The importance of metal ions in regulating chaperone activity has been well documented (Biswas and Das, 2008). It is known that α A- and α B-crystallins are involved in the maintenance of redox homeostasis (Ahmad et al., 2008; Sreekumar et al., 2012) and their interaction with metal ions such as Cu^{2+} modulate the chaperone activity (Ganadu et al., 2004; Moschini et al., 2006). A recent study demonstrated that α A-crystallin chaperone site is also a Cu^{2+} -binding site (Raju et al., 2011). We anticipate that further investigation will identify new interacting partners of these two major chaperone proteins and will reveal potential therapeutic approaches for retinal and other neurodegenerative and other aggregation diseases.

5.7. Interaction of α -crystallin with other small heat shock proteins

Substantial evidence exists on the interaction of α -crystallins between themselves and with other sHSPs. Studies show that the interaction between α A- and α B-crystallin is required to form structurally stable complexes (Srinivas et al., 2002). Although peptide scan studies provide evidence for two regions in α B-crystallin, spanning from residues 42–57 and 60–71, to be involved in interaction with α A-crystallin, mutation studies revealed that sequence 42–57 is the only major interacting region in α B- and α A-crystallin oligomer formation (Sreelakshmi et al., 2004). α B-crystallin is copurified with Hsp27, these proteins form tight complexes (Kato et al., 1992; Zantema et al., 1992) and the complexes formed by Hsp27 and α A- or α B-crystallin are detected both under *in vitro* and *in vivo* conditions (Bova et al., 2000; Kato et al., 1992; Zantema et al., 1992). It is hoped that a detailed analysis of the sHSP interactions will contribute to a better understanding of the biological role of sHSP complexes. The interactions of sHSPs with one another may be of significant medical relevance since mutations in sHSPs including α A-crystallin and α B-crystallin are associated with human cataracts, and desmin-related myopathy.

5.8. Amyloid β - α B-crystallin interaction and neurodegenerative diseases

Given the known similarity between brain degenerative disease (Alzheimer Disease) and retinal degenerative disease (AMD) with regard to the presence of amyloid β ($\text{A}\beta$), the interaction between $\text{A}\beta$ and α -crystallins is of considerable interest with the objective of developing a common therapeutic strategy (Ohno-Matsui, 2011). Visual problems have been observed during the initial stages of Alzheimer Disease (AD) (Sadun et al., 1987) with subsequent reduction in the number of ganglion cells and in the thickness of the nerve-fiber layer, increase in optic disc cupping, retinal vascular thinning and visual function impairment (Guo et al., 2010; Hinton et al., 1986). Evidence for the potential role of $\text{A}\beta$ in the pathogenesis of AMD has been shown in *in vitro* studies (Bruban et al., 2009; Luibl et al., 2006; Wang et al., 2008) as well as in different animal models (Bruban et al., 2009; Ding

et al., 2011; Wang et al., 2008; Yoshida et al., 2005). Beta amyloid, a 39–43 amino acid residue cleavage product of the amyloid precursor protein (APP), has been identified in the retinas of AD patients (Koronyo-Hamaoui et al., 2011) and is also one of the components of drusen (Anderson et al., 2004; Dentchev et al., 2003; Isas et al., 2010; Luibl et al., 2006; Wang et al., 2008). The aggregation of these peptides is thought to be critical to the pathogenesis of the diseases (Ohno-Matsui, 2011), however, α -crystallins are also found colocalized with $A\beta$ peptides (Liang, 2000; Muchowski and Wacker, 2005; Stege et al., 1999). Of special interest, α B-crystallin is associated with $A\beta$ deposition in supranuclear cataracts in lenses from patients with AD (Goldstein et al., 2003).

The observation that $A\beta$ and α B-crystallin are colocalized *in vivo* (Liang, 2000; Stege et al., 1999) has led to detailed investigations into the effect of α B-crystallin on $A\beta$ aggregation under *in vitro* experimental conditions. Several independent studies indicated that α -crystallin is capable of interacting with $A\beta$ (Liang, 2000; Raman et al., 2005; Santhoshkumar and Sharma, 2004; Shammas et al., 2011; Stege et al., 1999; Wilhelmus et al., 2006). Some of these studies showed that upon interaction, α B-crystallin enhanced β -sheet structure in $A\beta$ (Liang, 2000; Stege et al., 1999) and stabilized $A\beta$ into toxic β -sheet rich oligomers as evidenced by increased toxicity in cultured rat neuronal cells (Stege et al., 1999). In support, Narayanan et al. (2006) found that instead of preventing the cell from toxicity of $A\beta$, α B-crystallin induced an increased toxicity which could arise from the oxidation of the side chain of the Met35 in $A\beta$ by α B-crystallin. However, several other studies reported that α B-crystallin inhibited fibril formation (Dehle et al., 2010; Raman et al., 2005; Shammas et al., 2011; Wilhelmus et al., 2006) and presented evidence for a protective effect of α B-crystallin in $A\beta$ -induced cytotoxicity (Dehle et al., 2010; Wilhelmus et al., 2006). Similarly, α A-crystallin also inhibited fibril formation and protected neuronal cells from cytotoxicity (Santhoshkumar and Sharma, 2004). Controversy exists regarding the ability of α B-crystallin to inhibit the toxicity associated with $A\beta$ fibril formation (Ecroyd and Carver, 2009). The results are highly dependent on how the experiments are conducted, including the method of preparation, purification and handling of the $A\beta$ peptide, the incubation conditions and in particular whether $A\beta$ and α B-crystallin are pre-incubated before addition to the cell culture media. In a recent study Dehle et al. (2010) pre-incubated both α B-crystallin and $A\beta$ species prior to addition to the culture media and observed a correlation between the inhibition of cell toxicity and fibril inhibition. However, in previous studies both species have been simultaneously added to the cell culture media (Stege et al., 1999). Similar findings have been documented for kappa-casein (which forms fibrils) and α B-crystallin, implying that α B-crystallin's generic ability to inhibit fibril formation is coupled with its inhibition of cell toxicity (Dehle et al., 2010). It has to be noted that all the above studies were performed in different cell types and hence in addition to variations in experimental protocols, the observed effects may also be dependent on the type of cell line used. While α B-crystallin could prevent β -amyloid fibril formation, in a very recent study, Laganowsky et al. (2012) found that a segment of α B-crystallin called 'cylindrin' peptide formed a toxic amyloid small oligomer. However, it is intriguing that treatment with α B-crystallin for 3 weeks did not produce apparent toxicity either in *in vitro* RPE monolayer (Sreekumar et al., unpublished observations) or in animal models (Arac et al., 2011; Ousman et al., 2007; Pangratz-Fuehrer et al., 2011; Velotta et al., 2011). Thus, defining the precise beneficial role of α B-crystallin in β -amyloid fibril formation would require further study.

In vivo, the upregulation of α -crystallins has been reported in a variety of protein misfolding and neurodegenerative diseases (De et al., 2007; Guo et al., 2007; Kase et al., 2011; Kumar et al., 2005; Lowe et al., 1992; Nakata et al., 2005; Pountney et al., 2005; Rao et al., 2008; Renkawek et al., 1994; Shinohara et al., 1993; Wilhelmus et al. 2006), and α B-crystallin colocalization to amyloid deposits (Lowe et al., 1992; Pountney et al., 2005; Renkawek et al., 1994; Shinohara et al., 1993; Wilhelmus et al., 2006) may represent an important protective

effect to mitigate fibril formation and prevent the progression of some of these diseases involving A β - α B-crystallin interaction. A β is known to induce inflammation (Johnson et al., 2000; Wang et al., 2009b), alter mitochondrial functions (Bruban et al., 2009), regulate growth factors (Wang et al., 2009b; Yoshida et al., 2005), alter RPE tight junction proteins and RPE hypertrophy (Bruban et al., 2009; Ding et al., 2011; Luibl et al., 2006). α B-crystallin on the other hand, is anti-inflammatory (Arac et al., 2011; Ousman et al., 2007; Rothbard et al., 2012), and is antiapoptotic, in part, through elevation of mitochondrial antioxidants (Sreekumar et al., 2012), and growth factor regulation (Ghosh et al., 2007a; Kase et al., 2010) so that it can be hypothesized that α B-crystallin could be a potential therapeutic molecule for the treatment for A β associated retinal diseases and other neurodegenerative diseases.

5.9. Interaction of α -crystallin with ubiquitin-proteasome system

The quality control and maintenance of proteasome homeostasis is achieved mostly by molecular chaperones which assist in *de novo* protein folding or refolding and the ubiquitin-proteasome (UPS) and autophagy systems which mediate timely removal of aggregated or misfolded proteins (Hartl et al., 2011). Accordingly, the proteasome complex is involved in the cell cycle, signal transduction pathways, transcriptional regulation, stress responses, cell differentiation, and metabolic adaptation (Gottesman et al., 1997). Participation of α B-crystallin in protein degradation pathways has been described. α B-Crystallin is reported to interact both *in vitro* and *in vivo* with C8/ α 7, one of the 14 subunits of the 20S proteasome (Boelens et al., 2001). The interaction is highly specific as C8/ α 7 does not interact with α A-crystallin or HSP27 (Boelens et al., 2001). α B-Crystallin can also contribute to the degradation of some misfolded proteins which cannot be converted into their native state after repeated cycling through the chaperone systems by the proteasome (den Engelsman et al., 2004; Lin et al., 2006). When α B-crystallin is phosphorylated at Ser-19 and Ser-45, it can interact with FBX4, which is a component of ubiquitin-ligase SCF (den Engelsman et al., 2003). This combination is of particular interest because these serines are mainly phosphorylated during mitosis (Kato et al., 1998), suggesting that the interaction with FBX4 might play a role during cell division. Both FBX4 and α B-crystallin are required for rapid ubiquitination and degradation of cyclin D1 *in vivo* and that purified SCF-FBX4- α B-crystallin complexes can direct cyclin D1 ubiquitination *in vitro* (Lin et al., 2006). Loss of α B-crystallin resulted in cyclin D1 stabilization showing the requirement of the SCF-FBX4- α B-crystallin ligase for cyclin D1 proteolysis (Lin et al., 2006). Another target for α B-crystallin is VEGF. Selective disruption of UPR pathways (IRE1/XBP-1, ATF6) or knockdown of α B-crystallin significantly increased VEGF proteolytic degradation (Ruan et al., 2011). We have shown that in the absence of α B-crystallin, VEGF is degraded, resulting in decreased secretion (Kase et al., 2010). Inhibition of the 26S proteasome partially rescues VEGF secretion leading to increased retinal angiogenesis, suggesting that in the absence of α B-crystallin, misfolded, mono-ubiquitinated VEGF could be exported to the cytoplasm and undergoes ubiquitination and degradation (Kase et al., 2010).

Another recently discovered target for α B-crystallin ubiquitination is NF- κ B and this interaction is phosphorylation dependent and promotes cell survival (Adhikari et al., 2011). Oxidative stress by TNF- α caused phosphorylation of α B-crystallin at Ser-59, and phosphorylated α B-crystallin associates and enhances the kinase activity of the IKK complex, thereby facilitating phosphorylation and subsequent degradation of I κ B (Adhikari et al., 2011). Thus, the mutation and or absence/decreased expression of α B-crystallin could inhibit the proteasomal pathways and result in the accumulation of denatured proteins or lead to toxicity/cell death. Further understanding of these processes may allow the development of promising targets in the therapy of degenerative diseases.

5.10. Mechanism for the export of α B-crystallin

Identification of HSPs and sHSPs in extracellular fluids has been a puzzling finding since the majority of these proteins lack the consensus signal sequence required for secretion via the classical endoplasmic reticulum-golgi pathway. α -Crystallins are synthesized in the cytosol and exported to extracellular space by an active mechanism that is not blocked by typical inhibitors of the ER-Golgi pathway, such as brefeldin or tunicamycin. Consequently, we hypothesized that α B-crystallin could be released by an alternative mechanism mediated by the non-classical secretory or unconventional pathway. To test the hypothesis, we collected exosomes from RPE cultured in exosome-free medium, and isolated exosomes using high speed centrifugation (Thery et al., 2006) and characterized exosomes by immunogold labeling using known exosomal markers such as CD63 (Fig. 10). Our studies revealed that α B-crystallin localized to exosomes. This was confirmed by immunoblot analysis that verified positivity for CD63 and α B-crystallin. Exosomes are rich in cholesterol and sphingolipids (Simpson et al., 2008; Thery et al., 2009) and inhibition of lipid rafts caused a significant decrease in exosome release and extracellular α B-crystallin suggesting participation of exosomes in α B-crystallin secretion in RPE cells (Sreekumar et al., 2010). In addition, we investigated the polarized nature of α B-crystallin secretion since α B-crystallin was reported to be neuroprotective (Ousman et al., 2007). Our results using highly polarized human RPE monolayers revealed preferential secretion of α B-crystallin toward the apical photoreceptor facing neural retina which supported its neuroprotective function (Fig. 12A,B). A recent report using ARPE-19 cells showed similar results on exosomal release of α B-crystallin (Gangalum et al., 2011). Further, exogenous α B-crystallin is taken up by RPE cells and photoreceptors with oxidative stress (Fig. 12C,D). As is well known, exosomes have diverse functions in the immune response, antigen presentation, intracellular communication, and the transfer of RNA and proteins (Simpson et al., 2009). Exosomes also contain inactive forms of both mRNA and microRNAs that can be transferred to a neighboring cell, conferring new functional properties to the recipient cell (Mathivanan et al., 2012, 2010). Therefore, detailed studies on microRNA profiling of diseased cell-derived exosomes and exosomes circulating in blood and their possible regulation of crystallins are needed in order to assess their potential as diagnostic biomarkers of different neurodegenerative disorders.

5.11. Extracellular functions of α -crystallin

We will now turn our attention to the hypothesis that secreted sHSPs can act as signaling molecules in different cell types (eg. microglia, leukocytes and vascular endothelial cells) and can regulate immune and inflammatory mechanisms. Before addressing the biological relevance of extracellular sHSPs, it should be noted that a number of studies have demonstrated the extracellular presence of several HSPs (Campisi et al., 2003; Gangalum et al., 2011; Lai et al., 2006; Oh et al., 2012; Rothbard et al., 2012; Sreekumar et al., 2010). Furthermore, their release could be induced by endogenous and exogenous factors such as cell polarity and stress stimuli. As mentioned earlier, we have provided evidence that α B-crystallin is secreted by RPE cells via a non-classical pathway that involves exosomes (Sreekumar et al., 2010).

Several studies suggest that α -crystallins promote anti-inflammatory rather than pro-inflammatory innate immune response profiles (Arac et al., 2011; Masilamoni et al. 2006, 2005; Ousman et al., 2007; Pangratz-Fuehrer et al., 2011; Rao et al., 2012). Also α -crystallin has been shown to be upregulated in diseases such as AMD, and diabetic retinopathy (De et al., 2007; Kumar et al., 2005; Losiewicz and Fort, 2011; Nakata et al., 2005). However, in spite of the upregulation of α -crystallin, chronic inflammation persists or disease progression continues. Although oxidative stress resulted in the upregulation of α -crystallins (Kumar et al., 2005; Rao et al., 2008; Yaung et al., 2007), the amount of α -

crystallins synthesized and/or released may not be adequate for eliciting their anti-inflammatory role or protein modifications could compromise their functions resulting in inflammation to persist. For example, in diabetic retinopathy, α -crystallins showed significant upregulation, but the upregulated crystallins could not stop inflammation and subsequent apoptosis (Kumar et al., 2005). This hypothesis was further verified in multiple *in vivo* animal models such as experimental autoimmune encephalomyelitis (Ousman et al., 2007), ischemia-reperfusion (Pangratz-Fuehrer et al., 2011; Velotta et al., 2011), stroke (Arac et al., 2011) and experimental optic neuropathy (Pangratz-Fuehrer et al., 2011). Systemic or exogenous delivery of α B-crystallin had broad anti-inflammatory activity that counteracted the pro-inflammatory environment thereby providing a stable cellular homeostasis. In experimental autoimmune uveitis, systemic delivery of α A-crystallin ameliorated the disease by decreasing infiltration of immune cells into the retina and suppression of immune cell function (Rao et al., 2012). These authors showed that intravenous α A-crystallin administration caused a marked decrease in Th1 cytokines and inhibition of the expression of toll-like receptors (Rao et al., 2012). Intraperitoneal delivery of α -crystallin (a combination of α A- and α B-crystallin) in a silver nitrate-induced inflammatory model inhibited neuroinflammation by inhibiting synthesis and release of inflammatory cytokines and nitric oxide synthesis (Masilamoni et al., 2006, 2005). The above findings suggest that the effects of exogenous administration of α -crystallins are not limited to a single disease model but can be effective in multiple disease models where inflammation is involved in disease initiation or progression. Of note, irrespective of the models studied, either α A- or α B-crystallin or their combination inhibited cell death whether it is in the brain, retina or heart, demonstrating that exogenous delivery offers a unique approach for preventing diseases involving multiple cell/tissue types. To explore how α B-crystallin exerts its therapeutic effect, Rothbard et al. (2012) studied the proinflammatory proteins that bind with α B-crystallin. Binding of α B-crystallin in the plasma occurred with an array of proinflammatory proteins which influence both innate and adaptive immunity in a temperature dependent manner, thereby modulating inflammation. In experimental autoimmune uveitis, systemic injection of α A-crystallin resulted in the downregulation of Toll-like receptors; and NF κ B-related genes, including Ccl2, IFN- γ , NF κ B, TNF- α , and TNF- α receptors and retinas were free of inflammatory cell infiltration and were well preserved (Rao et al., 2009). It is also important to note in this context that α A-crystallin has been reported to be an endogenous ligand for TLR4 (Roelofs et al., 2006). While systemic delivery of either α A or α B-crystallin resulted in the inhibition of inflammation and apoptosis, the mechanism is different for the two crystallins. α A-crystallin acts through the TLR4–NF κ B pathway (Rao et al., 2012) and α B-crystallin by binding and reducing the amount of inflammatory plasma proteins (eg. IL-6) (Rothbard et al., 2012).

Receptor-mediated signaling by HSPs has been a subject of debate because the receptors (Toll-like receptors) can be activated by bacterial products that may be present in the recombinant preparations used in the experiments. This could lead to activation of NF- κ B and increased production of TNF α , IL-6 and several chemokines (Takeda and Akira, 2005). Despite earlier controversy, TLR2 and TLR4 are still considered candidate receptors for HSP60, HSP70 and α A-crystallin (Habich et al., 2006; Roelofs et al., 2006). Whether exogenous α A-crystallin is primarily responsible for induction or inhibition of inflammatory mediators is not quite clear. Dendritic cells treated with α A-crystallin induced secretion of IL-6, TNF- α , and to a lesser extent IL-10, which resembled the cytokine pattern induced by lipopolysaccharide stimulation. This may involve TLR4 since with blockade of TLR4 or in TLR4 KO mice this effect was clearly abrogated (Roelofs et al., 2006). This phenomenon appears to be specific for α A-crystallin since HSP27 and α B-crystallin did not induce cytokine production (Roelofs et al., 2006). In another study, activated retinal microglial cells treated with α -crystallin (mixture of α A- and α B-crystallin) in *in vitro* or intravitreal injection of α -crystallin after optic nerve crush, significantly reduced inflammation by

downregulating the expression and release of TNF- α and inducible nitric oxide synthase (Wu et al., 2009). However, it has to be noted that incubation of non-activated microglia with α -crystallin resulted in an increased production of TNF- α and inducible nitric oxide synthase (Wu et al., 2009). Therefore, the function of α A-crystallin as a pro- or anti-inflammatory protein depends among other things, on the stage or status of the cells, form of α -crystallin used, and the disease type.

Several questions remain unanswered. Further research is needed to define precisely the physiological functions of α -crystallins in the extracellular space. Second, it is not clear whether extracellular α -crystallins are capable of acting as chaperones. Finally, how extracellular α -crystallins enter cytosol from outside would need further investigation.

6. Changes in α B-crystallin expression in retina and relationship to retinal diseases

All the functions described for α B-crystallins above have distinct implications in the pathogenesis of AMD, DR and related disorders. In AMD tissue samples, α B-crystallin has been identified in drusen and is prominently expressed in the RPE associated with drusen (Fig. 4) (Crabb et al., 2002; Johnson et al., 2005; Nakata et al., 2005; De et al., 2007). This has led to the concept that increased α B-crystallin expression in RPE may be a “biomarker” of AMD (De et al., 2007). An increase in α B-crystallin expression was also seen in Muller glial cells, cones, and cone terminals overlying and adjacent to drusen (Johnson et al., 2005); this may be a stress response in the photoreceptors and Muller cells but could also represent increased uptake in retinal cells from exosomal secretion of α B-crystallin from RPE (Sreekumar et al., 2010). The localization of α B-crystallin in synaptic regions may be regulated by post-translational modifications as has been shown for Ser-59 phosphorylation of α B-crystallin in hippocampal neurons (Schmidt et al., 2012).

Kumar et al. (2005) showed that in streptozotocin (STZ) –induced diabetic rats both α A- and α B-crystallin increased in the retina with considerable degradation of α B-crystallin. An increase in α -crystallin expression was also reported by Fort et al. (2009) using retinal proteomics and immunostaining in the STZ rats. Elevated α -crystallin expression was found around the blood vessels of the ganglion cell layer as well as in activated Mueller cells. Kase et al. (2011) reported increased expression of α A-crystallin in the retina of diabetic retinopathy (DR) patients. This group also described an increased expression of α B-crystallin in the cytoplasm of endothelial cells of epiretinal membranes in proliferative DR where it co-localized with VEGF (Dong et al., 2012). Several other reports in animal models of DR confirm an increase in α A- and/or α B-crystallin in the retina (VanGuilder et al., 2011; Kandpal et al., 2012).

These observations suggest the importance of α -crystallins in retinal disorders. A compilation of several ocular and other neurological disorders that involve changes in α -crystallins is presented in Table 2.

7. α -Crystallin as a therapeutic molecule

Current therapies for ocular degenerative diseases such as AMD, diabetic retinopathy, and other neuroinflammatory diseases may be useful only in a small fraction of the patients and are ineffective in most patients thus underscoring a vital need for new therapies. α -Crystallins, especially α B-crystallin has shown to have pluripotent functions stemming from cell protection through preventing inflammation. Recombinant α B-crystallin or α A-crystallin or a combination of α -crystallin therapy was shown to be effective in multiple disease models such as experimental autoimmune encephalomyelitis, stroke, cardiac

ischemia-reperfusion, optic neuropathy, experimental autoimmune uveitis, and other inflammation-induced toxicity models. The route of administration of α -crystallin in these studies was either intravenous, intravitreal or intraperitoneal and irrespective of the route of administration, α -crystallin showed anti-inflammatory and neuroprotective functions and rescued the phenotype in all the models studied. However, the amount and duration of treatment varied based on the route of delivery; lower and single dose regimens were used in localized delivery while multiple and extended treatment was required for systemic or intraperitoneal delivery. Recombinant human α -crystallins were used in all published in vitro and in vivo studies. The major advantages of recombinant proteins are related to their higher specificity and to their safety since they are produced by recombinant DNA technology. However, there are disadvantages such as low bioavailability and short half-life, conformational changes and tendency to irreversible aggregation on long-term storage. Because of these limitations, different techniques have been developed for stabilizing the protein drugs using carriers whether in entrapped form, encapsulated in a semipermeable membrane, covalently bonded to a carrier or adsorbed to the carrier. As stated earlier, in the case of α -crystallin, specific regions within the parent proteins have similar chaperone, antiapoptotic properties and antifibrillogenic functions (Bhattacharyya et al., 2006; Ghosh et al., 2005; Kannan et al., 2010; Santhoshkumar and Sharma, 2004; Sharma et al., 2000). The 20-mer mini-chaperone peptide derived from either α A- crystallin or α B-crystallin protected human RPE cells from oxidant-induced stress by inhibiting caspase 3 activation (Kannan et al., 2010). Further, PLGA nanoparticles loaded with either α A- or α B-minichaperone peptide protected RPE cells from oxidative stress-induced cell death and more effectively than the peptide alone for the same doses, suggesting the efficacy of carrier mediated delivery. Polymeric nanoparticles have more advantage over other carrier molecules as they can be easily modified and conjugated to targeting proteins or peptides. For example, it has been reported that PLGA nanoparticles containing superoxide dismutase, effectively prevented H_2O_2 induced neuronal cell death when compared to superoxide dismutase alone (Reddy et al., 2008). An area of great interest to our laboratory is determining the therapeutic efficacy of free and nanoparticle coated α -crystallin minichaperone peptides in arresting disease progression in established animal models of ocular disease. However, more information needs to be gathered on establishing the best route and duration for administration of α -crystallins and their derivatives, as well as the selection of the ideal formulation of crystallins that is safe and non-toxic. In addition, whether α B-crystallin based therapy would inhibit or promote angiogenesis and whether α -crystallin combination therapy provide greater protective benefit than α -crystallin monotherapy are some other issues to consider before initiating clinical therapy.

8. Future Perspectives

From the extensive nature of the disorders listed in Table 2, one can appreciate how the initial research which was confined to the cataract-preventing and chaperone properties of α -crystallins in the lens has expanded greatly in the last decade. Our emphasis in the present review is on the multifaceted functions of α -crystallins in non-lenticular tissues particularly the retina. In the retina, participation of α -crystallins in a variety of cellular processes has been characterized using mainly RPE as the cell system. The multiple functions of α -crystallins known to date are shown schematically in Fig. 13. The basic and mechanistic findings outlined in Fig. 13 have laid the foundation and opened up new avenues for applied research in some or all of the categories listed with particular emphasis on the therapeutic potential of α -crystallins in ocular pathophysiology. Studies using human RPE and the retina have revealed that α -crystallins possess antiapoptotic properties and whether this function is independent of their chaperone activity or works in tandem with the antioxidative enzymes remains to be fully explored. In this context, the fact that α B-crystallin was found to be localized in mitochondria and is regulated by stress is relevant and suggests that

mitochondrial drug targeting has potential in arresting cell death. The localization of α B-crystallin in other organelles including the golgi and the endoplasmic reticulum opens up new areas for investigation. An important feature of α B-crystallin is that it readily interacts with a wide array of substrates as discussed earlier and summarized in Table 2. It is likely that this list will grow with the identification of more and more binding partners. The exact nature of this protein-protein interaction is important to understand since varying binding sites can have differing and contrasting cellular functions based on the amino acid sequence and chain length of the peptides involved. While α B-crystallin interacts with several growth factors, it will be of interest to determine the topology of this interaction and whether other membrane proteins, such as membrane receptors participate in this interaction. Autophagy has emerged as an important protective and pathologic mechanism in retinal development and disease. Future investigations will determine the nature and significance of the link between α B-crystallin and autophagy.

Another area of research that is certain to attract a lot of attention is the extracellular localization of α B-crystallin and its functional significance. While intracellular properties of α B-crystallin have been widely studied, its extracellular localization has only been recently identified. Further the functional role in extracellular space and on the surface of adjacent cells that take up α B-crystallin will be an area of active investigation. For example, the mechanisms of the anti-inflammatory actions of extracellular α B-crystallin are only now being elucidated. Recent findings that α B-crystallin is secreted via exosomes and is neuroprotective is important in this regard. Further research on how this secretion is controlled, what other components exist in exosomes and how α B-crystallin interacts with other exosomal proteins will prove to be of value. For example, it is known that several microRNAs which are responsible for regulating a number of cellular processes are secreted in exosomes (Mathivanan et al., 2010, 2012; Yuan et al 2009). Which of these microRNAs regulate α B-crystallin or vice versa in RPE may shed some light on modulating their extracellular therapeutic action. Identification of multiple short-chain peptides that have similar or even higher chaperone activity than the parent α -crystallins have provided researchers with a platform for evaluating the beneficial roles of these peptides in preventing ocular disease. These α B-crystallin-derived minichaperone peptides are antiapoptotic in addition to being efficient chaperones. Further research will be needed to find out their roles in other areas such as autophagy, inflammation, and angiogenesis. Generation of novel nanoparticle containing minichaperones of α -crystallins for efficient delivery to the retina would greatly advance the utility of these peptides as therapeutic agents.

One pathophysiological feature of AMD is the accumulation of toxic compounds in drusen among which β -amyloid peptide is an important component. α B-Crystallin was shown to inhibit β -amyloid fibril formation and thus reduce β -amyloid toxicity. However this interpretation gets complicated because of a very recent finding that while α B-crystallin can prevent fibrillogenesis, it can by itself form a toxic β -amyloid oligomer (Laganowsky et al., 2012). This important area calls for further work to address the controversy particularly with respect to precise conditions of β -amyloidogenesis in RPE, identify the actual α B-crystallin peptide sequence(s) that affect β -amyloid formation and toxicity and other primary or secondary factors that may play a part. It would also be valuable to perform such toxicity studies with both individual pro-and anti-chaperone peptides that have been discovered recently.

Recent finding of α B-crystallin expression by a subset of progenitor cells in the human fetal telencephalon suggests its importance in the developing human brain (Kida et al., 2010). Whether α B-crystallin plays a similar significant role in retinal development remains to be studied using retinal progenitor cells.

Similarly intriguing are the effects of α B-crystallin on angiogenesis. While deficiency of α B-crystallin strongly inhibits the angiogenic response, at least in part through loss of binding of VEGF, treatment with α B-crystallin could be either pro- or anti-angiogenic by promoting endothelial cell survival and VEGF expression or inhibiting pro-angiogenic inflammatory responses.

While α A- and α B-crystallins are differentially expressed in various retinal cell types, much needs to be learned about the mechanisms controlling the genetic and epigenetic regulation of crystallin gene expression and post-translational modifications in each of these cell types in normal retina and in disease. It is believed that as more and more functions of α -crystallins are unraveled, these versatile sHSPs are likely to serve as important therapeutic targets in ocular disease.

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References

- Acunzo J, Katsogiannou M, Rocchi P. Small heat shock proteins HSP27 (HspB1), α B-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. *Int J Biochem Cell Biol.* 2012 [Epub ahead of print].
- Adhikari AS, Singh BN, Rao KS, Rao ChM. α B-crystallin, a small heat shock protein, modulates NF- κ B activity in a phosphorylation-dependent manner and protects muscle myoblasts from TNF- α induced cytotoxicity. *Biochim Biophys Acta.* 2011; 1813:1532–1542. [PubMed: 21640763]
- Adhikari AS, SridharRao K, Rangaraj N, Parnaik VK, Rao ChM. Heat stress-induced localization of small heat shock proteins in mouse myoblasts: intranuclear lamin A/C speckles as target for α B-crystallin and Hsp25. *Exp Cell Res.* 2004; 299:393–403. [PubMed: 15350538]
- Aggeli IK, Beis I, Gaitanaki C. Oxidative stress and calpain inhibition induce α B-crystallin phosphorylation via p38-MAPK and calcium signalling pathways in H9c2 cells. *Cell Signal.* 2008; 20:1292–1302. [PubMed: 18420382]
- Ahmad MF, Raman B, Ramakrishna T, Rao ChM. Effect of phosphorylation on α B-crystallin: differences in stability, subunit exchange and chaperone activity of homo and mixed oligomers of α B-crystallin and its phosphorylation-mimicking mutant. *J Mol Biol.* 2008; 375:1040–1051. [PubMed: 18061612]
- Anderson DH, Talaga KC, Rivest AJ, Barron E, Hageman GS, Johnson LV. Characterization of beta amyloid assemblies in drusen: the deposits associated with aging and age-related macular degeneration. *Exp Eye Res.* 2004; 78:243–256. [PubMed: 14729357]
- Andley UP, Song Z, Wawrousek EF, Bassnett S. The molecular chaperone α A-crystallin enhances lens epithelial cell growth and resistance to UVA stress. *J Biol Chem.* 1998; 273:31252–31261. [PubMed: 9813033]
- Andley UP, Song Z, Wawrousek EF, Fleming TP, Bassnett S. Differential protective activity of α A- and α B-crystallin in lens epithelial cells. *J Biol Chem.* 2000; 275:36823–36831. [PubMed: 10967101]
- Andley UP. Crystallins in the eye: Function and pathology. *Prog Retin Eye Res.* 2007; 26:78–98. [PubMed: 17166758]
- Aquilina JA, Benesch JL, Ding LL, Yaron O, Horwitz J, Robinson CV. Phosphorylation of α B-crystallin alters chaperone function through loss of dimeric substructure. *J Biol Chem.* 2004; 279:28675–28680. [PubMed: 15117944]
- Arac A, Brownell SE, Rothbard JB, Chen C, Ko RM, Pereira MP, Albers GW, Steinman L, Steinberg GK. Systemic augmentation of α B-crystallin provides therapeutic benefit twelve hours post-stroke onset via immune modulation. *Proc Natl Acad Sci U S A.* 2011; 108:13287–13292. [PubMed: 21828004]

- Arrigo AP, Paul C, Ducasse C, Manero F, Kretz-Remy C, Viot S, Javouhey E, Mounier N, Diaz-Latoud C. Small stress proteins: novel negative modulators of apoptosis induced independently of reactive oxygen species. *Prog Mol Subcell Biol.* 2002; 28:185–204. [PubMed: 11908059]
- Arrigo AP, Simon S, Gibert B, Kretz-Remy C, Nivon M, Czekalla A, Guillet D, Moulin M, Diaz-Latoud C, Vicart P. Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. *FEBS Lett.* 2007; 581:3665–3674. [PubMed: 17467701]
- Arroyo AG, Iruela-Arispe ML. Extracellular matrix, inflammation, and the angiogenic response. *Cardiovasc Res.* 2010; 86:226–235. [PubMed: 20154066]
- Asomugha CO, Gupta R, Srivastava OP. Structural and functional properties of NH2-terminal domain, core domain, and COOH-terminal extensions of α A- and α B-crystallins. *Mol Vis.* 2011; 17:2356–2367. [PubMed: 21921988]
- Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science.* 2008; 319:916–919. [PubMed: 18276881]
- Barton KA, Hsu CD, Petrash JM. Interactions between small heat shock protein alpha-crystallin and galectin-related interfiber protein (GRIFIN) in the ocular lens. *Biochemistry.* 2009; 48:3956–3966. [PubMed: 19296714]
- Bauer NG, Richter-Landsberg C. The dynamic instability of microtubules is required for aggresome formation in oligodendroglial cells after proteolytic stress. *J Mol Neurosci.* 2006; 29:153–168. [PubMed: 16954605]
- Bennardini F, Wrzosek A, Chiesi M. Alpha B-crystallin in cardiac tissue. Association with actin and desmin filaments. *Circ Res.* 1992; 71:288–294. [PubMed: 1628387]
- Berengian AR, Parfenova M, Mchaourab HS. Site-directed spin labeling study of subunit interactions in the alpha-crystallin domain of small heat-shock proteins. Comparison of the oligomer symmetry in alphaA-crystallin, HSP 27, and HSP 16.3. *J Biol Chem.* 1999; 274:6305–6314. [PubMed: 10037719]
- Bhat SP, Nagineni CN. alpha B subunit of lens-specific protein alpha-crystallin is present in other ocular and non-ocular tissues. *Biochem Biophys Res Commun.* 1989; 158:319–325. [PubMed: 2912453]
- Bhattacharyya J, Padmanabha Udupa EG, Wang J, Sharma KK. Mini- α B-crystallin: A functional element of α B-crystallin with chaperone-like activity. *Biochemistry.* 2006; 45:3069–3076. [PubMed: 16503662]
- Biswas A, Das KP. Zn²⁺ enhances the molecular chaperone function and stability of alpha-crystallin. *Biochemistry.* 2008; 47:804–186. [PubMed: 18095658]
- Biswas A, Das KP. Role of ATP on the interaction of alpha-crystallin with its substrates and its implications for the molecular chaperone function. *J Biol Chem.* 2004; 279:42648–42657. [PubMed: 15292216]
- Björkdahl C, Sjögren MJ, Zhou X, Concha H, Avila J, Winblad B, Pei JJ. Small heat shock proteins Hsp27 or alphaB-crystallin and the protein components of neurofibrillary tangles: tau and neurofilaments. *J Neurosci Res.* 2008; 86:1343–1352. [PubMed: 18061943]
- Bluhm WF, Martin JL, Mestrl R, Dillmann WH. Specific heat shock proteins protect microtubules during simulated ischemia in cardiac myocytes. *Am J Physiol.* 1998; 275:H2243–2249. [PubMed: 9843825]
- Boelens WC, Croes Y, de Jong WW. Interaction between alphaB-crystallin and the human 20S proteasomal subunit C8/alpha7. *Biochim Biophys Acta.* 2001; 1544:311–319. [PubMed: 11341940]
- Bousette N, Chugh S, Fong V, Isserlin R, Kim KH, Volchuk A, Backx PH, Liu P, Kislinger T, MacLennan DH, Emili A, Gramolini AO. Constitutively active calcineurin induces cardiac endoplasmic reticulum stress and protects against apoptosis that is mediated by alpha-crystallin-B. *Proc Natl Acad Sci U S A.* 2010; 107:18481–18486. [PubMed: 20937869]
- Bova MP, McHaourab HS, Han Y, Fung BK. Subunit exchange of small heat shock proteins. Analysis of oligomer formation of alphaA-crystallin and Hsp27 by fluorescence resonance energy transfer and site-directed truncations. *J Biol Chem.* 2000; 275:1035–1042. [PubMed: 10625643]
- Bova MP, Ding LL, Horwitz J, Fung BK. Subunit exchange of alphaA-crystallin. *J Biol Chem.* 1997; 272:29511–29517. [PubMed: 9368012]

- Boyle DL, Takemoto L, Brady JP, Wawrousek EF. Morphological characterization of the Alpha A- and Alpha B-crystallin double knockout mouse lens. *BMC Ophthalmol.* 2003; 3:3. [PubMed: 12546709]
- Brady JP, Garland D, Douglas-Tabor Y, Robison WG Jr, Groome A, Wawrousek EF. Targeted disruption of the mouse alpha A-crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein alpha B-crystallin. *Proc Natl Acad Sci USA.* 1997; 94:884–889. [PubMed: 9023351]
- Brady JP, Garland DL, Green DE, Tamm ER, Giblin FJ, Wawrousek EF. AlphaB-crystallin in lens development and muscle integrity: a gene knockout approach. *Invest Ophthalmol Vis Sci.* 2001; 42:2924–2934. [PubMed: 11687538]
- Brennan LA, Lee W, Giblin FJ, David LL, Kantorow M. Methionine sulfoxide reductase A (MsrA) restores alpha-crystallin chaperone activity lost upon methionine oxidation. *Biochim Biophys Acta.* 2009; 1790:1665–1672. [PubMed: 19733220]
- Bruban J, Glotin AL, Dinet V, Chalour N, Sennlaub F, Jonet L, An N, Faussat AM, Mascarelli F. Amyloid-beta(1–42) alters structure and function of retinal pigmented epithelial cells. *Aging Cell.* 2009; 8:162–177. [PubMed: 19239420]
- Campisi J, Leem TH, Fleshner M. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. *Cell Stress Chaperones.* 2003; 8:272–286. [PubMed: 14984061]
- Carver JA, Guerreiro N, Nicholls KA, Truscott RJ. On the interaction of α -crystallin with unfolded proteins. *Biochim Biophys Acta.* 1995; 1252:251–260. [PubMed: 7578231]
- Carver JA, Lindner RA. NMR spectroscopy of alpha-crystallin. Insights into the structure, interactions and chaperone action of small heat-shock proteins. *Int J Biol Macromol.* 1998; 22:197–209. [PubMed: 9650074]
- Carver JA, Lindner RA, Lyon C, Canet D, Hernandez H, Dobson CM, Redfield C. The interaction of the molecular chaperone alpha-crystallin with unfolding alpha-lactalbumin: a structural and kinetic spectroscopic study. *J Mol Biol.* 2002; 318:815–827. [PubMed: 12054825]
- Chan SK, Lui PC, Tan PH, Yamaguchi R, Moriya T, Yu AM, Shao MM, Hliang T, Wong SI, Tse GM. Increased alpha-B-crystallin expression in mammary metaplastic carcinomas. *Histopathology.* 2011; 59:247–255. [PubMed: 21884203]
- Chen Q, Liu JB, Horak KM, Zheng H, Kumarapeli AR, Li J, Li F, Gerdes AM, Wawrousek EF, Wang X. Intracellular amyloidosis impairs proteolytic function of proteasomes in cardiomyocytes by compromising substrate uptake. *Circ Res.* 2005; 97:1018–1026. [PubMed: 16210548]
- Cheng C, Xia CH, Huang Q, Ding L, Horwitz J, Gong X. Altered chaperone-like activity of alpha-crystallins promotes cataractogenesis. *J Biol Chem.* 2010; 285:41187–41193. [PubMed: 20959464]
- Chiesa R, Gawinowicz-Kolks MA, Spector A. The phosphorylation of the primary gene products of alpha-crystallin. *J Biol Chem.* 1987; 262:1438–1441. [PubMed: 3805033]
- Chiou SH, Huang CH, Lee IL, Wang YT, Liu NY, Tsay YG, Chen YJ. Identification of in vivo phosphorylation sites of lens proteins from porcine eye lenses by a gel-free phosphoproteomics approach. *Mol Vis.* 2010; 16:294–302. [PubMed: 20182557]
- Civil A, van Genesen ST, Klok EJ, Lubsen NH. Insulin and IGF-I affect the protein composition of the lens fibre cell with possible consequences for cataract. *Exp Eye Res.* 2000; 70:785–794. [PubMed: 10843783]
- Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: cause or consequence? *Angiogenesis.* 2007; 10:149–166. [PubMed: 17457680]
- Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG, Hollyfield JG. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci USA.* 2002; 99:14682–14687. [PubMed: 12391305]
- Cuervo AM. Chaperone-mediated autophagy: selectivity pays off. *Trends Endocrinol Metab.* 2010; 21:142–150. [PubMed: 19857975]
- Das KP, Surewicz WK. Temperature induced exposure of hydrophobic surfaces and its effect on the chaperone activity of alpha-crystallin. *FEBS Lett.* 1995; 369:321–325. [PubMed: 7649280]

- de Jong WW, Caspers GJ, Leunissen JA. Genealogy of the alpha-crystallin-small heat-shock protein superfamily. *Int J Biol Macromol*. 1998; 22:151–162. [PubMed: 9650070]
- de Jong WW, Leunissen JA, Voorter CE. Evolution of the alpha-crystallin/small heat-shock protein family. *Mol Biol Evol*. 1993; 10:103–126. [PubMed: 8450753]
- De S, Rabin DM, Salero E, Lederman PL, Temple S, Stern JH. Human retinal pigment epithelium cell changes and expression of alphaB-crystallin: a biomarker for retinal pigment epithelium cell change in age-related macular degeneration. *Arch Ophthalmol*. 2007; 125:641–645. [PubMed: 17502503]
- Dehle FC, Ecroyd H, Musgrave IF, Carver JA. α B-Crystallin inhibits the cell toxicity associated with amyloid fibril formation by κ -casein and the amyloid- β peptide. *Cell Stress Chaperones*. 2010; 15:1013–1026. [PubMed: 20632140]
- den Engelsman J, Bennink EJ, Doerwald L, Onnekink C, Wunderink L, Andley UP, Kato K, de Jong WW, Boelens WC. Mimicking phosphorylation of the small heat-shock protein alphaB-crystallin recruits the F-box protein FBX4 to nuclear SC35 speckles. *Eur J Biochem*. 2004; 271:4195–4203. [PubMed: 15511225]
- den Engelsman J, Gerrits D, de Jong WW, Robbins J, Kato K, Boelens WC. Nuclear import of {alpha}B-crystallin is phosphorylation-dependent and hampered by hyperphosphorylation of the myopathy-related mutant R120G. *J Biol Chem*. 2005; 280:37139–37148. [PubMed: 16129694]
- den Engelsman J, Keijsers V, de Jong WW, Boelens WC. The small heat-shock protein alpha B-crystallin promotes FBX4-dependent ubiquitination. *J Biol Chem*. 2003; 278:4699–4704. [PubMed: 12468532]
- Dentchev T, Milam AH, Lee VM, Trojanowski JQ, Dunaief JL. Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. *Mol Vis*. 2003; 9:84–90.
- Deretic D, Aebersold RH, Morrison HD, Papermaster DS. Alpha A- and alpha B-crystallin in the retina. Association with the post-Golgi compartment of frog retinal photoreceptors. *J Biol Chem*. 1994; 269:16853–16861. [PubMed: 8207008]
- Derham BK, van Boekel MA, Muchowski PJ, Clark JI, Horwitz J, Hepburne-Scott HW, de Jong WW, Crabbe MJ, Harding JJ. Chaperone function of mutant versions of alpha A- and alpha B-crystallin prepared to pinpoint chaperone binding sites. *Eur J Biochem*. 2001; 268:713–721. [PubMed: 11168410]
- Derham BK, Harding JJ. Alpha-crystallin as a molecular chaperone. *Prog Retin Eye Res*. 1999; 18:463–509. [PubMed: 10217480]
- Dice JF. Chaperone-mediated autophagy. *Autophagy*. 2007; 3:295–299. [PubMed: 17404494]
- Dimberg A, Rylova S, Dieterich LC, Olsson AK, Schiller P, Wikner C, Bohman S, Botling J, Lukinius A, Wawrousek EF, Claesson-Welsh L. alphaB-crystallin promotes tumor angiogenesis by increasing vascular survival during tube morphogenesis. *Blood*. 2008; 111:2015–2023. [PubMed: 18063749]
- Ding JD, Johnson LV, Herrmann R, Farsiu S, Smith SG, Groelle M, Mace BE, Sullivan P, Jamison JA, Kelly U, Harrabi O, Bollini SS, Dilley J, Kobayashi D, Kuang B, Li W, Pons J, Lin JC, Bowes Rickman C. Anti-amyloid therapy protects against retinal pigmented epithelium damage and vision loss in a model of age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2011; 108:E279–287. [PubMed: 21690377]
- Djabali K, de Néchaud B, Landon F, Portier MM. AlphaB-crystallin interacts with intermediate filaments in response to stress. *J Cell Sci*. 1997; 110 (Pt 21):2759–2769. [PubMed: 9427392]
- Djabali K, Piron G, de Néchaud B, Portier MM. alphaB-crystallin interacts with cytoplasmic intermediate filament bundles during mitosis. *Exp Cell Res*. 1999; 253:649–662. [PubMed: 10585288]
- Doerwald L, van Rheede T, Dirks RP, Madsen O, Rexwinkel R, van Genesen ST, Martens GJ, de Jong WW, Lubsen NH. Sequence and functional conservation of the intergenic region between the head-to-head genes encoding the small heat shock proteins alphaB-crystallin and HspB2 in the mammalian lineage. *J Mol Evol*. 2004; 59:674–686. [PubMed: 15693623]

- Dong Z, Kase S, Ando R, Fukuhara J, Saito W, Kanda A, Murata M, Noda K, Ishida S. AlphaB-Crystallin expression in epiretinal membrane of human proliferative diabetic retinopathy. *Retina*. 2012 Feb 24. [Epub ahead of print].
- Dou G, Sreekumar PG, Spee C, He S, Ryan SJ, Kannan R, Hinton DR. Deficiency of α B-crystallin Augments ER Stress-Induced Apoptosis by Accentuating Mitochondrial Dysfunction in RPE Cells. *Invest Ophthalmol Vis Sci*. 2010; 51:E-Abstract 1438.
- Dutta T, Bhattacharjee A, Majumdar U, Ray SS, Sahoo R, Ghosh S. In vitro renaturation of alkaline family G/11 xylanase via a folding intermediate: alpha-crystallin facilitates refolding in an ATP-independent manner. *Appl Biochem Biotechnol*. 2010; 162:1238–1248. [PubMed: 20703955]
- Ecroyd H, Carver JA. Crystallin proteins and amyloid fibrils. *Cell Mol Life Sci*. 2009; 66:62–81. [PubMed: 18810322]
- Ecroyd H, Meehan S, Horwitz J, Aquilina JA, Benesch JL, Robinson CV, Macphee CE, Carver JA. Mimicking phosphorylation of alphaB-crystallin affects its chaperone activity. *Biochem J*. 2007; 401:129–141. [PubMed: 16928191]
- Edwards KL, Kueltzo LA, Fisher MT, Middaugh CR. Complex effects of molecular chaperones on the aggregation and refolding of fibroblast growth factor-1. *Arch Biochem Biophys*. 2001; 393:14–21. [PubMed: 11516157]
- Ellis RJ. The general concept of molecular chaperones. *Philos Trans R Soc Lond B Biol Sci*. 1993; 339:257–261. [PubMed: 8098529]
- Enomoto Y, Adachi S, Matsushima-Nishiwaki R, Niwa M, Tokuda H, Akamatsu S, Doi T, Kato H, Yoshimura S, Ogura S, Iwama T, Kozawa O. alphaB-crystallin extracellularly suppresses ADP-induced granule secretion from human platelets. *FEBS Lett*. 2009; 583:2464–2468. [PubMed: 19559024]
- Evans JR, Bosman JD, Brown-Endres L, Yehiely F, Cryns VL. Induction of the small heat shock protein alpha B crystallin genotoxic stress is mediated by p53 and p73. *Breast Cancer Res Treat*. 2010; 122:159–68. [PubMed: 19777343]
- Farahbakhsh ZT, Huang QL, Ding LL, Altenbach C, Steinhoff HJ, Horwitz J, Hubbell WL. Interaction of alpha-crystallin with spin-labeled peptides. *Biochemistry*. 1995; 34:509–516. [PubMed: 7819243]
- FitzGerald PG, Graham D. Ultrastructural localization of alpha A-crystallin to the bovine lens fiber cell cytoskeleton. *Curr Eye Res*. 1991; 10:417–436. [PubMed: 1889228]
- Fontaine JM, Sun X, Benndorf R, Welsh MJ. Interactions of HSP22 (HSPB8) with HSP20, alphaB-crystallin, and HSPB3. *Biochem Biophys Res Commun*. 2005; 337:1006–1011. [PubMed: 16225851]
- Fort PE, Freeman WM, Losiewicz MK, Singh RS, Gardner TW. The retinal proteome in experimental diabetic retinopathy: up-regulation of crystallins and reversal by systemic and periocular insulin. *Mol Cell Proteomics*. 2009; 8:767–779. [PubMed: 19049959]
- Frydman J. Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem*. 2001; 70:603–647. [PubMed: 11395418]
- Fu L, Liang JJ. Detection of protein-protein interactions among lens crystallins in a mammalian two-hybrid system assay. *J Biol Chem*. 2002; 277:4255–4260. [PubMed: 11700327]
- Fujita Y, Ohto E, Katayama E, Atomi Y. alphaB-Crystallin-coated MAP microtubule resists nocodazole and calcium-induced disassembly. *J Cell Sci*. 2004; 117:1719–1726. [PubMed: 15075233]
- Fukushima K, Mizuno Y, Takatama M, Okamoto K. Increased neuronal expression of alpha B-crystallin in human olivary hypertrophy. *Neuropathology*. 2006; 26:196–200. [PubMed: 16771174]
- Ganadu ML, Aru M, Mura GM, Coi A, Mlynarz P, Kozlowski H. Effects of divalent metal ions on the alphaB-crystallin chaperone-like activity: spectroscopic evidence for a complex between copper(II) and protein. *J Inorg Biochem*. 2004; 98:1103–1109. [PubMed: 15149821]
- Ganea E, Harding JJ. Molecular chaperones protect against glycation-induced inactivation of glucose-6-phosphate dehydrogenase. *Eur J Biochem*. 1995; 231:181–185. [PubMed: 7628468]

- Ganea E, Harding JJ. Inhibition of 6-phosphogluconate dehydrogenase by carbamylation and protection by alpha-crystallin, a chaperone-like protein. *Biochem Biophys Res Commun.* 1996; 222:626–631. [PubMed: 8670255]
- Gangalum RK, Atanasov IC, Zhou ZH, Bhat SP. AlphaB-crystallin is found in detergent-resistant membrane microdomains and is secreted via exosomes from human retinal pigment epithelial cells. *J Biol Chem.* 2011; 286:3261–3269. [PubMed: 21097504]
- Ghosh JG, Estrada MR, Clark JI. Interactive domains for chaperone activity in the small heat shock protein, human alphaB crystallin. *Biochemistry.* 2005; 44:14854–14869. [PubMed: 16274233]
- Ghosh JG, Houck SA, Clark JI. Interactive sequences in the stress protein and molecular chaperone human alphaB crystallin recognize and modulate the assembly of filaments. *Int J Biochem Cell Biol.* 2007b; 39:1804–1815. [PubMed: 17590381]
- Ghosh JG, Houck SA, Doneanu CE, Clark JI. The beta4-beta8 groove is an ATP-interactive site in the alpha crystallin core domain of the small heat shock protein, human alphaB crystallin. *J Mol Biol.* 2006; 364:364–375. [PubMed: 17022999]
- Ghosh JG, Shenoy AK Jr, Clark JI. Interactions between important regulatory proteins and human alphaB crystallin. *Biochemistry.* 2007a; 46:6308–6317. [PubMed: 17487982]
- Goldstein LE, Muffat JA, Cherny RA, Moir RD, Ericsson MH, Huang X, Mavros C, Coccia JA, Faget KY, Fitch KA, Masters CL, Tanzi RE, Chylack LT Jr, Bush AI. Cytosolic beta-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease. *Lancet.* 2003; 361:1258–1265. [PubMed: 12699953]
- Golenhofen N, Ness W, Koob R, Htun P, Schaper W, Drenckhahn D. Ischemia-induced phosphorylation and translocation of stress protein alpha B-crystallin to Z lines of myocardium. *Am J Physiol.* 1998; 274:H1457–1464. [PubMed: 9612350]
- Goplen D, Bougnaud S, Rajcevic U, Bøe SO, Skaftnesmo KO, Voges J, Enger PØ, Wang J, Tysnes BB, Laerum OD, Niclou S, Bjerkvig R. α B-crystallin is elevated in highly infiltrative apoptosis-resistant glioblastoma cells. *Am J Pathol.* 2010; 177:1618–1628. [PubMed: 20813964]
- Gottesman S, Wickner S, Maurizi MR. Protein quality control: triage by chaperones and proteases. *Genes Dev.* 1997; 11:815–823. [PubMed: 9106654]
- Graw J. Genetics of crystallins: cataract and beyond. *Exp Eye Res.* 2009; 88:173–189. [PubMed: 19007775]
- Guo L, Salt TE, Luong V, Wood N, Cheung W, Maass A, Ferrari G, Russo-Marie F, Sillito AM, Cheetham ME, Moss SE, Fitzke FW, Cordeiro MF. Targeting amyloid-beta in glaucoma treatment. *Proc Natl Acad Sci US A.* 2007; 104:13444–13449.
- Guo L, Duggan J, Cordeiro MF. Alzheimer's disease and retinal neurodegeneration. *Curr Alzheimer Res.* 2010; 7:3–14. [PubMed: 20205667]
- Gupta R, Srivastava OP. Identification of interaction sites between human betaA3- and alphaA/alphaB-crystallins by mammalian two-hybrid and fluorescence resonance energy transfer acceptor photobleaching methods. *J Biol Chem.* 2009; 284:18481–18492. [PubMed: 19401464]
- Gusev NB, Bogatcheva NV, Marston SB. Structure and properties of small heat shock proteins (sHsp) and their interaction with cytoskeleton proteins. *Biochemistry (Mosc).* 2002; 67:511–519. [PubMed: 12059769]
- Habich C, Kempe K, Gomez FJ, Lillicrap M, Gaston H, van der Zee R, Kolb H, Burkart V. Heat shock protein 60: identification of specific epitopes for binding to primary macrophages. *FEBS Lett.* 2006; 580:115–120. [PubMed: 16343489]
- Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature.* 2011; 475:324–332. [PubMed: 21776078]
- Haslbeck M, Franzmann T, Weinfurter D, Buchner J. Some like it hot: the structure and function of small heat-shock proteins. *Nat Struct Mol Biol.* 2005; 12:842–846. [PubMed: 16205709]
- Hauck SM, Schoeffmann S, Amann B, Stangassinger M, Gerhards H, Ueffing M, Deeg CA. Retinal Mueller glial cells trigger the hallmark inflammatory process in autoimmune uveitis. *J Proteome Res.* 2007; 6:2121–2131. [PubMed: 17444670]
- Head MW, Hurwitz L, Kegel K, Goldman JE. AlphaB-crystallin regulates intermediate filament organization in situ. *Neuroreport.* 2000; 11:361–365. [PubMed: 10674487]

- Heath MM, Rixon KC, Harding JJ. Glycation induced inactivation of malate dehydrogenase protection by aspirin and a lens molecular chaperone, alpha-crystallin *Biochim. Biophys Acta*. 1996; 1315:176–184.
- Hegen H, Wanschitz J, Ehling R, Deisenhammer F, Löscher WN, Reindl M, Berger T. Anti-alpha B-crystallin immunoreactivity in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. *J Peripher Nerv Syst*. 2010; 15:150–152. [PubMed: 20626779]
- Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-nerve degeneration in Alzheimer's disease. *N Engl J Med*. 1986; 315:485–487. [PubMed: 3736630]
- Hishiya A, Salman MN, Carra S, Kampinga HH, Takayama S. BAG3 directly interacts with mutated alphaB-crystallin to suppress its aggregation and toxicity. *PLoS One*. 2011; 6:e16828. [PubMed: 21423662]
- Hook DW, Harding JJ. Alpha-crystallin acting as a molecular chaperone protects catalase against steroid-induced inactivation. *FEBS Lett*. 1996; 382:281–284. [PubMed: 8605985]
- Hoover HE, Thuerauf DJ, Martindale JJ, Glembotski CC. alpha B-crystallin gene induction and phosphorylation by MKK6-activated p38. A potential role for alpha B-crystallin as a target of the p38 branch of the cardiac stress response. *J Biol Chem*. 2000; 275:23825–23833. [PubMed: 10816593]
- Horwitz J. Alpha-crystallin can function as a molecular chaperone. *Proc Natl Acad Sci USA*. 1992; 89:10449–10453. [PubMed: 1438232]
- Horwitz J. Alpha-crystallin. *Exp Eye Res*. 2003; 76:145–153. [PubMed: 12565801]
- Horwitz J, Bova MP, Ding LL, Haley DA, Stewart PL. Lens alpha-crystallin: function and structure. *Eye (Lond)*. 1999; 13:403–408. [PubMed: 10627817]
- Hoskins J, Lovell S, Blundell TL. An algorithm for predicting protein-protein interaction sites: Abnormally exposed amino acid residues and secondary structure elements. *Protein Sci*. 2006; 15:1017–1029. [PubMed: 16641487]
- Hu WF, Gong L, Cao Z, Ma H, Ji W, Deng M, Liu M, Hu XH, Chen P, Yan Q, Chen HG, Liu J, Sun S, Zhang L, Liu JP, Wawrousek E, Li DW. α A- and α B-crystallins interact with caspase-3 and Bax to guard mouse lens development. *Curr Mol Med*. 2012; 12:177–187. [PubMed: 22280356]
- Inaguma Y, Ito H, Iwamoto I, Saga S, Kato K. α B-crystallin phosphorylated at Ser-59 is localized in centrosomes and midbodies during mitosis. *Eur J Cell Biol*. 2001; 80:741–748. [PubMed: 11831387]
- Inaguma Y, Hasegawa K, Goto S, Ito H, Kato K. Induction of the synthesis of hsp27 and alpha B crystallin in tissues of heat-stressed rats and its suppression by ethanol or an alpha 1-adrenergic antagonist. *J Biochem (Tokyo)*. 1995; 117:1238–1243. [PubMed: 7490266]
- Isas JM, Luibl V, Johnson LV, Kaye R, Wetzel R, Glabe CG, Langen R, Chen J. Soluble and mature amyloid fibrils in drusen deposits. *Invest Ophthalmol Vis Sci*. 2010; 51:304–310.
- Ito H, Kamei K, Iwamoto I, Inaguma Y, Garcia-Mata R, Sztul E, Kato K. Inhibition of proteasomes induces accumulation, phosphorylation, and recruitment of Hsp27 and α B-crystallin to aggresomes. *J Biochem (Tokyo)*. 2002; 131:593–603. [PubMed: 11926998]
- Ito H, Kamei K, Iwamoto I, Inaguma Y, Nohara D, Kato K. Phosphorylation-induced change of the oligomerization state of alpha B-crystallin. *J Biol Chem*. 2001; 276:5346–5352. [PubMed: 11096101]
- Ito H, Okamoto K, Nakayama H, Isobe T, Kato K. Phosphorylation of alphaB-crystallin in response to various types of stress. *J Biol Chem*. 1997; 272:29934–29941. [PubMed: 9368070]
- Iwaki T, Kume-Iwaki A, Goldman JE. Cellular distribution of alpha B-crystallin in non-lenticular tissues. *J Histochem Cytochem*. 1990; 38:31–39. [PubMed: 2294148]
- Iwaki T, Kume-Iwaki A, Liem RK, Goldman JE. Alpha B-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. *Cell*. 1989; 57:71–78. [PubMed: 2539261]
- Jaenicke R. Folding and association of proteins. *Prog Biophys Mol Biol*. 1987; 49:117–237. [PubMed: 3327098]
- Jehle S, Rajagopal P, Bardiaux B, Markovic S, Kühne R, Stout JR, Higman VA, Kleivit RE, van Rossum BJ, Oschkinat H. Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers. *Nat Struct Mol Biol*. 2010; 17:1037–1042. [PubMed: 20802487]

- Jin JK, Whittaker R, Glassy MS, Barlow SB, Gottlieb RA, Glembotski CC. Localization of phosphorylated alphaB-crystallin to heart mitochondria during ischemia-reperfusion. *Am J Physiol Heart Circ Physiol*. 2008; 294:H337–344. [PubMed: 17993600]
- Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res*. 2000; 70:441–449. [PubMed: 10865992]
- Johnson PT, Brown MN, Pulliam BC, Anderson DH, Johnson LV. Synaptic pathology, altered gene expression, and degeneration in photoreceptors impacted by drusen. *Invest Ophthalmol Vis Sci*. 2005; 46:4788–4795. [PubMed: 16303980]
- Jones SE, Jomary C, Grist J, Thomas MR, Neal MJ. Expression of alphaB-crystallin in a mouse model of inherited retinal degeneration. *Neuroreport*. 1998; 9:4161–4165. [PubMed: 9926867]
- Kaarniranta K, Salminen A, Eskelinen EL, Kopitz J. Heat shock proteins as gatekeepers of proteolytic pathways—Implications for age-related macular degeneration (AMD). *Ageing Res Rev*. 2009; 8:128–139. [PubMed: 19274853]
- Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones*. 2009; 14:105–111. [PubMed: 18663603]
- Kamradt MC, Chen F, Cryns VL. The small heat shock protein alpha B-crystallin negatively regulates cytochrome c- and caspase-8 dependent activation of caspase-3 by inhibiting its autoproteolytic maturation. *J Biol Chem*. 2001; 276:16059–16063. [PubMed: 11274139]
- Kamradt MC, Chen F, Sam S, Cryns VL. The small heat shock protein alpha B-crystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation. *J Biol Chem*. 2002; 277:38731–38736. [PubMed: 12140279]
- Kamradt MC, Lu M, Werner ME, Kwan T, Chen F, Strohecker A, Oshita S, Wilkinson JC, Yu C, Oliver PG, Duckett CS, Buchsbaum DJ, LoBuglio AF, Jordan VC, Cryns VL. The small heat shock protein alpha B-crystallin is a novel inhibitor of TRAIL-induced apoptosis that suppresses the activation of caspase-3. *J Biol Chem*. 2005; 280:11059–11066. [PubMed: 15653686]
- Kandpal RP, Rajasimha HK, Brooks MJ, Nellissery J, Wan J, Qian J, Kern TS, Swaroop A. Transcriptome analysis using next generation sequencing reveals molecular signatures of diabetic retinopathy and efficacy of candidate drugs. *Mol Vis*. 2012; 18:1123–1146. [PubMed: 22605924]
- Kannan R, Sreekumar PG, Kannan N, Ryan SJ, Kompella UB, Sharma K, Hinton DR. Alpha Crystallin Derived Peptide Chaperone Protects Human RPE Cells From Oxidative Injury. *Invest Ophthalmol Vis Sci*. 2010; 51:E-Abstract 1441.
- Kannan R, Ouyang B, Wawrousek E, Kaplowitz N, Andley UP. Regulation of GSH in alphaA-expressing human lens epithelial cell lines and in alphaA knockout mouse lenses. *Invest Ophthalmol Vis Sci*. 2001; 42:409–416. [PubMed: 11157875]
- Kantorow M, Horwitz J, van Boekel MA, de Jong WW, Piatigorsky J. Conversion from oligomers to tetramers enhances autophosphorylation by lens alpha A-crystallin. Specificity between alpha A- and alpha B-crystallin subunits. *J Biol Chem*. 1995; 270:17215–17220.
- Kantorow M, Piatigorsky J. Alpha-crystallin/small heat shock protein has autokinase activity. *Proc Natl Acad Sci USA*. 1994; 91:3112–3116. [PubMed: 8159713]
- Kappé G, Leunissen JA, de Jong WW. Evolution and diversity of prokaryotic small heat shock proteins. *Prog Mol Subcell Biol*. 2002; 28:1–17. [PubMed: 11908054]
- Kapphahn RJ, Ethen CM, Peters EA, Higgins L, Ferrington DA. Modified alpha A crystallin in the retina: altered expression and truncation with aging. *Biochemistry*. 2003; 42:15310–15325. [PubMed: 14690441]
- Kase S, He S, Sonoda S, Kitamura M, Spee C, Wawrousek E, Ryan SJ, Kannan R, Hinton DR. alphaB-crystallin regulation of angiogenesis by modulation of VEGF. *Blood*. 2010; 115:3398–3406. [PubMed: 20023214]
- Kase S, Parikh JG, Rao NA. Expression of alpha-crystallin in retinoblastoma. *Arch Ophthalmol*. 2009; 127:187–192. [PubMed: 19204237]
- Kase S, Ishida S, Rao NA. Increased expression of α A-crystallin in human diabetic eye. *Int J Mol Med*. 2011; 28:505–511. [PubMed: 21617844]
- Kase S, Meghpara BB, Ishida S, Rao NA. Expression of α -crystallin in the retina of human sympathetic ophthalmia. *Mol Med Report*. 2012; 5:395–399. [PubMed: 22052021]

- Kato K, Ito H, Kamei K, Inaguma Y, Iwamoto I, Saga S. Phosphorylation of α B-crystallin in mitotic cells and identification of enzymatic activities responsible for phosphorylation. *J Biol Chem*. 1998; 273:28346–28354. [PubMed: 9774459]
- Kato K, Inaguma Y, Ito H, Iida K, Iwamoto I, Kamei K, Ochi N, Ohta H, Kishikawa M. Ser-59 is the major phosphorylation site in α B-crystallin accumulated in the brains of patients with Alexander's disease. *J Neurochem*. 2001; 76:730–736. [PubMed: 11158243]
- Kato K, Ito H, Kamei K, Inaguma Y, Iwamoto I, Saga S. Phosphorylation of α B-crystallin in mitotic cells and identification of enzymatic activities responsible for phosphorylation. *J Biol Chem*. 1998; 273:28346–28354. [PubMed: 9774459]
- Kato K, Shinohara H, Goto S, Inaguma Y, Morishita R, Asano T. Copurification of small heat shock protein with α B crystallin from human skeletal muscle. *J Biol Chem*. 1992; 267:7718–7725. [PubMed: 1560006]
- Kato K, Ito H, Kamei K, Iwamoto I. Selective stimulation of Hsp27 and α B-crystallin but not Hsp70 expression by p38 MAP kinase activation. *Cell Stress Chaperones*. 1999; 4:94–101. [PubMed: 10547059]
- Kato K, Shinohara H, Kurobe N, Goto S, Inaguma Y, Ohshima K. Immunoreactive α A crystallin in rat non-lenticular tissues detected with a sensitive immunoassay method. *Biochim Biophys Acta*. 1991; 1080:173–180. [PubMed: 1932094]
- Keezer SM, Ivie SE, Krutzsch HC, Tandle A, Libutti SK, Roberts DD. Angiogenesis inhibitors target the endothelial cell cytoskeleton through altered regulation of heat shock protein 27 and cofilin. *Cancer Res*. 2003; 63:6405–6412. [PubMed: 14559830]
- Kida E, Wierzbicka-Bobrowicz T, Palminiello S, Kaur K, Jarzabek K, Walus M, Albertini G, Golabek AA. Molecular chaperone α B-crystallin is expressed in the human fetal telencephalon at midgestation by a subset of progenitor cells. *J Neuropathol Exp Neurol*. 2010; 69:745–759. [PubMed: 20535031]
- Kim YH, Choi MY, Kim YS, Han JM, Lee JH, Park CH, Kang SS, Choi WS, Cho GJ. Protein kinase C δ regulates anti-apoptotic α B-crystallin in the retina of type 2 diabetes. *Neurobiol Dis*. 2007; 28:293–303. [PubMed: 17904375]
- Knoferle J, Koch JC, Ostendorf T, Michel U, Planchamp V, Vutova P, Tönges L, Stadelmann C, Brück W, Bähr M, Lingor P. Mechanisms of acute axonal degeneration in the optic nerve in vivo. *Proc Natl Acad Sci USA*. 2010; 107:6064–6069. [PubMed: 20231460]
- Koronyo-Hamaoui M, Koronyo Y, Ljubimov AV, Miller CA, Ko MK, Black KL, Schwartz M, Farkas DL. Identification of amyloid plaques in retinas from Alzheimer's patients and noninvasive in vivo optical imaging of retinal plaques in a mouse model. *Neuroimage*. 2011; 54:S204–217. [PubMed: 20550967]
- Koteiche HA, McHaourab HS. Mechanism of chaperone function in small heat-shock proteins. Phosphorylation-induced activation of two-mode binding in α B-crystallin. 2003; 278:10361–10367.
- Kozawa O, Matsuno H, Niwa M, Hatakeyama D, Kato K, Uematsu T. α B-crystallin, a low-molecular-weight heat shock protein, acts as a regulator of platelet function. *Cell Stress Chaperones*. 2001; 6:21–28. [PubMed: 11525239]
- Kumar PA, Haseeb A, Suryanarayana P, Ehtesham NZ, Reddy GB. Elevated expression of α A- and α B-crystallins in streptozotocin-induced diabetic rat. *Arch Biochem Biophys*. 2005; 444:77–83. [PubMed: 16309625]
- Laganowsky A, Liu C, Sawaya MR, Whitelegge JP, Park J, Zhao M, Pensalfini A, Soriaga AB, Landau M, Teng PK, Cascio D, Glabe C, Eisenberg D. Atomic view of a toxic amyloid small oligomer. *Science*. 2012; 335:1228–1231. [PubMed: 22403391]
- Laskowska E, Matuszewska E, Kuczyńska-Wiśnik D. Small heat shock proteins and protein-misfolding diseases. *Curr Pharm Biotechnol*. 2010; 11:146–157. [PubMed: 20166966]
- Launay N, Tarze A, Vicart P, Lilienbaum A. Serine 59 phosphorylation of α B-crystallin down-regulates its anti-apoptotic function by binding and sequestering Bcl-2 in breast cancer cells. *J Biol Chem*. 2010; 285:37324–37332. [PubMed: 20841355]

- Launay N, Goudeau B, Kato K, Vicart P, Lilienbaum A. Cell signaling pathways to alphaB-crystallin following stresses of the cytoskeleton. *Exp Cell Res*. 2006; 312:3570–3584. [PubMed: 16979163]
- Li R, Reiser G. Phosphorylation of Ser45 and Ser59 of α B-crystallin and p38/extracellular regulated kinase activity determine α B-crystallin-mediated protection of rat brain astrocytes from C2-ceramide- and staurosporine-induced cell death. *J Neurochem*. 2011; 118:354–364. [PubMed: 21615407]
- Li R, Rohatgi T, Hanck T, Reiser G. Alpha A-crystallin and alpha B-crystallin, newly identified interaction proteins of protease-activated receptor-2, rescue astrocytes from C2-ceramide- and staurosporine-induced cell death. *J Neurochem*. 2009; 110:1433–1444. [PubMed: 19558454]
- Liang JJ. Interaction between beta-amyloid and lens alphaB-crystallin. *FEBS Lett*. 2000; 484:98–101. [PubMed: 11068040]
- Lin DI, Barbash O, Kumar KG, Weber JD, Harper JW, Klein-Szanto AJ, Rustgi A, Fuchs SY, Diehl JA. Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4-alphaB crystallin) complex. *Mol Cell*. 2006; 24:355–366. [PubMed: 17081987]
- Liu JP, Schlosser R, Ma WY, Dong Z, Feng H, Lui L, Huang XQ, Liu Y, Li DW. Human alphaA- and alphaB-crystallins prevent UVA-induced apoptosis through regulation of PKC α , RAF/MEK/ERK and AKT signaling pathways. *Exp Eye Res*. 2004a; 79:393–403.
- Liu S, Li J, Tao Y, Xiao X. Small heat shock protein alphaB-crystallin binds to p53 to sequester its translocation to mitochondria during hydrogen peroxide-induced apoptosis. *Biochem Biophys Res Commun*. 2007; 354:109–114. [PubMed: 17222797]
- Liu B, Bhat M, Nagaraj RH. AlphaB-crystallin inhibits glucose-induced apoptosis in vascular endothelial cells. *Biochem Biophys Res Commun*. 2004b; 321:254–258. [PubMed: 15358243]
- Losiewicz MK, Fort PE. Diabetes impairs the neuroprotective properties of retinal alpha-crystallins. *Invest Ophthalmol Vis Sci*. 2011; 52:5034–5042. [PubMed: 21467180]
- Lowe J, McDermott H, Pike I, Spendlove I, Landon M, Mayer RJ. alpha B crystallin expression in non-lenticular tissues and selective presence in ubiquitinated inclusion bodies in human disease. *J Pathol*. 1992; 166:61–68. [PubMed: 1311375]
- Luibl V, Isas JM, Kaye R, Glabe CG, Langen R, Chen J. Drusen deposits associated with aging and age-related macular degeneration contain nonfibrillar amyloid oligomers. *J Clin Invest*. 2006; 116:378–385. [PubMed: 16453022]
- Lund AL, Smith JB, Smith DL. Modifications of the water-insoluble human lens alpha-crystallins. *Exp Eye Res*. 1996; 63:661–672. [PubMed: 9068373]
- Maddala R, Rao VP. alpha-Crystallin localizes to the leading edges of migrating lens epithelial cells. *Exp Cell Res*. 2005; 306:203–215. [PubMed: 15878345]
- Maeda A, Ohguro H, Maeda T, Nakagawa T, Kuroki Y. Low expression of alphaA-crystallins and rhodopsin kinase of photoreceptors in retinal dystrophy rat. *Invest Ophthalmol Vis Sci*. 1999; 40:2788–2794. [PubMed: 10549637]
- Magalhães J, Santos SD, Saraiva MJ. α B-crystallin (HspB5) in familial amyloidotic polyneuropathy. *Int J Exp Pathol*. 2010; 91:515–521. [PubMed: 20804537]
- Mailankot M, Howell S, Nagaraj RH. Kynurenine inhibits fibroblast growth factor 2-mediated expression of crystallins and MIP26 in lens epithelial cells. *Biochim Biophys Acta*. 2010; 1802:609–620. [PubMed: 20478381]
- Mallikarjuna K, Sundaram CS, Sharma Y, Deepa PR, Khetan V, Gopal L, Biswas J, Sharma T, Krishnakumar S. Comparative proteomic analysis of differentially expressed proteins in primary retinoblastoma tumors. *Proteomics Clin Appl*. 2010; 4:449–463. [PubMed: 21137063]
- Maloyan A, Sanbe A, Osinska H, Westfall M, Robinson D, Imahashi K, Murphy E, Robbins J. Mitochondrial dysfunction and apoptosis underlie the pathogenic process in alpha-B-crystallin desmin-related cardiomyopathy. *Circulation*. 2005; 112:3451–3461. [PubMed: 16316967]
- Mao YW, Liu JP, Xiang H, Li DW. Human alphaA- and alphaB-crystallins bind to Bax and Bcl-X(S) to sequester their translocation during staurosporine-induced apoptosis. *Cell Death Differ*. 2004; 11:512–526. [PubMed: 14752512]

- Mao YW, Xiang H, Wang J, Korsmeyer S, Reddan J, Li DW. Human bcl-2 gene attenuates the ability of rabbit lens epithelial cells against H₂O₂-induced apoptosis through down-regulation of the alpha B-crystallin gene. *J Biol Chem*. 2001; 276:43435–43445. [PubMed: 11546795]
- Martin JL, Mestrlil R, Hilal-Dandan R, Brunton LL, Dillmann WH. Small heat shock proteins and protection against ischemic injury in cardiac myocytes. *Circulation*. 1997; 96:4343–4348. [PubMed: 9416902]
- Masilamoni JG, Vignesh S, Kirubakaran R, Jesudason EP, Jayakumar R. The neuroprotective efficacy of alpha-crystallin against acute inflammation in mice. *Brain Res Bull*. 2005; 67:235–241. [PubMed: 16144660]
- Masilamoni JG, Jesudason EP, Baben B, Jebaraj CE, Dhandayuthapani S, Jayakumar R. Molecular chaperone alpha-crystallin prevents detrimental effects of neuroinflammation. *Biochim Biophys Acta*. 2006; 1762:284–293. [PubMed: 16443350]
- Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic Acids Res*. 2012; 40:D1241–1244. [PubMed: 21989406]
- Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics*. 2010; 73:1907–1920. [PubMed: 20601276]
- McGreal RS, Lee Kantorow W, Chauss DC, Wei J, Brennan LA, Kantorow M. α B-crystallin/sHSP protects cytochrome c and mitochondrial function against oxidative stress in lens and retinal cells. *Biochim Biophys Acta*. 2012; 1820:921–930. [PubMed: 22521365]
- McLachlin DT, Chait BT. Analysis of phosphorylated proteins and peptides by mass spectrometry. *Curr Opin Chem Biol*. 2001; 5:591–602. [PubMed: 11578935]
- Mehlen P, Schulze-Osthoff K, Arrigo AP. Small stress proteins as novel regulators of apoptosis. Heat shock protein 27 blocks Fas/APO-1- and staurosporine-induced cell death. *J Biol Chem*. 1996; 271:16510–16514. [PubMed: 8663291]
- Mellén MA, de la Rosa EJ, Boya P. The autophagic machinery is necessary for removal of cell corpses from the developing retinal neuroepithelium. *Cell Death Differ*. 2008; 15:1279–1290. [PubMed: 18369370]
- Menko AS, Andley UP. α A-Crystallin associates with α 6 integrin receptor complexes and regulates cellular signaling. *Exp Eye Res*. 2010; 91:640–651. [PubMed: 20709056]
- Mercatelli N, Dimauro I, Ciafré SA, Farace MG, Caporossi D. AlphaB-crystallin is involved in oxidative stress protection determined by VEGF in skeletal myoblasts. *Free Radic Biol Med*. 2010; 49:374–378. [PubMed: 20441791]
- Miyara N, Shinzato M, Yamashiro Y, Iwamatsu A, Kariya K, Sawaguchi S. Proteomic analysis of rat retina in a steroid-induced ocular hypertension model: potential vulnerability to oxidative stress. *Jpn J Ophthalmol*. 2008; 52:84–90. [PubMed: 18626730]
- Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008; 451:1069–1075. [PubMed: 18305538]
- Morozov V, Wawrousek EF. Caspase-dependent secondary lens fiber cell disintegration in alphaA-/alphaB-crystallin double-knockout mice. *Development*. 2006; 133:813–821. [PubMed: 16439475]
- Morrison LE, Hoover HE, Thuerauf DJ, Glembofski CC. Mimicking phosphorylation of alphaB-crystallin on serine-59 is necessary and sufficient to provide maximal protection of cardiac myocytes from apoptosis. *Circ Res*. 2003; 92:203–211. [PubMed: 12574148]
- Moschini R, Marini I, Malerba M, Cappiello M, Del Corso A, Mura U. Chaperone-like activity of alpha-crystallin toward aldose reductase oxidatively stressed by copper ion. *Arch Biochem Biophys*. 2006; 453:13–17. [PubMed: 16615933]
- Muchowski PJ, Clark JL. ATP-enhanced molecular chaperone functions of the small heat shock protein human alphaB crystallin. *Proc Natl Acad Sci USA*. 1998; 95:1004–1009. [PubMed: 9448275]
- Muchowski PJ, Valdez MM, Clark JL. AlphaB-crystallin selectively targets intermediate filament proteins during thermal stress. *Invest Ophthalmol Vis Sci*. 1999; 40:951–958. [PubMed: 10102292]
- Munemasa Y, Kwong JM, Caprioli J, Piri N. The role of alphaA- and alphaB-crystallins in the survival of retinal ganglion cells after optic nerve axotomy. *Invest Ophthalmol Vis Sci*. 2009; 50:3869–3875. [PubMed: 19279307]

- Nagaraj RH, Oya-Ito T, Bhat M, Liu B. Dicarbonyl stress and apoptosis of vascular cells: prevention by alphaB-crystallin. *Ann N Y Acad Sci.* 2005; 1043:158–165. [PubMed: 16037235]
- Nakata K, Crabb JW, Hollyfield JG. Crystallin distribution in Bruch's membrane-choroid complex from AMD and age-matched donor eyes. *Exp Eye Res.* 2005; 80:821–826. [PubMed: 15939038]
- Narayanan S, Kamps B, Boelens WC, Reif B. alphaB-crystallin competes with Alzheimer's disease beta-amyloid peptide for peptide-peptide interactions and induces oxidation of Abeta-Met35. *FEBS Lett.* 2006; 580:5941–5946. [PubMed: 17046756]
- Ngo JT, Klisak I, Dubin RA, Piatigorsky J, Mohandas T, Sparkes RS, Bateman JB. Assignment of the alpha B-crystallin gene to human chromosome 11. *Genomics.* 1989; 5:665–669. [PubMed: 2591958]
- Nicholl ID, Quinlan RA. Chaperone activity of α -crystallins modulates intermediate filament assembly. *EMBO J.* 1994; 13:945–953. [PubMed: 7906647]
- Noh SJ, Jeong WJ, Rho JH, Shin DM, Ahn HB, Park WC, Rho SH, Soung YH, Kim TH, Park BS, Yoo YH. Sensitization of RPE cells by alphaB-crystallin siRNA to SAHA-induced stage I apoptosis through abolishing the association of alphaB-crystallin with HDAC1 in SC35 speckles. *Invest Ophthalmol Vis Sci.* 2008; 49:4753–4759. [PubMed: 18552382]
- Oh JY, Choi H, Lee RH, Roddy GW, Ylöstalo JH, Wawrousek E, Prockop DJ. Identification of the HSPB4/TLR2/NF- κ B axis in macrophage as a therapeutic target for sterile inflammation of the cornea. *EMBO Mol Med.* 2012 Feb 22. [Epub ahead of print]. 10.1002/emmm.201200221
- Ohno-Matsui K. Parallel findings in age-related macular degeneration and Alzheimer's disease. *Prog Retin Eye Res.* 2011; 30:217–238. [PubMed: 21440663]
- Organisciak D, Darrow R, Barsalou L, Rapp C, McDonald B, Wong P. Light induced and circadian effects on retinal photoreceptor cell crystallins. *Photochem Photobiol.* 2011; 87:151–159. [PubMed: 21091955]
- Ousman SS, Tomooka BH, van Noort JM, Wawrousek EF, O'Connor KC, Hafler DA, Sobel RA, Robinson WH, Steinman L. Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination. *Nature.* 2007; 448:474–479. [PubMed: 17568699]
- Pal J, Bera S, Ghosh SK. The effect of glutathione upon chaperone activity of alpha-crystallin is probably mediated through target modulation. *Ophthalmic Res.* 1998; 30:271–279. [PubMed: 9704329]
- Pangratz-Fuehrer S, Kaur K, Ousman SS, Steinman L, Liao YJ. Functional rescue of experimental ischemic optic neuropathy with α B-crystallin. *Eye (Lond).* 2011; 25:809–817. [PubMed: 21475310]
- Parcellier A, Schmitt E, Brunet M, Hammann A, Solary E, Garrido C. Small heat shock proteins HSP27 and alphaB-crystallin: cytoprotective and oncogenic functions. *Antioxid Redox Signal.* 2005; 7:404–413. [PubMed: 15706087]
- Pasupuleti N, Matsuyama S, Voss O, Doseff AI, Song K, Danielpour D, Nagaraj RH. The anti-apoptotic function of human α A-crystallin is directly related to its chaperone activity. *Cell Death Dis.* 2010; 1:e31. [PubMed: 21364639]
- Pattison JS, Robbins J. Autophagy and proteotoxicity in cardiomyocytes. *Autophagy.* 2011; 7:1259–1260. [PubMed: 21677510]
- Pattison JS, Osinska H, Robbins J. Atg7 induces basal autophagy and rescues autophagic deficiency in CryAB^{R120G} cardiomyocytes. *Circ Res.* 2011; 109:151–160. [PubMed: 21617129]
- Pineda R 2nd, Chan CC, Ni M, Hayden BJ, Johnson MA, Nickerson J, Chader GJ. Human retinoblastoma cells express alpha B-crystallin in vivo and in vitro. *Curr Eye Res.* 1993; 12:239–245. [PubMed: 8482112]
- Piras A, Gianetto D, Conte D, Bosone A, Vercelli A. Activation of autophagy in a rat model of retinal ischemia following high intraocular pressure. *PLoS One.* 2011; 6:e22514. [PubMed: 21799881]
- Piri N, Song M, Kwong JM, Caprioli J. Modulation of alpha and beta crystallin expression in rat retinas with ocular hypertension-induced ganglion cell degeneration. *Brain Res.* 2007; 1141:1–9. [PubMed: 17316577]
- Plater ML, Goode D, Crabbe MJ. Effects of site-directed mutations on the chaperone-like activity of alphaB-crystallin. *J Biol Chem.* 1996; 271:28558–28566. [PubMed: 8910485]

- Pountney DL, Treweek TM, Chataway T, Huang Y, Chegini F, Blumbergs PC, Raftery MJ, Gai WP. Alpha B-crystallin is a major component of glial cytoplasmic inclusions in multiple system atrophy. *Neurotox Res.* 2005; 7:77–85. [PubMed: 15639800]
- Prescott AR, Sandilands A, Hutcheson AM, Carter JM, Quinlan RA. The intermediate filament cytoskeleton of the lens: an ever changing network through development and differentiation. A minireview. *Ophthalmic Res.* 1996; 28:58–61. [PubMed: 8727967]
- Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. *Nat Neurosci.* 2009; 12:44–52. [PubMed: 19060896]
- Quax-Jeuken Y, Quax W, van Rens G, Khan PM, Bloemendal H. Complete structure of the alpha B-crystallin gene: conservation of the exon-intron distribution in the two nonlinked alpha-crystallin genes. *Proc Natl Acad Sci US A.* 1985; 82:5819–5823.
- Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, Zhang XQ, Stevenson TJ, Peshock RM, Leopold JA, Barry WH, Loscalzo J, Odelberg SJ, Benjamin IJ. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. *Cell.* 2007; 130:427–439. [PubMed: 17693254]
- Raju M, Santhoshkumar P, Henzl TM, Sharma KK. Identification and characterization of a copper-binding site in α A-crystallin. *Free Radic Biol Med.* 2011; 50:1429–1436. [PubMed: 21300147]
- Raman B, Ban T, Sakai M, Pasta SY, Ramakrishna T, Naiki H, Goto Y, Rao ChM. AlphaB-crystallin, a small heat-shock protein, prevents the amyloid fibril growth of an amyloid beta-peptide and beta2-microglobulin. *Biochem J.* 2005; 392:573–581. [PubMed: 16053447]
- Raman B, Rao CM. Chaperone-like activity and quaternary structure of alpha-crystallin. *J Biol Chem.* 1994; 269:27264–27268. [PubMed: 7961635]
- Raman B, Ramakrishna T, Rao CM. Rapid refolding studies on the chaperone-like alpha-crystallin. Effect of alpha-crystallin on refolding of beta- and gamma-crystallins. *J Biol Chem.* 1995; 270:19888–19892. [PubMed: 7650002]
- Rao NA, Saraswathy S. Alpha A Crystallin Down Regulates Innate and Adaptive Immune Response in Experimental Autoimmune Uveitis. *Invest Ophthalmol Vis Sci.* 2009; 50:E-Abstract 6025.
- Rao NA, Saraswathy S, Pararajasegaram G, Bhat SP. Small heat shock protein alpha crystallin prevents photoreceptor degeneration in experimental autoimmune uveitis. *PLoS One.* 2012; 7(3):e33582. Epub 2012 March 30. [PubMed: 22479415]
- Rao NA, Saraswathy S, Wu GS, Katselis GS, Wawrousek EF, Bhat S. Elevated retina-specific expression of the small heat shock protein, alphaA-crystallin, is associated with photoreceptor protection in experimental uveitis. *Invest Ophthalmol Vis Sci.* 2008; 49:1161–1171. [PubMed: 18326745]
- Ray PS, Martin JL, Swanson EA, Otani H, Dillmann WH, Das DK. Transgene overexpression of alphaB crystallin confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion. *FASEB J.* 2001; 15:393–402. [PubMed: 11156955]
- Reddy MK, Wu L, Kou W, Ghorpade A, Labhasetwar V. Superoxide dismutase loaded PLGA nanoparticles protect cultured human neurons under oxidative stress. *Appl Biochem Biotechnol.* 2008; 151:565–577. [PubMed: 18509606]
- Regini JW, Grossmann JG, Burgio MR, Malik NS, Koretz JF, Hodson SA, Elliott GF. Structural changes in alpha-crystallin and whole eye lens during heating, observed by low-angle X-ray diffraction. *J Mol Biol.* 2004; 336:1185–1194. [PubMed: 15037078]
- Rekas A, Adda CG, Andrew Aquilina J, Barnham KJ, Sunde M, Galatis D, Williamson NA, Masters CL, Anders RF, Robinson CV, Cappai R, Carver JA. Interaction of the molecular chaperone alphaB-crystallin with alpha-synuclein: effects on amyloid fibril formation and chaperone activity. *J Mol Biol.* 2004; 340:1167–1183. [PubMed: 15236975]
- Renkawek K, de Jong WW, Merck KB, Frenken CW, van Workum FP, Bosman GJ. alpha B-crystallin is present in reactive glia in Creutzfeldt-Jakob disease. *Acta Neuropathol.* 1992; 83:324–327. [PubMed: 1373027]
- Renkawek K, Voorter CE, Bosman GJ, van Workum FP, de Jong WW. Expression of alpha B-crystallin in Alzheimer's disease. *Acta Neuropathol.* 1994; 87:155–60. [PubMed: 8171966]
- Robinson ML, Overbeek PA. Differential expression of alpha A- and alpha B-crystallin during murine ocular development. *Invest Ophthalmol Vis Sci.* 1996; 37:2276–2284. [PubMed: 8843924]

- Rodríguez-Muela N, Germain F, Mariño G, Fitze PS, Boya P. Autophagy promotes survival of retinal ganglion cells after optic nerve axotomy in mice. *Cell Death Differ.* 2012; 19:162–169. [PubMed: 21701497]
- Roelofs MF, Boelens WC, Joosten LA, Abdollahi-Roodsaz S, Geurts J, Wunderink LU, Schreurs BW, van den Berg WB, Radstake TR. Identification of small heat shock protein B8 (HSP22) as a novel TLR4 ligand and potential involvement in the pathogenesis of rheumatoid arthritis. *J Immunol.* 2006; 176:7021–7027. [PubMed: 16709864]
- Rothbard JB, Kurnellas MP, Brownell S, Adams CM, Su L, Axtell RC, Chen R, Fathman CG, Robinson WH, Steinman L. Therapeutic effects of systemic administration of the chaperone alpha B crystallin associated with binding proinflammatory plasma proteins. *J Biol Chem.* 2012; 287:9708–9721. [PubMed: 22308023]
- Ruan Q, Han S, Jiang WG, Boulton ME, Chen ZJ, Law BK, Cai J. α B-crystallin, an effector of unfolded protein response, confers anti-VEGF resistance to breast cancer via maintenance of intracrine VEGF in endothelial cells. *Mol Cancer Res.* 2011; 9:1632–1643. [PubMed: 21984182]
- Ryhänen T, Hyttinen JM, Kopitz J, Rilla K, Kuusisto E, Mannermaa E, Viiri J, Holmberg CI, Immonen I, Meri S, Parkkinen J, Eskelinen EL, Uusitalo H, Salminen A, Kaarniranta K. Crosstalk between Hsp70 molecular chaperone, lysosomes and proteasomes in autophagy-mediated proteolysis in human retinal pigment epithelial cells. *J Cell Mol Med.* 2009; 13:3616–3631. [PubMed: 19017362]
- Sabrkhan S, Griffioen AW, Oude Egbrink MG. The role of blood platelets in tumor angiogenesis. *Biochim Biophys Acta.* 2011; 1815:189–196. [PubMed: 21167916]
- Sacconi S, Féasson L, Antoine JC, Pécheux C, Bernard R, Cobo AM, Casarin A, Salvati L, Desnuelle C, Urtizberea A. A novel CRYAB mutation resulting in multisystemic disease. *Neuromuscul Disord.* 2012; 22:66–72. [PubMed: 21920752]
- Sadun AA, Borchert M, DeVita E, Hinton DR, Bassi CJ. Assessment of visual impairment in patients with Alzheimer's disease. *Am J Ophthalmol.* 1987; 104:113–120. [PubMed: 3618708]
- Sakaguchi H, Miyagi M, Darrow RM, Crabb JS, Hollyfield JG, Organisciak DT, Crabb JW. Intense light exposure changes the crystallin content in retina. *Exp Eye Res.* 2003; 76:131–133. [PubMed: 12589783]
- Salvador-Silva M, Ricard CS, Agapova OA, Yang P, Hernandez MR. Expression of small heat shock proteins and intermediate filaments in the human optic nerve head astrocytes exposed to elevated hydrostatic pressure in vitro. *J Neurosci Res.* 2001; 66:59–73. [PubMed: 11599002]
- Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kaye R, Maloyan A, Robbins J. Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. *Proc Natl Acad Sci USA.* 2004; 101:10132–10136. [PubMed: 15220483]
- Santhoshkumar P, Raju M, Sharma KK. α A-crystallin peptide SDRDKFVIFLDVKHF accumulating in aging lens impairs the function of α -crystallin and induces lens protein aggregation. *PLoS One.* 2011; 6:e19291. [PubMed: 21552534]
- Santhoshkumar P, Sharma KK. Phe71 is essential for chaperone-like function in alpha A-crystallin. *J Biol Chem.* 2001; 276:47094–47099. [PubMed: 11598124]
- Santhoshkumar P, Sharma KK. Inhibition of amyloid fibrillogenesis and toxicity by a peptide chaperone. *Mol Cell Biochem.* 2004; 267:147–155. [PubMed: 15663196]
- Saraswathy S, Rao NA. Mitochondrial proteomics in experimental autoimmune uveitis oxidative stress. *Invest Ophthalmol Vis Sci.* 2009; 50:5559–5566. [PubMed: 19578012]
- Sarnat HB, Flores-Sarnat L. Alpha-B-crystallin as a tissue marker of epileptic foci in paediatric resections. *Can J Neurol Sci.* 2009; 36:566–574. [PubMed: 19831124]
- Sarnat HB, Flores-Sarnat L, Hader W, Bello-Espinosa L. Mitochondrial “hypermetabolic” neurons in paediatric epileptic foci. *Can J Neurol Sci.* 2011; 38:909–917. [PubMed: 22030431]
- Schaefer H, Chamrad DC, Herrmann M, Stuwe J, Becker G, Klose J, Blueggel M, Meyer HE, Marcus K. Study of posttranslational modifications in lenticular alphaA-Crystallin of mice using proteomic analysis techniques. *Biochim Biophys Acta.* 2006; 1764:1948–1962. [PubMed: 17157567]

- Schmidt T, Bartelt-Kirbach B, Golenhofen N. Phosphorylation-dependent subcellular localization of the small heat shock proteins HspB1/Hsp25 and HspB5/ α B-crystallin in cultured hippocampal neurons. *Histochem Cell Biol*. 2012 May 23. [Epub ahead of print].
- Selcen D. Myofibrillar myopathies. *Neuromuscul Disord*. 2011; 21:161–171. [PubMed: 21256014]
- Shammas SL, Waudby CA, Wang S, Buell AK, Knowles TP, Ecroyd H, Welland ME, Carver JA, Dobson CM, Meehan S. Binding of the molecular chaperone α B-crystallin to A β amyloid fibrils inhibits fibril elongation. *Biophys J*. 2011; 101:1681–1689. [PubMed: 21961594]
- Sharma KK, Kaur H, Kester K. Functional elements in molecular chaperone α -crystallin: Identification of binding sites in α B-crystallin. *Biochem Biophys Res Commun*. 1997; 239:217–222. [PubMed: 9345298]
- Sharma KK, Kumar RS, Kumar GS, Quinn PT. Synthesis and characterization of a peptide identified as a functional element in α A-crystallin. *J Biol Chem*. 2000; 275:3767–3771. [PubMed: 10660525]
- Shin JH, Kim SW, Lim CM, Jeong JY, Piao CS, Lee JK. α B-crystallin suppresses oxidative stress induced astrocyte apoptosis by inhibiting caspase-3 activation. *Neurosci Res*. 2009; 64:355–361. [PubMed: 19379782]
- Shinohara H, Inaguma Y, Goto S, Inagaki T, Kato K. Alpha B crystallin and HSP28 are enhanced in the cerebral cortex of patients with Alzheimer's disease. *J Neurol Sci*. 1993; 119:203–208. [PubMed: 8277336]
- Shu E, Matsuno H, Akamatsu S, Kanno Y, Suga H, Nakajima K, Ishisaki A, Takai S, Kato K, Kitajima Y, Kozawa O. α B-crystallin is phosphorylated during myocardial infarction: involvement of platelet-derived growth factor-BB. *Arch Biochem Biophys*. 2005; 438:111–118. [PubMed: 15907784]
- Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. *Proteomics*. 2008; 8:4083–4099. [PubMed: 18780348]
- Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. *Expert Rev Proteomics*. 2009; 6:267–283. [PubMed: 19489699]
- Sinclair C, Mirakhur M, Kirk J, Farrell M, McQuaid S. Up-regulation of osteopontin and α B-crystallin in the normal-appearing white matter of multiple sclerosis: an immunohistochemical study utilizing tissue microarrays. *Neuropathol Appl Neurobiol*. 2005; 31:292–303. [PubMed: 15885066]
- Singh BN, Rao KS, Rao ChM. Ubiquitin-proteasome-mediated degradation and synthesis of MyoD is modulated by α B-crystallin, a small heat shock protein, during muscle differentiation. *Biochim Biophys Acta*. 2010; 1803:288–299. [PubMed: 20005263]
- Singh BN, Rao KS, Ramakrishna T, Rangaraj N, Rao ChM. Association of α B crystallin, a small heat shock protein, with actin: role in modulating actin filament dynamics in vivo. *J Mol Biol*. 2007; 366:756–767. [PubMed: 17196975]
- Smith JB, Sun Y, Smith DL, Green B. Identification of the posttranslational modifications of bovine lens α B-crystallins by mass spectrometry. *Protein Sci*. 1992; 1:601–608. [PubMed: 1304359]
- Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, D'Amore PA. Oxygen-induced retinopathy in the mouse. *Invest Ophthalmol Vis Sci*. 1994; 35:101–111. [PubMed: 7507904]
- Spector A, Chiesa R, Sredy J, Garner W. cAMP-dependent phosphorylation of bovine lens α -crystallin. *Proc Natl Acad Sci USA*. 1985; 82:4712–4716. [PubMed: 2991889]
- Sreekumar, PG.; Hinton, DR.; Kannan, R. Glutathione metabolism and its contribution to antiapoptotic properties of α -crystallin in the retina. In: Stratton, RD.; Hauswirth, WW.; Gardner, TW., editors. *Studies on Retinal and Choroidal Disorders, Oxidative Stress in Applied Basic Research and Clinical Practice*. Springer Science; New York: 2012b. p. 181–201.
- Sreekumar PG, Kannan R, Kitamura M, Spee C, Barron E, Ryan SJ, Hinton DR. α B crystallin is apically secreted within exosomes by polarized human retinal pigment epithelium and provides neuroprotection to adjacent cells. *PLoS One*. 2010; 5:e12578. [PubMed: 20949024]

- Sreekumar PG, Spee C, Ryan SJ, Cole SPC, Kannan R, Hinton DR. Mechanism of RPE Cell Death in α -Crystallin Deficient Mice: A Novel and Critical Role for MRP1-Mediated GSH Efflux. *PLoS ONE*. 2012; 7:e33420. [PubMed: 22442691]
- Sreekumar PG, Hinton DR, Kannan R. Methionine sulfoxide reductase A: Structure, function and role in ocular pathology. *World J Biol Chem*. 2011; 2:184–192. [PubMed: 21909460]
- Sreekumar PG, Kannan R, Yaung J, Spee CK, Ryan SJ, Hinton DR. Protection from oxidative stress by methionine sulfoxide reductases in RPE cells. *Biochem Biophys Res Commun*. 2005; 334:245–253. [PubMed: 15993845]
- Sreelakshmi Y, Santhoshkumar P, Bhattacharyya J, Sharma KK. AlphaA-crystallin interacting regions in the small heat shock protein, alphaB-crystallin. *Biochemistry*. 2004; 43:15785–15795. [PubMed: 15595834]
- Sreelakshmi Y, Sharma KK. Recognition sequence 2 (residues 60–71) plays a role in oligomerization and exchange dynamics of alphaB-crystallin. *Biochemistry*. 2005; 44:12245–12252. [PubMed: 16142923]
- Srinivas V, Santhoshkumar P, Sharma KK. Effect of trifluoroethanol on the structural and functional properties of alpha-crystallin. *J Protein Chem*. 2002; 21:87–95. [PubMed: 11934279]
- Srinivasan AN, Nagineni CN, Bhat SP. alpha A-crystallin is expressed in non-ocular tissues. *J Biol Chem*. 1992; 267:23337–23341. [PubMed: 1429679]
- Stege GJ, Renkawek K, Overkamp PS, Verschuure P, van Rijk AF, Reijnen-Aalbers A, Boelens WC, Bosman GJ, de Jong WW. The molecular chaperone alphaB-crystallin enhances amyloid beta neurotoxicity. *Biochem Biophys Res Commun*. 1999; 262:152–156. [PubMed: 10448084]
- Sun Y, MacRae TH. Small heat shock proteins: molecular structure and chaperone function. *Cell Mol Life Sci*. 2005; 62:2460–2476. [PubMed: 16143830]
- Takeda K, Akira S. Toll-like receptors in innate immunity. *Int Immunol*. 2005; 17:1–14. [PubMed: 15585605]
- Takemoto LJ. Differential phosphorylation of alpha-A crystallin in human lens of different age. *Exp Eye Res*. 1996; 62:499–504. [PubMed: 8759518]
- Tanaka N, Tanaka R, Tokuhara M, Kunugi S, Lee YF, Hamada D. Amyloid fibril formation and chaperone-like activity of peptides from alphaA-crystallin. *Biochemistry*. 2008; 47:2961–2967. [PubMed: 18232642]
- Thedieck C, Kalbacher H, Kratzer U, Lammers R, Stevanovic S, Klein G. alpha B-crystallin is a cytoplasmic interaction partner of the kidney-specific cadherin-16. *J Mol Biol*. 2008; 378:145–153. [PubMed: 18343407]
- Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol*. 2006; Chapter 3(Unit 3.22)
- Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol*. 2009; 9:581–593. [PubMed: 19498381]
- Tomokane N, Iwaki T, Tateishi J, Iwaki A, Goldman JE. Rosenthal fibers share epitopes with alpha B-crystallin, glial fibrillary acidic protein, and ubiquitin, but not with vimentin. *Immunoelectron microscopy with colloidal gold*. *Am J Pathol*. 1991; 138:875–885. [PubMed: 1707236]
- van den Ijssel PR, Overkamp P, Knauf U, Gaestel M, de Jong WW. Alpha A-crystallin confers cellular thermoresistance. *FEBS Lett*. 1994; 355:54–56. [PubMed: 7957962]
- van Noort JM, Bsibsi M, Nacken P, Gerritsen WH, Amor S. The link between small heat shock proteins and the immune system. *Int J Biochem Cell Biol*. 2012 Jan 5. [Epub ahead of print].
- van Noort JM, van Sechel AC, Bajramovic JJ, el Ouagmiri M, Polman CH, Lassmann H, Ravid R. The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis. *Nature*. 1995; 375:798–801. [PubMed: 7596414]
- VanGuilder HD, Bixler GV, Kutzler L, Brucklacher RM, Bronson SK, Kimball SR, Freeman WM. Multi-modal proteomic analysis of retinal protein expression alterations in a rat model of diabetic retinopathy. *PLoS One*. 2011; 6:e16271. [PubMed: 21249158]
- Velotta JB, Kimura N, Chang SH, Chung J, Itoh S, Rothbard J, Yang PC, Steinman L, Robbins RC, Fischbein MP. α B-crystallin improves murine cardiac function and attenuates apoptosis in human endothelial cells exposed to ischemia-reperfusion. *Ann Thorac Surg*. 2011; 91:1907–1913. [PubMed: 21619989]

- Verschuure P, Croes Y, van den Ijssel PR, Quinlan RA, de Jong WW, Boelens WC. Translocation of small heat shock proteins to the actin cytoskeleton upon proteasomal inhibition. *J Mol Cell Cardiol.* 2002; 34:117–128. [PubMed: 11851352]
- Voorter CE, Mulders JW, Bloemendal H, de Jong WW. Some aspects of the phosphorylation of alpha-crystallin A. *Eur J Biochem.* 1986; 160:203–210. [PubMed: 3769919]
- Vorum H, Østergaard M, Rice GE, Honoré B, Bek T. Identification of differentially regulated proteins in a patient with Leber's Congenital Amaurosis—a proteomic study. *Proteome Sci.* 2007; 5:5. [PubMed: 17326818]
- Vos MJ, Hageman J, Carra S, Kampinga HH. Structural and functional diversities between members of the human HSPB, HSPH, HSPA, and DNAJ chaperone families. *Biochemistry.* 2008; 47:7001–7011. [PubMed: 18557634]
- Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, Neufeld AH. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. *PLoS One.* 2009a; 4:e4160. [PubMed: 19129916]
- Wang J, Ohno-Matsui K, Yoshida T, Kojima A, Shimada N, Nakahama K, Safranov O, Iwata N, Saido TC, Mochizuki M, Morita I. Altered function of factor I caused by amyloid beta: implication for pathogenesis of age-related macular degeneration from Drusen. *J Immunol.* 2008; 181:712–720. [PubMed: 18566438]
- Wang J, Ohno-Matsui K, Yoshida T, Shimada N, Ichinose S, Sato T, Mochizuki M, Morita I. Amyloid-beta up-regulates complement factor B in retinal pigment epithelial cells through cytokines released from recruited macrophages/microglia: Another mechanism of complement activation in age-related macular degeneration. *J Cell Physiol.* 2009b; 220:119–128. [PubMed: 19277984]
- Wang K, Ma W, Spector A. Phosphorylation of alpha-crystallin in rat lenses is stimulated by H₂O₂ but phosphorylation has no effect on chaperone activity. *Exp Eye Res.* 1995; 61:115–124. [PubMed: 7556464]
- Wang YD, Wu JD, Jiang ZL, Wang YB, Wang XH, Liu C, Tong MQ. Comparative proteome analysis of neural retinas from type 2 diabetic rats by two-dimensional electrophoresis. *Curr Eye Res.* 2007; 32:891–901. [PubMed: 17963109]
- Wang K, Spector A. alpha-crystallin prevents irreversible protein denaturation and acts cooperatively with other heat-shock proteins to renature the stabilized partially denatured protein in an ATP-dependent manner. *Eur J Biochem.* 2000; 267:4705–4712. [PubMed: 10903503]
- Wang T, Lao U, Edgar BA. TOR-mediated autophagy regulates cell death in Drosophila neurodegenerative disease. *J Cell Biol.* 2009c; 186:703–711. [PubMed: 19720874]
- Wanschitz J, Ehling R, Löscher WN, Künz B, Deisenhammer F, Kuhle J, Budka H, Reindl M, Berger T. Intrathecal anti-alphaB-crystallin IgG antibody responses: potential inflammatory markers in Guillain-Barré syndrome. *J Neurol.* 2008; 255:917–924. [PubMed: 18712293]
- Waudby CA, Knowles TP, Devlin GL, Skepper JN, Ecroyd H, Carver JA, Welland ME, Christodoulou J, Dobson CM, Meehan S. The interaction of alphaB-crystallin with mature alpha-synuclein amyloid fibrils inhibits their elongation. *Biophys J.* 2010; 98:843–851. [PubMed: 20197038]
- Whiston EA, Sugi N, Kamradt MC, Sack C, Heimer SR, Engelbert M, Wawrousek EF, Gilmore MS, Ksander BR, Gregory MS. alphaB-crystallin protects retinal tissue during Staphylococcus aureus-induced endophthalmitis. *Infect Immun.* 2008; 76:1781–1790. [PubMed: 18227158]
- Whittaker R, Glassy MS, Gude N, Sussman MA, Gottlieb RA, Glembotski CC. Kinetics of the translocation and phosphorylation of alphaB-crystallin in mouse heart mitochondria during ex vivo ischemia. *Am J Physiol Heart Circ Physiol.* 2009; 296:H1633–1642. [PubMed: 19252088]
- Wilhelmus MM, Otte-Höller I, Wesseling P, de Waal RM, Boelens WC, Verbeek MM. Specific association of small heat shock proteins with the pathological hallmarks of Alzheimer's disease brains. *Neuropathol Appl Neurobiol.* 2006; 32:119–130. [PubMed: 16599941]
- Wisniewski T, Goldman JE. Alpha B-crystallin is associated with intermediate filaments in astrocytoma cells. *Neurochem Res.* 1998; 23:385–392. [PubMed: 9482251]
- Wu N, Wang YH, Zhao HS, Liu DN, Ying X, Yin ZQ, Wang Y. alpha-Crystallin downregulates the expression of TNF-alpha and iNOS by activated rat retinal microglia in vitro and in vivo. *Ophthalmic Res.* 2009; 42:21–28. [PubMed: 19478537]

- Xi J, Farjo R, Yoshida S, Kern TS, Swaroop A, Andley UP. A comprehensive analysis of the expression of crystallins in mouse retina. *Mol Vis.* 2003; 9:410–419. [PubMed: 12949468]
- Xi JH, Bai F, McGaha R, Andley UP. Alpha-crystallin expression affects microtubule assembly and prevents their aggregation. *FASEB J.* 2006; 20:846–857. [PubMed: 16675842]
- Yaung J, Jin M, Barron E, Spee C, Wawrousek EF, Kannan R, Hinton DR. alpha-Crystallin distribution in retinal pigment epithelium and effect of gene knockouts on sensitivity to oxidative stress. *Mol Vis.* 2007; 13:566–577. [PubMed: 17438522]
- Yaung J, Kannan R, Wawrousek EF, Spee C, Sreekumar PG, Hinton DR. Exacerbation of retinal degeneration in the absence of alpha crystallins in an in vivo model of chemically induced hypoxia. *Exp Eye Res.* 2008; 86:355–365. [PubMed: 18191123]
- Yoshida T, Ohno-Matsui K, Ichinose S, Sato T, Iwata N, Saido TC, Hisatomi T, Mochizuki M, Morita I. The potential role of amyloid beta in the pathogenesis of age-related macular degeneration. *J Clin Invest.* 2005; 115:2793–2800. [PubMed: 16167083]
- Yuan A, Farber EL, Rapoport AL, Tejada D, Denishkin R, Akhmedov NB, Farber DB. Transfer of microRNAs by embryonic stem cell microvesicles. *PLoS One.* 2009; 4(3):e4722. Epub 2009 Mar 6. [PubMed: 19266099]
- Zantema A, Verlaan-De Vries M, Maasdam D, Bol S, van der Eb A. Heat shock protein 27 and alpha B-crystallin can form a complex, which dissociates by heat shock. *J Biol Chem.* 1992; 267:12936–12941. [PubMed: 1618790]
- Zhou L, Xinling L, Koh SK, Xiaorong L, Beuerman RW. Quantitative Proteomic Analysis of Retina in Oxygen-Induced Retinopathy Mice using iTRAQ with 2D NanoLC-nanoESI-MS/MS. *JIOMICS.* 2011; 1:226–235.
- Zhu W, Qi X, Ren S, Jia C, Song Z, Wang Y. AlphaA crystallin in the pathogenesis and intervention of experimental murine corneal neovascularization. *Exp Eye Res.* 2012; 98C:44–51. [PubMed: 22465406]
- Zigler JS Jr, Zhang C, Grebe R, Sehrawat G, Hackler L Jr, Adhya S, Hose S, McLeoc DS, Bhutto I, Barbour W, Parthasarathy G, Zack DJ, Sergeev Y, Luttly GA, Handa JT, Sinha D. Mutation in the β A3/A1-crystallin gene impairs phagosome degradation in the retinal pigmented epithelium of the rat. *J Cell Sci.* 2011; 124:523–531. [PubMed: 21266465]

Figure 1.

Structural organization of α-crystallin protein. α-Crystallins have a crystallin core domain flanked by variable N-terminal domain and C-terminal extension. Three known phosphorylation sites on αB-crystallin are located in the N-terminal domain and can be activated by p44/42 and p38 MAPkinases as well as other yet to be determined pathways.

Figure 2.

Diagrammatic ribbon representation of the lowest-energy solid state NMR structure of the α B-crystallin dimer core domain and sites of the beta (β) sheet strands. Figure modified and redrawn from Jehle S., et al. Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers. *Nat Struct Mol Biol* 2010; 17: 1037–1042.

Figure 3.

Immunofluorescent localization of α B-crystallin in retina and in RPE (arrows) of a 129 S6/SvEvTac mouse (10 weeks of age). Abbreviations used are OS, outer segment; IS, inner segment, ONL, outer nuclear layer; OPL, outer plexiform layer, INL, inner nuclear layer and IPL, inner plexiform layer. Bar indicates 20 microns. For inset, bar indicates 10 microns.

Figure 4.

Expression of α B-crystallin in RPE associated with drusen from a patient with age-related macular degeneration. Confocal immunofluorescent image shows increased expression of α B-crystallin (FITC-green) in RPE (arrow), especially overlying drusen (*).

Autofluorescent signal (eg. from lipofuscin) was digitally subtracted from the image. DAPI nuclear counterstain (blue).

Figure 5.

Localization of α B-crystallin in mitochondria of RPE. Confocal micrographs showing α B-crystallin expression in human RPE mitochondria with and without H_2O_2 treatment.

Mitochondria tracker (Mito-Tracker; red) allowed visualization of mitochondria in the RPE (i-l). α B-Crystallin expression was upregulated with low dose H_2O_2 treatment indicated by an increase in fluorescent intensity. In composite images, the cellular distribution pattern of α B-crystallin was found to show an increase in mitochondrial localization (j). The staining for α B-crystallin decreased in mitochondria with increasing dose of H_2O_2 (k,l). Bar represents 50 μm . α B-Crystallin staining is green, mitochondria red, colocalization of α -crystallin, and mitochondria shown in yellow in merged panels. *Reproduced with permission from Yaung et al. alpha-Crystallin distribution in retinal pigment epithelium and effect of gene knockouts on sensitivity to oxidative stress. Mol Vis. 2007, 13:566–77.*

Figure 6.

Attenuation of retinal neovascularization in oxygen-induced retinopathy (OIR) in α B-crystallin knockout (KO) mice. In (a–h), FITC-Isolectin-B4 angiography in wild-type (a–d) and α B-crystallin KO (e–h) mice at P12 (a,e) and P17 (b–d, f–h) with OIR are shown. At P12, avascular areas are identified with arrows. In panel H, the α B-crystallin KO flat mount is seen at greater magnification with additional DAPI nuclear staining. Bars indicate 1 mm in panels a,b,e, and f; 200 μ m in panels c, d, and g; and 50 μ m in panel h. In panels i–l, hematoxylin and eosin staining in wild-type (i,j) and α B-crystallin KO (k,l) mice at P12 (i,k) and P17 (j,l) with OIR. At P12, occluded existing retinal vessels identified with white arrows. At P17, neovascular tufts identified with black arrows. Bars indicate 100 μ m in panels i–l. In panels m and n, immunofluorescent staining with isolectin-B4 (green) demonstrates prominent neovascularization (white arrows) in wild-type mice (M) and infrequent neovascularization in α B-crystallin KO mice (n). Bar indicates 50 μ m in panels m–p. “This research was originally published in *Blood*. Kase et al. *alphaB-crystallin regulation of angiogenesis by modulation of VEGF*. *Blood* 2010; 115: 3398–3406, © The American Society of Hematology.”

Figure 7.

Increased apoptosis in RPE cells from α -crystallin KO mice (**A,B**) and attenuation of apoptosis in ARPE-19 cells overexpressing αA - or αB -crystallin (**C**). RPE from αB -crystallin KO mice grown to confluency were treated with 100 μM or 200 μM H_2O_2 for 24 h and stained for apoptosis by TUNEL and quantified by flow cytometry. Cell death of RPE increased in a dose dependent manner and αA -crystallin KO (**A**) and αB -crystallin KO (**B**) RPE showed an increased sensitivity to apoptosis from H_2O_2 treatment compared to wild type cells. **C**. Protection from cell death in α -crystallin overexpressing ARPE-19 cells. Cells were incubated in serum free medium and cell death was analyzed by TUNEL staining. Blue: DAPI nuclear staining; Red: TUNEL positive cells. Bar indicates 20 microns. *Fig. A & B reproduced with permission from Yaung et al. alpha-Crystallin distribution in retinal pigment epithelium and effect of gene knockouts on sensitivity to oxidative stress. Mol Vis. 2007;13:566–77. Fig. C reproduced with permission from Sreekumar et al. Mechanism of RPE Cell Death in α -Crystallin Deficient Mice: A Novel and Critical Role for MRP1-Mediated GSH Efflux. PLoS ONE 7(3): e33420. doi:10.1371/journal.pone.0033420.*

Figure 8.

Accelerated CoCl₂-induced retinal degeneration in α A-crystallin KO and α B-crystallin KO mice, 96 hour post-injection. With 12 nmol (low dose) CoCl₂ α A-crystallin KO and α B-crystallin KO retina revealed no apparent change compared to wild type retina (panels A,D,G). At 33 nmol (intermediate dose), α A-crystallin KO and α B-crystallin KO retina showed selective degeneration in both inner nuclear layer and outer nuclear layers while the wild type retina appeared comparatively intact (panels B,E,H). At 60 nmol (high dose), retina from both α A-crystallin KO and α B-crystallin KO mice exhibited extensive degeneration in all layers, while the wild type retina showed early changes of retinal degeneration (panels C,F,I).

GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium. Bar indicates 75 microns. *Reproduced with permission from Yaung et al. Exacerbation of retinal degeneration in the absence of alpha crystallins in an in vivo model of chemically induced hypoxia. Exp Eye Res. 2008; 86:355–65.*

Figure 9.

Exogenously added recombinant human (rh) αB-crystallin protects RPE cells from apoptosis. (A) Human RPE cells were treated with 500 μM H₂O₂ or 500 μM H₂O₂ plus 25 μg/ml rhαB-crystallin for 3 h, and apoptosis was assessed by TUNEL staining and cleaved caspase 3 staining. Apoptosis and caspase 3 staining were significantly higher in H₂O₂-only treated cells when compared with cells co-treated with rhαB-crystallin and H₂O₂. (B) Quantification of TUNEL-positive cells. One and two asterisks indicate *p<0.05, ** p<0.01. Reproduced with permission from Sreekumar et al. *αB Crystallin Is Apically Secreted within Exosomes by Polarized Human Retinal Pigment Epithelium and Provides Neuroprotection to Adjacent Cells*. *PLoS ONE* 2010; 5(10): e12578.

Figure 10.

Evidence for secretion of αB-crystallin via exosomes. (A) Western blot analysis showing preferential secretion of αB-crystallin into the apical domain of RPE cells. Secretion was measured in polarized human RPE monolayers with high trans-epithelial resistance in apical and basolateral medium. (B) Evidence by transmission electron microscopy showing that αB crystallin is present in exosomes of RPE cells. Exosomes averaged 100 nm in size. Immunogold labeling of αB-crystallin (15 nm gold particles) within exosomes is shown by arrows. (C) Immunofluorescence staining of αB-crystallin in RPE monolayers showing predominant apical staining as compared to central and basal regions. (D) Co-localization of αB-crystallin (green) with the exosomal marker CD63 (red) in polarized RPE. *Panels A, C and D are reproduced with permission from Sreekumar et al. αB Crystallin Is Apically Secreted within Exosomes by Polarized Human Retinal Pigment Epithelium and Provides Neuroprotection to Adjacent Cells. PLoS ONE 2010; 5(10): e12578.*

Figure 11.

Protein-protein interaction of α -crystallin with methionine sulfoxide reductase A (MsrA) in retinas of patients with age-related macular degeneration and vascular endothelial growth factor (VEGF) in oxygen-induced retinopathy (OIR). (A) Immunohistochemical localization of α A-crystallin (red), α B-crystallin (red) and MsrA (green). Arrows indicate co-localization (yellow) of α A-crystallin or α B-crystallin and MsrA. *: Drusen. Bar indicates 50 microns. (B). Western blot for α B-crystallin, VEGF, and VEGFR-2 in anti-VEGF-A immunoprecipitates from wild-type and α B-crystallin KO mice at P12 and P17 in OIR. α B-Crystallin binds to VEGF-A at P12 and P17 in wild-type mice but not in α B-crystallin KO mice. Knockout of α B-crystallin does not affect VEGFR-2 binding to VEGF-A. (C). Localization of α B crystallin and VEGF in cultured RPE cells after hypoxia (a-d) - Expression of α B-crystallin and VEGF in cultured RPE cells after cobalt chloride (150 μ M) treatment for 6 hr (b-d). Nuclei are identified with DAPI (a; blue); along with staining for α B-crystallin (b; red) and VEGF (c; green). Double staining for α B-crystallin and VEGF is seen in merged (d; yellow). Bar indicates 20 microns. *Panel (A). Reproduced with permission from Sreekumar et al. Methionine sulfoxide reductase A: Structure, function and role in ocular pathology. World J. Biol. Chem.2011 2(184–192). Panels (B& C) “This research was originally published in Blood. Kase et al. alphaB-crystallin regulation of angiogenesis by modulation of VEGF. Blood 2010; 115: 3398–3406, © The American Society of Hematology.”*

Figure 12.

Extracellular localization of αB-crystallin in murine interphotoreceptor matrix in retinal tissue sections, and uptake of exogenous αB crystallin in RPE and photoreceptors *in vitro*. (A). Double staining is shown for interphotoreceptor retinoid-binding protein (IRBP, red) and αB-crystallin (green) demonstrating partial colocalization in the merged image. Arrows indicate yellow staining in the merged image for αB crystallin and IRBP in the inter photoreceptor matrix (IPM). (B) Transmission electron microscopy of murine retinal sections shows distribution of αB-crystallin (15 nm gold particles, arrows in the figure and in inset) in the IPM and photoreceptor inner (IS) and outer segments (OS). (C) Uptake of fluorescein labeled recombinant human (rh) αB-crystallin in the presence or absence of oxidative stress (150 or 500 μM H₂O₂). Nuclear and cytoplasmic uptake of αB-crystallin

(green) is seen after 1 h treatment which is more pronounced with 500 μM H_2O_2 . **(D)** Uptake of fluorescein labeled 25 $\mu\text{g/ml}$ rh α B-crystallin in photoreceptors of mouse retinal explant cultures co-treated with 500 μM H_2O_2 for 30 min (arrows in right panel and in inset). Unstressed control is shown in the left panel. INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; OS, outer segment.

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Scheme depicting novel roles of α B-Crystallin

Figure 13.

Cartoon depicting novel roles of α B-crystallin. Several cellular functions of α B-crystallin are highlighted followed by the mechanism of action of α B-crystallin marked in red. The diseases associated with the various functions are shown in green. DR, diabetic retinopathy; CNV, choroidal neovascularization; ROP, retinopathy of prematurity; AMD, age-related macular degeneration; PVR, proliferative vitreoretinopathy; EAE, autoimmune encephalomyelitis; CMA autophagy, chaperone-mediated autophagy.

Table 1Proteins interacting with α -crystallins

Functional categories	Crystallin	Interacting protein	Study Type (Cell/tissue)	References
Apoptosis – related	α A	Caspase-3	ARPE-19, Lens epithelial cells	Hu et al., 2012; Mao et al., 2004
		Caspase 6	Lens extract	Morozov and Wawrousek, 2006
		Bax	ARPE-19, Lens epithelial cells	Mao et al., 2004
		Bcl-X(S)	ARPE-19, Lens epithelial cells	Mao et al., 2004
		Protease-activated receptor-2 (PAR-2)	Astrocytes	Li et al., 2009
		Methionine sulfoxide reductase (MsrA)	Retina	Sreekumar et al., 2011
	α B	Caspase-3	ARPE-19, Lens epithelial cells	Hu et al., 2012; Kamradt, et al., 2001; Mao et al., 2001, 2004
		Bax	ARPE-19, Lens epithelial cells	Hu et al., 2012; Mao et al., 2004
		Bcl-X(S)	ARPE-19, Lens epithelial cells	Mao et al., 2004
		Bcl-2-associated athanogene (BAG)	Cardiomyocytes	Hishiya et al., 2011
		Protease-activated receptor-2 (PAR-2)	Astrocytes	Li et al., 2009
		Methionine sulfoxide reductase (MsrA)	Retina	Sreekumar et al., 2011
		HSP22	COS7 cells	Fontaine et al., 2005
		Hsp27	HeLa cells	Fu and Liang, 2002.
Apoptosis – related	α A and α B crystallin	Methionine sulfoxide reductase (MsrA)	Human lens epithelial cells	Brennan et al., 2009
Cytoskeletal	α A	Actin	Lens Epithelial cells	Maddala and Rao, 2005
		Fiber cell cytoskeleton complex	Bovine lens cortex	FitzGerald and Graham, 1991
	α B	Actin	Cardiac tissue; Physico-chemical	Bennardini et al., 1992; Ghosh et al., 2007b; Singh et al., 2007
		Peripherin	Lens cell homogenate; Optic nerve head-astrocytes	Djabali et al., 1997
		Vimentin	Lens cell homogenate; Optic nerve head-astrocytes	Djabali et al., 1997; Muchowski et al., 1999 Salvador-Silva et al., 2001
		Filensin (CP115)	Lens cell homogenate	Muchowski et al., 1999
		Desmin filaments	Cardiac tissue; Physico-chemical	Bennardini et al., 1992; Ghosh et al., 2007a
		α -synuclein	Physico-chemical	Rekas et al., 2004; Waudby et al., 2010
		Glial fibrillary acidic protein (GFAP)	Astrocytoma cells, Optic nerve head astrocytes	Salvador-Silva et al., 2001;

Functional categories	Crystallin	Interacting protein	Study Type (Cell/tissue)	References
				Wisniewski and Goldman, 1998
		Microtubules	L6E9 myoblasts cells, Lens epithelial cells, Oligodendroglial cell line	Bauer and Richter-Landsberg, 2006; Fujita et al., 2004; Xi et al., 2006
		Phakinin (CP49)	Lens cell homogenate	Muchowski et al., 1999
Cytoskeletal	α A and α B crystallin	Vimentin	Physiochemical	Nicholl and Quinlan, 1994
Growth factors	α B	Fibroblast growth factor 1	Physico-chemical	Edwards et al., 2001
		Platelet-derived growth factor (PDGF)	Cardiac myocytes	Shu et al., 2005
		FGF-2	Physico-chemical	Ghosh et al., 2007a
		NGF- β	Physico-chemical	Ghosh et al., 2007a
		VEGF	Physico-chemical, RPE cells	Ghosh et al., 2007a; Kase et al., 2010
Signaling	α A	α 6 integrin receptor complexes	Lens fractions	Menko and Andley, 2010
		E-cadherin	Lens epithelial cells	Menko and Andley, 2010
	α B	β -catenin	Len epithelial cells, physico-chemical	Ghosh et al., 2007a; Maddala and Rao, 2005
		Protein kinase C δ (PKC δ)	Retina	Kim et al., 2007
		F-box protein FBX4	HeLa cells	den Engelsman et al., 2003
		Cyclin D1	NIH3T3 cells/293T cells	Lin et al., 2006
		Kidney-specific cadherin-16	Kidney lysates	Thedieck et al., 2008
Amyloid associated	α A	Amyloid- β (A β) peptides	Physiochemical, PC12 cells	Santhoshkumar and Sharma, 2004; Tanaka et al., 2008
	α B	Amyloid- β (A β) peptides	Physico-chemical, Brain	Ecroyd and Carver, 2009; Raman et al., 2005; Shamma et al., 2011
Other Crystallins	α A	β A3-crystallin	HeLa cells	Gupta and Srivastava, 2009
		β B2 crystallin	HeLa cells	Fu and Liang, 2002
		C-crystallins	HeLa cells	Fu and Liang, 2002
	α B	α A crystallin	Physico-chemical	Sreelakshmi et al., 2004
		β A3-crystallin	HeLa cells	Gupta and Srivastava, 2009
		β B2 crystallin	HeLa cells	Fu and Liang, 2002
		β H crystallin	Physico-chemical	Ghosh et al., 2005
		γ D crystallin	Physico-chemical	Ghosh et al., 2005
		δ C-crystallins	HeLa cells	Fu and Liang, 2002

Functional categories	Crystallin	Interacting protein	Study Type (Cell/tissue)	References
Others	αA	Galectin-related interfiber protein (GRIFIN)	Intact lens	Barton et al., 2009
	αB	Insulin	Physico-chemical	Ghosh et al., 2007a
		Alcohol dehydrogenase	Physico-chemical	Ghosh et al., 2005
		Citrate synthase	Physico-chemical	Ghosh et al., 2005
		HSP22	COS7 cells	Fontaine et al., 2005
		Survival motor neuron (SMN) protein	HeLa cells	den Engelsman et al., 2005
		Intranuclear lamin A/C speckles	Myoblasts	Adhikari et al., 2004
		Splicing factor SC-35	Myoblasts	Adhikari et al., 2004
		IKK β	Myoblasts	Adhikari et al., 2011
	αA and αB crystallin	Vimentin	Physico chemical	Nicholl and Quinlan, 1994

Table 2Pathological Disorders Associated with α α -Crystallin

Disease	Species	Tissue/crystallin	Change in level	Reference (s)
Ocular Disorders				
Age-related macular degeneration	Human	Retina/ α A & α B crystallin	Increase	Nakata et al., 2005
Age-related macular degeneration	Human	Retina/ α B crystallin	Increase	De et al., 2007; Johnson et al., 2005
Diabetic retinopathy	Human	Retina/ α A crystallin	Increase	Kase et al., 2011
Proliferative diabetic retinopathy	Human	Epiretinal membranes/ α B crystallin	Increase	Dong et al., 2012
Retinoblastoma	Human	Retina/ α A crystallin	Increase	Kase et al., 2009; Mallikarjuna et al., 2010
Retinoblastoma	Human	Retina/ α B crystallin	Increase	Pineda et al., 1993
Leber Congenital Amaurosis	Human	Retina/ α A crystallin	Increase	Vorum et al., 2007
Sympathetic Ophthalmia	Human	Retina α A crystallin)	Increase	Kase et al., 2012
Experimental diabetes	Rat	Retina/ α A and α B crystallin	Increase	Fort et al., 2009; Kumar et al., 2005; VanGuilder et al., 2011
Experimental diabetes	Rat	Retina/ α A crystallin	Increase	Wang et al., 2007
Experimental diabetes	Mouse	Retina/ α A crystallin, α B crystallin	Increase	Losiewicz and Fort, 2011
Retinal degeneration	rd Mouse	Retina/ α B crystallin	Increase	Jones et al., 1998
Retinitis pigmentosa	RCS Rat	Retina/ α A crystallin	Decrease	Maeda et al., 1999
Staphylococcus aureus-induced endophthalmitis	Mouse	Retina/ α B crystallin	Increase	Whiston et al., 2008
Experimental Autoimmune uveitis	Mouse	Retina/ α A crystallin	Increase	Rao et al., 2008
Retinal ganglion cell (RGC) degeneration model	Rat	Retina/ α A & α B crystallin	Decrease	Munemasa et al., 2009
Retinal neovascularization	Mouse	Retina/ α A & α B crystallin	Decrease	Zhou et al., 2011
Retinal neovascularization	Mouse	Retina/ α B crystallin	Increase	Kase et al., 2010
Experimental axotomy induced retinal ganglion cell degeneration	Rat	Retina/ α A & α B crystallin	Decrease	Munemasa et al., 2009
Spontaneous equine recurrent uveitis	Horse	Retina/ α A crystallin	Increase	Hauck et al., 2007
Steroid induced ocular hypertension model	Rat	Retina/ α A crystallin	Decrease	Miyara et al., 2008
CoCl ₂ -induced retinal degeneration (intermediate dose)	Mouse	Retina/ α A & α B crystallin	Increase	Yaung et al., 2008
CoCl ₂ -induced retinal degeneration (High dose)	Mouse	Retina/ α A & α B crystallin	Decrease	Yaung et al., 2008
Light Injury	Rat	Retina/ α A & α B crystallin	Increase	Organisciak et al., 2011; Sakaguchi et al., 2003
Neurologic disorders				
Alexander disease	Human	Brain/ α B crystallin	Increase	Iwaki et al., 1989; Kato et al., 2001
Creutzfeldt-Jacob disease	Human	Brain/ α B crystallin	Increase	Rekas et al., 2004; Renkawek et al., 1992

Disease	Species	Tissue/crystallin	Change in level	Reference (s)
Alzheimer disease	Human	Brain/ α B crystallin	increase	Björkdahl et al., 2008; Renkawek et al., 1994
Parkinson disease	Human	Brain/ α B crystallin	Increase	Waudby et al., 2010
Olivary hypertrophy	Human	Brain/ α B crystallin	Increase	Fukushima et al., 2006
Pediatric Epilepsy	Human	Brain/ α B crystallin	Increase	Sarnat and Flores-Sarnat 2009; Sarnat et al., 2011
Amyloidotic polyneuropathy	Human	Brain/ α B crystallin	Increase	Magalhães et al., 2010
Multiple sclerosis	Human	Brain/ α B crystallin	Increase	Sinclair et al., 2005; van Noort et al., 1995
Experimental autoimmune encephalomyelitis (EAE)	Mouse	Brain/ α B crystallin	Increase	Ousman et al., 2007