

α B-crystallin as a promising target in pathological conditions – A review

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Abstract

Introduction and objective. α B-crystallin belongs to the ubiquitous family of small heat-shock proteins. It was discovered as a physiological protein of the eye lens, maintaining its liquid-like property. Furthermore, α B-crystallin was proved to play a bipolar role in both physiological and pathophysiological conditions. This review discusses current knowledge about the biology and genetics of α B-crystallin, and summarizes recent advances in understanding its role in ophthalmic and neurological disorders, as well as breast cancer, renal cancer and other malignancies.

State of knowledge. α -crystallins are established as important elements of the protein quality control network, and consequently their defects are related to multiple human diseases. New studies highlight α B-crystallin's involvement in proliferative diabetic retinopathy angiogenesis and point out its therapeutic potential in age-related macular degeneration. α B-crystallin is thought to be associated with the disease-causing protein aggregates, leading to its connection with such neurological disturbances as anaplastic astrocytoma, Parkinson disease, aging deficits in the peripheral nervous system and multiple sclerosis. In breast cancer, it was proven to be a marker of aggressive behavior and cerebral metastases. Strong expression of α B-crystallin promoted growth and migration of clear cell renal cell carcinoma cells and was correlated with lower overall survival rate. Considering other malignancies, its various roles were established in colorectal and gastric cancers, head and neck squamous cell carcinomas and osteosarcomas.

Conclusions. Further studies concerning α B-crystallin seem to be enormously promising, as they might improve our understanding of common human pathologies as well as contemporary diagnostics and treatment.

Key words

α B-crystallin, small heat-shock proteins, breast cancer, renal cell carcinoma

INTRODUCTION AND OBJECTIVE

α B-crystallin, called also HspB5, belongs to the ubiquitous family of small heat-shock proteins (sHsps) [1]. sHsps is a class of ATP-independent chaperones, whose characteristic feature is the presence of a conserved α -crystallin domain consisting of two β -sheets flanked by a non-conserved long N-terminal extension and a short C-terminal segment [2]. α B-crystallin is a part of the Class I of sHsps along with Hsp20, Hsp22 and Hsp27, which have been identified in various tissues – eye lens, muscles, heart, etc. [3]. Members of sHsps family play a pleiotropic role in maintaining protein homeostasis and, moreover, their role has been verified in protein folding diseases and cancers [4]. Data about wide substrate spectra and functions of sHsps *in vivo* remains unclear since most of our knowledge is based on *in-vitro* models [2].

Because of their connection to human diseases and novel protein dynamics, sHsps are an area of interest in biology and biochemistry [5]. α B-crystallin was discovered as a physiological protein of the eye lens maintaining its

liquid-like property [6]. Afterwards, scientists investigated its role in proliferative diabetic retinopathy and missense mutation in the α B-crystallin gene that leads to autosomal dominant congenital lamellar cataract [7, 8]. Recently, α B-crystallin has been identified as a marker of aggressive breast cancer behavior and resistance to neoadjuvant chemotherapy, regulator of angiogenesis, enhancer of cell migration and inductor of rapamycin-resistance in clear cell renal cell carcinoma (ccRCC) [9–11].

Due to the wide range of the role of α B-crystallin in the organism, further investigation is necessary. Nowadays, medicine faces new difficulties associated with the treatment of various diseases (including neural diseases) and cancers; thus, scientists need to search for new markers, and enhance knowledge about those which have been newly discovered. This review discusses the well-established knowledge about the biology and genetics of α B-crystallin, and summarized recent advances in understanding its role in ophthalmic and neurological disorders, as well as breast cancer, renal cancer and other malignancies. To-date, according to the best knowledge of the authors, similar reviews encompassing the described area have not been published.

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STATE OF KNOWLEDGE

Biology. α B-crystallin belongs to the family of sHsps, based on the presence of a conserved 90-residue-long α -crystallin domain [12]. α -crystallins form large oligomers with more than 20 subunits, tending to be extremely labile and rapidly exchange association and dissociation. Jehle *et al.* (2011) suggested the following organization of three-domain α B-crystallin:

- 1) an N-terminal domain of approximately 60 residues;
- 2) a central α -crystallin domain of about 90 residues involved in dimerization;
- 3) a C-terminal domain of 25 residues with hydrophobic motif I-X-I.

Interaction between two α -crystallin domains forms a dimer. Three dimers connected by their C-terminal regional define a hexameric unit, and higher-order multimers are formed by variable interactions involving the N-terminal region [13, 14]. HspB5 uses its structural plasticity to expose different binding interfaces [15].

Stress, disease or mutation may lead to protein aggregation representing a major threat to the whole organism. α B-crystallin as a part of sHsps poses an ATP-independent chaperone function and can bind partially unfolded proteins in order to prevent their accumulation. Thus, α -crystallins have proved to be important elements of the protein quality control network, and consequently, their defects cause multiple human diseases [5]. α B-crystallin is placed as the first responder to cell stress due to being capable of immediately binding unfolding proteins. Discussed substrate binding is facilitated by a huge increase in the available hydrophobic surface on the α -crystallins [16]. The potency of sHsps to suppress aggregation is correlated with their ability to form stable substrate complexes – HspB5 binds tightly to variety of proteins. In the study by Mymrikov *et al.* (2017), HspB5 bounded one of the highest numbers of different substrates (a total of 88) [2]. Research by Peschek *et al.* (2013) proved that the chaperone activity of α B-crystallin protected more than 300 proteins from aggregation, as shown using HeLa cells exposed to heat stress [17]. HspB5 preferentially binds relatively large (50–100kDa) and slightly acidic (pH 5.4–6.8) proteins. Noticeably, α -crystallins are suspected to be the most active and promiscuous sHsps in temperature-induced aggregation assays due to forming large oligomers, although, the ability to form large oligomer does not seem to influence reduction-induced aggregation [2, 18].

α B-crystallin has proved to play a crucial role in the biogenesis of multipass transmembrane proteins (TMPs) by assisting their folding from the cytosolic face of the endoplasmic reticulum (ER) [19]. Furthermore, Ciano *et al.* (2016) established that phosphorylation of serine, resident at position 59, finely regulates the chaperone activity of HspB5 with studied TMPs [20]. Recent studies demonstrated the competence of HspB5 to interact with membrane channels. Analysis by Huang *et al.* (2016) validated the interaction between $\text{Na}_v1.5$ (the pore-forming α subunit of the cardiac voltage-gated Na^+ channel complex) and α B-crystallin. The crystallin increases I_{Na} densities and, what is more, knock-out of its expression significantly decreases cell surface of $\text{Na}_v1.5$ [21]. In some studies, α B-crystallin showed affinity to Bax and Bcl-X_s – proapoptotic agents. HspB5 suppresses the mitochondrial translocation of these agents and, as a result,

prevents apoptosis. Regarding cancers cells, overexpression of HspB5 and its antiapoptotic function may be connected with faster progression and worse prognosis [22].

Genetics. Various mutations of *CRYAB* gene have been described and associated with human pathologies (Tab. 1). Ngo *et al.* (1989) localized the α B-crystallin gene at chromosome 11 locus q22.3–23.1 [23]. *CRYAB* gene is 3–4 kb long with three exons and an open reading frame of 175 codons [24]. Diverse expression of α B-crystallin between different tissues, both physiologically and under the influence of stress factors, requires sophisticated regulatory mechanisms at the transcriptional level. Dubin *et al.* (1991) studied the murine *CRYAB* gene and described a promoter expanding from position -661 to +44, as well as an upstream enhancer (at positions -426/–257) especially active in murine myoblasts and myotubes [25]. *CRYAB* contains a canonical heat shock promoter, which shows significant conservation of nucleotide sequence when compared between humans and rats [26]. Due to presence of the heat shock element (HSE), it is a target of heat shock transcription factors HSF1 and HSF4; nevertheless, the outcome of such interaction varies between different cell-types [27]. Moreover, Sadamitsu *et al.* (2001) linked HSF2 with increased expression of the *CRYAB* gene in human glioma U-251MG cells in response to high extracellular concentrations of potassium [28]. In addition to those highly universal HSE sequences, Jing *et al.* (2014) identified a 10-bp gene-specific promoter sequence (GPS), deletion of which resulted in the absence of α B-crystallin expression in transgenic mice [29]. Gopal-Srivastava *et al.* (1994) showed that proximal sequence (-164/+44) of the *CRYAB* gene is not sufficient for the expression of α B-crystallin in the skeletal muscle and cardiomyocytes, while allowing the expression of α B-crystallin in the lens of transgenic mice. This sequence is referred to as lens-specific regulatory region (LSR), and is activated synergically by paired box protein PAX6 and its second form PAX6(5a), which play a crucial role in eye development [30–32]. LSR is also targeted by retinoic acid receptor RAR and retinoid X receptor RXR [33]. In later studies, LSR was found to be also sufficient for *CRYAB* expression in corneal epithelial cells [34].

Enhancer of *CRYAB* gene is crucial for α B-crystallin expression in structures other than the lens and cornea. There is ongoing search for signaling pathways leading to the regulation of *CRYAB* enhancer activity, thus influencing α B-crystallin expression in various tissues. Several control elements have been described among enhancer sequences: α BE-1, α BE-2 and α BE-3, showing activity both in lens and muscle cell lines; MRF containing E-box, specific for the skeletal muscle and α BE-4 required for the expression of α B-crystallin in cardiomyocytes [35–37]. MRF control element, crucial for *CRYAB* transcription in skeletal muscles, is known to bind via E-box to the MyoD, myogenin or a different member of this protein family [35]. Increased *CRYAB* expression was shown in cardiomyocytes in response to biochemical stress stimuli [38]. In their study on mice with hypertrophic hearts, Manukyan *et al.* (2010) proposed a signaling pathway activating α BE-4 control element of *CRYAB* enhancer. They observed that transcription factors NFAT, Nished and STAT3 form a dynamic ternary complex in the presence of hypertrophic stimuli, and interact with the α BE4 promoter element, indicating the important role

Table 1. Mutations of *CRYAB* gene in combination with corresponding changes in the protein product and related diseases

| Report | Mutation | Probable impact on the resulting protein | AD/AR | Associated disease |
|---|------------------|--|-------|--|
| Chen <i>et al.</i> , 2009 [40] | R11H | alteration of pI and electrostatic potential, changed tertiary structure | AD | congenital nuclear cataract |
| Jiao <i>et al.</i> , 2015 [41] | R11C, R12C | distortion of the electrostatic potential | AR | cataract |
| Xia <i>et al.</i> , 2014 [42] | P20R | alteration of the stability and solubility | AD | PPC |
| Li <i>et al.</i> , 2008 [43] | P20S | decreased subunit-exchange rate and chaperone activity, increased ability to trigger apoptosis | AD | PPC |
| Del Bigio <i>et al.</i> , 2011 [44] | S21AfsX24 | highly truncated non-functional product | AR | infantile muscular dystrophy |
| Safieh <i>et al.</i> , 2009 [45], Khan <i>et al.</i> , 2010 [46] | R56W | disturbance in interaction with α A-crystallin | AR | juvenile cataract, predisposition for retinal degeneration |
| Sun <i>et al.</i> , 2011 [47] | R69C | damaging effect on highly conserved residues | AD | cataract |
| Sacconi <i>et al.</i> , 2012 [48] | D109H | affects an aspartate residue causing change in surface charge | AD | cardiomyopathy, MM, PPC |
| Fichna <i>et al.</i> , 2017 [49] | D109A | aggregates formation | AD | MM |
| Brodehl <i>et al.</i> , 2017 [50] | D109G | aggregates formation | AR | restrictive cardiomyopathy |
| Forrest <i>et al.</i> , 2011 [51] | S115PfsX14 | aberrant protein consisting of 127 residues | AR | infantile onset myofibrillar myopathy |
| Vicart <i>et al.</i> , 1998 [52] | R120G | aggregates formation | AD | desmin-related myopathy |
| Liu <i>et al.</i> , 2006 [8] | D140N | alterations in tertiary and/or quaternary structure; aggregates formation | AD | congenital lamellar cataract |
| Berry <i>et al.</i> , 2001 [53] | K150fs | aberrant protein consisting of 184 residues | AD | PPC |
| Selcen <i>et al.</i> , 2003 [54] | Q151X, P155RfsX9 | impairment of solubilization and chaperone activity | AD | MM |
| Pilotto <i>et al.</i> , 2006 [55]; Reilich <i>et al.</i> , 2010 [56] | G154S | damaging effect on highly conserved residues | AD | DC, late-onset distal vacuolar myopathy |
| Inagaki <i>et al.</i> , 2006 [57] | R157H | decreased binding to titin/connectin heart-specific N2B domain | AD | DC |
| Devi <i>et al.</i> , 2008 [58] | A171T | disturbance in the chaperone function | AD | lamellar cataract |
| Van der Smagt <i>et al.</i> , 2014 [59] | X176WfsX19 | elongation of the normal protein with 19 amino acid residues | AD | PPC, adult onset DC |

AD - autosomal dominant; AR - autosomal recessive, DC - dilating cardiomyopathy, MM - myofibrillar myopathy; pI - isoelectric point; PPC - posterior polar cataract

of calcineurin/NFAT and Jak/STAT signaling pathways in the transcription of *CRYAB* gene in cardiomyocytes [39].

Ophthalmic disorders. Despite being widely expressed in many tissues, α B-crystallin is the ubiquitous structural protein of lens and retina. It is considered to be soluble and obtain high concentration, enabling creation of the phenotype of transparency during development of the ocular lens [60, 61]. The non-crystallin/catalytic functions of this protein remain unknown; thus, on the basis of finding α B-crystallin in Golgi in the human glioblastoma cell line U373, Gangalum *et al.* (2009) investigated a Golgi-related function for this protein in the lens. They confirmed that the location of the Golgi determines the location of α B-crystallin in the lens and, what is more, the physical state of the Golgi determines its status - membrane bound or soluble [62, 63].

α B-crystallin was proven to be expressed in proliferative diabetic retinopathy (PDR) membranes and neovessels [64]. New studies highlight its involvement in PDR angiogenesis. Research by Chen *et al.* (2017) suggests a significant increase of this protein in the vitreous fluid of patients with PDR, and additionally investigated the association between α B-crystallin and VEGF with angiogenic activity [65]. Correlation between this crystallin and VEGF was previously proved in a study from 2016 in which Dong *et al.* stated that phosphorylation of α B-crystallin may play a role as a molecular chaperon for VEGF in the pathogenesis of epiretinal membranes in PDR [7].

α B-crystallin is being taken into consideration in the cataractogenesis. In 2016, Yang *et al.* identified a reduction of HspB5 mRNA and soluble protein levels in the lens

epithelium of age-related and congenital cataracts. What is more, the studied reduction was more significant in the congenital cataract, and it was assumed that differences in reduction might be one of the factors causing different cataract presentation [66]. Since cataract is a major cause of childhood blindness worldwide, scientists try to figure-out the genetic background of this disease. Javadiyan *et al.* (2016) described a case report in which they identified recurrent mutation in the *CRYAB* gene associated with inherited paediatric cataract in a South Australian family [67]. Song *et al.* (2018) described a cataract caused by novel mutation in another member of an α -crystallin family gene - *CRYAA* gene, in which an arginine was substituted at position 12 in the N-terminal region of α A-crystallin. Approximately half of all congenital cataracts are supposed to have a genetic basis, thus searching for this mutations seems to be crucial [68].

Given age-related macular degeneration (AMD), α B-crystallin is proven to have a therapeutic potential. Retinal pigment epithelial cells (RPE) play a crucial role in the pathogenesis of AMD since they are constantly exposed to photo-oxidative stress. α B-crystallin protects RPE from oxidative and ER stress injury and autophagy which plays a vital role in cellular homeostasis. A study by Kannan *et al.* (2016) indicated the presence of mRNA for HspB5 in exosomes from RPE cells; therefore, they may become suitable for drug delivery. In the future, these researchers want to precisely explore the therapeutic potential of exosomes, especially in retinal degenerative disorders [69]. On the contrary, mutation of the α B-crystallin R120G variant induces protein aggregation that takes part in AMD pathomechanism. In 2018, Cai *et al.* discovered that overexpression of

microRNA-29 reduces the formation of protein aggregates by mutant HspB5. In contrast, deficiency of miR-29 may lead to inhibition of autophagy and AMD progression. Regarding these discoveries, miR-29 overexpression seems to have therapeutic potential in AMD [70].

Neurological disorders. One of the most important cytoprotective mechanism enabling survival in times of recurring cellular stress events in the brain, is the upregulation of heat shock proteins, including α B-crystallin. Its expression was found in glia and neurons, and its overexpression was detected in certain cell types at pathophysiological conditions. α B-crystallin is said to be associated with the disease-causing protein aggregates, thus its connection with such neurological disturbances as anaplastic astrocytoma, Parkinson disease, aging deficits in the peripheral nervous system and multiple sclerosis [71–75].

Gliomas are the most common primary tumours of the brain and scientists are trying to figure out mutations implicated in gliomagenesis. One of them is isocitrate dehydrogenase (IDH) 1 mutation, resulting in gain-of-function enzymatic activity and associated with longer survival. Apparently, the impact of this mutation on protein expression is not well characterized; therefore, Avliyakov *et al.* (2014) investigated the differences in protein expression levels in tumours with and without IDH1 mutations. They investigated 34 differentially expressed proteins in mutant and wild type IDH1 tumours, including α B-crystallin. A novel C-terminal truncated form of α B-crystallin was identified as being highly expressed only in mutant astrocytomas. This finding establishes the role of α B-crystallin in gliomagenesis, but there is a need for further research to verify how exactly IDH1 mutation affects this process [72].

Another disease connected with the upregulation of α B-crystallin is Parkinson disease (PD). Iwaki *et al.* proved in 1992 that HspB5 is upregulated in PD brains in different cell types [76]. Due to the fact that PD is characterized by selective degeneration of dopaminergic neurons in substantia nigra, scientists try to establish the mechanism of this neurodegeneration. Since the discovery of HspB5 upregulation in PD, this has become one of the target points. One of the newest researches conducted by Liu *et al.* (2015) showed that α B-crystallin was markedly upregulated in the substantia nigra of PD patients and present in glia cell inclusions. They assumed that the aggregation of HspB5 contributes to the dysfunction of glial cells and neurodegeneration; nevertheless, they pointed out the need for further research to determine when α B-crystallin inclusions are formed, and how exactly this protein is sequestered into the glial inclusion [73].

α B-crystallin is constitutively expressed by the peripheral nervous system (PNS) axons and Schwann cells. It was proved to be important for recovery after PNS nerve injury [77]. In 2017, Lim *et al.* observed a reduction in α B-crystallin expression one day after injury; they therefore assumed that it is a negative regulator of early events, such as axon degradation, Schwann cell differentiation and proliferation. On the other hand, it was established as a positive modulator of late events – regeneration and remyelination, due the fact that its level increased 28 days after crush. What is more, the contribution of α B-crystallin to remyelination of peripheral axons was confirmed by its absence in attenuated myelin formation [78]. Furthermore, Lim *et al.* (2017) examined the

correlation between expression of HspB5 and aging deficits in mice PNS. First of all, they observed thinner myelin sheaths at 28 day after injury in mice without expression of α B-crystallin, compared to mice with expression, which proved α B-crystallin to be involved in remyelination. In both young and older animals, HspB5 may be important in ensuring optimal myelin thickness. What is more, a significant decrease was found in HspB5 after one month of mice age and therefore assumed that the crystallin was inversely correlated with the increased deficits typical of the aging PNS [74].

α B-crystallin and a few other small heat shock proteins have been shown in demyelinating plaques of multiple sclerosis (MS) brains [75]. The crystallin was established as one of the triggers responsible for microglia and macrophages activation, playing a crucial role in demyelination during MS. Bsibsi *et al.* (2014) suggest that INF- γ can act like a cofactor, reprogramming local microglia and macrophages, causing α B-crystallin-triggered release of TNF- α , IL-6, IL-12 and drive of demyelination and oxidative damage [79]. In 2015, van Noort *et al.* described therapeutic intervention in MS with α B-crystallin. HspB5 was applied in relapsing-remitting MS using doses sufficient to support its protective effects, but low enough to avoid triggering T-cell response. This proved the favourable safety profile of HspB5, and the MRI data demonstrated progressively suppressive effects on lesions development in patients, which was not observed in the placebo group [80].

Breast cancer. In the human mammary epithelial cells, overexpression of α B-crystallin leads to neoplastic-like changes by disrupting mammary acinar morphology. Moyano *et al.* (2006) observed that cells with overexpression of α B-crystallin had higher levels of total and phosphorylated ERK1/2, Akt, and p38. Additionally, overexpression conferred EGF- and anchorage-independent growth and enhanced MEK-dependently migration and invasiveness [81]. Afterwards, α B-crystallin proved to be a sensitive and specific marker for aggressive breast cancer behaviour [9]. In a study by Tsang *et al.* (2012) of 395 cases of breast carcinoma, α B-crystallin demonstrated high sensitivity and specificity as a triple negative breast cancer (TNBC) and basal-like breast cancer (BLBC) marker, and in 2011, Chan *et al.* highlighted it as an adjunct marker of mammary metaplastic carcinoma [82, 83]. In 2015, Kim *et al.* studied 82 breast tissue samples obtained from patients with stage IA – IIIC infiltrating ductal carcinoma. Their study showed both in the univariate and multivariate analysis of α B-crystallin expression a shorter overall survival rate (OS), suggesting that α B-crystallin is an independent prognostic factor of infiltrating ductal carcinoma [84]. Also, Kabbage *et al.* (2012) confirmed the upregulation of α B-crystallin in infiltrating ductal breast carcinoma [85].

Due to the fact that α B-crystallin is highly expressed in TNBC and BLBC, recent studies focused on the discovery of its molecular inhibitor. In 2014, Chen *et al.* investigated a small potent molecular inhibitor – NCI-41356, which disrupted the interaction between α B-crystallin and VEGF165. *In vitro* it triggered anti-tumour cell proliferation and invasive effects. Moreover, in *in vivo* breast cancer xenograft models it inhibited the tumour growth and vascularization [86].

For a long time, α B-crystallin has been known as a marker of lymph nodal involvement and metastases [87]. Only recently it proved to be a promising predictor of breast cancer

metastases to the brain. This connection was discovered in two independent studies. In 2014, Malin *et al.* verified an association between α B-crystallin expression and TNBC, and studied its high expression in breast cancer brain metastases. *In vitro* they proved that overexpression of α B-crystallin in TNBC enhanced adhesion to human brain microvascular endothelial cells, transendothelial migration and blood-brain barrier [88]. In 2015, Voduc *et al.* determined α B-crystallin expression as the strongest predictor of brain metastasis and the only independent predictor of brain metastasis as the first site of distant relapse. They suggested that α B-crystallin may become a marker for identifying patients with breast cancer who are at high risk connected with brain metastasis [89].

Renal cell carcinoma (RCC). α B-crystallin was found to be abundantly expressed in the epithelial tissues of healthy kidneys. Its amount increased from the renal cortex to the inner medulla, where it constitutes approximately 2% of the protein mass. α B-crystallin expression is not homogeneous, concentrated in thin limbs of Henle, proximal convoluted tubules and collecting ducts, while being absent in glomerular components [90, 91].

Michl *et al.* (2006) investigated the influence of environmental factors on α B-crystallin expression in rat kidneys and canine kidney cell culture. They found a strong positive correlation between the level of α B-crystallin expression and increasing osmolarity of the environment [90]. Thus, its role in the renal physiology is currently considered to be a cell-protective agent in the hyperosmotic environment of renal medulla, given the chaperone and anti-apoptotic activity of α B-crystallin [90, 92]. A different study showed that the level of α B-crystallin, among many other proteins, increased in the kidneys of mice fetuses exposed *in utero* to cigarette smoke [93]. Lou *et al.* (2016) conducted a study on mice and cultured renal proximal tubular cells with or without knockdown of heat shock factor 1 (HSF1) treated with nephrotoxic cisplatin. Exposure to cisplatin induced the expression of α B-crystallin in an Hsf1+ specimen, showing cytoprotective activity. HSF1 knockdown led to decreased α B-crystallin expression and susceptibility to the nephrotoxic activity of cisplatin [94]. These results suggest that α B-crystallin protects tubular cells against apoptosis while exposed to toxic agents, and that this activity is mediated by HSF1 [93, 94].

The presence of α B-crystallin in renal tissues provoked an investigation into its role in neoplastic processes occurring in the kidneys. α B-crystallin expression in RCC varies among different subtypes based on their place of origin [91]. The most common of them, clear-cell and papillary types, which originate from proximal convoluted tubules, were observed to show a strong expression of α B-crystallin [11, 91]. Chromophobe subtype of RCC develops from cortical collecting ducts and are reported to show no expression of α B-crystallin similarly to uroepithelial carcinomas. Thus, immunohistochemical staining for α B-crystallin may prove as a useful tool in differentiating between subtypes of RCC and uroepithelial carcinoma, especially in poorly differentiated cases [91].

Anti-apoptotic activity of α B-crystallin, crucial for proper functioning of cells, in a challenging environment of renal medulla, becomes a double-edged sword when neoplastic processes occur. Strong expression of α B-crystallin promoted growth and migration of ccRCC cells and was correlated

with lower OS in patients with clear-cell RCC [11]. On the contrary, Shi *et al.* (2004) associated a higher expression level of α B-crystallin with lower grade RCC tumors; nevertheless, the material included only 11 RCC samples [95].

α B-crystallin is suggested to be involved in the development of rapamycin resistance in patients diagnosed with RCC, and treated with mTOR targeted therapies [11]. However, different study have not confirmed a significant correlation with OS; therefore, further investigation is necessary [91].

The dieck *et al.* (2008) proposed another role of α B-crystallin in renal tissue, observing its co-localization with kidney-specific cadherin (Ksp-cad) at the basal side of collecting duct cells [96]. Ksp-cad belongs to a small family of 7D-cadherins that do not interact with cytoplasmatic catenins. It plays an important role in maintaining proper architecture of tissues by regulating cell adhesion. α B-crystallin was suggested to be a partner of Ksp-cad involved in its signaling pathways. In renal-cell carcinomas, reduced Ksp-cadherin expression, possibly due to dysregulation of its interaction with α B-crystallin, causes disturbed cell adhesion leading to distant metastases [97].

Other malignancies. The role of α B-crystallin in tumorigenesis is currently being studied in various types of malignancies, producing evidence of its crucial effect on the aggressiveness of the disease and its prognosis (Tab. 2). Shi *et al.* (2014) studied 118 samples obtained from patients with colorectal cancer and showed an increased level of α B-crystallin protein and mRNA, compared with healthy tissue. Moreover, the expression of HspB5 protein was significantly correlated with distant metastases and poor OS [98]. Another study by Shi *et al.* (2017), conducted on colorectal cancer cell line SW480 transfected with lentiviral vector inhibiting CRYAB expression, showed decreased expression of markers of epithelial-mesenchymal transition (EMT), such as E-cadherin, fibronectin and vimentin. Suppression of CRYAB gene not only inhibited EMT, but also increased apoptosis and G1 arrest, as well as reduced migration capability of the colorectal cancer cells [99]. Similar results were obtained by Li *et al.* (2017) in a study conducted on 70 samples of colorectal cancer; they found up-regulation of α B-crystallin and its positive association with tumour stage and level of matrix metalloproteinase-7 (MMP-7). They also provided evidence that EMT induced by α B-crystallin may be regulated by ERK, PI3K and p38 signaling pathways [100].

Wu *et al.* (2018) studied CRYAB C-802G (rs14133) polymorphism in association with colorectal cancer risk and survival. CG/GG genotype carriers were associated with increased risk of CRC in comparison of CC genotype carriers [101].

ERK signaling pathway involvement in increased aggressiveness and MPP-9 expression was described in osteosarcoma cell lines and samples obtained from biopsies. Similar to colorectal cancer, a high expression of CRYAB was correlated with shorter OS and earlier relapse in patients surgically-treated with osteosarcoma [102]. In a study by Wang *et al.* (2017), the up-regulation of α B-crystallin in patients with osteosarcoma was associated with a decreased level of serum miR-491, which is correlated with increased metastases, worse response to chemotherapy, and lower survival rate [103].

α B-crystallin's expression was also studied by Chen *et al.* (2018) in gastric cancer. Their results were similar to

Table 2. Collation of α B-crystallin's up-regulation and its role in various carcinomas

| Type of cancer: | Up-regulation of α B-crystallin: |
|--|--|
| Breast cancer | Marker of aggressive cancer behaviour [9]; |
| | marker of TNBC, BLBC [82]; |
| | marker of mammary metaplastic carcinoma [83]; |
| | independent negative prognostic factor of infiltrating ductal carcinoma – lower OS [84, 85]; |
| | marker of lymph nodal involvement and metastases [87]; |
| | the strongest predictor of brain metastasis and the only independent predictor of brain metastasis as the first site of distant relapse [89]. |
| Renal cell carcinoma (RCC) | Differentiation between subtypes of RCC and uroepithelial carcinoma [11, 91]: |
| | • clear-cell and papillary types – strong expression |
| | • chromophobe type and uroepithelial carcinoma – no expression; |
| | negative prognostic factor of ccRCC – lower OS [11]; |
| | probable involvement in development of rapamycin resistance in RCC [11]. |
| Colorectal cancer | Marker of distant metastases [98]; |
| | negative prognostic factor – lower OS [98]; |
| | association with higher tumor stage and level of MMP-7 [100]. |
| | Negative predictive factor – lower OS and earlier relapse in surgically- treated patients [102]; |
| Osteosarcoma | association with decreased level of serum miR-491 – which correlates with: |
| | • increased metastases • worse response to chemotherapy • lower OS [103]. |
| Gastric cancer | Marker of distant metastases [104]; |
| | negative prognostic factor – lower OS [104]. |
| | Negative prognostic factor – lower disease specific survival of patients with oral SCC [105]; |
| Head and neck squamous cell carcinomas (HNSCC) | correlation between expression of α B-crystallin and markers of hypoxia associated with therapeutic resistance of HNSCC and presence of distant metastases [107]. |
| Non-small cell lung cancer (NSCLC) | Negative predictive factor – lower OS [108]; |
| | lack of statistical significance between expression of α B-crystallin and patients' outcome [109]. |
| Ovarian cancer | Independent negative prognostic factor – lower OS, lower recurrence free survival (RFS) [110]; |
| | negative regulator of TRAIL and cisplatin-induced apoptosis on ovarian cancer cell survival [110]. |
| Hepatocellular carcinoma | Independent negative prognostic factor – lower OS [111]. |

previously mentioned studies, showing up-regulation of CRYAB in close correlation with distant metastases and poorer OS. Moreover, high expression of CRYAB promoted epithelial-mesenchymal transition of cancer cells via the NF- κ B signaling pathway, thus increasing cells migration and invasion abilities [104].

α B-crystallin has also been suggested as a negative prognostic marker in head and neck squamous cell carcinomas (HNSCC). In a study by Annertz *et al.* (2014) conducted on samples obtained from 55 patients with oropharyngeal and oral SCC, disease specific survival of patients with oral SCC was correlated with the presence of α B-crystallin expression (27.3 months for CRYAB-negative tumours, but only 7.5 months for CRYAB-positive tumours) [105]. Up-regulation of α B-crystallin was also described by Yilmaz *et al.* (2014) in laryngeal SCC, although without any correlation with the presence of distant metastases or tumour TNM stage [106]. A different study conducted on HNSCC showed correlation between the expression of α B-crystallin and markers of hypoxia, well-associated with therapeutic resistance of HNSCC and presence of distant metastases. Further *in vitro* studies on HNSCC cell lines indicated that α B-crystallin up-regulation in hypoxic environment is not associated with lack of oxygen, but more likely with ROS formation [107].

CONCLUSIONS

As the very first responder to cell stress and an extremely important element of protein quality control network, α B-crystallin plays a bipolar role both in physiological and pathophysiological conditions. Recent studies have proved its involvement in the development of many disorders, including cancers. Unfortunately, the connection between its biology, genetics and pathological condition remains unclear. Nevertheless, α B-crystallin seems to be an enormously promising protein, which may improve contemporary diagnostics and treatment. It has already been suggested as a potential therapeutic target in AMD and trialed in relapsing-remitting MS with positive reports.

REFERENCES

- Boelens WC. Cell biological roles of α B-crystallin. *Progress in Biophysics and Molecular Biology*. 2017; 115(1): 3–10.
- Mymrikov EV, Daake M, Richter B, Haslbeck M, Buchner J. The Chaperone Activity and Substrate Spectrum of Human Small Heat Shock Proteins. *J Biol Chem*. 2017; 292(2): 672–684. <https://doi.org/10.1074/jbc.M116.760413>
- Bakthisaran R, Tangirala R, Rao Ch M. Small heat shock proteins: Role in cellular functions and pathology. *Biochim Biophys Acta*. 2015; 1854(4): 291–319. <https://doi.org/10.1016/j.bbapap.2014.12.019>

4. Kriehuber T, Rattei T, Weinmaier T, Bepperling A, Haslbeck M, Buchner J. Independent evolution of the core domain and its flanking sequences in small heat shock proteins. *FASEB J*. 2010; 24(10): 3633–3642. <https://doi.org/10.1096/fj.10-156992>
5. Basha E, O'Neill H, Vierling E. Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. *Trends Biochem Sci*. 2012; 37(3): 106–117. <https://doi.org/10.1016/j.tibs.2011.11.005>
6. Liu Z, Wang C, Li Y, Zhao C, Li T, Li D, et al. Mechanistic insights into the switch of alphaB-crystallin chaperone activity and self-multimerization. *J Biol Chem*. 2018; 293(38): 14880–14890. <https://doi.org/10.1074/jbc.RA118.004034>
7. Dong Y, Dong Z, Kase S, Ando R, Fukuhara J, Kinoshita S, et al. Phosphorylation of alphaB-crystallin in epiretinal membrane of human proliferative diabetic retinopathy. *Int J Ophthalmol*. 2016; 9(8): 1100–1105. <https://doi.org/10.18240/ijo.2016.08.03>
8. Liu Y, Zhang X, Luo L, Wu M, Zeng R, Cheng G, et al. A novel alphaB-crystallin mutation associated with autosomal dominant congenital lamellar cataract. *Invest Ophthalmol Vis Sci*. 2006; 47(3): 1069–1075. <https://doi.org/10.1167/iov.05-1004>
9. Koletsis T, Stavridi F, Bobos M, Kostopoulos I, Kotoula V, Eleftheraki AG, et al. alphaB-crystallin is a marker of aggressive breast cancer behavior but does not independently predict for patient outcome: a combined analysis of two randomized studies. *BMC Clin Pathol*. 2014; 14: 28. <https://doi.org/10.1186/1472-6890-14-28>
10. Kase S, He S, Sonoda S, Kitamura M, Spee C, Wawrousek E, et al. alphaB-crystallin regulation of angiogenesis by modulation of VEGF. *Blood*. 2010; 115(16): 3398–3406. <https://doi.org/10.1182/blood-2009-01-197095>
11. Ho PY, Chueh SC, Chiou SH, Wang SM, Lin WC, Lee IL, et al. AlphaB-crystallin in clear cell renal cell carcinoma: tumor progression and prognostic significance. *Urol Oncol*. 2013; 31(7): 1367–1377. <https://doi.org/10.1016/j.urolonc.2012.01.015>
12. Kappe G, Leunissen JA, de Jong WW. Evolution and diversity of prokaryotic small heat shock proteins. *Prog Mol Subcell Biol*. 2002; 28: 1–17.
13. Jehle S, Vollmar BS, Bardiaux B, Dove KK, Rajagopal P, Gonen T, et al. N-terminal domain of alphaB-crystallin provides a conformational switch for multimerization and structural heterogeneity. *Proc Natl Acad Sci U S A*. 2011; 108(16): 6409–6414. <https://doi.org/10.1073/pnas.1014656108>
14. Braun N, Zacharias M, Peschek J, Kastenmuller A, Zou J, Hanzlik M, et al. Multiple molecular architectures of the eye lens chaperone alphaB-crystallin elucidated by a triple hybrid approach. *Proc Natl Acad Sci U S A*. 2011; 108(51): 20491–20496. <https://doi.org/10.1073/pnas.1111014108>
15. Mainz A, Peschek J, Stavropoulou M, Back KC, Bardiaux B, Asami S, et al. The chaperone alphaB-crystallin uses different interfaces to capture an amorphous and an amyloid client. *Nat Struct Mol Biol*. 2015; 22(11): 898–905. <https://doi.org/10.1038/nsmb.3108>
16. Mymrikov EV, Seit-Nebi AS, Gusev NB. Large potentials of small heat shock proteins. *Physiol Rev*. 2011; 91(4): 1123–1159. <https://doi.org/10.1152/physrev.00023.2010>
17. Peschek J, Braun N, Rohrberg J, Back KC, Kriehuber T, Kastenmuller A, et al. Regulated structural transitions unleash the chaperone activity of alphaB-crystallin. *Proc Natl Acad Sci U S A*. 2013; 110(40): E3780–3789. <https://doi.org/10.1073/pnas.1308898110>
18. Carra S, Rusmini P, Crippa V, Giorgetti E, Boncoraglio A, Cristofani R, et al. Different anti-aggregation and pro-degradative functions of the members of the mammalian sHSP family in neurological disorders. *Philos Trans R Soc Lond B Biol Sci*. 2013; 368(1617): 20110409. <https://doi.org/10.1098/rstb.2011.0409>
19. D'Agostino M, Lemma V, Chesi G, Stornaiuolo M, Cannata Serio M, D'Ambrosio C, et al. The cytosolic chaperone alpha-crystallin B rescues folding and compartmentalization of misfolded multipass transmembrane proteins. *J Cell Sci*. 2013; 126(Pt 18): 4160–4172. <https://doi.org/10.1242/jcs.125443>
20. Ciano M, Allocca S, Ciardulli MC, Della Volpe L, Bonatti S, D'Agostino M. Differential phosphorylation-based regulation of alphaB-crystallin chaperone activity for multipass transmembrane proteins. *Biochem Biophys Res Commun*. 2016; 479(2): 325–330. <https://doi.org/10.1016/j.bbrc.2016.09.071>
21. Huang Y, Wang Z, Liu Y, Xiong H, Zhao Y, Wu L, et al. alphaB-Crystallin Interacts with Nav1.5 and Regulates Ubiquitination and Internalization of Cell Surface Nav1.5. *J Biol Chem*. 2016; 291(21): 11030–11041. <https://doi.org/10.1074/jbc.M115.695080>
22. Mao YW, Liu YP, Xiang H, Li WC. Human α A- and α B-crystallins bind to Bax and Bcl-XS to sequester their translocation during staurosporine-induced apoptosis. *Cell Death & Differentiation* 2004; 11: 512–526.
23. Ngo JT, Klisak I, Dubin RA, Piatigorsky J, Mohandas T, Sparkes RS, et al. Assignment of the alpha B-crystallin gene to human chromosome 11. *Genomics*. 1989; 5(4): 665–669.
24. Wistow G. The human crystallin gene families. *Hum Genomics*. 2012; 6: 26. <https://doi.org/10.1186/1479-7364-6-26>
25. Dubin RA, Gopal-Srivastava R, Wawrousek EF, Piatigorsky J. Expression of the murine alpha B-crystallin gene in lens and skeletal muscle: identification of a muscle-preferred enhancer. *Mol Cell Biol*. 1991; 11(9): 4340–4349.
26. Srinivasan AN, Bhat SP. Complete structure and expression of the rat alpha B-crystallin gene. *DNA Cell Biol*. 1994; 13(6): 651–661. <https://doi.org/10.1089/dna.1994.13.651>
27. Jing Z, Gangalum RK, Lee JZ, Mock D, Bhat SP. Cell-type-dependent access of HSF1 and HSF4 to α B-crystallin promoter during heat shock. *Cell Stress Chaperones*. 2013; 18(3): 377–387. <https://doi.org/10.1007/s12192-012-0386-7>
28. Sadamitsu C, Nagano T, Fukumaki Y, Iwaki A. Heat shock factor 2 is involved in the upregulation of alphaB-crystallin by high extracellular potassium. *Journal of biochemistry*. 2001; 129(5): 813–820.
29. Jing Z, Gangalum RK, Mock DC, Bhat SP. A gene-specific non-enhancer sequence is critical for expression from the promoter of the small heat shock protein gene alphaB-crystallin. *Hum Genomics*. 2014; 8: 5. <https://doi.org/10.1186/1479-7364-8-5>
30. Gopal-Srivastava R, Piatigorsky J. Identification of a lens-specific regulatory region (LSR) of the murine alpha B-crystallin gene. *Nucleic Acids Res*. 1994; 22(7): 1281–1286.
31. Gopal-Srivastava R, Cvekl A, Piatigorsky J. Pax-6 and alphaB-crystallin/small heat shock protein gene regulation in the murine lens. Interaction with the lens-specific regions, LSR1 and LSR2. *J Biol Chem*. 1996; 271(38): 23029–23036.
32. Chauhan BK, Yang Y, Cveklóvá K, Cvekl A. Functional interactions between alternatively spliced forms of Pax6 in crystallin gene regulation and in haploinsufficiency. *Nucleic Acids Res*. 2004; 32(5): 1696–1709. <https://doi.org/10.1093/nar/gkh334>
33. Gopal-Srivastava R, Cvekl A, Piatigorsky J. Involvement of retinoic acid/retinoid receptors in the regulation of murine alphaB-crystallin/small heat shock protein gene expression in the lens. *J Biol Chem*. 1998; 273(28): 17954–17961.
34. Gopal-Srivastava R, Kays WT, Piatigorsky J. Enhancer-independent promoter activity of the mouse α B-crystallin/small heat shock protein gene in the lens and cornea of transgenic mice. *Mechanisms of Development*. 2000; 92: 125–134.
35. Gopal-Srivastava R, Piatigorsky J. The murine alpha B-crystallin/small heat shock protein enhancer: identification of alpha BE-1, alpha BE-2, alpha BE-3, and MRF control elements. *Mol Cell Biol*. 1993; 13(11): 7144–7152.
36. Liu S, Piatigorsky J. Regulation of mouse small heat shock protein α B-crystallin gene by aryl hydrocarbon receptor. *PLoS One*. 2011; 6(4): e17904. <https://doi.org/10.1371/journal.pone.0017904>
37. Gopal-Srivastava R, Haynes JL, 2nd, Piatigorsky J. Regulation of the murine alpha B-crystallin/small heat shock protein gene in cardiac muscle. *Mol Cell Biol*. 1995; 15(12): 7081–7090.
38. Xiao X, Benjamin IJ. Stress-response proteins in cardiovascular disease. *Am J Hum Genet*. 1999; 64(3): 685–690. <https://doi.org/10.1086/302305>
39. Manukyan I, Galatioto J, Mascareno E, Bhaduri S, Siddiqui MA. Cross-talk between calcineurin/NFAT and Jak/STAT signalling induces cardioprotective alphaB-crystallin gene expression in response to hypertrophic stimuli. *J Cell Mol Med*. 2010; 14(6B): 1707–1716. <https://doi.org/10.1111/j.1582-4934.2009.00804.x>
40. Chen Q, Ma J, Yan M, Mothobi ME, Liu Y, Zheng F. A novel mutation in CRYAB associated with autosomal dominant congenital nuclear cataract in a Chinese family. *Mol Vis*. 2009; 15: 1359–1365.
41. Jiao X, Khan SY, Irum B, Khan AO, Wang Q, Kabir F, et al. Missense Mutations in CRYAB are Liable for Recessive Congenital Cataracts. *PLoS one*. 2015; 10(9): e0137973. <https://doi.org/10.1371/journal.pone.0137973>
42. Xia XY, Wu QY, An LM, Li WW, Li N, Li TF, et al. A novel P20R mutation in the alpha-B crystallin gene causes autosomal dominant congenital posterior polar cataracts in a Chinese family. *BMC Ophthalmol*. 2014; 14: 108. <https://doi.org/10.1186/1471-2415-14-108>
43. Li H, Li C, Lu Q, Su T, Ke T, Li DW, et al. Cataract mutation P20S of alphaB-crystallin impairs chaperone activity of alphaA-crystallin and induces apoptosis of human lens epithelial cells. *Biochim Biophys Acta*. 2008; 1782(5): 303–309. <https://doi.org/10.1016/j.bbadis.2008.01.011>

44. Del Bigio MR, Chudley AE, Sarnat HB, Campbell C, Goobie S, Chodirker BN, et al. Infantile muscular dystrophy in Canadian aboriginals is an alphaB-crystallinopathy. *Ann Neurol*. 2011; 69(5): 866–871. <https://doi.org/10.1002/ana.22331>
45. Safieh LA, Khan AO, Alkuraya FS. Identification of a novel CRYAB mutation associated with autosomal recessive juvenile cataract in a Saudi family. *Mol Vis*. 2009; 15: 980–984.
46. Khan AO, Abu Safieh L, Alkuraya FS. Later retinal degeneration following childhood surgical aphakia in a family with recessive CRYAB mutation (p.R56W). *Ophthalmic Genet*. 2010; 31(1): 30–36. <https://doi.org/10.3109/13816810903452047>
47. Sun W, Xiao X, Li S, Guo X, Zhang Q. Mutation analysis of 12 genes in Chinese families with congenital cataracts. *Mol Vis*. 2011; 17: 2197–2206.
48. Sacconi S, Feasson L, Antoine JC, Pecheux C, Bernard R, Cobo AM, et al. A novel CRYAB mutation resulting in multisystemic disease. *Neuromuscul Disord*. 2012; 22(1): 66–72. <https://doi.org/10.1016/j.nmd.2011.07.004>
49. Fichna JP, Potulska-Chromik A, Miszta P, Redowicz MJ, Kaminska AM, Zekanowski C, et al. A novel dominant D109A CRYAB mutation in a family with myofibrillar myopathy affects alphaB-crystallin structure. *BBA Clin*. 2017; 7: 1–7. <https://doi.org/10.1016/j.bbaci.2016.11.004>
50. Brodehl A, Gaertner-Rommel A, Klauke B, Grewe SA, Schirmer I, Peterschroder A, et al. The novel alphaB-crystallin (CRYAB) mutation p.D109G causes restrictive cardiomyopathy. *Hum Mutat*. 2017; 38(8): 947–952. <https://doi.org/10.1002/humu.23248>
51. Forrest KM, Al-Sarraj S, Sewry C, Buk S, Tan SV, Pitt M, et al. Infantile onset myofibrillar myopathy due to recessive CRYAB mutations. *Neuromuscul Disord*. 2011; 21(1): 37–40. <https://doi.org/10.1016/j.nmd.2010.11.003>
52. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, et al. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet*. 1998; 20(1): 92–95. [10.1038/1765](https://doi.org/10.1038/1765)
53. Berry V, Francis P, Reddy MA, Collyer D, Vithana E, MacKay I, et al. Alpha-B crystallin gene (CRYAB) mutation causes dominant congenital posterior polar cataract in humans. *Am J Hum Genet*. 2001; 69(5): 1141–1145. <https://doi.org/10.1086/324158>
54. Selcen D, Engel AG. Myofibrillar myopathy caused by novel dominant negative alpha B-crystallin mutations. *Ann Neurol*. 2003; 54(6): 804–810. <https://doi.org/10.1002/ana.10767>
55. Pilotto A, Marziliano N, Pasotti M, Grasso M, Costante AM, Arbustini E. alphaB-crystallin mutation in dilated cardiomyopathies: low prevalence in a consecutive series of 200 unrelated probands. *Biochem Biophys Res Commun*. 2006; 346(4): 1115–1117. <https://doi.org/10.1016/j.bbrc.2006.05.203>
56. Reilich P, Schoser B, Schramm N, Krause S, Schessl J, Kress W, et al. The p.G154S mutation of the alpha-B crystallin gene (CRYAB) causes late-onset distal myopathy. *Neuromuscul Disord*. 2010; 20(4): 255–259. <https://doi.org/10.1016/j.nmd.2010.01.012>
57. Inagaki N, Hayashi T, Arimura T, Koga Y, Takahashi M, Shibata H, et al. Alpha B-crystallin mutation in dilated cardiomyopathy. *Biochem Biophys Res Commun*. 2006; 342(2): 379–386. <https://doi.org/10.1016/j.bbrc.2006.01.154>
58. Devi RR, Yao W, Vijayalakshmi P, Sergeev YV, Sundaresan P, Hejtmancik JF. Crystallin gene mutations in Indian families with inherited pediatric cataract. *Mol Vis*. 2008; 14: 1157–1170.
59. van der Smagt JJ, Vink A, Kirkels JH, Nelen M, ter Heide H, Molenschot MM, et al. Congenital posterior pole cataract and adult onset dilating cardiomyopathy: expanding the phenotype of alphaB-crystallinopathies. *Clin Genet*. 2014; 85(4): 381–385. <https://doi.org/10.1111/cge.12169>
60. Wistow GJ, Piatigorsky J. Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. *Annu Rev Biochem*. 1988; 57: 479–504. [10.1146/annurev.bi.57.070188.002403](https://doi.org/10.1146/annurev.bi.57.070188.002403)
61. Clark JL. Functional sequences in human alphaB crystallin. *Biochim Biophys Acta*. 2016; 1860(1 Pt B): 240–245. <https://doi.org/10.1016/j.bbagen.2015.08.014>
62. Gangalum RK, Bhat SP. AlphaB-crystallin: a Golgi-associated membrane protein in the developing ocular lens. *Invest Ophthalmol Vis Sci*. 2009; 50(7): 3283–3290. <https://doi.org/10.1167/iovs.08-3052>
63. Gangalum RK, Schibler MJ, Bhat SP. Small heat shock protein alphaB-crystallin is part of cell cycle-dependent Golgi reorganization. *J Biol Chem*. 2004; 279(42): 43374–43377. <https://doi.org/10.1074/jbc.C400371200>
64. Dong Z, Kase S, Ando R, Fukuhara J, Saito W, Kanda A, et al. AlphaB-crystallin expression in epiretinal membrane of human proliferative diabetic retinopathy. *Retina*. 2012; 32(6): 1190–1196. <https://doi.org/10.1097/IAE.0b013e318233ab9c>
65. Chen W, Lu Q, Lu L, Guan H. Increased levels of alphaB-crystallin in vitreous fluid of patients with proliferative diabetic retinopathy and correlation with vascular endothelial growth factor. *Clin Exp Ophthalmol*. 2017; 45(4): 379–384. <https://doi.org/10.1111/ceo.12891>
66. Yang J, Zhou S, Guo M, Li Y, Gu J. Different alpha crystallin expression in human age-related and congenital cataract lens epithelium. *BMC Ophthalmol*. 2016; 16: 67. [10.1186/s12886-016-0241-1](https://doi.org/10.1186/s12886-016-0241-1)
67. Javadiyan S, Craig JE, Souzeau E, Sharma S, Lower KM, Pater J, et al. Recurrent mutation in the crystallin alpha A gene associated with inherited paediatric cataract. *BMC Res Notes*. 2016; 9: 83. <https://doi.org/10.1186/s13104-016-1890-0>
68. Song Z, Si N, Xiao W. A novel mutation in the CRYAA gene associated with congenital cataract and microphthalmia in a Chinese family. *BMC Med Genet*. 2018; 19(1): 190. <https://doi.org/10.1186/s12881-018-0695-5>
69. Kannan R, Sreekumar PG, Hinton DR. Alpha crystallins in the retinal pigment epithelium and implications for the pathogenesis and treatment of age-related macular degeneration. *Biochim Biophys Acta*. 2016; 1860(1 Pt B): 258–268. <https://doi.org/10.1016/j.bbagen.2015.05.016>
70. Cai J, Zhang H, Zhang YF, Zhou Z, Wu S. MicroRNA-29 enhances autophagy and cleanses exogenous mutant α B-crystallin in retinal pigment epithelial cells. *Experimental Cell Research*. 2019; 374(1): 231–248.
71. Golenhofen N, Bartelt-Kirbach B. HspB5/ α B-Crystallin in the Brain. In: Tanguay R, Hightower L, editors. *The Big Book on Small Heat Shock Proteins*. 8: Springer; 2015
72. Avliyakov NK, Rajavel KS, Le KM, Guo L, Mirsadraei L, Yong WH, et al. C-terminally truncated form of alphaB-crystallin is associated with IDH1 R132H mutation in anaplastic astrocytoma. *J Neurooncol*. 2014; 117(1): 53–65. <https://doi.org/10.1007/s11060-014-1371-z>
73. Liu Y, Zhou Q, Tang M, Fu N, Shao W, Zhang S, et al. Upregulation of alphaB-crystallin expression in the substantia nigra of patients with Parkinson's disease. *Neurobiol Aging*. 2015; 36(4): 1686–1691. <https://doi.org/10.1016/j.neurobiolaging.2015.01.015>
74. Lim EF, Musa A, Frederick A, Ousman SS. AlphaB-crystallin expression correlates with aging deficits in the peripheral nervous system. *Neurobiol Aging*. 2017; 53: 138–149. <https://doi.org/10.1016/j.neurobiolaging.2017.01.006>
75. Cwiklinska H, Mycko MP, Luvsannorov O, Walkowiak B, Brosnan CF, Raine CS, et al. Heat shock protein 70 associations with myelin basic protein and proteolipid protein in multiple sclerosis brains. *Int Immunol*. 2003; 15(2): 241–249.
76. Iwaki T, Wisniewski T, Iwaki A, Corbin E, Tomokane N, Tateishi J, et al. Accumulation of alpha B-crystallin in central nervous system glia and neurons in pathologic conditions. *Am J Pathol*. 1992; 140(2): 345–356.
77. Ma CH, Omura T, Cobos EJ, Latremoliere A, Ghasemlou N, Brenner GJ, et al. Accelerating axonal growth promotes motor recovery after peripheral nerve injury in mice. *J Clin Invest*. 2011; 121(11): 4332–4347. <https://doi.org/10.1172/JCI58675>
78. Lim EF, Nakanishi ST, Hoghooghi V, Eaton SE, Palmer AL, Frederick A, et al. AlphaB-crystallin regulates remyelination after peripheral nerve injury. *Proc Natl Acad Sci U S A*. 2017; 114(9): E1707–E1716. <https://doi.org/10.1073/pnas.1612136114>
79. Bsibsi M, Peferoen LA, Holtman IR, Nacken PJ, Gerritsen WH, Witte ME, et al. Demyelination during multiple sclerosis is associated with combined activation of microglia/macrophages by IFN-gamma and alpha B-crystallin. *Acta Neuropathol*. 2014; 128(2): 215–229. <https://doi.org/10.1007/s00401-014-1317-8>
80. van Noort JM, Bsibsi M, Nacken PJ, Verbeek R, Venneker EH. Therapeutic Intervention in Multiple Sclerosis with Alpha B-Crystallin: A Randomized Controlled Phase IIa Trial. *PLoS One*. 2015; 10(11): e0143366. <https://doi.org/10.1371/journal.pone.0143366>
81. Moyano JV, Evans JR, Chen F, Lu M, Werner ME, Yehiely F, et al. AlphaB-crystallin is a novel oncoprotein that predicts poor clinical outcome in breast cancer. *J Clin Invest*. 2006; 116(1): 261–270. <https://doi.org/10.1172/JCI25888>
82. Tsang JY, Lai MW, Wong KH, Chan SK, Lam CC, Tsang AK, et al. alphaB-crystallin is a useful marker for triple negative and basal breast cancers. *Histopathology*. 2012; 61(3): 378–386. <https://doi.org/10.1111/j.1365-2559.2012.04234.x>
83. Chan SK, Lui PC, Tan PH, Yamaguchi R, Moriya T, Yu AM, et al. Increased alpha-B-crystallin expression in mammary metaplastic carcinomas. *Histopathology*. 2011; 59(2): 247–255. <https://doi.org/10.1111/j.1365-2559.2011.03882.x>

84. Kim MS, Lee HW, Jun SY, Lee EH. Expression of alpha B crystallin and BCL2 in patients with infiltrating ductal carcinoma. *Int J Clin Exp Pathol.* 2015; 8(8): 8842–8856.
85. Kabbage M, Trimeche M, Ben Nasr H, Hammann P, Kuhn L, Hamrita B, et al. Expression of the molecular chaperone alphaB-crystallin in infiltrating ductal breast carcinomas and the significance thereof: an immunohistochemical and proteomics-based strategy. *Tumour Biol.* 2012; 33(6): 2279–2288. <https://doi.org/10.1007/s13277-012-0490-4>
86. Chen Z, Ruan Q, Han S, Xi L, Jiang W, Jiang H, et al. Discovery of structure-based small molecular inhibitor of alphaB-crystallin against basal-like/triple-negative breast cancer development in vitro and in vivo. *Breast Cancer Res Treat.* 2014; 145(1): 45–59. <https://doi.org/10.1007/s10549-014-2940-8>
87. Chelouche-Lev D, Kluger HM, Berger AJ, Rimm DL, Price JE. alphaB-crystallin as a marker of lymph node involvement in breast carcinoma. *Cancer.* 2004; 100(12): 2543–2548. <https://doi.org/10.1002/cncr.20304>
88. Malin D, Strelakova E, Petrovic V, Deal AM, Al Ahmad A, Adamo B, et al. alphaB-crystallin: a novel regulator of breast cancer metastasis to the brain. *Clin Cancer Res.* 2014; 20(1): 56–67. <https://doi.org/10.1158/1078-0432.CCR-13-1255>
89. Voduc KD, Nielsen TO, Perou CM, Harrell JC, Fan C, Kennecke H, et al. alphaB-crystallin Expression in Breast Cancer is Associated with Brain Metastasis. *NPJ Breast Cancer.* 2015; 1. <https://doi.org/10.1038/nnpjbcancer.2015.14>
90. Michl M, Ouyang N, Fraek ML, Beck FX, Neuhofer W. Expression and regulation of alphaB-crystallin in the kidney in vivo and in vitro. *Pflugers Arch.* 2006; 452(4): 387–395. <https://doi.org/10.1007/s00424-005-0033-6>
91. Kim MS, Lee HW, Lee EH. Renal tumor with alpha B crystallin expression. *Int J Clin Exp Pathol.* 2015; 8(8): 9383–9389.
92. Dasgupta S, Hohman TC, Carper D. Hypertonic stress induces alpha B-crystallin expression. *Exp Eye Res.* 1992; 54(3): 461–470.
93. Canales L, Chen J, Kelty E, Musah S, Webb C, Pisano MM, et al. Developmental cigarette smoke exposure: liver proteome profile alterations in low birth weight pups. *Toxicology.* 2012; 300(1–2): 1–11. <https://doi.org/10.1016/j.tox.2012.04.016>
94. Lou Q, Hu Y, Ma Y, Dong Z. Heat shock factor 1 induces crystallin-alphaB to protect against cisplatin nephrotoxicity. *Am J Physiol Renal Physiol.* 2016; 311(1): F94-F102. <https://doi.org/10.1152/ajprenal.00201.2016>
95. Shi T, Dong F, Liou LS, Duan ZH, Novick AC, DiDonato JA. Differential protein profiling in renal-cell carcinoma. *Mol Carcinog.* 2004; 40(1): 47–61. <https://doi.org/10.1002/mc.20015>
96. 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(1): 145–153. <https://doi.org/10.1016/j.jmb.2008.02.008>
97. Horstmann M, Geiger LM, Vogel U, Schmid H, Hennenlotter J, Kuehs U, et al. Kidney-specific cadherin correlates with the ontogenetic origin of renal cell carcinoma subtypes: an indicator of a malignant potential? *World J Urol.* 2012; 30(4): 525–531. <https://doi.org/10.1007/s00345-011-0763-3>
98. Shi C, He Z, Hou N, Ni Y, Xiong L, Chen P. Alpha B-crystallin correlates with poor survival in colorectal cancer. *Int J Clin Exp Pathol.* 2014; 7(9): 6056–6063.
99. Shi C, Yang X, Bu X, Hou N, Chen P. Alpha B-crystallin promotes the invasion and metastasis of colorectal cancer via epithelial-mesenchymal transition. *Biochem Biophys Res Commun.* 2017; 489(4): 369–374. <https://doi.org/10.1016/j.bbrc.2017.05.070>
100. Li Q, Wang Y, Lai Y, Xu P, Yang Z. HspB5 correlates with poor prognosis in colorectal cancer and prompts epithelial-mesenchymal transition through ERK signaling. *PLoS One.* 2017; 12(8): e0182588. <https://doi.org/10.1371/journal.pone.0182588>
101. Wu X, Zheng YZ, Han B, Wang K. Alpha B-crystallin C-802G polymorphism and colorectal cancer susceptibility and clinical outcome in Chinese population. *Sci Rep.* 2018; 8(1): 11731. <https://doi.org/10.1038/s41598-018-29589-y>
102. Shi QM, Luo J, Wu K, Yin M, Gu YR, Cheng XG. High level of α B-crystallin contributes to the progression of osteosarcoma. *Oncotarget.* 2016; 7(8): 9007–9016. <https://doi.org/10.18632/oncotarget.6928>
103. Wang SN, Luo S, Liu C, Piao Z, Gou W, Wang Y, et al. miR-491 Inhibits Osteosarcoma Lung Metastasis and Chemoresistance by Targeting α B-crystallin. *Molecular therapy: the journal of the American Society of Gene Therapy.* 2017; 25(9): 2140–2149. <https://doi.org/10.1016/j.ymthe.2017.05.018>
104. Chen D, Cao G, Qiao C, Liu G, Zhou H, Liu Q. Alpha B-crystallin promotes the invasion and metastasis of gastric cancer via NF- κ B-induced epithelial-mesenchymal transition. *J Cell Mol Med.* 2018; 22(6): 3215–3222. <https://doi.org/10.1111/jcmm.13602>
105. Annertz K, Enoksson J, Williams R, Jacobsson H, Coman WB, Wennerberg J. Alpha B-crystallin – a validated prognostic factor for poor prognosis in squamous cell carcinoma of the oral cavity. *Acta Otolaryngol.* 2014; 134(5): 543–550. <https://doi.org/10.3109/00016489.2013.872293>
106. Yilmaz M, Karatas OF, Yuceturk B, Dag H, Yener M, Ozen M. Alpha-B-crystallin expression in human laryngeal squamous cell carcinoma tissues. *Head Neck.* 2015; 37(9): 1344–1348. <https://doi.org/10.1002/hed.23746>
107. van de Schootbrugge C, Schults EM, Bussink J, Span PN, Grenman R, Pruijn GJ, et al. Effect of hypoxia on the expression of alphaB-crystallin in head and neck squamous cell carcinoma. *BMC Cancer.* 2014; 14: 252. <https://doi.org/10.1186/1471-2407-14-252>
108. Cherneva R, Petrov D, Georgiev O, Slavova Y, Toncheva D, Stamenova M, et al. Expression profile of the small heat-shock protein alpha-B-crystallin in operated-on non-small-cell lung cancer patients: clinical implication. *Eur J Cardiothorac Surg.* 2010; 37(1): 44–50. <https://doi.org/10.1016/j.ejcts.2009.06.038>
109. Campbell-Lloyd AJ, Mundy J, Deva R, Lampe G, Hawley C, Boyle G, et al. Is alpha-B crystallin an independent marker for prognosis in lung cancer? *Heart Lung Circ.* 2013; 22(9): 759–766. <https://doi.org/10.1016/j.hlc.2013.01.014>
110. Volkman J, Reuning U, Rudelius M, Hafner N, Schuster T, Becker VRA, et al. High expression of crystallin alphaB represents an independent molecular marker for unfavourable ovarian cancer patient outcome and impairs TRAIL- and cisplatin-induced apoptosis in human ovarian cancer cells. *Int J Cancer.* 2013; 132(12): 2820–2832. <https://doi.org/10.1002/ijc.27975>
111. Tang Q, Liu YF, Zhu XJ, Li YH, Zhu J, Zhang JP, et al. Expression and prognostic significance of the alpha B-crystallin gene in human hepatocellular carcinoma. *Hum Pathol.* 2009; 40(3): 300–305. <https://doi.org/10.1016/j.humpath.2008.09.002>